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Başıbüyük, Maltepe, İstanbul, Turkey
Tel: +90 216 4144734, Faks: +90 216 4144731
E-mail: mmj@marmara.edu.tr

Publisher
Marmara University Press
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Original Articles

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Original Article

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Molecular analysis of human adenoviral keratoconjunctivitis cases: Results of a 2-year survey

Ayfer GUNER[1], Rabia CAN SARINOGLU[2], Fahri Onur AYDIN[3], Semra AKKAYA TURHAN[3], Mert Ahmet KUSKUCU[4], Ayse Ebru TOKER[3], Aysegul KARAHASAN YAGCI[1]

[1] Department of Medical Microbiology, School of Medicine, Marmara University, Istanbul, Turkey.
[2] Department of Medical Microbiology, School of Medicine, Bahcesehir University, Istanbul, Turkey.
[3] Department of Ophthalmology, School of Medicine, Marmara University, Istanbul, Turkey.
[4] Department of Medical Microbiology, School of Medicine, Cerrahpasa School of Medicine, Istanbul University, Istanbul, Turkey.

Corresponding Author: Rabia CAN SARINOGLU
E-mail: rabia.cansarinoglu@med.bau.edu.tr

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ABSTRACT

Objective: This study aimed to determine the adenovirus genotypes and their epidemiological features between January 2018 and November 2019, in Istanbul, Turkey.

Material and Methods: Conjunctival swab samples were obtained from patients who were clinically diagnosed with keratoconjunctivitis. Samples were screened with an Adeno Detector kit (Rapid Pathogen Screening, RPS Inc., South Williamsport, PA). Nucleic acid extraction and amplification were performed with the ADENOVIRUS ELITe MGB® kit in the ELITe In Genius instrument (Elitech Group, Torino, Italy). For subtyping of the strains, sequencing primers targeted the 'Hypervariable Region 7' (HVR-7) of the hexon gene were used. DNA sequence analysis (n:72) was performed with ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, USA), and subtyping was done by BLAST analysis.

Results: The median viral load in the samples (n: 77) was 7 log10 copies/mL (IQR: 4.5-7.4 log10 copies/mL). The clinical finding score was found to be significantly higher in the high viral load group (Adenovirus DNA≥6 Log 10 copies/mL) than in the low viral load group (Adenovirus DNA<6 Log 10 copies/mL) (p = 0.031).

Conclusion: Our study analyzing hAdV strains collected in 2018 and 2019 revealed that genotype 8 is the dominant type (94.0%) in our region. Molecular methods are very useful for future epidemiological studies and the selection of a vaccine strain.

Keywords: Adenovirus, Genotyping, Viral conjunctivitis, Epidemiological analyzes, Epidemic keratoconjunctivitis

1. INTRODUCTION

Human adenoviruses (hAdVs) are icosahedral, non-enveloped, double-stranded deoxyribonucleic acid (DNA) viruses that can cause an array of diseases including conjunctivitis, gastroenteritis, hepatitis, myocarditis, and pneumonia [1]. There are 51 serotypes of hAdVs based on neutralization assays which are classified into seven species HAdV-A to –G [2]. Over 60 types of the genotypes of hAdV have been identified based on sequence homologies as reported by Robinson et al. [3] or 88 as reported by Dhingra et al. [4].

Adenoviruses are the etiologic agents of the most commonly occurring ocular viral infections worldwide. Adenoviral infection in the eye can be in the form of epidemic keratoconjunctivitis (EKC), pharyngoconjunctival fever, and non-specific conjunctivitis [5]. Subgenus D consists of 32 serotypes including Ad8, Ad19, and Ad37, the main agents of EKC, and Ad9 and Ad15, which cause acute follicular conjunctivitis [6]. HAdV-associated follicular conjunctivitis or pharyngoconjunctival fever is relatively mild and short-term. In contrast, EKC is a highly contagious and more serious disease involving the cornea and conjunctiva, with potential long-term consequences on visual acuity leading to decreased quality of life and possible economic consequences. The modes of transmission are mainly through hand-to-eye contact, ocular secretions, respiratory droplets, and contact with medical instruments. Adenovirus is extremely hardy when deposited on environmental surfaces and may be detected on plastic and metal surfaces for more than 30 days [7]. Thus, the elimination of adenovirus from inanimate surfaces and ophthalmic instruments is essential in preventing outbreaks of EKC.

Various methods such as viral culture, antigen detection, serological tests, and nucleic acid detection can be used in the

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laboratory diagnosis of adenovirus infections. In recent years, the development and application of molecular methods using DNA amplification by polymerase chain reaction (PCR) have increased the sensitivity and enabled rapid diagnosis.

PCR primers for the hexon gene, fiber gene, or virally associated ribonucleic acid (RNA) I and II regions are usually preferred because they have some areas that are highly conserved among serotypes.

Identification of the adenovirus genotype is required to understand the geographical distribution of the virus and to improve the knowledge of the relation between a specific genotype and clinical presentation. Epidemiological studies determining genotypes can help to understand the nature of the epidemics and take effective infection control measures [8, 9]. Type-based hAdV surveillance has three objectives: 1) to monitor patterns of circulation for hAdV subtypes over time; 2) to assist with recognition and confirmation of outbreaks associated with circulating types; and 3) to inform the development or use of diagnostics tests, therapeutics, and vaccines [10]. Since, there is limited data about the subtyping of adenoviruses in Turkey, we analyzed hAdV strains obtained from conjunctival swab samples sent from the ophthalmology clinic.

2. PATIENTS and METHODS

Patients

Adult patients whose clinical signs and symptoms were compatible with acute adenoviral keratoconjunctivitis who tested positive for adenoviral antigen at the Marmara University Hospital Ophthalmology Clinic between January 2018 and November 2019 were included in the study. Clinical findings score for each patient was given by evaluation of the presence of eyelid edema, conjunctival injection, and chemosis by an ophthalmologist within the first 72 hours after the onset of symptoms. Rapid pathogen screening (RPS) adeno detector plus (RPS INC., Sarasota, Florida, USA) kit as a rapid immunoassay test for in vitro qualitative detection of adenoviral antigens (hexon protein) was used to detect antigens directly from human tears [11]. The test was performed by an ophthalmologist during a clinical examination.

Conjunctival swab samples (n: 77) obtained from patients clinically diagnosed as having keratoconjunctivitis and confirmed by a rapid antigen test were sent to the clinical microbiology laboratory for molecular analysis.

DNA extraction and real-time PCR Assay

DNA extraction was performed with the extraction cartridges Elite InGenius® SP 200 (ELITech Group, Torino, Italy) and adenovirus DNA was detected and quantified with ADENOVIRUS ELITe MGB® kit, in the fully automated ELITe In Genius TM instrument (ELITech Group, Torino, Italy) by using quantitative real-time PCR method according to the manufacturer's instructions. In each well, two amplification reactions are performed for a specific reaction of the Hexon protein gene and a specific reaction of the human beta Globin gene (Internal Control of inhibition). Measurement range of the assay is 250 to 25,000,000 copies of DNA. All specimens and viral DNA extracts were aliquoted and stored at – 80°C until further testing. One specimen per patient was selected.

Sequencing PCR

Primers targeted 605-629 base pairs including the conserved segments of "Hypervariable Region 7" (HVR-7) that differ according to different genotypes used [12]. The sequences of the sense and antisense primers were 5' – CTG ATG TAC TAC AAC AGC ACT GGC AAC ATG GG-3' and 5' – GCG TTG CGG TGG TGG TTA AAT GGG TTT ACG TTG TCC AT-3', respectively. The total volume of the reaction was 25 µl. Each reaction contained 2.5 µl 10X PCR buffer, 1.5 µl 25mM MgCl2, 0.2 µl of 25mM dNTP mix (Thermo Fisher Scientific, USA), 0.7 µl of each primer, 0,25 µl hot start Taq DNA polymerase (Thermo Fisher Scientific, USA), 16.15 µl distilled water and 3 µl DNA. PCR was performed using a T100™ Thermal Cycler (Bio-Rad, USA). The cycling parameters were as follows: an initial denaturing step of 15 min at 95˚C, 40 cycles consisting of denaturation at 95˚C for 1 min, annealing at 52˚C for 1 min, and elongation at 72˚C for 1min, with a final incubation at 72˚C for 10 min. After this, 5 µl of the reaction mixture was subjected to electrophoresis on a 1.5% agarose gel containing ethidium bromide. The bands were visualized with a UV transilluminator and then evaluated.

Sequence Analysis

ExoSAP-IT® mixture was used for the enzymatic removal of primers and dNTPs that were not bound in PCR products. The PCR purification reaction cycle was carried out at 37° C for 15 minutes and at 80° C for 15 minutes and the products were ready for the sequence stage. The sequencing reaction was performed by using the primers of HVR-7 sequencing PCR with T100™ Thermal Cycler (Bio-Rad, USA). Each 20 µl reaction contained 4 µl of Big Dye Terminator v 3.1 reaction mix (Thermo Fisher Scientific, USA), 2 µl of 5X sequence buffer (Thermo Fisher Scientific, USA), 0.7 µl of 5 pmol primers, 11.3 µl of distilled water and 2 µl of PCR product. The electrophoresis process of sequence products was performed in the automated DNA sequence analysis instrument of the ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, USA). The obtained hAdV DNA sequences were typed by BLAST analysis and the genotypes were identified by using the reference hAdV sequences of the NCBI [13]. The phylogenetic tree was drawn with the "neighbor-joining" method using the "MEGA-X" program including reference adenovirus sequences of genotypes 1 to 54 obtained from GenBank [14, 15]. The reliability of the phylogenetic tree was tested using the bootstrap test with 1000 replicates.

Statistical analyses

Statistical analysis was performed using SPSS v 22.0 (Statistical Package for Social Sciences, IBM, USA) software package. The distribution in SPSS according to Shapiro-Wilk was not normal. Therefore, non-parametric tests were used. The Fisher's Exact test was used in the analysis of categorized data. Data is

categorized according to the Adenovirus viral load detected in conjunctival swab samples. Adenovirus viral load was categorized as low (Adenovirus DNA<6 Log 10 copies/mL) and high (Adenovirus DNA≥6 Log 10 copies/mL). Total clinical finding score was compared between high and low viral load groups. The correlation between Adenovirus viral loads and total clinical finding scores was calculated using Spearman's rho test.

3. RESULTS

Demographic data and clinical finding scores of the patients were given in the Table. The median age (IQR) of the patients was 38 years (18-76 years) and 34 (45.3%) of them were male. Detection and quantification of hAdV DNA were performed in the conjunctival swab (n: 77) samples by quantitative real-time PCR. The median viral load in the samples was 7 log10 copies/mL (IQR: 4.5-7.4 log10 copies/mL) (Table). When the data was categorized according to the Adenovirus viral load, the clinical finding score was found to be significantly higher in the high viral load group than in the low viral load group (p = 0.031). The correlation between Adenovirus viral loads and total clinical finding scores was found to be statistically significant (r: 0.348, p = 0.002).

Table. *Characteristics of the patients (n=77)*

Age (years), median (IQR)	38 (18-76)
Sex, male (%)	33 (42.9)
Clinical finding score, median (IQR)	3 (1-7)
Adenovirus viral load in conjunctival swab samples (Log10 copies/mL), median (IQR)	7 (4.5-7.4)

Examination at the diagnosis
Slit lamp inspection
Eyelid edema (0 = absent, 1 = mild, 2 = medium, 3 = severe)
Conjunctival injection (0 = absent, 1 = mild, 2 = moderate, 3 = severe)
Chemosis (0 = absent, 1 = mild, 2 = medium, 3 = severe)
Clinical finding score was determined by evaluation of the presence of eyelid edema, conjunctival injection and chemosis.

After sequencing PCR, amplicons showing a band present in the gel electrophoresis (n: 72) were included in the DNA sequence analysis. Three samples could not be genotyped. Samples were numbered as day/month/year according to the date of admission to the hospital. A phylogenetic analysis based on the obtained sequences classified three genotypes (shown in Figs. 1-2). Three different genotypes, hADV-8 (97.1%, n=67), hADV-4 (1.4%, n=1) (sample no: 2.16_12.07.2019) and hADV-19 (1.4%, n=1) (sample no: 2.9_18.07.2019) were detected (Fig. 1). There were 4 clusters in genotype 8 (Fig. 2)

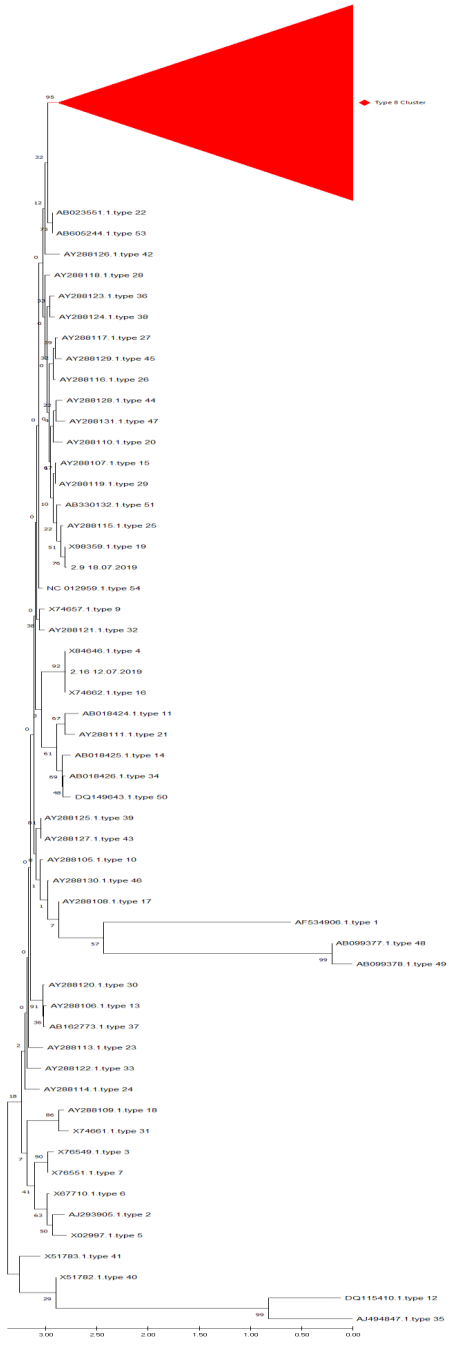

Figure 1. *Phylogenetic tree of hAdV strains*

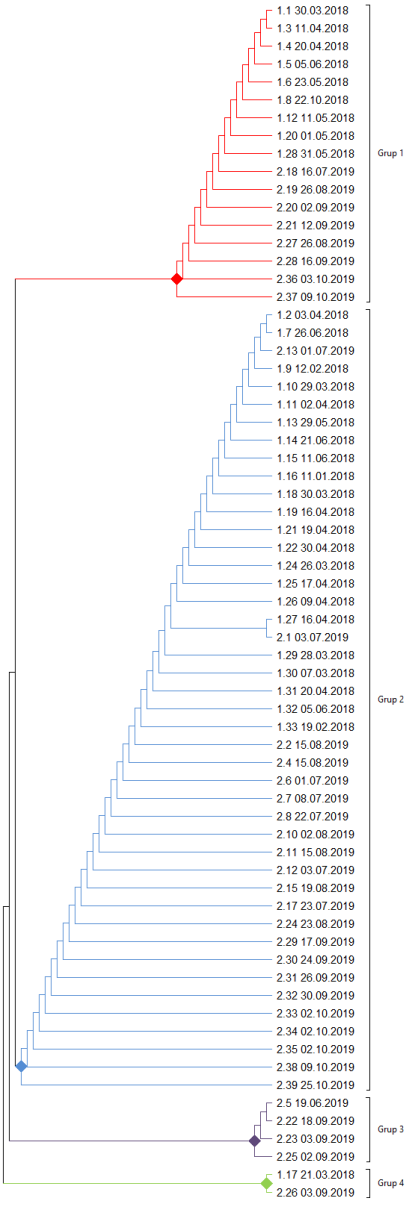

Figure 2. *Adenovirus type 8 cluster groups*

4. DISCUSSION

In our study, HAdV-8 was the most common genotype (97.1%) followed by hAdV-4 (1.4%) and hADV-19 (1.4%). The median viral load in the samples (n: 77) was 7 log10 copies/mL (IQR: 4.5-7.4 log10 copies/mL). The clinical finding score was found to be significantly higher in the high viral load group (Adenovirus DNA≥6 Log 10 copies/mL) than in the low viral load group (Adenovirus DNA<6 Log 10 copies/mL) (p = 0.031). HAdV-8 was the most common genotype (97.1%) followed by hAdV-4 (1.4%) and hADV-19 (1.4%).

The incidence of adenovirus eye infections varies worldwide. In the USA 20 million cases per year were reported whereas the incidence of adenovirus conjunctivitis cases is 0.2 to 0.8 per 100,000 population in Germany [16]. In Russia, more than 300,000 people are annually diagnosed with epidemic keratoconjunctivitis [17]. National Infectious Diseases Surveillance Center of Japan reports hAdV serotypes 3, 8, and 37 as the most common serotypes [18]. In Tunisia, North Africa between 2012 and 2013, hADV-8 (87.6%) was identified as the dominant genotype and this is followed by hADV-4 (6.8%), hADV-3 (3.5%) [19]. In the study conducted in patients with acute conjunctivitis in West India between 2013-2014, hAdV-8 (78.6%), hADV-37 (7.2%), hAdV – 3 (7.2%) and hAdV – 4 (7.2%) serotypes were detected [20].

There are very limited data on the ocular adenovirus infection and genotype distribution in Turkey. The first study published in 2010 reported hAdV genotypes 3, 4, and 8 from conjunctival swab samples (n: 9) collected in 2003 and 2004 [21]. An outbreak of adenovirus conjunctivitis in a neonatal intensive care unit was related to genotype 8 only that were obtained from 14 patients [22]. During the 5-year study period between 2006 to 2010, in adenovirus-positive patients with conjunctivitis (n: 101) type 8 was the dominant genotype (66.3%) followed by genotype 4 (24.7%) [23]. Tezcan et al. [24] included conjunctival swab samples from patients with acute conjunctivitis (n: 100) and from healthy individuals (n: 50) between September 2014-July 2017. A total of 5 genotypes were identified and the most common genotypes were hAdV-8 (n: 17, 63%) and followed by hAdV-53 (n: 4, 14.8%), hAdV-4 (n: 4, 14.8%).

Our study analyzing hAdV strains collected in 2018 and 2019 revealed that genotype 8 is the dominant type (94.0%) in our region. HAdV-8 has been the commonest genotype both in sporadic infections and during epidemics possibility related to high tropism for conjunctival cells produces severe clinical manifestations and pathologic alterations [25]. HAdV-8 is also an important cause of healthcare-associated outbreaks and has been associated with contaminated ocular instruments and ophthalmologic solutions [26]. We could not detect an epidemiologic relation among our isolates. The incidence of hAdV-4 in ocular infection is rare [23, 26]. HAdV – 4 outbreak was demonstrated in a group of fifty patients who had used the same swimming pool [27].

In a multicenter US study, hAdV was detected in 390 (78%) conjunctival swab samples of 500 participants with a 6.52 mean viral load in log10 copies/mL, and high viral load at presentation was associated with poorer clinical outcomes [28]. The mean viral load in our samples was 7 log10 copies/ mL. Measuring viral load in repetitive samples is important to detect the efficacy of treatment in viral blood-borne pathogens like HIV, CMV, and HBV. Latent adenovirus reactivation or transmission during transplantation can be responsible for disseminated infection and graft loss and viral load monitoring is essential to quickly set up an appropriate therapy [29]. We found a significant relationship between the severity of clinical findings and Adenovirus viral load (p=0.03).

Quantification of hAdV in ocular infections by measuring viral load in clinical samples could be investigated to evaluate the efficacy of the treatment, especially for patients having recurrent conjunctivitis.

Conclusion

To our knowledge, this is the first study from Turkey that detects hAdV viral load in clinical samples and analyses epidemiological relations between strains. Our study analyzing hAdV strains collected in 2018 and 2019 revealed that genotype 8 is the dominant type (94.0%) followed by hAdV-4 (1.4%) and hADV-19 (1.4%) in our region. We presented only a small amount of strains since the high cost was a limiting factor. Molecular methods are very useful for future epidemiological studies and the selection of a vaccine strain.

Compliance with the Ethical Standards

Ethical Approval: The study protocol was approved by the Institutional Review Board and the Ethics Committee of Marmara University School of Medicine (Protocol number: 4.01.2019-09).

Human and animal rights

The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. No animals were used in this research. All humans research procedures followed were in accordance with the standards set forth in the Declaration of Helsinki principles of 1975, as revised in 2008 (http://www.wma.net/en/20 activities/10ethics/10helsinki/). General written consent including the laboratory tests to be made was obtained from patients who admitted to our hospital as a routine application.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

AG: Doing PCR and sequencing tests, analyse results, manuscript preparation, RCS: Method determination, doing real time PCR tests, analyse real time PCR results, manuscript preparation, AKY: Method determination, analyse PCR and sequencing results, manuscript preparation, FOA: Conception and design, data collection, analysis and interpretation of data, critical revision of the manuscript, SAT: Conception and design, data collection, analysis and interpretation of data, critical revision of the manuscript, MAK: Analyse PCR and sequencing results, doing phylogenetic analyses, manuscript preparation, AET: Conception and design, data collection, analysis and interpretation of data, critical revision of the manuscript,

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Are neighborhood characteristics associated with physical activity levels among school children?

Gulin KAYA[1], Pinar AY[2], Seyhan HIDIROGLU[2]

[1] Public Health, Maltepe District Health Directorate, Istanbul, Turkey.
[2] Department of Public Health, School of Medicine, Marmara University, Maltepe, Istanbul, Turkey.

Corresponding Author: Pinar AY
E-mail: npay@marmara.edu.tr

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ABSTRACT

Objective: The objective of this study was to determine the prevalence of insufficient physical activity (IPA) and neighborhood characteristics associated with it, among school children. The impact of sociodemographic characteristics and ownership of electronic devices on physical activity (PA) were also evaluated.

Materials and Methods: This was a cross-sectional study carried out among 5-7th grade students attending schools and their parents. IPA was defined as having moderate-vigorous activity for <60 minutes per week. Sociodemographic factors, ownership of electronic devices and characteristics of the neighborhoods were also evaluated.

Results: A total of 334 students participated in the study. The prevalence of IPA was 79.3% (95%CI:75.0-83.7%). IPA was associated with ownership of mobile phones (OR:1.96, 95%CI:1.01-3.78), not being a member of a sports team (OR:2.83, 95%CI:1.21-6.58) and having ≤1 day of physical education classes at school (OR:2.10, 95%CI:1.08-4.09). Neighborhood characteristics were not associated with IPA (p>0.05).

Conclusion: The prevalence of IPA is alarmingly high among school children. The impact of neighborhood characteristics on PA might be obscured since both variables were measured subjectively. Devices related information/communication technologies increase IPA; we need to find novel ways to use these devices for PA promotion. There is also a need to increase structured PA opportunities.

Keywords: Physical activity, Inactivity, Students, Neighborhood characteristics

1. INTRODUCTION

Adequate physical activity (PA) is essential for physical, psychosocial and cognitive well-being of children and adolescents [1,2]. For this reason, World Health Organization (WHO) recommends at least an average of 60 minutes of moderate-to-vigorous intensity PA (MVPA) daily for children aged 5-17 years [2,3]. However studies indicate the majority of children cannot achieve the recommended activity levels [4,5]. Worldwide 81% of 11-17 year old school children are physically inactive, e.g. they are not able to perform at least 60 minutes of MVPA daily [5]. Research indicates that PA levels are also very low among Turkish children. In Turkey, the inactivity rate is reported as 82% among the 11-17 year olds [5].

Physical activity can be performed both in structured and unstructured contexts. While structured activity is a planned and a repetitive activity led often by an adult in physical education classes or sports teams, unstructured PA is a form of spontaneous activity (e.g. playing in parks, yards, streets or walking to school and back) which is not supervised by a trainer. Studies indicate that both physical and social characteristics of the neighborhood are among the determinants of PA levels, particularly for unstructured contexts [6-17]. A systematic review determined that the walkability level of the neighborhood, traffic density, proximity to recreation facilities, land-use mix, and residential density were associated with activity levels among children [6]. Social characteristics of the neighborhood also influence activity; the structure of social networks, trust and solidarity among neighbors/friends, cohesion of neighborhood residents and their sense of belonging, social norms in the neighborhood and

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safety are associated with PA levels in children [15-17]. Hence, both the physical and social attributes of the neighborhood shape the activity levels of children.

Studies evaluating the impact of environmental attributes on PA have been increasing throughout the recent years, yet most of this research comes from North America and Western Europe [6-17]. To our knowledge, there is only limited number of studies in Turkey assessing the impact of environmental factors on the activity level of children [18-20]. These studies highlight the importance of street network connectivity, parents' perceptions of condition of sidewalks and shade-casting street trees and also the green areas around the home as correlates of activity [18,19].

Every community is unique in terms of its neighborhood characteristics, so local studies are essential in identifying the environmental factors enabling PA. Hence, the main objective of this study was to determine the prevalence of insufficient PA (IPA) and neighborhood characteristics associated with it among children aged 9-13 years living in a district of Istanbul. The impact of sociodemographic characteristics and ownership of electronic devices on PA were also evaluated.

2. MATERIALS and METHODS

This was a cross-sectional study carried out among 5-7th grade students attending public schools and their parents in a district of Istanbul. We wanted to capture neighborhoods with diverse physical and social characteristics, so we used land values in determining the study area. In Turkey, land values are published yearly by the Revenue Administration Office [21]. Based on the median value of the district, neighborhoods with high and low land value were stratified. A total of four schools; two from neighborhoods with low and two from high land values were selected randomly. From each school one branch from the 5th, 6th and 7th grades were determined through simple random sampling.

Sample size was calculated as 344 assuming a difference of IPA prevalence of 15% (IPA of 55% and 70% in positive vs. negative neighborhood characteristics), an alpha error of 0.05 and a power of 80%.

Measures

IPA: The PA level was determined by the child's self-report through a question as the number of days in the previous week that the child performed MVPA for at least 60 minutes. The question was adapted from the Youth Risk Behavior Surveillance System (YRBSS) [22]. IPA was defined as less than 60 minutes of daily MVPA in accordance with the WHO recommendations [3].

Sociodemographic factors, ownership of electronic devices and neighborhood characteristics: Sociodemographic factors, ownership of electronic devices and characteristics of the neighborhood were evaluated by questionnaires applied to both the parents and children. The parental questionnaire assessed; age; gender; educational status of parents; ownership of a mobile phone, tablet of the child, presence of computer at home, electronic equipment in the child's room. The physical and social characteristics of the neighborhood were evaluated with the parental questionnaire through some selected questions adapted from the Built Environment and Active Play (BEAP) study [23]. Questions regarding the presence of a yard, living at a dead-end street, perceived safety of yards/streets were also included in the parental questionnaire. The child's questionnaire evaluated the number of days the child had participated in physical education classes during an average week and the number of sports teams that the child had played during the last 12 months.

The questionnaire was applied to the students in the classrooms under the supervision of the teachers. The students were asked to take home the parental questionnaire. Thus, the student-parent matching was achieved.

Statistical Analysis

Descriptive data were presented as mean, standard deviation, median, percentile and percentages. Categorical data were analyzed by the Chi-square and Fisher's tests. Binary logistic regression (backward LR method) was used to control for the confounders. Variables having a p-value less than 0.10 in the univariate analyzes, were evaluated for multicollinearity and added to the binary logistic regression models. Prevalence and ORs were reported with 95% Confidence Intervals (CIs). A p value of less than 0.05 was considered as statistically significant.

3. RESULTS

A total of 334 students from four schools participated in the study. The response rate was 96%. The median age was 11 years and 52.9% were girls. Among the respondents 70.4% were mothers, 25.5% were fathers and 4.0% were other family members. The characteristics of the children are presented on Table I.

Only 20.7% of the children (95% CI: 16.4-25.0%) reported sufficient PA; participating in daily MVPA for at least 60 minutes within the previous week (Figure 1). IPA prevalence was 79.3% (95%CI: 75.0-83.7%).

In the univariate analyzes; grade, having a mobile phone, presence of computer at home, participation in organized and unorganized sports activities, the number of days the child had participated in physical education classes in an average week, the number of sports teams the child had played during the last 12 months and presence of convenient yards for playing had a significant association with IPA ($p<0.05$ for all) (Tables II and III). The children who had parents with low level of acquaintance of neighbors had higher inactivity compared to the ones with better social ties ($p=0.054$).

Table I. *Sociodemographic characteristics and ownership of electronic devices*

		n	%
Gender	Girl	176	52.9
	Boy	157	47.1
Grade	5th	107	32.0
	6th	112	33.5
	7th	115	34.4
Mother's educational level	University	49	14.8
	High school	132	39.8
	Secondary school	43	13.0
	Primary school or lower	108	32.5
Father's educational level	University	71	21.5
	High school	132	40.0
	Secondary school	51	15.5
	Primary school or lower	76	23.0
Mobile phone	Yes	162	49.4
	No	166	50.6
Tablet	Yes	179	54.2
	No	151	45.8
Presence of a computer at home	Yes	255	77.5
	No	74	22.5
Presence of a television in the child's bedroom	Yes	46	14.0
	No	282	86.0
Presence of a computer in the child's bedroom	Yes	158	47.9
	No	172	52.1

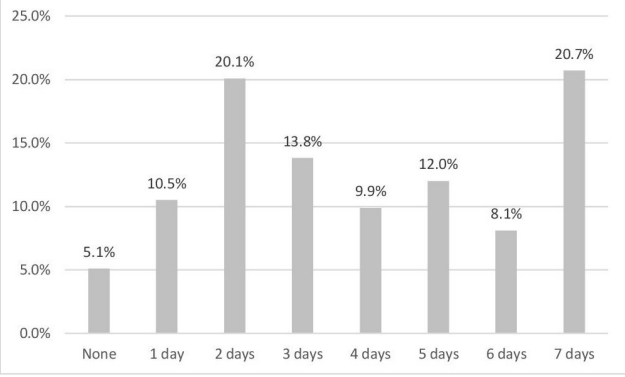

Figure 1. *Number of days in engaging MVPA for at least 60 Minutes within the previous week*

Table II. *The association of IPA with sociodemographic characteristics, ownership of electronic devices and participation in sports activities, univariable analysis*

		IPA		p value
		n	%	
Gender	Girl	144	81.8	0.283
	Boy	121	77.1	
Grade	5th	80	74.8	**0.044**
	6th	85	75.9	
	7th	100	87.0	
Mother's educational level	High school or higher	145	80.1	0.660
	Secondary school or lower	118	78.1	
Father's educational level	High school or higher	167	82.3	0.073
	Middle school or lower	94	74.0	
Mobile phone	Yes	138	85.2	**0.009**
	No	122	73.5	
Tablet	Yes	142	79.3	0.975
	No	120	79.5	
Presence of a computer at home	Yes	209	82.0	**0.029**
	No	52	70.3	
Presence of a television in the child's bedroom	Yes	35	76.1	0.566
	No	225	79.8	
Presence of a computer in the child's bedroom	Yes	128	81.0	0.411
	No	133	77.3	
Participation in organized sports activities	Yes	84	73.0	**0.037**
	No	178	82.8	
Participation in unorganized sports activities	Yes	118	73.8	**0.022**
	No	138	84.1	
Number of sports teams that the child had participated in the last 12 months	None	129	86.0	**<0.001**
	1	66	84.6	
	2	44	72.1	
	≥3	26	57.8	
Number of days with physical education classes in a week	≤1 day	132	87.4	**0.001**
	≥2 days	133	72.7	

IPA: insufficient physical activity

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Table III. *The association of IPA with physical and social neighborhood characteristics, univariable analysis*

		IPA		p value
		n	%	
PHYSICAL CHARACTERISTICS				
Presence of a yard convenient for playing	Agree	127	74.7	**0.046**
	Disagree	133	83.6	
Living on a dead-end street	Agree	17	73.9	0.429
	Disagree	244	80.3	
Presence of hilly areas	Agree	82	78.1	0.751
	Disagree	172	79.6	
Presence of heavy traffic	Agree	169	80.5	0.486
	Disagree	88	77.2	
Presence of traffic signs and pedestrian crossings	Agree	86	76.8	0.413
	Disagree	171	80.7	
Presence of high street connectivity	Agree	200	79.7	0.882
	Disagree	56	78.9	
Presence of convenient sidewalks	Agree	170	78.3	0.616
	Disagree	84	80.8	
Presence of bikeways	Agree	28	73.7	0.367
	Disagree	228	80.0	
Presence of sufficient lighting	Agree	190	78.5	0.601
	Disagree	65	81.3	
Presence of playgrounds and fields	Agree	147	77.8	0.477
	Disagree	111	81.0	
Presence of free sports halls	Agree	116	80.0	0.733
	Disagree	142	78.5	
SOCIAL CHARACTERISTICS				
There are knit neighborhood relationships	Agree	135	79.9	0.652
	Disagree	123	77.8	
Neighbors get along well	Agree	165	77.1	0.290
	Disagree	92	82.1	
Neighbors are willing to help each other	Agree	135	77.6	0.555
	Disagree	122	80.3	
Neighbors share similar social norms	Agree	117	82.4	0.159
	Disagree	139	76.0	
Neighbors can be trusted	Agree	135	81.8	0.182
	Disagree	122	75.8	
Parents' level of acquaintance of neighbors	None at all	17	89.5	**0.054**
	Slightly	64	88.9	
	Moderately	102	74.5	
	Well	78	76.5	

IPA: insufficient physical activity

All variables with a p-value less than 0.10 were evaluated by multivariate analysis. In the multivariable analysis, six predictors remained in the model and the model explained 18.8% of the variance. IPA was lower among children who were participating in unorganized sports activities (OR:0.54, 95% CI: 0.26-1.00). IPA was associated with ownership of mobile phones (OR: 1.96, 95% CI: 1.01-3.78), not being a member of a sports team (OR: 2.83, 95% CI: 1.21-6.58) and having ≤1 day of physical education classes at school (OR: 2.10, 95% CI: 1.08-4.09). The other variables which remained in the last step of the model were grade and presence of a computer at home, but they were not statistically significant (p>0.05 for both).

Table IV. *Factors associated with IPA, multivariable analysis*

		p value	IPA OR	95% CI	
				Min	Max
Grade	7th	0.112	1.93	0.86	4.34
	6th	0.700	0.86	0.41	1.81
	5th		reference		
Mobile phone	Yes	**0.046**	1.96	1.01	3.78
	No		reference		
Presence of computer at home	Yes	**0.055**	1.95	0.99	3.85
	No		reference		
Participation in unorganized sports activities	Yes	**0.051**	0.54	0.26	1.00
	No		reference		
Number of sports teams that the child had participated in the last 12 months	None	**0.016**	2.83	1.21	6.58
	1	**0.037**	2.79	1.06	7.33
	2	0.640	1.26	0.477	3.34
	≥3		reference		
Number of days with physical education classes in a week	≤1 day	**0.029**	2.10	1.08	4.09
	≥2 days		reference		

Variables included in the model: Gender, grade, father's educational level, mobile phone, presence of computer at home, participation in unorganized sports activities, participation in organized sports activities, number of sports teams that the child had participated in the last 12 months, number of days with physical education classes in a week, presence of a yard convenient for play and parents' level of acquaintance of neighbors.

IPA: insufficient physical activity, OR: odds ratio, CL: confidence interval

4. DISCUSSION

Our survey revealed that four fifths of the children had IPA, which is very similar to the rates reported by WHO and other studies [4,5,24]. These are alarmingly high rates given that inactivity is an important determinnat for the development of coronary heart disease, type 2 diabetes, breast and colon cancer and premature death during adulthood [25]. There is an urgent need to develop policies and programs to adress inactivity among schoolchildren in Turkey.

Studies show that gender is associated with activity among children and girls are less physically active [4,5,24,26]. We also observed that IPA prevalance was higher among girls compared to the boys (81.8% vs 77.1%), yet multivariable analyzes did not show a statistically significant association (p>0.05). This might be related to the limited sample size and thus to type 2 error in our study.

Research indicates that youth become more inactive with increasing age [26,27]. WHO reported the highest prevalence of PA among children aged 11-13-15 was in the 15-year-old group in the European Region [26]. Similarly our study revealed that the prevalence of IPA increased with grade in the univariate analyses. However, this association lost its significance in the

multivariate model. We suggest that this finding was related to the mobile phone ownership; mobile phone ownership incresed with grade (data not shown) and having a mobile phone was an independent predictor of inactivity. So when the mobile phone ownership was controlled in the multivaraite model, age lost its significance.

The factors associated with IPA were mainly related to the presence of electronic devices. In the multivariable analysis; having a mobile phone and presence of a computer at home each increased the OR of inactivity almost two folds. This finding is in line with other studies [28-33]. Use of electronic devices is very prevalent and their problematic use serves as a critical barrier for achieving PA among schoolchildren. We suggest that children use mobilephones and computers for mostly sedentary activities (e.g. watching movies, playing games and listening to music) [28]. We cannot eliminate the use of these devices totally, so we need to find novel methods to promote PA through using electronic devices.

Structured programs carried out both in the community and in schools are a good opportunity for increasing PA levels among children. In a systematic review, PA was shown as being positively associated with community sports participation in the 13-18 age group [27]. A cross-sectional study of 1223 children aged 8-9 years in 47 schools in the UK found that children who had participated in school sports teams 3-4 days a week and those who had participated in out-of-school sports teams 5 days a week had more MVPA than those who did not [34]. Similarly physical education classes held at schools had an impact on activity. In a study conducted with 17 776 adolescents, it was found that attending physical education classes 1-4 times a week increased MVPA by 1.21 times and attending five times a week increased by 2.21 times [35]. Another study conducted with adolescents in Brazil revealed that not attending physical education classes was a risk factor for physical inactivity [36]. Our analysis also revelaed that both the number of sports teams that the child had participated in the last 12 months and the number of physical education classes that the child had attended in a week were associated with PA. While the number of physical education classes per academic year in Denmark, Portugal, Germany and France were reported to be 60, 90, 85 and 108 hours respectively, it was 24 hours in Turkey [37]. School-based interventions as motivating children to participate in sports teams and increasing physical education classes seem as practical and achievable strategies to combat IPA.

Previous research indicates an association of activity levels with both physical and social characterists of the meighborhood. A systematic review of 103 studies examining the relationship between PA and environmental factors in children and adolescents reported that PA was associated with objective measures of walkability, traffic speed/volume, access/proximity to recreation facilities, land-use mix, and residential density [6]. In a study conducted with children aged 6-11 years and their parents in the United States; the parent reported proximity to the play areas was associated with both accelerometery MVPA and the parent reported PA. In the same study, the lower street connectivity and higher neighborhood aesthetics, safety from crime and walk and cycle facilities were positively correlated with reported PA [13]. In the BEAP study conducted in the United States, the parents of physical active children reported higher esthetics, active play areas, walkability and safety of the neighborhood than parents of non-active children [23]. Studies in Turkey indicated that some neighborhood characteristics as street network connectivity, condition of sidewalks, shade-casting street trees and also the green areas were important correlates of activity [18,19]. In our survey, we did not find any statistically significant association between the physical and social characteristics of the neighborhood and the PA levels of children. Although, the prevalence of IPA was lower in the families who had reported presence of available yards for playing in the univariable comparisons, this association lost its significance in the multivariate analysis. However our findings should be interpreted with caution; some associations might be obscured because we measured both the neighborhood characteristics and PA levels only subjectively, and also we did not determine PA as spesific domains of walking, transportation-related PA and leisure-related PA.

Our study is one of the few studies conducted in our country that examine the PA of school children according to the WHO recommendations. Still we have some limitations; the PA levels have been evaluated based on self report, which is a subjective measurement. There might also be recall bias in reporting the PA levels of the previous week. Another limitation is that our data were collected in February-April, we are aware that PA levels might be different in warmer months. We assessed the perceived physical and social characteristics of the neighborhoods by the parental reports, which is also a subjective measure. In addition some variables that might be predictors of IPA as parents' PA levels, parental support or peer support for PA, children's self-efficacy and psychological, cognitive conditions were not evaluated in our study [38,39].

The prevalence of IPA was considerably high among schoolchildren. Devices related to information and communication technologies as mobile phones and computers increased the IPA. Since it seems unrealistic to keep children totally away from mobile phones and computers, we need to find innovative ways to use these devices for PA promotion. We also showed that organized activities were more important in determining PA behavior compared to the unstructured ones. So the effectiveness of increasing structured physical activity levels at schools by utilizing sports teams and physical education classes should be evaluated in future studies for Turkey.

Compliance with Ethical Standards

Ethical Approval: The study was approved by the Marmara University, School of Medicine Ethics Committee (Number: 09.2016.569) and the Provincial Directorate of Ministry of Education This study was conducted in accordance with the Declaration of Helsinki. Informed consents were obtained from both the students and their parents.

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Authors' Contribution: GK, PA and SH: Study conception and design, GK and SH: Data collection, GK and PA: Analysis and interpretation of results, GK and PA: Draft manuscript preparation. All authors reviewed the results and approved the final version of the manuscript.

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The failure on the effectiveness of formalin on cadaver disinfection and alternative methods

Ozgur YANILMAZ[1], Mehmet Mucahit GUNCU[2], Mehmet Burak AKSU[3], Mazhar OZKAN[4], Umit Suleyman SEHIRLI[4]

[1] Medical Microbiology Laboratory, Marmara University, Pendik Training and Research Hospital, Pendik, Istanbul, Turkey.
[2] Department of Microbiology and Medical Microbiology, Institute of Health Sciences, Marmara University, Maltepe, Istanbul, Turkey.
[3] Department of Medical Microbiology, School of Medicine, Marmara University, Maltepe, Istanbul, Turkey.
[4] Department of Anatomy, School of Medicine, Marmara University, Maltepe, Istanbul, Turkey.

Corresponding Author: Ozgur YANILMAZ
E-mail: dryanilmaz@gmail.com

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ABSTRACT

Objective: It was aimed to identify the contaminant and determine the alternative disinfectant detection in the microbial growth observed in various parts of the cadaver stored in the formalin tank in the dissection laboratory of Marmara University Anatomy Department. We also performed a literature review of this unusual pathogen.

Materials and Methods: Swab samples were inoculated on agar mediums. After incubation, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis was used to identify the isolate from the detected uniform colonies. Sample solution from the cadaver tank, freshly prepared 5% formalin and 0.55% ortho-phthalaldehyde were used to determine the disinfectant sensitivity of the isolate.

Results: According to 16s rDNA sequence analysis, it was concluded as *Skermanella aerolata* with 99% similarity. In the disinfectant susceptibility test, it was observed that *S. aerolata* and control bacteria could grow in 5% formalin taken from the cadaver tank. No growth was detected in other disinfectants.

Conclusion: To prevent cadaver contamination in anatomy laboratories, the quality control of the embalming solutions and indoor air filtration of the dissection rooms should be checked at regular intervals. Members of *Skermanella* genus have been identified as environmental organisms in several studies, however, recent researches reported this bacterium as a human pathogen.

Keywords: *Skermanella aerolata*, Cadaver, Anatomy, Formalin.

1. INTRODUCTION

Human cadavers are essential educational and research materials for students and anatomists in medical anatomy education.

The very first proofs for use of human cadavers to examine the human body can be traced back to ancient Egypt [1].

Today, several studies have concluded that the use of cadavers is a valuable tool for anatomy education, clinical training, and development of surgical skills [2, 3].

Embalming and preservation process of cadavers used for dissection studies is essential to prevent tissue loss, decomposition and pathogen contamination. Nowadays, the main approach for embalming applications involve the use of fixative agents including formalin, phenol, thymol and glycerin [4, 5].

Among these chemicals, formalin is considered to be the most commonly used agent in embalming solutions in anatomy departments worldwide [6].

Formalin exerts its effects on tissue proteins by cross-linking the amine groups; by the way tissue becomes resistant to microbial contamination and decaying process. This chemical is bactericidal, sporicidal and virucidal; it has been reported that formalin-embalmed cadavers could be used for over a 12-months period without tissue decay [7].

Several studies have shown that despite the implementation of fixative solutions, bacterial, fungal and viral agents may contaminate the preserved cadavers [8, 9]. The detected organisms include a broad range of pathogens and non-pathogens

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such as *Mycobacterium tuberculosis*, Enterobacterales members, hepatitis B and C viruses, and HIV; *Bacillus* spp., *Streptomyces* spp., *Aspergillus flavus, Geotrichum candidum, Penicillium* spp. [10, 11].

The Anatomy Laboratory is set up on the basement floor of the School of Medicine. Dissection laboratory contained formalin-fixed human cadavers for study and research purposes. The smooth, sticky, whitish substance indicating microbial growth was detected on the soft tissues and open spongy ends of the bones of the male cadaver stored in the formalin tank.

The aims of this study are to find out the cause of failure of formalin disinfection on anatomy cadaver, to determine the effectiveness of various disinfectants for the decontamination process and to detect the microbial contaminant.

2. MATERIALS and METHODS

Sampling

Embalming solution samples (20 mL) were collected from the cadaver pool to determine the effectiveness of formalin against bacteria. The affected male cadaver stored in 5% formalin had white-yellow tissue deterioration on the facial soft tissue surfaces and the spongy parts of the facial bones (Figure 1). Ten swab samples were taken from these sites with suspicion of microbial colonization.

Figure 1. *Presentation of contamination on the superficial soft tissues and spongy parts of bone tissue of the anatomy cadaver. Arrows indicate whitish-colored substance on cadaver superficial tissues.*

Microbial Isolation and Identification

Swab samples taken from cadaver surface were immediately inoculated on 5% sheep blood agar media (BioMérieux, France) and incubated at 35℃ in an aerobic environment. After 72 hours of incubation, visible uniform colonies were detected on cultured media. Gram staining performed and the isolate was determined as Gram-negative bacillus. Matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis (Vitek-MS, BioMérieux, France) was used to identify the isolate [12].

However, no matching organism could be detected within the peptide profile library of the system, and microbial identification could not be made.

Genomic deoxyribonucleic acid (DNA) of the isolate was extracted by boiling method. Bacterial 16s rDNA was amplified by Polymerase Chain Reaction (PCR) using universal primers [13].

PCR product was sequenced and the Basic Local Alignment Search Tool (BLAST) was applied on resulting 16s rDNA sequence for bacterial identification [14].

Disinfectant Sensitivity Determination

In order to determine the disinfectant activity, sample solution taken from the cadaver tank, freshly prepared 5% formalin (Merck, Germany) and 0.55% ortho-phthalaldehyde (OPA, Nuova Farmec, Italy) as an alternative disinfectant were used in the disinfectant sensitivity testing.

Escherichia coli ATCC 25922 was used as the control strain. Bacterial suspensions were prepared at a density of 10^8 cfu/ml by using spectrophotometry. The bacterial suspensions and disinfectant samples were mixed at a ratio of 1/1 (v/v) and incubated at room temperature. At the 15th minute and 8th hour of the incubation, 100 µl samples were taken from the mixtures and inoculated on MacConkey agar medium (BioMérieux, France). The colonies grown on media were evaluated after 24-72 hours of incubation under aerobic conditions at 35℃ [15].

Statistical Analysis

All tests were two-tailed; p values of <0.05 were considered statistically significant. Statistical analyses were performed by using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA).

Ethical Approval

This study was approved by Marmara University, School of Medicine Ethics Committee with the following date and numbers: 07.01.2022, 09.2022.88.

3. RESULTS

Microbial Identification

Samples taken from the facial tissues of the cadaver revealed round, convex, pink-colored colonies on 5% sheep blood agar medium (Figure 2).

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Table I. *Disinfectant sensitivity test results.*

Bacteria	PBS*		Cadaver Tank		Formalin 5%		OPA** 0.55%	
	15 m	8 h	15 m	8 h	15 m	8 h	15 m	8 h
E. coli ATCC 25922	Growth	Growth	Growth	No-growth	No-growth	No-growth	No-growth	No-growth
S.aerolata isolate	Growth	Growth	Growth	No-growth	No-growth	No-growth	No-growth	No-growth

** Phosphate-buffered saline; ** Ortho-phthalaldehyde; p<0.05 (compared to formalin 5% and OPA 0.55%)*

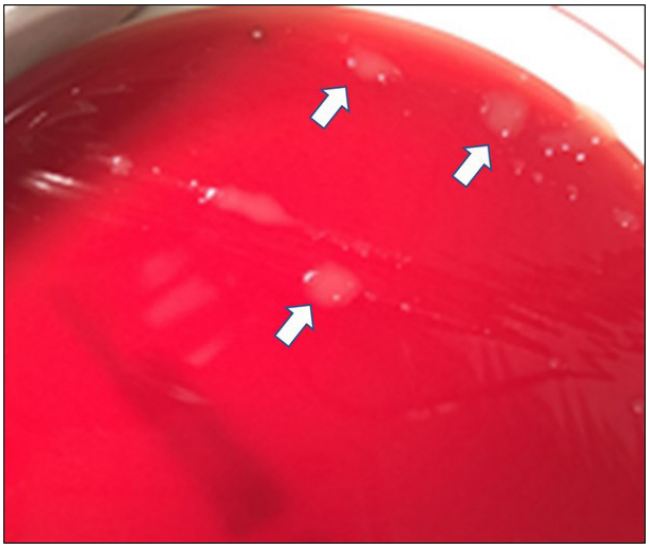

Figure 2. *Colony morphology of S. aerolata has grown on 5% sheep blood agar plate. Arrows indicate pink-colored S. aerolata colonies.*

Microscopic examination of the Gram stained smears prepared from colonies revealed Gram negative bacilli. MALDI-TOF MS analysis resulted with no match with any microorganism.

The result of the BLAST search conducted on 16s rDNA sequence of the isolate was *Skermanella aerolata* with 99% similarity with Genbank deposited reference sequence (Accession No. JX841089).

Disinfectant Sensitivity

In the disinfectant sensitivity test, it was observed that both *S. aerolata* and *E. coli* ATCC 25922 strains had grown in the cultures prepared at the 15th minute of incubation of bacteria with 5% formalin taken from the cadaver tank. Bacteria did not grow in the cultures of the same sample after 8 hours of incubation. Comparison with other substances, freshly prepared 5% formalin, and 0.55% OPA resulted with no growth of either bacterial strains after 15 minutes and 8 hours of incubation (Table I).

4. DISCUSSION

The human cadaver has been identified as a distinct educational material with unique features, such as a three-dimensional model presenting with a low health hazard and high quality of experience, and moderate cost. In a comparison of medical educational materials, human cadavers are classified as unique teaching tools without viable alternatives [3, 16].

Preservation of the cadaver is among the most important principles for the use of the human body as a teaching tool. The process of preservation is accepted as adequate if the cadaver is kept safe from contamination, destruction or decomposition. This is maintained by the cadaver treatment with embalming solutions such as formalin [3].

However, environmental conditions (dryness, humidity, etc.), errors in preparation of embalming solutions, and storage problems may cause microbial contamination of cadavers.

Microorganisms identified on the cadavers can be endogenous (related with body microbiota members) or exogenous.

In this study, we tested the disinfectant sensitivity of the *S. aerolata* isolate and *E. coli* ATCC25922 to embalming solution (5% formalin) from cadaver tank in anatomy laboratory, freshly prepared formalin (5%) and OPA 0.55% which is commonly used as a high-level disinfectant for medical instruments. Both bacterial strains were found to be resistant to embalming solution from cadaver tank in 15 min exposure time. These findings suggest that there is a problem with the embalming solution sampled from the cadaver tank, such as an error in preparation or activity loss due to environmental conditions.

In addition, the bacterial agent obtained from cadaver's superficial tissues was identified as *S. aerolata.* The genus *Skermanella* was first described and classified in the genus *Conglomeromonas* in 1983 by Skerman et al.[17].

Later, this bacterium was determined to be a new genus and was named in honor of Skerman who made the first identification [18].

Skermanella aerolata was first isolated from air samples in South Korea in 2007 by Weon et al. The bacteria has Gram-negative bacillus morphology, obligate aerobe with polar or subpolar flagella. Colonies are light pink in color, convex, rounded shape and have open margins. It can express high salt tolerance (up to 5%) and can grow at low temperatures (down to 5°C) [19].

Skermanella aerolata was accepted as an environmental microorganism till it was isolated from a human breast milk specimen [20].

In the second report, *S. aerolata* was detected as the cause of necrotizing fasciitis on the lower extremity of the patient [21].

In conclusion, we reported that a potentially pathogenic bacterium present in nature may cause cadaver contamination. Effective measures such as quality control of embalming solutions, indoor air filtration of dissection halls should be implied to prevent

contamination of cadavers in anatomy laboratories which in turn may threat students and anatomists studying with cadaver.

Compliance with Ethical Standards

Ethical Approval: The study was approved by the Marmara University, School of Medicine Ethics Committee.

Financial Support: The authors have no relevant financial information to disclose.

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

Authors' Contributions: OY and BA: Designed the study, OY, MMG, and MO: Performed the laboratory work and analysed the data, BA and MMG: performed the statistical analysis, OY, BA, MMG and USS: wrote the manuscript. All authors approved the final manuscript.

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Original Article

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Burden, depression and fatigue in caregivers of lung transplantation candidates

Sehnaz OLGUN YILDIZELI[1], Asli TUFAN CINCIN[2], Huseyin ARIKAN[1], Emel ERYUKSEL[1]

[1] Department of Pulmonology and Intensive Care, School of Medicine, Marmara University, Pendik Training and Research Hospital, Pendik, Istanbul, Turkey
[2] Division of Geriatrics, Department of Internal Medicine, School of Medicine, Pendik Teaching and Research Hospital, Pendik, Istanbul, Turkey

Corresponding Author: Sehnaz OLGUN YILDIZELI
E-mail: drsehnazolgun@yahoo.com

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ABSTRACT

Objective: A great deal of social support is often provided by the caregiver of the patient. The purpose of this study is to evaluate the burden, fatigue and depression of the primary caregiver of patients with pulmonary transplantation candidates.

Patients and Method: The primary caregivers of patients who were admitted to our hospital's pulmonary transplant outpatient clinic with end-stage pulmonary disease and no definite contraindications for transplantation, were included in the study. Zarit Burden Scale, Beck Depression Inventory and Short-Form (SF)-36 – Vitality questionnaires were applied to participants.

Results: Thirty-nine patients and their caregivers were evaluated. Caregivers experienced low levels of depression. Mean score for Beck Depression Inventory was 12.7±10.1. Caregivers generally experienced medium levels of burden. Mean score for Zarit Burden Scale was 26.9±14.2. Majority of caregivers experienced clinically significant fatigue. Mean score for SF-36 – Vitality was 61±16.7. Zarit Burden Scale scores and Beck Depression Inventory scores showed a statistically significant positive correlation (r:0.962 p<0.01).

Conclusion: Increased burden of caregivers of lung transplant candidates was associated with depression and fatigue. The presence of depressive symptoms is noteworthy even in the mild to moderate levels of burden. Supporting caregivers, has a great importance in terms of patient care and quality.

Keywords: Lung transplantation, Caregivers, Burden, Depression, Fatigue

1. INTRODUCTION

Lung transplantation has emerged as a remedy for some of the end-stage pulmonary diseases in the world over the last 25 years. Although, progress was slow for 15 years following first transplantation in 1968, the number of procedures and centers implementing lung transplantation since the beginning of the '80s has increased rapidly [1-3]. According to the International Society for Heart and Lung Transplantation (ISHLT) data, between 1993 and 2000, the number of annual pulmonary transplants worldwide reached 2000, and in 2012 more than 3,700 pulmonary transplants were performed [4].

To become a candidate for lung transplantation, in addition to medical parameters it is necessary that there is a full family support and the psychosocial state is stable [5]. When patients with transplantation necessity are assessed, it is known that the vast majority are in need of physical support during daily activities and administration of treatments. After transplantation, especially in the early period, caregiver support may often be required during complications and intense routine follow-up [5].

In the follow-up of chronic patients, the caregiver concept is defined as the primary person undertaking the care of the patient [6]. Studies have shown that the stress, burnout and fatigue of the caregiver directly affect both the physical and mental state of the patient requiring care [7, 8].

There are very few studies on caregiver burden, fatigue, burnout, and depression in pre-transplant caregivers of lung transplant candidates, as well as studies on the burden and depression of caregivers for some chronic advanced organ failure and terminal diseases.

The aim of this study is to evaluate the care burden, fatigue, burnout syndrome and depression of the primary caregiver in cases with pulmonary transplant candidates.

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2. PATIENTS and METHODS

Participants

Caregivers of patients who were evaluated for lung transplantation at our hospital lung transplantation outpatient clinic between December 2016 and September 2018 were recruited for this study. A caregiver has been identified for each patient. If more than one person within family members is included in care of patient, the eligible individual is asked to be selected by family members. Caregivers who are younger than 18 years old or who cannot understand/speak Turkish are excluded from study. Characteristics of patients were recorded. Patients were classified according to their care needs by clinicians as almost independent, partially dependent, greatly dependent and completely dependent according to Barthel index [9]. Barthel index was validated in Turkish [10]. The questionnaire block was administered during the first visit if the primary caregiver of the patient was with him/her. If not, primary caregiver was requested to come to the next appointment. All participants provided an informed consent. This study was approved by the Institutional Review Board of Marmara University School of Medicine local ethics committee (approval no:09.2016.441).

Measures

1. **Assessment of Care Burden**: Zarit Burden Scale was developed in 1980 by Zarit et al [11]. It is a measure used to assess the stress experienced by caregivers. This scale can be used to assess relationship between patents and caregiver, caregiver's health status, psychological comfort, social life and economic burden. Zarit Burden scale was validated in Turkish and has shown good reliability in clinical practice [12, 13]. The scale has a Likert-type rating that ranges from 0 to 4 corresponding to never, rarely, sometimes, frequently, or almost always. 0-20 points indicate light burden, 21-40 points indicate medium burden and > 40 points indicate heavy burden. The higher the scale score, the more intense the experience burden.
2. **Assessment of Depression**: The Beck depression inventory is a self-reported inventory consisting of 21 questions and was developed by Beck in 1961 [14]. Basically, it is based on evaluating the characteristics and symptoms of depression. Each question is scored from 0 to 3 points, with 0-9 minimal depression, 10-16 mild depression, 17-29 medium depression and 30-63 severe depression. It is validated in Turkish [15].
3. **Assessment of Fatigue**: Short Form (SF)-36 provided internal consistency, reliability, and content validity criteria that were tested in various populations [16]. SF-36 Turkish validated formula was used to evaluate the fatigue of the caregiver [17]. The four questions in the survey asks how much time they spent in last week: 1) felt full of pep; 2) had a lot of energy; 3) felt worn out; or 4) felt tired. The answers were sorted from "always" to "never". Score ranges from 0 to 100. The scores at 45 and below represented clinically significant fatigue.

Statistical Analysis

Descriptive statistics were reported for all variables as mean±standart deviation or n (%) when appropriate. Kruskal-Wallis test was used for comparing continuous variables of more than two groups. Pearson's r was used to measure correlation of two continuous variable. The sample size was not calculated because lung transplantation was performed on a very limited number of patients. All eligible patients and their caregivers were included in the study. P value <0.05 was accepted as a sign of statistical significance. Statistical analyses were performed using the PSPP version 1.0.1 (GNU Project Development; San Carlos, CA, USA).

3. RESULTS

Demographic Characteristics

Thirth-nine patients and their caregivers were evaluated. Caregivers consisted of 31 (79.5%) female and 7 (21.5%) males. Mean age was 41.8±9.8. Most of them were married (92.3%) and were at least primary school graduates (92.3%). Majority of caregivers were either spouse or child of the patient (56.4%). Only 4 (10.2%) caregivers received professional help for their patients care. Demographic characteristics of caregivers and demographic and clinical characteristics of lung transplant candidates are shown in Table I.

Table I. *Characteristics of patients and caregivers*

Caregiver Characteristics		Patient Characteristics	
Age, mean ± SD	41.8 ± 9.8	Age, mean ± SD	41.2 ± 13.3
Female, n (%)	31 (79.5)	Male, n (%)	23 (59)
Caregiving Duration, n (%)		Smoking, packs/year, mean ± SD	6.9 ± 9.5
< 1 year	1 (2.6)	BMI, kg/m^2, mean ± SD	29 (6.8)
1-3 years	13 (33.3)	Primary Diagnosis, n (%)	
> 3 years	25 (64.1)	COPD	11 (28.2)
Income, n (%)		Interstitial Lung Disease	12 (30.8)
Very High	4 (10.2)	Bronchiectasis	9 (23.1)
High	3 (7.7)	Cystic Fibrosis	5 (12.8)
Middle	12 (30.8)	Idiopathic Pulmonary Arterial Hypertension	2 (5.1)
Low	15 (38.5)	Care Need, n (%)	
Very low	5 (12.8)	1 – almost independent	4 (10.3)
Educational Status, n (%)		2 – partially dependent	13 (33.3)
Illiterate	3 (7.7)	3 – greatly dependent	19 (48.7)
Primary School	14 (35.9)	4 – completely dependent	3 (7.7)
Secondary School	9 (23.1)	Duration of Primary Diagnosis Years, mean ± SD	9.5 (6.3)
High School	9 (23.1)	Oxygen Support, n (%)	30 (76.9)
University	4 (10.3)	NIV Support, n (%)	10 (25.6)
		6MWT, meters, mean ± SD	264 (100.3)

SD: Standard Deviation, BMI: Body Mass Index, COPD: Chronic Obstructive Pulmonary Disease, NIV: Non-Invasive Ventilation, 6MWT: 6 Minutes Walking Test

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Level of Burden, Depression and Fatigue

Caregivers experienced low levels of depression. Mean score for Beck Depression Inventory was 12.7±10.1. Cutoff point for Beck Depression score to determine clinically significant emotional distress was identified as ≥14 in the literature. Among participants 16 (41%) were having clinically significant emotional distress. Caregivers generally experienced medium levels of burden. Mean score for Zarit Burden Scale was 26.9±14.2. Majority of caregivers experienced clinically significant fatigue. Mean score for SF-36 Vitality was 61±16.7.

Distribution of levels of burden, depression and fatigue are described in Table II.

Table II. *Distribution of levels of burden, depression and fatigue*

Depression n (%)		Burden of Care n (%)		SF-36 Vitality n (%)	
Minimal	20 (51.3)	No burden	16 (41)	≤45 Fatigue	10 (25.6)
Mild	6 (15.4)	Moderate Burden	13 (33,3)	>45	29 (74.4)
Moderate	9 (23.1)	Severe Burden	10 (25.6)		
Severe	4 (10.3)				

Correlation between measures is shown in Figure 1. Zarit Burden Scale scores and Beck Depression Inventory scores showed a statistically significant positive correlation (r:0.962 p<0.01). SF-36 Vitality scores found to be negatively correlated with both Zarit Burden Scale scores and Beck Depression Inventory score (r: – 0.854 p<0.01; r: – 0.87 p<0.01 respectively).

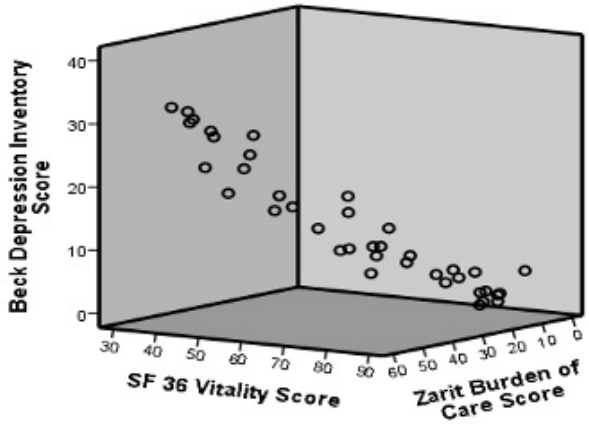

Figure 1. *Correlation between burden, depression and fatigue*

SF-36: Short Form 36

Educational status did not influence Zarit Burden Scale, Beck Depression Inventory and SF-36 Vitality scores (p=0.705, p=0.584 and p=0.365 respectively). But there was a statistically significant difference among income groups for Zarit Burden Scale, Beck Depression Inventory and SF-36 Vitality scores (p=0.001, p=0.002 and p=0.003 respectively). Figure 2 shows distribution of all 3 scores among care givers according to their income.

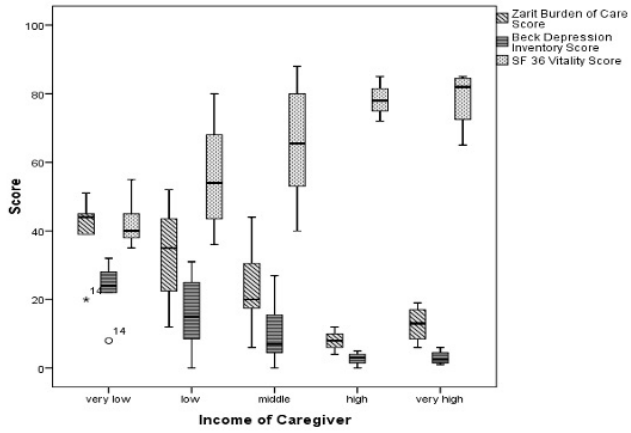

Figure 2. *Evaluation of burden, depression and fatigue among caregivers according to income*

SF-36: Short Form 36

Patients were grouped according to their care needs. In this case 1 referred to almost independent, 2 referred to partially care dependent, 3 referred to greatly care dependent and 4 referred to completely care dependent. Figure 3 shows distribution of all 3 scores among caregivers according to their patient's care need. There was a statistically a significant difference among income groups according to Zarit Burden Scale, Beck Depression Inventory and SF-36 Vitality scores (p=0.001, p=0.001 and p<0.001 respectively).

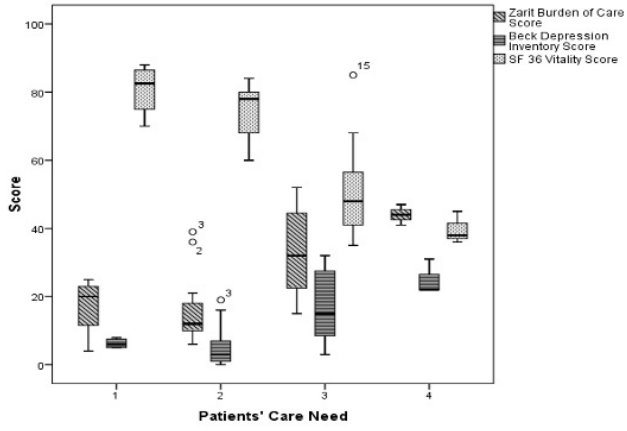

Figure 3*. Evaluation of burden, depression and fatigue among caregivers according to their patient's care need*

Care need was defined as: 1. almost independent, 2. partially dependent, 3. greatly dependent, 4. completely dependent, SF-36: Short Form 36

4. DISCUSSION

In this study, the symptoms of fatigue, depression and caregiver burden were evaluated in primary caregivers of lung transplantation candidates. As a result; the increased burden of

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caregivers in the group of patients with predominantly COPD and ILD, resulted in an increase in depressive symptoms and fatigue.

Today, lung transplantation is not close to the desired success in terms of long-term outcomes compared to other solid organ transplants [18]. Because most of the patients are in the end-stage in the pre-transplant period, a large proportion of patients fail to reach the targeted exercise capacity in the early post-transplant period due to a number of systemic changes secondary to chronic respiratory failure, and expected recovery time may be prolonged [19]. Even in stable cases; close monitoring at home (such as spirometry and vitals), frequent hospital inspections, general life changes (diet, exercise) and lifelong drug treatment which require close follow-up are needed. In addition, there may be medical conditions in some patients such as infections, ongoing chest pain, intestinal dysfunction, dyspnea, anxiety, which require treatment and support after transplantation. Therefore, when compared to other organ transplantations, the need for care of these patients in the preoperative period can continue for a while in the post-operative period.

At present, the caregiver concept is mainly used for chronic and terminal medical conditions, and patient groups are mainly composed of stroke, dementia, chronic care patients after ICU and cancer patients [20-22]. It has been shown that regardless of the underlying disease, individuals who do not do caregiving professionally choose to have socialize less, change their lifestyles and habits, choose half-time work, change jobs or quit, show depressive symptoms [23]. In addition; lack of social support, and low socioeconomic status have significant effects on Quality of Life. It has been reported that the greatest sources of stress in caregivers of palliative care patients are patient's treatments, dietary needs, doctor's appointments, and psychosocial status of the patient [24]. The number of studies in specific chronic organ failures is less than in the other groups. One study compared COPD, congestive heart failure and patients with chronic renal failure and found that physical burden and stress in COPD caregivers were greater than in the other groups [7]. The caregiver's burden was assessed in COPD-diagnosed patients and it was determined that the most important factor that increased the burden in caregivers was the patient caregiver incompatibility. Caregivers' SF-36 survey indicated that the worst scoring was in the mental status, vitality and general health status sections [25]. Again, in the same study, as supported by others, it is emphasized that caregivers feel sicker and need to go to the doctor more than other individuals with the same characteristics [26].

Studies have also shown that in the caregivers of solid organ transplantations, physical health deterioration was associated with psychosocial stress [27]. In addition, it has been argued that if the patient is married and spouse is the caregiver, postoperative long-term outcomes are better due to good care [28, 29].

While, there is no data on lung transplantation caregivers, in a study of caregivers of renal transplant cases; it has been reported that psychosocial support to caregivers resulted in reduction of depression and facilitated care [30].

Lung transplantation cannot always provide the expected improvement in the quality of life, or it can take time [31]. Like our study, Claar et al., assessed quality of life and emotional burden of the caregiver of lung transplantation candidates in the waiting list. Caregiver burden and emotional stress was found to be less than expected. They speculated that caregivers fear of patient not receiving psychosocial support impression resulted in underestimation by caregiver. While accepting the patient's illness in caregivers was associated with depression, caregivers who did not accept the illness were reported to have high anxiety rates [32].

When caregivers are family members, patient related future concerns, responsibilities and financial reasons have been reported to be an additional source of stress for the caregiver [33]. Mollberg et al., have found that primary caregivers have an impact on long-term transplantation success in cases of lung transplantation. Unlike previous studies in transplant patients they report that being married was not associated with good care; especially in post-transplant care, caregiver's psychological and physiological health was the most important factor in good care [34].

When we look at the general profile of caregivers in our study; most of them were family caregivers and most of the caregivers were the patient's wife or daughter. This can be considered as a risk factor for emotional maladjustment of the medical problems or worsening of the patient at the same time. Patients who were cared by their spouses or daughters could not be assessed thoroughly because of the lack of validated questionnaires in Turkish. When the socioeconomic status was evaluated, it was determined that the middle-poor income group was the forerunner. Educational status is predominantly primary school graduate, parallel to income situation. Since, most caregivers are in the low socioeconomic status, it may be difficult to find sufficient financial support for the problems that arise and being at a lower educational level may make it difficult to reach solutions.

The burden of care was directly related to depression scoring and fatigue assessment in our study. Although, cases were mostly in the mild group; depressive symptoms were found to be higher than workload, and these results were associated with the fact that the caregiver was mostly close family members like the study of Dew et al.[33].

There is no special unit for social services in the centers where transplantation is performed in our country. Because of the lack of social workers that may be helpful in the programming of the medical services or non-medical problems this rises as an additional burden on caregivers. A supportive service can be an effective way to reduce emotional stress and fatigue, especially when caregivers are supported by these institutions or individuals in groups that require high cost and labor, such as transplantation. Improvements in caregiver conditions can improve the patient's quality of care before and after the transplant and the long-term survival of the patient.

One of the limitations of our study is small sample size. Also, post-transplant evaluation could not be done due to the small

number of transplanted cases. The risk assessment for depression and fatigue in caregivers is insufficient due to the low number of cases. Assessment of coping strategies for depressive symptoms identified in caregivers in similar studies is not included in this study. The marital status of the patient and the relationship with the caregiver on care were not assessed due to the low number of cases after the distribution. Comprehensive evaluations of the caregiver were not implemented because some questionnaires were not validated in Turkish. Additionally, we did not have a control group for burden comparison which may provide insight for other chronic respiratory diseases.

In conclusion, in this study, increased burden of caregivers of lung transplant candidates was associated with depression and fatigue. The presence of depressive symptoms is noteworthy even in the mild to moderate levels of burden. Lung transplantation is a long-lasting and exhausting process. Post-operative burden is relatively high. We think that the psychosocial support applied to the primary caregivers in the pre-operative and post-operative period will provide a positive contribution to the long-term outcomes of the patients.

Compliance with the Ethical Standards

Ethical Approval: This study was approved by the Institutional Review Board of Marmara University School of Medicine local ethics committee (approval no:09.2016.441).

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Conflict of Interest: The authors have no potential conflicts of interest to disclose.

Author Contributions: SOY and ATC: Conceived the study, SOY and HA: Data collection; HA: Managed the data, including quality control, analyzed the data, SOY, ATC, HA, and EE: Drafted the manuscript, EE: provided critical revision of the manuscript and all authors contributed substantially to its revision, SOY: Takes responsibility for the paper.

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Problematic social media use, digital gaming addiction and excessive screen time among Turkish adolescents during remote schooling: implications on mental and academic well-being

Gresa CARKAXHIU BULUT, Sebla GOKCE

[1] Department of Child and Adolescent Psychiatry, School of Medicine, Maltepe University, Maltepe, Istanbul, Turkey.

Corresponding Author: Gresa CARKAXHIU BULUT
E-mail: gresacarkaxhiu@gmail.com

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ABSTRACT

Objective: The present study aimed to describe screen use patterns among Turkish adolescents during the coronavirus disease 19 (COVID-19) pandemic with a special emphasis on social media and digital gaming addiction, and explore how these are linked to adolescents' mental and academic well-being.

Materials and Method: The study sample consisted of 9th to 12th grade students from three public high-schools in Istanbul, Turkey (n=201). Participants were required to complete a comprehensive questionnaire which gathered information about various domains including problematic screen use, attitudes towards online education, and mental/academic well-being during the lockdown period.

Results: The overall screen time exhibited a significant increase during the lockdown, with the most common discretionary screen activities being social media use, communication, and watching movies/series ($p<0.001$). The students spent significantly less time on physical activities ($p=0.003$) and face-to-face meetings with their friends ($p<0.001$). Male students presented with higher scores on gaming addiction ($p<0.001$), whereas a significantly higher proportion of the female students (28.57% vs. 14.81%) were classified as at-risk for social media addiction ($p=0.046$). Both gaming addiction and social media addiction were associated with higher depression scores ($p=0.003$ and $p<0.001$ respectively).

Conclusion: Screen use patterns may have diverse consequences for youth's well-being during the pandemic. The addiction risk and other detrimental outcomes are likely to be associated with the qualitative features of screen activities, rather than just the amount of time spent on digital media by the adolescents.

Keywords: Screen time, Social media use, Digital gaming addiction, Adolescents, COVID-19, Well-being.

1. INTRODUCTION

The umbrella term screen dependency disorders (SDD) has been increasingly used by mental health professionals to describe the addictive pattern of engagement with a variety of overlapping screen activities. The well-known examples include internet addiction disorder (IAD), social media addiction (SMA), digital gaming addiction (DGA), and mobile phone addiction (MPA) [1, 2]. Although, prolonged screen use may be problematic at any age, adolescents constitute a particularly vulnerable group for developing SDD. One possible explanation is that, in addition to their natural tendency to use digital media, adolescents have more flexible schedules and freedom from parental control compared to other age groups [3, 4].

Screen dependency disorder has become a topic of increasing interest since the coronavirus disease 19 (COVID-19) pandemic, which brought unprecedented restrictions in youth's life in all aspects. In most countries, the in-person teaching has been suspended, with the decision to organize online education until further notice. The stay-at-home policies mandated most of the daily social interactions to be replaced with digital meetings on online platforms. Although, the scope of the restrictions may have varied between countries, research collectively suggests that most youth have faced the necessity to spend excessive amounts of time in front of screens during this particular period [5-8]. For example, a nationwide survey from China pointed out that 46.8% of the participants presented with internet overuse as a consequence of the pandemic [9]. The daily time spent on video games was found to have increased from 79.2 to 138.6 minutes among German youth after the lockdown

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[10]. A two-cohort design study, which investigated screen time during lockdown and one year after school closure among Dutch children, reported that the average screen time increased in both cohorts, with a mean of 35 minutes and one hour per day respectively [11]. Similarly, a cross-sectional survey conducted on children and adolescents from the U.S. during the first year of the pandemic reported excessive amounts of recreational screen time, which also correlated with higher rates of mental health problems [12]. Finally, Nagata et al., reported significantly increased screen times among adolescents from the U.S. during the early period of the pandemic [6]. These and several other reports have sparked the ongoing debate about the detrimental impacts of prolonged screen time on the well-being of children and adolescents during the COVID-19 pandemic. In terms of SDD, the emphasis has been placed particularly on SMA and DGA, which both have been consistently linked by previous research to a variety of adverse mental health outcomes including depression, anxiety, suicide-related behaviours, sleep and attention problems, eating disorder, as well as poor academic performance [13-18].

Considering that the pandemic is here to stay for a while and its impacts are likely to last for years, there is a need for better understanding of the problems arising from screen overuse in particular subpopulations. Given the relative lack of data on Turkish population, our motive in this cross-sectional study was to provide a multi-dimensional picture of the screen use patterns among Turkish adolescents within the context of stay-at-home orders during the first year of the pandemic, and assess whether and how these were related to their mental and academic well-being. More specifically, the main research objectives were as follows: 1) examine the students' screen use patterns and the rates of SMA and DGA, 2) explore whether and how their screen time and daily activities were affected by the pandemic, 3) identify their experienced difficulties during online class and their views on different aspects of the stay-at-home measures, 4) examine relationships between participants' demographic characteristics, screen use tendencies and psychological well-being.

2. MATERIALS and METHODS

Participants

The participants were recruited among the 9th to 12th grade students (aged between 14-18) from three public high-schools in the eastern side of Istanbul, Turkey. An invitation e-mail containing a survey link was sent to the students who had priorly expressed willingness to be contacted for research investigations (n=653). Potential participants and their parents (those who replied positively to the study invitation, n=267) were provided more detailed information about the study protocol and required to give online informed consent to move on to the study procedure. Data collection was performed in November 2020 (approximately seven months after the implementation of curfew restrictions and online education), during a three-week period of the lockdown. In line with the methodological design, participants were required to respond to each item in the questionnaire to submit the finalized form (those who left without completing the procedure were excluded from the data analysis). Accordingly, of 215 students who gave consent and started filling out the forms, 201 completed the entire questionnaire and were included in the final sample. The median age of the participants was 16 (14-18), and 73.13% (n=147) were females. The study protocol was approved by the Ethical ommittee of Maltepe University (2021/900/27). The correspondence and the experimental procedure were performed in accordance with the official authorization of the Istanbul Maltepe Provincial Directorate of National Education.

Materials

The questionnaire was prepared by the authors in parallel with the methodology of some previous studies about the impacts of COVID-19 on different populations [19]. The questionnaire consisted of 20 sections (some of them containing several items or scales) which gathered information within various fields of interest including (1) students' sociodemographic characteristics, (2) ownership status for a range of digital devices and accessibility to digital media, (3) the pattern of use of the available devices, 4) time spent on screen and screen-free activities before and during the pandemic, 5) students' attitudes towards online education and experienced difficulties during online class, 6) screen related problems and experienced difficulties, 7) social media addiction, 8) digital gaming addiction, and 9) depressive symptoms. The initial five domains were assessed by specific questions and items developed for the current study while the last three fields were assessed by means of the following scales:

Bergen Social Media Addiction Scale

The Bergen Social Media Addiction Scale (BSMAS) developed by Andreassen and colleagues is based on Griffiths' criteria for addiction (salience, mood modification, tolerance, withdrawal, conflict, and relapse) [20], and is widely used in research [21]. The scale consists of six items rated on a 5-point Likert scale ranging from 1 (very rarely) to 5 (very often). The higher total score reflects stronger addiction to the social media, and the BSMAS score over 19 indicates that an individual is at-risk of developing problematic social media use [22]. The Turkish adaptation and the reliability study of the scale was performed by Demirci et al. [23]. The scale's internal consistency was found acceptable (Cronbach's α=0.83).

Gaming Addiction Scale

Developed by Lemmens et al., the 7-item Gaming Addiction Scale (GAS) is a brief instrument based on DSM criteria to assess gaming addiction. The seven items in the GAS are rated using a five-point Likert scale ranging from 1 (never) to 5 (very often). A higher score on the GAS indicates more problematic digital gaming [24]. The Turkish adaptation and the reliability studies of the scale were performed by Irmak et al., and Baysak et al. [25, 26]. The scale's internal consistency was found considerably high (Cronbach's α=0.88).

Kutcher Depression Scale-11

The Kutcher Depression Scale-11 Items (KADS-11) is a self-report, diagnostic instrument measuring depression and suicidal thoughts in adolescents and young adults. The scale was introduced for clinical practice as a sensitive and specific instrument to aid in diagnosis and monitoring the change in severity of symptoms during the course of treatment. It is easily and quickly completed, diagnostically valid and demonstrates reasonable reliability [27, 28] The KADS-11 consists of items with an ordinal and polytomous scoring scale, ranging from 0 *(*hardly ever*)* to 3 *(*all of the time*)* [29]. The Turkish adaptation and the reliability study for the KADS-11 was performed by Balcı Çelik-Uysal Atabay [30]. The scale's internal consistency was found acceptable (Cronbach's α=0.82).

Statistical Analysis

Statistical analyses were performed using SPSS (version 24.0). Descriptive statistics are given as counts, percentages, means, standard deviations, medians, and ranges. Shapiro-Wilk was used to assess normal distribution. Pearson Chi Square was used to compare the distribution of categorical variables between two independent groups (e.g., males and females). Mann–Whitney U test was used to compare non-normally distributed continuous variables (e.g., BSMAS, GAS, KADS-11 scores) between two independent groups. Wilcoxon's signed-rank test was used to compare non-normally distributed continuous variables between two related groups (e.g., before and during the pandemic). Spearman correlation coefficient was used to evaluate bivariate associations between corresponding variables. The statistical significance level was set as α=0.05.

3. RESULTS

The demographic characteristics of the study sample (n=201) are shown in Table I. The vast majority of the students (94.03%) owned a smartphone. 61.69% of the students reported to have constant internet access both at home and outside. The most common intended uses for the digital devices were social media and communication (86.06% for both). Male students were significantly more likely to play digital games and surf the internet compared to females (p<0.001, and p= 0.004 for both) (Table II).

The reported durations for discretionary screen and screen-free activities before and during lockdown are shown in Table III. Notably, screen times for digital gaming, social media use, communication and watching movies/series have all significantly increased during the pandemic period (p=0.005, p<0.001, p=0.012, and p<0.001 respectively) whereas the opposite was true for the screen-free activities. The students spent significantly less time on homework (p<0.001), physical activities (p=0.003) and face-to-face meetings with their friends (p<0.001) during the lockdown period, while their contact with the family members has increased (p=0.022). Our results additionally showed that males spent significantly more time on digital games (p<0.001) whereas the average time spent on social media was significantly higher in females (p=0.003) during this period.

Students' attitudes and subjective experiences in regard with online education are displayed in Table IV. 60.69% of the participants were dissatisfied with not being able to chat with their friends during online class as before. Notably, 24.37% of the students reported being pleased that they did not have to go to school, and 13.93% that they got rid of the bullying.

In terms of the perceived problems with excessive screen activities (Table V), the majority of the students (69.64%) reported significantly increased screen time during the pandemic. 42.28% of the participants stated that their families complained about their excessive screen time, while 26.36% had arguments with family members for this reason. 18.40% reported a decrease in their academic performance due to prolonged screen use.

Figure 1 displays the gender differences in BSMAS, GAS, and KADS-11 scores. The median value for the BSMAS was 14 (6-30) for the whole sample, 15 (6-30) for the female and 12.50 (6-27) for the male students, with no significant difference between genders (p=0.088). With a cut-off score of 19 and above, 24.87% of the participants were classified as at-risk of problematic social media use. The rate of problematic social media use was %28.57 among females and %14.81 among males, with a statistically significant difference between genders (p=0.046). The median value for the GAS was 11 (7-33) for the whole sample, 15 (7-32) for the males and 8 (7-33) for the females, with the difference being statistically significant (p<0.001). Finally, the median value for the KADS-11 was 14 (0-33) for the whole sample, 14 (0-33) for the females and 11 (0-30) for the males. Again, a statistically significant difference was found between genders (p=0.045).

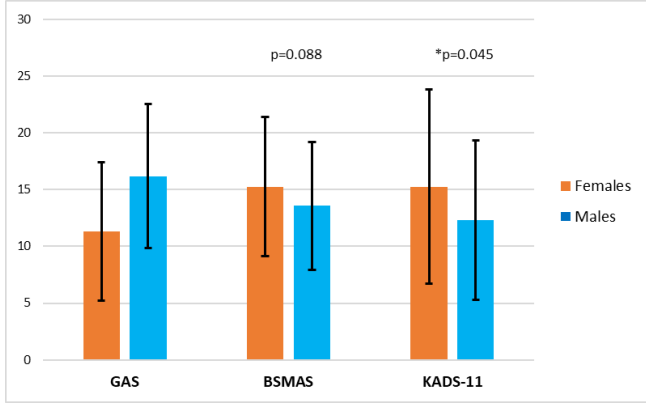

Figure 1. *Gender differences in gaming addiction, social media addiction, and depression scores. GAS: Gaming Addiction Scale, BSMAS: Bergen Social Media Addiction Scale, KADS-11: 11-Item Kutcher Adolescent Depression Scale.*

The SMA, DGA and KADS-11 scores were strongly and positively inter-correlated (p<0.001 for SMA-DGA, p<0.001 for SMA-KADS; p=0.003 for KADS-DGA) (Table VI). The age and the time spent in physical activities were found to negatively correlate with the SMA (p=0.006, and p=0.040), whereas time spent in face-to-face communication with peers negatively correlated with DGA (p=0.038).

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Table 1. *Demographic characteristics of the participants (n = 201)*

		Number (n)	*Percentage (%)*
Gender	*Female*	147	73.13
	Male	54	26.86
Age	*14-15*	86	42.8
	16-18	115	57.2
Grade	*9*	62	30.84
	10	79	39.30
	11	43	21.39
	12	17	8.46
Number of siblings	*Single child*	20	9.95
	2-3	150	74.63
	4-5	28	13.93
	6 and more	3	1.49
Household population	*1-3*	39	19.40
	4-5	138	68.65
	6-7	24	11.94
Maternal employment status	*N/A*	6	2.98
	Unemployed	137	68.16
	Employed	58	28.86
Paternal employment status	*N/A*	7	3.48
	Unemployed	19	9.45
	Employed	175	87.06
Digital devices (ownership)	*Smartphone*	189	94.03
	PC	88	43.78
	Tablet	41	20.39
	Game console	17	8.46
	TV	26	12.93
Digital devices (shared use)	*Smartphone*	10	4.97
	PC	68	33.8
	Tablet	39	19.40
	Game console	23	11.44
	TV	165	82.09
Internet access	*Outside and home-constant*	124	61.69
	Only at home-constant	72	35.82
	Only at home-limited time	2	0.99
	No available connection	3	1.49
Intended use of digital media	*Gaming*	101	50.24
	Social media	173	86.07
	Communication	173	86.07
	Internet surfing	116	57.71
	Watching movies-series	164	81.59

Table II. *Gender differences in digital media usage patterns*

	Rate (%)				
	Males	Females	df	χ2	p value
Intended use of digital media					
• Digital gaming	85.18%	37.41%	1	36.050	<0.001
• Social media use	88.88%	85.03%	1	0.489	0.484
• Internet surfing	74.07%	51.70%	1	8.100	0.004
• Communication	88.88%	85.03%	1	0.489	0.484
• Watching movies/series	75.92%	83.67%	1	1.578	0.209
Being at-risk for social media addiction	14.81%	28.57%	1	3.999	0.046

df: degrees of freedom

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Table III. *Daily time spent on screen and screen-free activities before and during COVID-19 (n=201)*

Screen activities						Screen-free activities				
		Before COVID-19	During COVID-19	Test	*p*		Before COVID-19	During COVID-19	Test	*p*
Digital gaming	Almost never	42.28%	39.80%	-2.82[a]	**0.005**	***Face-to-face interaction with friends***	8.95%	28.85%	-7.89[a]	**<0.001**
	Up to one hour	20.89%	18.41%				11.44%	19.40%		
	1 to 2 hours	23.38%	18.90%				29.85%	26.86%		
	2-3 hours	10.44%	15.92%				23.38%	14.43%		
	More than 3 hours	2.98%	6.96%				26.36%	10.45%		
Social media	Almost never	6.96%	6.47%	-4.22[a]	**<0.001**	***Doing homework***	5.97%	28.35%	-7.29[a]	**<0.001**
	Up to one hour	23.38%	14.43%				24.37%	26.86%		
	1 to 2 hours	38.80%	41.79%				46.76%	30.34%		
	2-3 hours	21.89%	20.89%				19.40%	11.44%		
	More than 3 hours	8.95%	16.42%				3.48%	2.98%		
Communication	Almost never	4.97%	3.48%	-2.52[a]	**0.012**	***Hobbies***	19.40%	27.36%	-1.01[a]	0.313
	Up to one hour	28.36%	26.86%				29.85%	22.88%		
	1 to 2 hours	40.29%	35.32%				30.34%	28.85%		
	2-3 hours	19.40%	22.38%				14.92%	14.92%		
	More than 3 hours	6.96%	11.94%				5.47%	5.97%		
Internet surfing	Almost never	18.41%	19.40%	-1.29[a]	0.198	***Interaction with family members***	6.46%	5.97%	-2.29[a]	**0.022**
	Up to one hour	37.31%	33.83%				18.90%	21.39%		
	1 to 2 hours	30.35%	26.86%				37.81%	30.84%		
	2-3 hours	11.44%	16.91%				26.86%	25.37%		
	More than 3 hours	2.49%	2.98%				9.95%	16.42%		
Watching movies/series	Almost never	15.92%	13.93%	-4.64[a]	**<0.001**	***Physical activities***	14.42%	26.86%	-2.97[a]	**0.003**
	Up to one hour	21.39%	13.93%				31.34%	28.36%		
	1 to 2 hours	35.32%	31.34%				29.85%	24.38%		
	2-3 hours	22.39%	29.35%				17.91%	15.42%		
	More than 3 hours	4.97%	11.44%				6.46%	4.97%		

[a] *Wilcoxon signed-rank test. Significant differences are shown in bold font*

Table IV. *Students' attitudes and subjective experiences in regard with online education (n=201)*

	Strongly disagree	Disagree	Nor agree or disagree	Agree	Strongly agree
I am dissatisfied with not being able to chat with friends during online class as before	8.95%	10.94%	19.40%	24.37%	36.31%
I am pleased that I do not have to go to school	22.38%	18.90%	34.32%	10.44%	13.93%
I am content that I got rid of the bullying in school	53.23%	17.41%	15.42%	8.45%	5.47%
I feel more comfortable in expressing my self in front of the screen	45.77%	19.90%	18.90%	7.96%	7.46%
It is more difficult for me to raise hands and speak up during online class	30.34%	17.41%	16.41%	15.92%	19.90%
I prefer to keep my camera off during online class because I feel uncomfortable	27.36%	15.42%	15.42%	16.41%	25.37%
I prefer to keep my camera off during online class because I do not want to be seen doing other thing	51.74%	22.88%	16.91%	3.98%	4.47%

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Table V. *Student's perceived problems regarding the excessive times on screen activities (n=201)*

	Strongly disagree	Disagree	Nor agree or disagree	Agree	Strongly agree
My screen time has significantly increased during COVID-19	8.95%	8.45%	12.93%	24.37%	45.27%
I spend excessive amounts of time in playing digital games during COVID-19	31.34%	17.91%	16.91%	14.42%	19.40%
I spend excessive amounts of time in social media during COVID-19	12.43%	9.45%	16.91%	33.83%	27.36%
I spend excessive amounts of time in watching movies/series during COVID-19	14.92%	11.44%	17.91%	30.34%	25.37%
Family members and people around me complain that I spend too much time in front of the screen during COVID-19	19.90%	17.91%	19.90%	20.39%	21.89%
I am getting into frequent arguments with my parents due to spending a lot of time in front of the screen	32.83%	22.88%	17.91%	12.43%	13.93%
I cannot find anything else to do when I am away from the screen	19.90%	19.40%	24.37%	13.93%	22.38%
I no longer enjoy the things/activities I used to do	24.37%	20.39%	18.40%	20.39%	16.41%
I cannot help thinking of my phone/computer/tablet even when I'm away from the screen	27.86%	21.89%	23.38%	13.93%	12.93%
My school performance decreased due to spending a lot of time in front of the screen	29.35%	24.37%	27.86%	9.45%	8.95%
My contact with others (family, friends etc.) decreased due to spending a lot of time in front of the screen	41.29%	21.39%	19.40%	7.46%	10.44%

Table VI. *Correlations between screen-free activities, gaming addiction, social media addiction and depression scores*

		1	2	3	4	5	6
1. Age	r_s	-					
	p	-					
2. Time spent in physical activities	r_s	0.050	-				
	p	0.483	-				
3. Time spent in face-to-face communication with peers	r_s	0.044	0.388	-			
	p	0.538	**<0.001**	-			
4. KADS-11	r_s	-0.066	-0.109	0.027	-		
	p	0.351	0.125	0.703	-		
5. GAS	r_s	-0.108	0.001	-0.146	0.206	-	
	p	0.126	0.997	**0.038**	**0.003**	-	
6. BSMAS	r_s	-0.192	-0.145	0.068	0.418	0.280	-
	p	**0.006**	**0.040**	0.335	**<0.001**	**<0.001**	-

Significant correlations are shown in bold font. rs : Spearman's correlation coefficient. KADS-11: 11-Item Kutcher Adolescent Depression Scale, GAS: Gaming Addiction Scale, BSMAS: Bergen Social Media Addiction Scale,

4. DISCUSSION

The findings from this cross-sectional, exploratory study may provide some insight into different aspects of the problematic screen use among Turkish adolescents within the context of the stay-at-home restrictions of the pandemic, and how these may be related to their well-being. Not surprisingly, the students' overall discretionary screen time was found to have increased significantly compared to the pre-pandemic period. Notably, social media use (aside with communication) was reported to be the most common screen activity in our sample with one quarter of the participants additionally being found "at risk" for SMA. This is strikingly high compared to the findings of Bányai et al.'s reference study which reported a prevalence of 4.50% among Hungarian adolescents [22]. Using the same scale with

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a modified cut-off score, the estimated 12-month prevalence of SMA among Chinese adolescents has been reported as 3.50% [31]. Although, the cross-sectional design of our study prevents us from drawing firm conclusions, the observed high rate in our sample may partly be attributed to the ongoing impacts of the COVID-19 restrictions. Indeed, findings from most recent studies similarly point out that the SMA is becoming increasingly prevalent among youth during the pandemic [32, 33].

Another remarkable finding in our study was the significantly higher rates of problematic social media use among female students compared to males. As also suggested by previous research, one possible reason may be that females are more prone to develop addiction towards activities involving social interaction [34]. Accordingly, Banyai et al. reported that participants who were found to be at-risk for SMA were mostly female [22]. Other studies have reported similar gender differences in problematic social media use [35-37]. Findings from a national survey conducted on Norwegian adults indicated that, aside from low self-esteem and higher narcissism, addictive use of social media was associated with being young, female, and single [34]. Finally, problematic social media use was linked to lower age and female gender among Bangladeshi college students during the pandemic, which was also the case in our study [32].

Our results also revealed that, unlike in the case of SMA, male students were significantly more prone to digital gaming compared to their female counterparts. The gender difference in screen activities was further reflected by the significantly divergent GAS scores. These findings are in line with previous work which suggests two to three folds higher rates of digital gaming among male adolescents [38]. A large population based study from the U.S. additionally showed that male gamers presented with higher rates of addictive gaming behaviours than females [39]. Males have also been reported to be more motivated to play, start playing games earlier in life and spend more time in digital games [40]. Despite the strong evidence underpinning the concept, the underlying reasons for the predominance of males in digital gaming remain elusive. More recent studies underscored the complexity of the phenomenon by pointing out that several gender-sensitive factors might contribute to the observed difference in gaming behaviours among male and female adolescents. These include but are not limited to impulsivity and coping styles [41], bullying victimization and presence of meaning in life [42], game genres, gaming motives, depression [43], and hyperactivity/inattention symptoms [44].

Taken together, our findings support the notion of gender-specific screen use tendencies among adolescents, implying that male and female students may develop SDD through different pathways. However, the design of our study does not allow inferences to be made as to why and how this difference occurs.

The significant increase in screen time, a predictable consequence of the lockdown restrictions, seemed to be associated with several negative outcomes among adolescents including more frequent arguments in the family, feelings of emptiness and boredom, as well as a significant loss of pleasure towards discretionary activities during screen-free time. On the other hand, it should be noted that the lockdown might have affected students' psychological well-being in more direct ways, due to the severe restrictions in their daily activities.

Notably, our findings suggest considerable diversity in the students' attitudes towards online education. The majority were dissatisfied with the lack of peer-interaction and/or reported difficulty expressing themselves during online class. Almost half of them reported keeping their camera off due to the feelings of shame and discomfort. On the other hand, about a quarter of the students were content with not being obliged to go to the school, whereas a considerable proportion (around 14%) were relieved that they were no more exposed to bullying. These latter topics (i.e., students' experienced difficulties during online class and their views on different aspects of online education) remain relatively unexplored by previous research. In line with our findings, a recent study from the U.S. showed that the vast majority of students had their video cameras off at least some of the time during remote class meetings, with the most frequently reported reasons being concerns about appearance or about other people being seen in the background. Not wanting to be seen doing other things was reported by less then 10% of the students, which was in line with our results [45]. Our findings on the exposure to bullying were also partly consistent with the limited evidence from previous research. While a recent study conducted on Canadian adolescents reported lower rates of bullying involvement [46], some evidence indicates that cyberbullying may have become more of a problem during this time period [47].

Not surprisingly, our findings indicate a strong correlation between BSMAS, GAS and KADS-11 scores. Time spent on major non-screen activities (e.g., physical exercises, face-to-face contact with peers) was also inversely associated with addictive social media use and/or digital gaming. The link between excessive screen time and depression among adolescents has been well recognized, with a substantial body of evidence from the last decade. One dramatic example is the nationally representative surveys of the 8-12th graders in the U.S., which pointed out a sudden decrease in the psychological well-being of the students after 2012. The same reports also suggested that adolescents who spent more time on digital communication and less time on screen-free activities presented with lower mental well-being [48]. A meta-analytic review of 19 studies indicated that higher screen time was associated with significantly elevated risk of depression [49]. Furthermore, a limited number of studies conducted during the pandemic underline the fact that both SMA [32, 50] and DGA [51-53] emerge as increasingly common mental health problems among children and adolescents, with potentially distinguishing features inherent to the context of the pandemic.

The link between SDD and depression is likely to be reciprocal. For instance, it has been found that adolescents have a higher chance to develop a greater depressed mood when they browse more often through Instagram, as well as a higher chance to post more on Instagram when they have higher levels of depressed mood [54]. Similarly, Romer at. al reported that while the heavy use of the internet and videogames were associated with

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an increase in depression among youth, increased depression also predicted greater use of these media as well as withdrawal from screen-free activities [55, 56].(50) Some research findings additionally suggest that the nature of this association may largely depend on gender. For example, Liang et al. found that depression predicted subsequent internet addiction among male adolescents whereas the opposite was true for females [57]. Although our results hinder drawing such further implications, it seems plausible that the observed relationship between the adolescents' screen activities and psychological well-being was not straightforward in nature but was mediated through several different mechanisms.

Limitations and Strengths

The present findings should be addressed within the methodological limitations of our study. The categorical data (expressed in ranges) prevented a more precise determination of the students' average screen times. Given that the recruitment was conducted online, voluntary response bias may have negatively affected the representativeness of the sample. The self-report questionnaires might also have been subject to recall-bias as the screen activities were questioned retrospectively regarding the period prior to the pandemic. Due to the cross-sectional design, reported associations cannot be used to infer causal relationship between COVID-19 related problems, screen activities and mental well-being. Finally, the fact that the participants were recruited from three public high-schools located in the eastern site of Istanbul and the relatively small sample size may limit the generalizability of the findings. On the other hand, one substantial strength of the present study was its comprehensive framework which assessed adolescents' screen activities, mental well-being and COVID-19 related problems through a series of standardized measures together with a qualitative perspective of their subjective experiences. These findings may serve as a reference for future research addressing various aspects of the relationship between SDD and the COVID-19 pandemic. These include a wider range of psychological problems (e.g., school refusal, attention problems) as well as physical health outcomes such as obesity, metabolic diseases and sleep disturbances among children and adolescents. Finally, the additional data on the participants' smartphone ownership and/or digital media accessibility may allow more objective comparability with similar studies on this topic.

Conclusion

The COVID-19 pandemic and subsequent public health measures including stay-at-home policies, online education and social distancing have led to an increasing dependence on digital technologies in several substantial domains of the adolescents' lives. Although the restrictions are being gradually lifted, their impacts on the adolescents' screen use habits are likely to last for some time. Research suggests that different screen use patterns may be associated with diverging (positive or negative) outcomes on the youth's well-being during the pandemic. The addiction risk and other detrimental consequences appear to be associated with the qualitative features of screen activities, rather than just the amount of time spent on digital media by the adolescents. It seems thus imperative for mental health professionals and decision makers to adopt multi-dimensional strategies aiming to prevent SDD among children and adolescents, through a balanced view of the risks and benefits of the use of digital technologies in the era of the pandemic.

Compliance with Ethical Standards

Ethical Approval: Approval for the study was obtained from the Ethics Committee of Maltepe University, School of Medicine with the protocol number 2021.900.27.

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Investigating the impact of endometrial compaction on clinical pregnancy rate in artificial frozen-thawed embryo transfer cycles

Kadriye ERDOGAN[1], Nazlı Tunca SANLIER[2], Emine UTLU OZEN[1], Serdar DILBAZ[1], Inci KAHYAOGLU[1], Yaprak Engin USTUN[1]

[1] Department of Obstetrics and Gynecology, University of Health Sciences, Etlik Zübeyde Hanim Women's Health Education and Research Hospital, Ankara, Turkey.
[2] Obstetrics and Gynecology Clinic, Ankara City Hospital, Ankara, Turkey.

Corresponding Author: Kadriye ERDOGAN
E-mail: opdrkadriye.erdogan@outlook.com

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ABSTRACT

Objective: The aim of our study was to evaluate sonographic endometrial thickness succeeding the estrogen-only stage and on the day when embryo transfer (ET) occurred in artificial frozen embryo transfer (FET) cycles to delve into the effect of endometrial compaction (EC) on clinical pregnancy rate (CPR).

Patients and Methods: In the first group endometrial thickness diminished when ET occurred when compared to the end of the estrogen-only phase (n:37). Endometrial thickness increased/did not alter for the second group (n:70).

Demographic characteristics were recorded and the following were studied: in vitro fertilization (IVF) treatment indications, hormone levels, total antral follicle count, duration of infertility, embryo quality, embryo-fundus distance, endometrial thickness at the end of estrogen-only phase and on ET day, luteal support, CPR.

Results: No significant difference occurred in CPRs (n:107). ET, on day 5 was higher in the first group ($p<0.05$). Regression analysis revealed EC was 8.000 times higher in those with ET day 5 than those with 3.

Conclusion: Endometrial compaction is non-relevant to the rate of clinical pregnancy. The day of ET affected the presence of EC.

Keywords: Endometrial compaction, Artificial frozen embryo transfer cycles, Clinical pregnancy rate, Day of embryo transfer

1. INTRODUCTION

Endometrium changes its surface epithelium, vascular network as well as expressing certain glycoproteins, integrins, receptors, along with chemokines to prepare endometrial environment for embryo implantation. Endometrial receptivity is common during the implantation window which occurred on days 20-24 in a 28-day cycle [1]. Recent research has revealed the findings pertaining to that endometrial thickness and pattern carry a pivotal role for embryo transfer (ET) outcomes at the end of the proliferative phase [2,3]. When the endometrial thickness was measured less than 7-8 mm or greater than 13 mm, the clinical pregnancy rate (CPR) and live birth rate lower both for fresh and frozen embryo transfer (FET) cycles [4,5]. Whilst, the assessment of endometrial thickness on the human chorionic gonadotropin (hCG) trigger day in fresh cycles or subsequent to the estrogen-only phase in FET cycles is a key factor to decide the ET, evaluating endometrial thickness on the day of ET is also deemed critical [6].

The decrease in endometrial thickness, boost in density and hyperechoic appearance on ultrasound by progesterone initiation between the closing of the estrogen-only phase and the time of ET is entitled endometrial compaction (EC). The relation between EC and the rate of pregnancy of clinical sort is controversial, although, some studies unveiled a relation between CPR and EC, some actually did not [7-9].

In this direction, we intended to evaluate sonographic endometrial thickness in the wake of the estrogen-only phase

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and on the day when ET occurred in artificial FET cycles with a view to scrutinizing the effect of EC on CPR.

2. PATIENTS and METHODS

This retrospective research was conducted with 107 women in the in-vitro fertilization (IVF) clinic of Etlik Zübeyde Hanim Women's Health Training and Research Hospital, Ankara, Turkey. The study protocol was approved by the said hospital's Ethics Committee (Clinical study 06.07.2022/2022/94). The present research had two groups and in the first one there were women whose endometrial thickness was spotted to diminish at the time of ET when compared to that of the end of the estrogen-only phase (n:37). The second group involved women whose endometrial thickness increased or did not alter (n:70).

Solely patients with artificial FET cycles and single ET were included in the study. History of chronic diseases, the patients who received mild or natural cycle protocols, the ones with fresh cycles, with more than one embryo transfer, the use of preimplantation genetic diagnosis, oocyte and embryo recipients were all excluded in this regard.

Characteristics that are pertinent to demographic information of the participants maternal age, body mass index (BMI), gravidity, abortion, and living child were documented. IVF treatment indications e.g., male factor, diminished ovarian reserve and others, day 3 (D3) estradiol (E2), levels belonging to follicle stimulating hormone (FSH), luteinizing hormone (LH) and anti-Müllerian hormone (AMH), total antral follicle count, duration of infertility, transferred embryo quality [10], embryo-fundus distance, E2 level on ET, progesterone level on ET, day of ET, endometrial thickness following the estrogen-only phase and on the very day when ET took place, luteal support, CPR were calculated. Clinical pregnancy was considered as the determination of gestational sac via ultrasound.

All the patients received oral E2 pill (Estrofem 2 mg pill, Novo Nordisk Pharma, Denmark) twice daily, on day 2–3 of the menstrual cycle, and ultrasound evaluation of endometrial thickness and pattern was executed 7 days after the initiation of E2. All ultrasound evaluations were performed by the same clinician in the IVF clinic by LOQIC A5 ultrasonography. On the condition that endometrial thickness was located as < 7 mm, the dose was elevated three times per day (6 mg E2). The endometrium was re-assessed on the 10-12 day after starting E2, and provided it was measured as >7-8 mm through a trilaminar pattern, the progesterone was initiated, if it was still < 7 mm, estrogen administration was prolonged for up to 28 days with maximum 6 mg E2 dosage per day [11].

Intramuscular progesteron (100 mg daily, Progestan, Koçak Pharma, Türkiye) and oral dydrogesteron (10 mg 3 times a day, Duphaston, Abbott Pharma, USA) or intramuscular progesteron (100 mg daily) and vaginal progesterone (90 mg 2 times a day, Crinone gel, Merck Pharma, Germany) were used and the endometrial thickness were measured on the day of embryo ET. Embryo freezing was performed using the vitrification method (RapidVit Omni, Vitrolife, Sweeden) on days 3 or 5. Embriyos were loaded onto straw (Kitazato Cryotop, Spain) for freezing. The thawing procedure (RapidWarm Omni, Vitrolife) was applied for thawed embryos. The embryos were transferred by using soft ET catheter (ET catheter, Laboratoire CCD, France) to the uterus via transabdominal ultrasound guidance [10]. Blastocyst stage embryo was transferred on the 6th day of progesterone administration, the cleavage stage embryo was transferred on the 4th day of progesterone administration.

Statistical Analysis

Statistical work of the data was implemented using the Statistical Package for Social Sciences (SPSS) software v.24 (SPSS Inc., Chicago, IL, USA). To be able to examine variables of continuous nature, the data coming from the two independent groups were contrasted resorting to the Independent Samples T-Test for normal distribution and the Mann-Whitney U test was referred to for non-normal distribution. "Pearson-χ2 crosstabs" were used to examine the relationships between two qualitative variables. A multiple linear regression analysis was utilized so as to observe the relationship between day of embryo transfer and EC group. Parameter estimates, alongside 95% confidence intervals were noted. A two-sided p-value < 0.05 was found out statistically significant for all the analyses realized.

3. RESULTS

A total of 107 women participated in this study. We found no significant difference in demographic and obstetric characteristics, D3 FSH level, D3 LH level, D3 E2 level, AMH level, total antral follicle count, duration of infertility, transferred embryo quality, embryo-fundus distance, E2 level on ET, progesterone level on ET between the groups (p>0.05) (Table I).

No difference of significant sort was figured out either in male factor, tubal factor, diminished ovarian reserve, unexplained infertility, luteal support, and CPR between the two groups (p>0.05). The day 5 of ET was significantly higher for the first group (p<0.05) (Table II).

As a result of the backward endeavor and the LR logistic regression analysis according to the existence of EC, all insignificant parameters were removed and ET day was determined as the only significant parameter in the model; the optimal model was given in Table III.

In the current model it was discerned that the day of ET was a crucial factor affecting the presence of EC (p<0.05). The existence of EC was 8,000 times more in those with ET day 5 than those with 3.

***Table I**. Comparison of demographic, laboratory, and cycle characteristics*

Variables	Study group (n=37)		Control group (n=70)		Statistical analysis* P value
	X̄ ±S.S.	Median [Min-Max]	X̄ ±S.S.	Median [Min-Max]	
Age (years)	29.33±3.19	29.0 [23.0-36.0]	2.32±3.45	28.5 [2.0-34.0]	Z=-1.305 p=0.192
BMI (kg/m²)	26.01±5.39	23.9 [16.7-37.2]	25.29±4.34	24.7 [18.6-36.0]	Z=-0.362 p=0.717
Gravida	0.53±0.85	0.0 [0.0-4.0]	0.59±0.91	0.0 [0.0-4.0]	Z=-0.077 p=0.939
Living child	0.08±0.28	0.0 [0.0-1.0]	0.06±0.29	0.0 [0.0-2.0]	Z=-0.789 p=0.430
Abortion	0.44±0.84	0.0 [0.0-4.0]	0.31±0.61	0.0 [0.0-2.0]	Z=-0.759 p=0.448
AMH (ng/mL)	3.68±3.31	3.0 [0.2-10.4]	4.77±4.71	3.7 [0.2-23.7]	Z=-1.058 p=0.290
D3 FSH (mIU/mL)	6.53±2.26	6.2 [2.2-11.0]	6.83±2.86	6.3 [1.5-22.0]	Z=-0.103 p=0.918
D3 LH(mIU/mL)	6.19±4.56	5.4 [0.4-23.1]	5.24±2.51	5.0 [0.2-15.2]	Z=-0.540 p=0.589
D3 E2(pg/mL)	46.50±25.83	39.8 [13.0-161.0]	43.82±20.23	42.2 [5.0-118.5]	Z=-0.033 p=0.973
Total antral follicul count	17.41±8.54	17.0 [3.0-30.0]	18.07±9.12	17.5 [0.0-30.0]	Z=-0.304 p=0.761
Duration of infertility (month)	63.24±36.13	60.0 [12.0-180.0]	70.35±42.71	60.0 [12.0-192.0]	Z=-0.606 p=0.545
Transferred embryo quality	1.50±0.63	1.0 [1.0-3.0]	1.48±0.64	1.0 [1.0-3.0]	Z=-0.181 p=0.856
Embryo – fundus distance (mm)	9.64±3.67	10.0 [1.6-19.0]	9.77±3.38	9.9 [0.5-16.7]	t=-0.174 p=0.862
ET Progesteron (ng/mL)	11.60±11.34	8.7 [0.5-60.0]	8.55±5.96	8.9 [0.1-33.6]	Z=-0.776 p=0.438
ET E2 (pg/mL)	377.21±468.29	265.0 [31.4-2810.0]	300.30±229.34	244.5 [27.9-1458.7]	Z=-0.762 p=0.446

*BMI: body mass index, AMH: anti-mullerian hormone, FSH: follicle stimulating hormone, LH: luteinizing hormone, E2: estradiol, ET: embryo transfer. S.D: standard deviation, Independent Sample-t test (t-table value), Mann-Whitney U test (Z-table value) statistics. *P-value of less than 0.05 was considered to be statistically significant*

***Table II.** Comparison of IVF parameters*

Variables	Study group (n=37)		Control group (n=70)		Statistical analysis* P value
	n	%	n	%	
Male factor					
Yes	14	37.8	30	42.9	χ^2=0.252 p=0.616
Tubal factor					
Yes	1	2.7	4	5.7	χ^2=0.493 p=0.483
Unexplained infertility					
Yes	16	43.2	25	35.7	χ^2=0.581 p=0.446
Diminished ovarian reserve					
Yes	5	17.9	9	15.3	χ^2=0.095 p=0.758
Luteal support					
Crinone gel	25	67.6	51	72.9	χ^2=0.329
Duphaston pill	12	32.4	19	27.1	p=0.566
Clinical pregnancy					
Yes	15	40.5	26	37.1	χ^2=0.118 p=0.731
Day of embryo transfer					
day 3	1	3.4	14	22.2	χ^2=5.129
day 5	28	96.6	49	77.8	**p=0.024**

*Pearson χ2 crosstabs. *P-value of less than 0.05 was considered to be statistically significant*

Table III. *The logistic regression model according to the presence of endometrial compaction*

Variables	B	S.E.	Wald	df	p	OR	95% Odds Ratio (OR)	
							Min	Max
Day of embryo transfer*	2.079	1.062	3.835	1	**0.049**	8.000	1.124	22.143
Constant	-2.639	1.035	6.500	1	0.011	0.071		

**Reference category: day of 3. B: beta coefficient, S.E: standard error, df: degrees of freedom*

4. DISCUSSION

It is noteworthy to highlight here that we did not find any significant difference between EC and CPR in artificial FET cycles. Though, in the literature, there were conflicting results about the impact of EC on CPR [7,12].

A review of the line of literature signals various results. To cite an example, in a study by Haas et al., the continuing pregnancy rate was significantly higher in EC group whereas Jin et al. reported that the CPR was higher in cycles that had no EC [7,13]. In contrast with Jin et al., Kaye showed that the CPR was higher in EC group [14]. In accordance with our study, Olgan determined that CPR did not differ between EC and non-compaction groups [15].

In the past, a fair number of studies concentrated upon the endometrial lining thickness rather than EC [16,17]. Recently, considering the endometrial pathophysiology, clinicians have begun to focus on whether EC may have an impact on pregnancy rate. Plentiful factors come into play in relation to the presence or absence of EC. In regular menstrual cycles, endometrial proliferation halts for 2–3 days posterior to ovulation and becomes denser by progesterone effect [18]. The continuation of endometrial proliferation during the luteal phase might be due to insufficient progesterone effect that cannot be observed by measuring serum progesterone levels. In the research of Usadi et al., it was announced that histological endometrial dating did not reflect circulating progesterone concentrations, the duration of progesterone exposure was more essential than serum progesterone concentrations on the quality of luteal function [19]. In addition to these, the continuation of endometrial proliferation during the luteal phase which manifested itself with absence of EC can emerge in the existence of progesterone receptor insufficiency or resistance in the endometrium. Several studies have underpinned that the activation of KRAS gene and the over-expression of Sirtuin 1 (SIRT1), BCL6 (B Cell Lymphoma 6) and escalated progesterone receptor (PGR) expression were associated with progesterone resistance [20, 21].

As a result of this study, we recognized that EC was more common in women undergoing day of 5 ET. As has been mentioned earlier, the duration of progesterone exposure on the endometrium was more influential than serum concentration. Blastocyst stage embryo was transferred on day 6 of progesterone initiation thereby, progesterone exposure on the endometrium was higher than the day of 3 ET.

In a nutshell, it would be fair to italicize that EC seems not to be linked to CPR, and the results of our study entails validation by prospective studies.

Compliance with Ethical Standards

Ethical Approval: The ethics committee of Etlik Zübeyde Hanim Women's Health Training and Research Hospital of Ankara, Turkey, approved the study on July 06, 2022, with decision number 2022/94.

Financial Support: No special funding was obtained.

Conflict of Interest: The authors declare that there are no conflicts of interest.

Authors' Contributions: KE: visualization, investigation, methodology, supervision, writing-review and editing, NTS: investigation, conceptualization, writing – original draft, EUO: investigation, conceptualization, writing – original draft, SD: conceptualization, writing – original draft, IK: investigation, conceptualization, writing – original draft, YEU: investigation, conceptualization, writing – original draft. All authors approved the final version of the article.

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Effect of whey protein derivatives on cell viability, cell migration and cell cycle phases in MCF-7 cells

F. Tutku AKSOY[1], Ayse Mine YILMAZ[2], Gokhan BICIM[3], A. Suha YALCIN[2]

[1] Department of Medical Biochemistry, School of Medicine, Altinbas University, Istanbul, Turkey
[2] Department of Biochemistry, School of Medicine, Marmara University, Istanbul, Turkey * Genetic and Metabolic Diseases Research and Investigation Center, Marmara University, Istanbul, Turkey
[3] Department of Basic Sciences, School of Dentistry, Istanbul Kent University, Istanbul, Turkey

Corresponding Author: A. Suha YALCIN
E-mail: asyalcin@marmara.edu.tr

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ABSTRACT

Objective: This study aimed to obtain protein derivatives after treatment of whey proteins with hazelnut oil and olive oil and determined their effects on MCF-7 cells.

Materials and Methods: Whey proteins obtained from 6% whey powder were treated with hazelnut oil (HO) and olive oil (OO) at a protein to lipid ratio of 1:10 at 60 ℃ for 120 minutes. The protein derivatives formed with whey protein and HO or OO were applied to MCF-7 cancer cells and healthy fibroblasts. The effects of protein derivatives on cell viability, apoptosis, reactive oxygen species (ROS) production, wound healing, cell cycle phase distribution and cell cycle related proteins Akt and p21(Waf1/Cip1) expressions were investigated.

Results: Cell viability decreased significantly after 24 h of incubation with WP:OO. The percentage of apoptotic or necrotic cells varied between 5-10% and no statistically significant effect was observed. There was no statistically significant difference in ROS production and colony formation between controls and WP:HO or WP:OO groups. Treatment of cells with WP:OO for 24 h significantly decreased cell migration compared to the control group. G2/M phase was significantly suppressed in WP:OO group compared to the control group. WP:OO also increased the expression of p21(Waf1/Cip1) significantly when compared with the control group.

Conclusion: Our results showed that whey protein derivatives applied to MCF-7 cells are cytotoxic and may be useful in breast cancer treatment.

Keywords: Whey proteins, Oleic acid, Cell survival, Apoptosis, Cell cycle

1. INTRODUCTION

The remaining liquid after precipitation of casein in cheese production is called whey or milk serum. This by-product contains soluble proteins that are grouped into major and minor fractions [1,2]. Major whey proteins are α-lactalbumin (α-La, 20%), β-lactoglobulin (β-Lg, 50%), serum albumin (10%), immunoglobulins (10%) and proteose-peptones (10%). Among minor proteins are lactoperoxidase, lactoferrin, insulin-like growth factor and γ-globulins [3,4]. Whey proteins are important in terms of both their biological value and their high content of sulfur-containing amino acids [5,6].

The human α-La and oleic acid (OA) complex called human alpha-lactalbumin made lethal to tumor cells (HAMLET) exhibits remarkable apoptotic activity [7-9]. A similar complex BAMLET, which is made with bovine α-La and oleic acid, can induce cell death in transformed cells [10, 11] and it has been used for the treatment of skin papilloma [12] and bladder cancer [13]. It was also reported that the lactoferrin-oleic acid complex showed similar apoptotic activity on cancer cells [14].

HAMLET-like cytotoxic complexes can be formed in different ways, in which α-La is assumed to be in partially unfolded conformation and able to bind OA at various stoichiometries [15]. OA has an important role in these complexes whereas the protein component acts as a vehicle for transporting toxic OA into cells and keeping OA in solution. Although, the protein/OA molar ratio was initially reported to be 1:1, recent data have shown that the OA complex is delivered by an oligomeric protein capable of binding a large number of OA molecules per protein monomer [16]. In general, the number of HAMLET-like

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complexes with proteinaceous component is not a prerequisite for their effectiveness. The cytotoxicity of these complexes is mostly because of OA [17].

Oleic acid is present in both hazelnut oil (HO) and olive oil (OO) in high amounts (70-80%). In addition to fatty acids, OO contains many phenolic compounds, i.e. hydroxytyrosol, tyrosol, oleuropein, oleocanthal, luteolin, apigenin, caffeic acid and ferulic acid [18]. The antioxidant, anti-inflammatory, immunomodulatory and antineoplastic activities of OO's phenolic components, as well as the effects of fatty acids, are of great biological importance [19,20]. These compounds show anticancer properties by promoting apoptosis, modulating epigenetic patterns, blocking the cell cycle, and reducing cell migration and angiogenesis [21–23]. Among the phenolic components oleochantal suppresses STAT3 activation and prevents malignant cell migration, and luteolin causes apoptosis with suppression in the G2/M phase [24,25]. In HO, there are main components such as waxes, sterols, methyl-sterols, aliphatic and terpenic alcohols, tocopherols, tocotrienols and hydrocarbons, as well as triacylglycerides and fatty acids. The lipid profile of HO appears to be very similar to OO [26].

In this study, HO and OO were used as the source of OA, which has an important role in the structure of HAMLET. Both oils contain various long-chain fatty acids and additional bioactive molecules. It was important to investigate the effect of these different molecules in forming possible protein derivatives with whey proteins and to observe their effects on viability of MCF-7 cells. We therefore aimed to obtain cytotoxic protein complexes of whey proteins, HO and OO, and determined their possible effects on apoptosis, wound healing, reactive oxygen species (ROS) production, colony formation, cell cycle phase distribution and cell cycle related protein expressions.

2. MATERIALS and METHODS

Whey powder with high protein content (11%) was used for the stock solution of whey and was supplied from Malkara Birlik (Malkara Birlik A.Ş., Malkara-Turkey). HO and OO were from Çotanak (Altaş, Ordu-Turkey) and Komili (Bunge Gıda A.Ş., İstanbul-Turkey), respectively. A stock solution of whey (6%) containing 116 µM α-La was prepared using 0.01 M phosphate-buffered saline (PBS) and whey powder [27]. Stock solutions of HO and OO containing 1160 µM OA were prepared using Dulbecco's modified Eagle's medium (DMEM) containing dimethyl sulfoxide (DMSO). Final concentration of DMSO was kept below 0.1%. These two solutions were mixed at a ratio of 1:1 and incubated at 60°C for 120 minutes. In this way cytotoxic whey protein complexes (WP:HO or WP:OO) containing 58 µM α-La and 580 µM OA with a protein:fatty acid ratio of 1:10 was prepared [28].

Determination of cell viability

Human breast cancer cells (MCF-7) and human foreskin fibroblast cells (HFF) obtained from American Type Culture Collection (ATCC, USA) were used. Cell lines were cultured in DMEM supplemented with 10% fetal bovine serum (Sigma, USA) containing 1% penicillin and streptomycin in a 5% CO_2 incubator at 37°C. The effect of whey protein derivatives on cell viability was determined by MTT Cell Viability Assay (Roche, Mannheim, Germany). Whey protein complexes (WP:HO or WP:OO) were applied to 96 well-plates, each well containing $1x10^4$ cells, and incubated at 37°C for 24 h. After incubation, MTT assay was applied to determine cell viability. Cell viability of each group was calculated from absorbance at 560 nm measured in a microplate reader. Measurements were repeated 3 times.

Determination of apoptotic and necrotic cell death

The effect of whey protein complexes on cell death was evaluated by flow cytometry using the FITC Annexin V Apoptosis kit (BioLegend). For this purpose, $1x10^5$ cells/well were seeded into a 6-well plate. Six mL of each sample was then added to each well and incubated for 24 h. Cells were harvested, washed with PBS, and stained with Annexin-V / PI according to the user instructions. Annexin-V-negative and PI-negative cells represented the healthy cells, while Annexin-V-positive and PI-negative cells corresponded to early apoptotic cells, Annexin-V-positive and PI-positive cells corresponded to late apoptotic cells and Annexin-V-negative and PI-positive cells corresponded to necrotic cells. Stained cells were detected by FACS Calibur flow cytometer, and the results were calculated with Cell Search software (BD Biosciences, USA).

Measurement of ROS

Reactive oxygen species were detected using 5-(and-6)-chloromethyl-20,70-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA) staining. Cells were seeded into 6-well plates ($1x10^5$ cells/well) and treated with whey protein derivatives for 24 h. After treatment cells were washed and incubated with phenol red-free DMEM medium containing 10 µM CM-H_2DCFDA for 30 min at 37°C, in the dark. Stained cells were analyzed in FACS Calibur flow cytometer. The analysis was performed by comparing the stained and unstained cells.

Wound healing assay

To determine the effect of whey protein derivatives on cell migration, a wound healing assay was performed [29]. MCF-7 cells were grown in 6-well plates until 70–80% confluency was reached. At this time a 100 µL pipette tip was used to create a scratch/wound with clear edges across the width of a well. Wells were treated either with vehicle control (DMSO) or whey protein derivatives and photomicrographs were taken at 24 and 48 h. A Nikon TS100 inverted microscope was used to measure and photograph cell migration from the wound/scratch edge. Experiments were performed in triplicate.

Colony formation

Colony formation was performed to determine the efficiency in inhibiting colony formation of the cells [29]. MCF-7 cells were seeded ($1x10^5$cells/well) in 6 well plates containing 2 mL of medium and waited for cell adhesion. Following incubation of

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cells for 24 h, the colonies were stained with 0.5% crystal violet and counted.

Cell cycle analysis

Cells were seeded at ($1x10^5$ cells/well) in 6-well plates containing 2 mL of medium. Analysis was performed 24 h after the application of whey protein derivatives. The collected cells were fixed with 70% ethanol and DNA extraction was performed in phosphate-citric acid buffer (0.2M, pH 7.8). The extracted DNA were stained with PI and incubated for 15 minutes at room temperature. Cell cycle phase distribution was analyzed using FACS Calibur flow cytometer.

Western blot analysis

Expression of p21(Waf1/Cip1) and AKT was analyzed by Western blot analysis. Cells were seeded in 10 cm culture dishes ($1x10^6$ cells). Protein was extracted from cells with lysis buffer (50 mM Tris–HCl, pH 6.8, 15 mM EDTA, 15 mM $MgCl_2$, 50 mM Glycerol, 150 mg/mL digitonin containing 1 mM dithiothreitol, and 100 mM PMSF). Samples were kept on ice for 15 min and the supernatant was collected after centrifugation at 18,000 x g for 10 min. Protein concentration was determined with BCA assay (Pierce Chemical, USA). Forty µg of total protein was loaded into each well. After SDS-PAGE, proteins were transferred to nitrocellulose membranes by wet-transfer. Membranes were blocked with 5% BSA in Tris-buffered saline containing 0.1% Tween 20 and immunoblotted overnight at 4°C with corresponding primary antibodies (GAPDH, p21(Waf1/Cip1), AKT) followed by horseradish peroxidase-linked secondary antibody. Detection was performed using the West Pico chemiluminescent substrate kit (Thermo Scientific, USA) and the ChemiDoc MP System (Bio-Rad, USA).

Ethical approval for the study was obtained from the Clinical Research Ethical Committee of Altınbaş University (Approval number: 121/27.5.2022).

Statistical Analysis

Results were presented as mean ± standard deviation and statistical analysis was performed using Graphpad Prism 8.0.1 software. The limit of significance was $p<0.05$ using the ANOVA test. The significance level between groups was evaluated by the Tukey test.

3. RESULTS

The effect of whey protein derivatives (WP:HO and WP:OO) on viability of MCF-7 cells is shown in Figure 1. Cell viability was decreased after 24 h of incubation with WP:OO and WP:HO, but the effect was significant only for WP:OO. We then investigated the effects of WP:HO and WP:OO on apoptotic and/or necrotic cell death by flow cytometry. The percentage of apoptotic or necrotic cells varied between 5-10% and although there was a slight difference between WP:HO and WP:OO no statistically significant effect was observed (Figure 2).

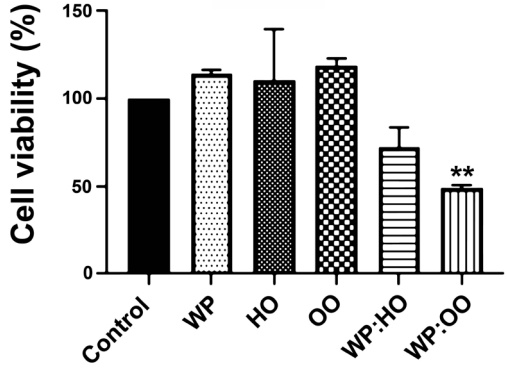

Figure 1. *Viability of MCF-7 cells after treatment with WP (58 µM α-LA), HO (580 µM oleic acid), OO (580 µM oleic acid), WP:HO (58 µM α-LA+580 µM oleic acid) and WP:OO (58 µM α-LA+580 µM oleic acid) for 24 h.*

Control group (Control) received no treatment. Cell viability was measured by MTT assay. Data presented as the mean ± SD of three independent experiments.
***p<0.001 compared with contro*

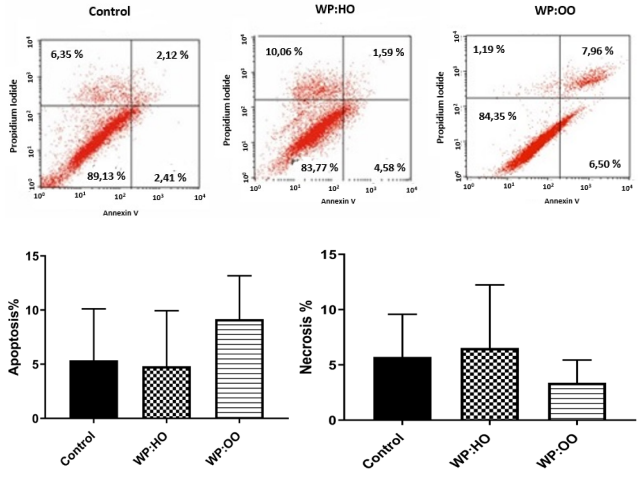

Figure 2. *Flow cytometric analysis for apoptosis and necrosis in MCF-7 cells after treatment with whey protein derivatives.*

Control group received no treatment. Percentage of viable cells (Annexin-V negative/PI negative), early apoptotic cells (Annexin-V positive/PI negative), late apoptotic cells (Annexin-V positive/PI positive) and necrotic cells (Annexin-V negative/PI positive) were calculated.

Data presented as the mean of at least three independent experiments.

$p < 0.05$ compared with control.

The effect of whey protein derivatives on the production of ROS was investigated by CM-H_2DCFDA staining in MCF-7 cells (Figure 3). No statistically significant difference was found between controls and WP:HO or WP:OO groups. We have also performed wound healing assay to determine whether whey protein derivatives

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decrease cell viability through their effect on cell migration (Figure 4). Treatment of cells with WP:HO and WP:OO for 24 h decreased cell migration compared to the control group. Here again the decrease was significant only for WP:OO. Next we determined colony formation which is also related to cell proliferation. However, there was no significant change in colony formation when the protein mixtures were applied for 24 h (Figure 5).

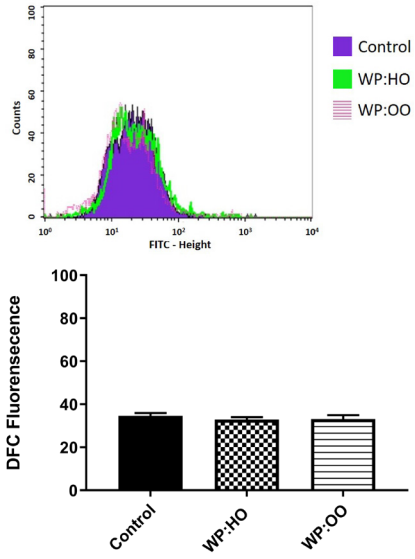

Figure 3. *Effect of whey protein derivatives on ROS production in MCF-7 cells.*

ROS production was investigated by flow cytometry.
Data presented as the mean of at least three independent experiments.
$p < 0.05$ compared with control.

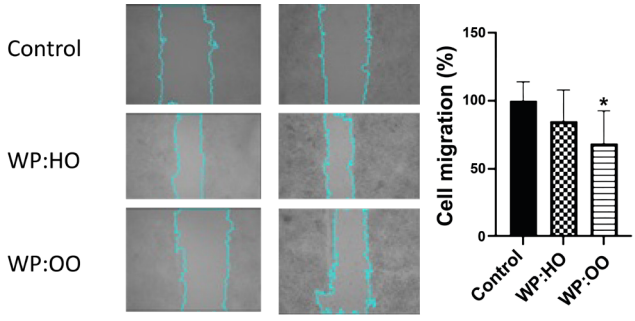

Figure 4. *Effect of whey protein derivatives on migration in MCF-7 cells.*

Cell migration was investigated by wound healing assay.
Data presented as the mean ± SD of three independent experiments.
**$p < 0.05$ compared with control.*

Finally, we have investigated the effect of whey protein derivatives on cell cycle phase distribution. It was determined that G2/M phase was significantly suppressed in WP:OO group compared to control group (Figure 6). Western blot analysis for the expression of cell cycle related proteins Akt and p21(Waf1/Cip1) showed that WP:HO mixture decreased the expression of AKT and increased the expression of p21(Waf1/Cip1) significantly. Additionally, it was observed that WP:OO increased expression of p21(Waf1/Cip1) was more than that of WP:HO (Figure 7).

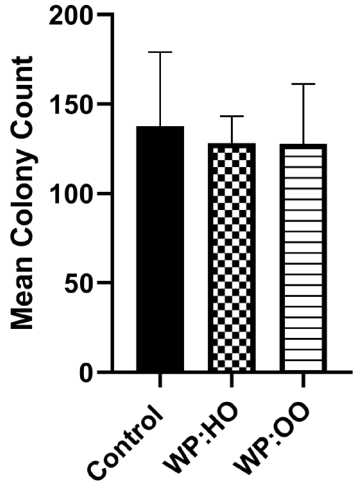

Figure 5. *Effect of whey protein derivatives on colony formation in MCF-7 cells.*

Results were obtained by colony formation assay as described in the methods.
Data presented as the mean ± SD of three independent experiments.
$p < 0.05$ compared with control.

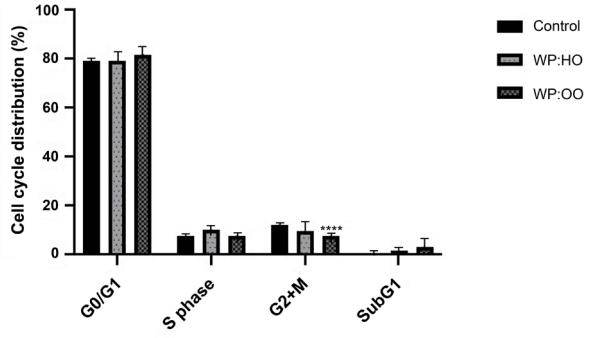

Figure 6. *Effect of whey protein derivatives on cell cycle distribution of MCF-7 cells.*

Cell cycle progression was measured by flow cytometry. Histogram shows representative distribution of G0/G1, S, G2/M and apoptotic phases after treatment with whey protein derivatives.
Data presented as the mean ± SD of at least three independent experiments.
*****$p<0.0001$ compared with control*

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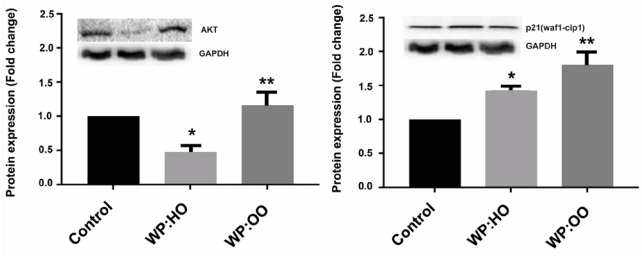

Figure 7. *Effect of whey protein derivatives on cell cycle related protein expressions.*

MCF-7 cells were treated with WP:HO and WP:OO for 24 h. Control group received no treatment. Protein lysates were analyzed by Western blotting as described in materials and methods. GAPDH was used as loading control.
Protein expression levels are given as fold changes of each protein compared to loading control.
Data presented as the mean ± SD of at least three independent experiments.
***p<0.001 compared with control*

4. DISCUSSION

Several different approaches to obtain cytotoxic complexes of whey proteins and OA exist in the literature [15]. In this study, we have used high protein whey powder and HO or OO to prepare whey protein derivatives. The polyunsaturated fatty acid contents of HO and OO were 15% and 11%, respectively. The ratio of whey proteins to the OA sources was 1:10 and whey protein derivatives were obtained by applying heat treatment [15]. We have determined that the effects of WP:OO on cell viability, cell migration ability, G2/M cell cycle phase and expression of cell cycle-related proteins AKT and p21(Waf1/Cip1) was significantly more and different compared to those of WP:HO.

Zhang et al., reported that both OA and linoleic acid induced apo-B/La intermediate to form amorphous aggregates at pH 4.0-4.5 [30]. The aggregates formed were structurally similar to HAMLET and BAMLET, a complex of partially unfolded α-La with OA. Their cell viability assays showed that apo-B/La aggregates induced by OA and linoleic acid showed significant dose-dependent cytotoxicity to human lung tumor cells. A simple 2-step method for purification of calcium free α-La was developed and the purified protein was used in another study to generate a complex with OA using 3 different cell lines and 2 types of cell viability assays [31]. It was determined that both bovine and human α-La showed comparable cytotoxic activity.

Cell adhesion is tightly regulated by certain molecular interactions. Dissociation from extracellular matrix affects cellular activities such as proliferation and survival. HAMLET is a protein-lipid complex that also triggers tumor cell detachment, both in vitro and in vivo [15, 30, 31]. A549 lung cancer cell membrane extracts treated with HAMLET binds α-actinins and adherent tumor cells were rolled and separated in response to HAMLET [32]. As a result, the cytoskeletal organization was disrupted and decreased cell migration observed in wound healing assay might be related to this change. Another factor might be the presence of various phenolic components in OO [18].

Increasing evidence has shown that natural ingredients, including lactoferrin, OA, docosahexaenoic acid (DHA), and linolenic acid have anti-inflammatory and anti-tumor activities. Yao et al., examined the viability, migration, invasion and apoptosis of HT29 cells to select appropriate doses of these components [33]. BALB/c nude mice bearing colorectal tumors were used to investigate the role of the chosen combination in inhibiting tumor growth. Their results showed that lactoferrin at 6.25 mM, OA at 0.18 mM, DHA at 0.18 mM and linolenic acid at 0.15 mM significantly inhibited the viability of HT29 cells. The combination of lactoferrin + linolenic acid exhibited the strongest activity in inhibiting migration and invasion of HT29 cells. Moreover, the combination of lactoferrin + linolenic acid activates p-AMPK and p-JNK and apoptosis of HT29 cells. The study was the first to demonstrate that the combination of lactoferrin+linolenic acid inhibits HT29 tumorigenesis by activating the AMPK/JNK-related pathway. It is possible that similar compounds may exist in our WP:OO mixture.

Interaction of OA with albumins isolated from human, bovine and camel milk results in the formation of complexes with high antitumor activity against Caco-2, HepG-2, PC-3 and MCF-7 tumor cells [34]. The antitumor effect of these complexes was mostly due to OA. The viability of tumor cells as assessed by the MTT method was inhibited in a dose-dependent manner by albumin-OA complexes. The strong induction of apoptosis in tumor cells after administration of the complexes caused morphological change in MCF-7 cells, showed an apoptotic effect around 50% and suppressed the G2/M cell cycle phase. Trullson et al., showed that HAMLET binds to α-actin, which is a cytoskeletal protein, and disrupts the integrin-dependent cell adhesion signal, and as a result the integrity of tumor cells is impaired [32]. In our study, although, we had a lower apoptotic effect there were similar changes in MCF-7 cells and suppression in the G2/M phase. As stated in the introduction, among of the phenolic components of OO, oleochantal prevents malignant cell migration, and luteolin causes apoptosis with suppression in the G2/M phase. This may explain our cell cycle and cell migration results.

Bovine β-Lg was shown to gain cytotoxicity against tumor cells upon binding to OA and increasing the molar ratio also increased the cytotoxicity of the complex [35]. In another study in which α-La and OA complexes were prepared, cytotoxic effects against both cancer cells and non-cancer primary cells were observed [36]. Flow cytometry was used to observe the cell killing mechanisms, apoptotic and/or necrotic effects of the complex and OA alone. Jurkat cells derived from T-cell leukemia mainly underwent apoptosis-like cell death, whereas THP1 cells derived from monocytic leukemia adopted a more necrotic-like cell death.

Considering all of the above-mentioned studies by various groups that are consistent with our results, we conclude that OA complexes of whey protein are indeed cytotoxic to cancer cells. Different OA protein complexes seem to be responsible for the

observed changes in viability, migration and cell cycle phases of MCF-7 cells. However, we must admit that the role of other ingredients present in OO cannot be excluded and additional studies are required to elucidate the structures of the specific components involved in the observed changes.

Compliance with the Ethical Standards

Ethical Approval: Ethical approval for the study was obtained from the Clinical Research Ethical Committee of Altınbaş University (Approval number: 121/27.5.2022).

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

Authors' Contributions: FTA and ASY: Generated the initial idea and experimental design, FTA, GB and AMY: Performed the experiments and analyzed data, FTA and ASY: Wrote the manuscript and all authors contributed to the critical revision and gave final approval to the submitted version.

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Glaucoma awareness in Family Health Centers

Miray SANCAKTAR DEMIROZ[1], Seyhan HIDIROGLU[1], Dilajla ORAOVCANIN[2], Merve AKBAS[2], Annisha Condace SKINNER[2], Sumeyye KARAPINAR[2], Ayse SARI[1], Melda KARAVUS[1]

[1] Department of Public Health, School of Medicine, Marmara University, Istanbul, Turkey
[2] School of Medicine, Marmara University, Istanbul, Turkey

Corresponding Author: Miray SANCAKTAR DEMIROZ
E-mail: miray.sanc@gmail.com

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ABSTRACT

Objective: This study aims to evaluate the glaucoma knowledge and awareness in individuals who visit to two Family Health Centers in a district of Istanbul.

Patients and Method: This descriptive study, using a three-part questionnaire consisting of 20 questions, was carried out on individuals who applied to two Family Health Centers in a district of Istanbul between May and June 2019. Data from 260 were collected through face to face interviews.

Results: A total of 44 (16.9%) participants had heard the word glaucoma before, while 179 (68.8%) said that glaucoma was treatable, 78 (30%) knew about asymptomatic course glaucoma. In addition, 47(18.1%) believed that eyes with glaucoma could not be operated, 152 (58.5%) thought that blindness resulting from glaucoma was reversible. Interestingly, 167(68.7%) participants thought that routine ophthalmologic visits should be done at least once a year, whereas 222 (86.9%) consulted an ophthalmologist less than 10 times in their lifetime.

Conclusion: There was no statistically significant difference between individuals with family members that have glaucoma and those that do not, when asked about glaucoma being asymptomatic, the preventability of blindness and whether or not they have heard of glaucoma before. The study findings stress the need to spread awareness about glaucoma for prevention of glaucoma related blindness.

Keywords: Awareness, Glaucoma, Knowledge

1. INTRODUCTION

Glaucoma is a chronic and progressive optic neuropathy that frequently presents with elevated intraocular pressure and results in visual field defects [1, 2]. Glaucoma, a possible cause of irreversible blindness, affects approximately six million people worldwide [3]. With the increase of life expectancy in recent years, the prevalence of glaucoma is predicted to increase. Visual loss associated with glaucoma is preventable if it is diagnosed early and properly treated. In the condition of glaucoma having an early, subtle clinical course, diagnosis in early phase and optimum treatment are often difficult. Therefore, glaucoma mostly remains either undiagnosed or undertreated [4].

There have been very few studies showing the association of late presenting glaucoma with social variables and poor awareness [5, 6]. Currently there is no feasible screening test and the only way to detect glaucoma early is to increase awareness among the population [7, 8]. Studies conducted in developing countries have highlighted the lack of this quality in public [9-11].

The prevalence can show variance among different ethnicities and different age groups. While anyone is at risk of developing glaucoma or being born with glaucoma, the population group older than 40 carries the highest risk. It is shown that overall 3,54% of people aged 40-80 years old are having glaucoma [12]. Despite these facts, US Preventive Services Task Force (USPSTF) did not reach the conclusion that wide screening among general population for glaucoma was evidently necessary [13] but this decision was regarded as debatable since it was planned only for primary care services, but not ophthalmology departments. However, American Academy of Ophtalmology guidelines stated that glaucoma screening may be beneficial among the high risk populations like African Americans or Hispanic communities [14].

Glaucoma is a prominent cause of blindness in Turkey. In a study done by Karahan et al. (2021), among 340 bilaterally blind patients, glaucoma was the cause in 9.6% [15]. Glaucoma

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is a preventable cause of blindness, thus it should be prevented through increased awareness and screening, since late presentation leads to blindness [16].

The present study is focusing on this group of population, while questioning how much the respondents know about the disease and its deceptive course; it is also designed to check on their health-seeking behaviour in the ophthalmologic field.

2. PATIENTS and METHODS

The study was designed as a descriptive study. The study was approved by a public university ethics committee (approval number; 09.2019.418) and adhered to the principles of the Helsinki declaration. This descriptive study, using a questionnaire comprised of 3 parts and 20 questions, was conducted at the individuals applying to two Family Health Centers in a county of Istanbul, between May 20, 2019 and June 30, 2019. Patients and their intimates were informed about the study, and those volunteered to participate in the study were enrolled in the study after their verbal approvals were obtained.

The questionnaire was designed using some questions taken from the studies of Rewri et al., (2014) [7], Gyawali et al., [17] and Çelebi et al., (2018) [18].

The questionnaire was formed and translated to Turkish [7, 17, 18]. The researchers live in Istanbul, Turkey. Since they knew the culture of that region well and had done awareness studies in that community before, they searched the literature and added questions suitable for the public. As public health experts working in the region, we shaped the questions according to the culture of that region. The study was conducted in Pendik, Istanbul by 2 study coordinators and 4 trained interviewers for data gathering. The data gathering team was divided into two groups, each group going to a different family health center (2 centers in total). This translated version was applied to 260 patients or their attendant(s) aged 40 years and older, coming to the forementioned health centers. Data were gathered during face-to-face interviews.

The questionnaire, which was applied through face to face interview method, had three sections: The first section pertaining to information about the patient's demographic characteristics (age, gender, occupation) and any screening undertaken by the participants as well as their attitudes towards the screening of newborns and school aged children. This information was used to assess the practice pattern, defined as activity undertaken to protect oneself from the disease. Section two, pertained to the patient's awareness and knowledge about glaucoma, through 25 questions (nine questions evaluating their awareness and sixteen assessing their knowledge). Each question had three options (yes, no, I do not know), and according to the respondent's answer, interviewer was to tick (√) the response of their choice. Questions asked to assess knowledge required information and understanding of the subject gained through some source or learning, unlike the questions assessing awareness, which merely required information, without the need of understanding. They included questions regarding the symptoms of glaucoma, its treatment options, nature of the disease, type of visual impairments in glaucoma and risk factors for glaucoma. The third section had six questions dealing with the source of information about glaucoma and the participants' glaucoma status and treatment modality. Reading assistance to the illiterate participants, as well as explanations, were provided to the participants by the researchers when necessary; taking maximum care not to influence their response. An evaluation which encompasses several comparisons between the demographic variables, answers to the questionnaire and scale were carried out to understand the behavioral tendencies of the public in this matter.

Statistical Analysis

The chi-square test was used to evaluate associations between knowledge/awareness of glaucoma and the individual characteristics of the study subjects, including age, gender, glaucoma status and presence of a diagnosed glaucoma patient in their surroundings as well as their occupation. P values <0.05 were considered statistically significant.

3. RESULTS

A total of 260 enrolled participants from the two family health centers undertook the survey. Responses from all were analyzed. Table I shows the demographic characteristics of participants. The mean age (SD) was 51.81 (9.839) (age range 40-89) years. Females constituted the majority (%63.5) of the participants. In addition, 20 (%7.7) of the participants were healthcare workers.

Table I. *Characteristics of the study population*

		n	%
Age	**Mean ± Standard deviation**	51.81 ± 9.839	
	40-59	208	80
	≥60	52	20
Gender	**Female**	165	63.5
	Male	95	36.5
Occupation*	**Health worker**	20	7.7
2 missing	**Non health worker**	238	91.5
**These data contain some missing data.*			

Table II illustrates participant's awareness and knowledge about glaucoma. 18 participants had been previously diagnosed with glaucoma, 44 had a family member that was diagnosed glaucoma and 74 knew someone that was diagnosed with glaucoma. A total of 44 (16.9%) participants have heard the word "glaucoma" before. The questionnaire continued by mentioning eye pressure with those who did not hear the word glaucoma. While 179 (68.8%) of the participants said that glaucoma was treatable, 47 (18.1%) believed that eyes with glaucoma could not be operated. A total of 107 (41.2%) participants understood the risk of familial predisposition to glaucoma and 78 (30%) of respondents knew about the asymptomatic course glaucoma. Awareness about the irreversible nature of vision loss in glaucoma was noted in 152 (58.5%) responses. Furthermore, 29 (11.2%) of the respondents believed that glaucoma resulted from

mature cataracts. Interestingly, 167 (68.7%) participants think that routine ophthalmological visits should be done at least once per year, however, 222 (86.9%) have seen an ophthalmologist less than 10 times during their lifetime, 21 (8.1%) have never consulted an ophthalmologist during their lifetime.

Source of information about glaucoma was also queried during the survey. When the source of information was questioned, 72 (27.7%) respondents received their glaucoma information from the media (newpapers, television, radio, internet), 53 (20.4%) received information from visiting hospitals, medical personnel or other health care resources. The largest source of information was "word of mouth" from family or friends, 98 (37.7%) respondents and 37 (14.2%) did not specify any source of information.

Table II. *Glaucoma awareness and knowledge (n=260)*

		n	%
Have you ever heard of the word glaucoma before?*	**Yes**	44	16.9
	No	215	82.7
If left untreated is the result in blindness reversible?	**Yes**	152	58.5
	No	7	2.7
	I do not know	101	38.8
Is glaucoma treatable?*	**Yes**	179	68.8
	No	18	6.9
	I do not know	60	23.1
Is blindness preventable?*	**Yes**	118	45.4
	No	26	10
	I do not know	116	46.6
Have you ever been to the ophthalmolo gist?*	**Yes**	227	87.3
	No	30	11.5
Why did you go to the ophthalmologist?*	**Near/far sightedness**	131	50.4
	Burning/redness/foreign body	34	13.1
	Blurred vision	20	7.7
	Other	49	18.8
Where did you get your glaucoma information?*	**Television/Radio/ Newspaper/Internet**	72	27.7
	Hospital/Family Doctor	53	20.4
	Family/Relative/Friend	98	37.7

**These data contain some missing data.*

Table III illustrates comparison between demographic variables with whether the participants had ever heard the word glaucoma. There was a statistically significant difference between glaucomatous and non-glaucomatous participants with regards to having heard of glaucoma before and knowing that it is an asymptomatic disease (p= 0.005, p=0.000 relatively). However, there was no statistically significant difference when preventable blindness was queried. Additionally, there was a statistically significant difference between people that knew of glaucomatous individuals and people that did not when asked if they have heard the term glaucoma before (p<0.05). The knowledge about the existence of glaucoma increases with age. Over 60 years of age, significantly more participants knew about the word (p<0.05). When asked if it was an asymptomatic disease and if the resulting blindness was preventable, the results were statistically insignificant (p>0.05). In addition, there was no statistical significance found between individuals with family members that have glaucoma and those that do not when asked about glaucoma being asymptomatic, the preventability of blindness and whether or not they have heard of glaucoma before (p>0.05), as shown in tables III, IV and V. There was no statistically significant difference found between genders with regards to glaucoma knowledge and awareness (p>0.05). There was no statistically significant difference found between health care workers and non-health care workers with regards to glaucoma knowledge and awareness (p>0.05).

Table III. *Associations between individual characteristics and hearing the word 'glaucoma' before*

		Have you ever heard the word 'glaucoma' before?				p Value*
		Yes		No		
		n	%	n	%	
Gender	**Female**	33	20.1	131	79.9	0.078
	Male	11	11.6	84	88.4	
Age	**40-59**	29	14.0	178	86.0	0.011
	≥ 60	15	28.8	37	71.2	
Glaucomatous	**Yes**	8	44.4	10	55.6	0.001
	No	34	14.4	202	85.6	
Healthcare worker	**Yes**	7	35.0	13	65.0	0.023
	No	36	15.2	201	84.8	
Know someone with glaucoma	**Yes**	20	27.0	54	73.0	0.009
	No	24	13.3	156	86.7	
Having a relative with glaucoma	**Yes**	11	25.0	33	75.0	0.127
	No	33	15.5	180	84.5	

** p<0.05 statistically significant. Categorical variables are reported n (%).*

Table IV. *Associations between individual characteristics and knowledge about symptom status in glaucoma*

		Is glaucoma asymptomatic?						P Value*
		Yes		No		I do not know		
		n	%	n	%	n	%	
Gender	**Female**	51	31.1	53	32.3	60	36.6	
	Male	27	28.4	36	37.9	32	33.7	
Age	**40-59**	61	29.5	70	33.8	76	36.7	0.724
	≥ 60	17	32.7	19	36.5	16	30.8	
Glaucomatous	**Yes**	13	72.2	2	11.1	3	16.7	
	No	63	26.7	86	36.4	87	36.9	
Healthcare worker	**Yes**	5	25.0	10	50.0	5	25.0	0.316
	No	73	30.8	79	33.3	85	35.9	
Know someone with glaucoma	**Yes**	26	35.1	25	33.8	23	31.1	0.424
	No	49	27.2	64	35.6	67	37.2	
Having a relative with glaucoma	**Yes**	17	38.6	9	20.5	18	40.9	0.097
	No	60	28.2	79	37.1	74	34.7	

** p<0.05 statistically significant. Categorical variables are reported n (%).*

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Table V. *Associations between individual characteristics and knowledge about blindness in glaucoma*

		Is the blindness preventable in glaucoma?						P Value*
		Yes		No		I do not know		
		n	%	n	%	n	%	
Gender	**Female**	68	41.2	17	10.3	80	48.5	0.078
	Male	50	52.6	9	9.5	36	37.9	
Age	**40-59**	91	43.8	18	8.7	99	47.6	0.102
	≥ 60	27	51.9	8	15.4	17	32.7	
Glaucomatous	**Yes**	11	61.1	2	11.1	5	27.8	
	No	106	44.7	24	10.1	107	45.1	
Healthcare worker	**Yes**	9	45.0	2	10.0	9	45.0	0.999
	No	108	45.4	24	10.1	106	44.5	
Know someone with glaucoma	**Yes**	38	51.4	11	14.9	25	33.8	0.045
	No	76	42.0	15	8.3	90	49.7	
Having a relative with glaucoma	**Yes**	25	56.8	4	9.1	15	34.1	0.212
	No	91	42.5	22	10.3	101	47.2	

** p <0.05 statistically significant. Categorical variables are reported n (%).*

4. DISCUSSION

The study assesses the awareness and knowledge about glaucoma among people visiting their family physicians at the two family health centers in question. The intent of this study was to evaluate how much the participants knew about the disease and its subtle clinical course while also checking on their health-seeking behavior in the ophthalmologic field. Low levels of awareness and knowledge of glaucoma highlight the need for public education regarding this disease. The focus in this study was not on to evaluate the anatomical, physiological and technical aspect of the term "glaucoma". In fact, the word glaucoma was only used in the third question to ask the participants if they have ever heard about it but only 16.9% responded as yes. The responders who depicted that they have not heard about glaucoma, were not stopped from continuing the survey. For this reason, the local term "göz tansiyonu (eye tension in English)" was used in the rest of the survey. Since 80% of the population was found to be between 40 and 59 years old and only 20% was over 60 years old, the awareness and knowledge of this two age groups were not statistically suitable to compare. Even though this seems as a limitation of our study in terms of age comparison, in a study similar to this one which was conducted in India, there was no relationship found between age and glaucoma awareness [7]. Also, our study did not find any statistically significant difference between genders in glaucoma knowledge or awareness. This finding was consistent with the results of a study done in Acıbadem Hospital, in Istanbul [2].

In a study conducted with 502 participants in Switzerland on glaucoma awareness, it was found that 383 (76%) did not know the term "glaucoma" at all, and only 123 (24.7%) could define "glaucoma" as an eye disease [19]. In another glaucoma awareness study conducted with 340 people participating in ophthalmic examination social assistance services in Ethiopia, only 8 people had information about glaucoma [11]. In other studies previously published studies on glaucoma knowledge, it is estimated that 29% [12] to 59% [20]. In this aspect, our study population showed comparable but far from adequate knowledge and awareness about this disease. In general, less than one third (30%) of the responders knew about the asymptomatic course of glaucoma which is thrice the value (10%) found in a research done in China [3]. Lack of awareness could often lead to under-diagnosis and late presentation, as noted in several previous studies [4, 5] and therefore, adversely affected the eye care–seeking behavior [5]. Awareness about the irreversible nature of the vision loss was more than expected as 58.5% of the participants knew about it. In contrast, as a result of a research in Southwestern Ethiopia only 12.5% of the responders knew that blindness due to glaucoma was irreversible [6]. Knowledge about these two aspects of the disease, the subtle clinical course and the irreversible but preventable vision loss, was thought to be the main determinant of people's attitude and concern about glaucoma. In this study the awareness of the participants about the disease was evaluated with twelve questions (two from the first part, nine from the second part and one from the third part of the questionnaire) and knowledge regarding the condition was assessed through the remaining 16 questions in the second part. While comparing certain groups in the study three main questions were selected which were asking the participants if they have ever heard about glaucoma, if the disease has an asymptomatic nature and if the blindness caused by it can be prevented. The main groups of people whose responses were analyzed further and included in a comparison were the glaucomatous or non-glaucomatous participants, the ones who had a positive or negative family history of the diseases, the ones who knew or did not know of someone with glaucoma, females or males and health care workers or others. There was a statistically significant difference between glaucomatous and non-glaucomatous participants with regards to having heard of glaucoma before and knowing that it is an asymptomatic disease which was hypothesized. However, there was no significant difference when preventable blindness was asked to these two groups. So, even though people were diagnosed with the disease, they did not know about the possibility of an effective screening. And it was found that the awareness among people who had a close acquaintance with glaucoma was higher but these people did not know better about the asymptomatic nature and preventable effects of the disease. Interestingly, responders with a positive family history did not show a higher level of awareness of glaucoma. This could be due to the fact that even though people have not heard the term glaucoma they knew about it as "göz tansiyonu". Even if there were discrepancies regarding the terminology, it was found that all participants from different comparison groups did not know if glaucoma was an asymptomatic disease and if the resulting blindness was preventable. Finally, there was no statistically significant difference between health workers and people from other professions regarding the main points touched above.

Through the questions in the first part of the survey attendants" attitude towards screening and their behavior were assessed and it was found out that 68.7% of them had the idea that routine ophthalmological visits should be at least once a year. In contrast, 86.9% of them had seen an ophthalmologist less than 10 times in their lives. Respondents' largest source of information was found to be "word of mouth" from family and friends which was the same case for many other studies [7-10]. This information points out to two facts, first the potential benefit that could be accrued from using glaucoma patients as a source of awareness to society and second the need for health care workers, health-related agencies and media to be more involved with the burning issue. World glaucoma day is March 12th. There could be workshops during that period or posters and pamphlets being handed out at various health care centers around the country as well as small advertisements on the radio or television to help spread awareness to the population most at risk.

There are studies that show that low blood pressure glaucoma is more common in patients with migraine [21-23]. Primary care physicians can be educated about glaucoma and risk factors, and patients with family history of glaucoma and migraine can be referred to early diagnosis. It is also crucial for family physician to ask patients over the age of 40 whether they had their eye pressure measured before. Because the population over the age of 40 will consider the family physician's recommendation as they feel close to them. One of the strong features of our study is that it was conducted in primary care since the prevalence of glaucoma increases over the age of 40 and the average age of the population applying to primary care is high. This can be seen as missed opportunities from a public health perspective. By placing tonometer devices in primary health care centers, eye pressure can be scanned for the population coming there.

When asked if the glaucoma was treatable, approximately 68% of our study population gave the answer "yes" to the question. In a study made by Manhas et al. (2019) the answer "yes" was nearly 30% and 53% of the population gave the answer "I do not know" (sample size=230). The percentage of the population who was aware of a disease called glaucoma, the percentages were 16,9% in our population and 26.1% in Indian population. In the evaluation of the speculation of this information, even though the awareness of the disease in the public were more in Indian study, knowledge of whether it is a treatable illness showed more than two fold increase in our population. When it is asked "is blindness preventable with the treatment of glaucoma", 45% of our study population said "yes", yet this percentage was 20% in the Indian population [24]. Lastly, when it is asked "if left untreated is the result in blindness reversible?" the answer "yes" hold a remarkable percentage of 59% in our population. This brings up the question: Is the global intercommunication causing partial and/or misinformation about these prevalent disorders? We think that simplified but fact checked scientific information should be given to public to prevent misinformation, because in this state, only knowing what is glaucoma is not enough to prevent its consequences, but knowing its irreversible complications is needed to avoid a potential underestimation among public.

There are some limitations that should be taken into consideration. The descriptive nature of the study is a limitation. Interviewer bias could not be completely eliminated as an individual's expression, and style of explanation may affect the response of the participant. The initial intent was to have the participants fill out the questionnaires on their own while providing assistance when needed, however there was a lack of willingness to participate which resulted in them having to be interviewed. Since presbyopia starts over the age of 40 and therefore the participants may not be able to see close by without glasses, they may have been reluctant to answer the questionnaire themselves. The participants of the survey were individuals who were visiting the family health centers instead of a more random sample of the population and as a result of this, there may have been a higher prevalence of illnesses that affect the eye. This may lead to a difference in behaviour, knowledge and beliefs when it comes to glaucoma and visits to the ophthalmologist and may be seen as selection bias. Finally, as we mentioned, it is both an advantage and a disadvantage that the study is conducted in a primary health care center. Since, our study was conducted with people who came to the health institution at that time, it does not reflect the whole society. But we can comment that even among people who are conscious enough to apply to a health institution, if the awareness is this low, it will be much lower in the general society.

Compliance with Ethical Standards

Ethical Approval: This study was approved by the Marmara University, School of Medicine Clinical Research Ethics Committee (approval number; 09.2019.418). The study adhered to the principles of the Helsinki declaration.

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The relationship between renal functions and multi-drug resistant organisms in patients with ventilator-associated pneumonia

Omur ILBAN[1], Aysegul ILBAN[2]

[1] Intensive Care Unit, Konya Numune Hospital, Selcuklu, Konya, Turkey.
[2] Department of Microbiology,Konya Numune Hospital, Selcuklu, Konya, Turkey.

Corresponding Author: Omur ILBAN
E-mail: droilban@gmail.com

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ABSTRACT

Objective: Despite the increase in the frequency of multi-drug resistant organism (MDRO) colonisation and infection in dialysis patients, it is not well known whether the risk of multi-drug resistant (MDR) pneumonia increases in mild-to-severe chronic kidney disease patients not undergoing dialysis. Therefore, we aimed to evaluate the relationship between renal functions and the risk of MDR ventilator-associated pneumonia (VAP) and the specific microbial pattern.

Patients and Methods: A total of 133 patients who developed VAP were divided according to their renal function into two groups, an estimated glomerular filtration rate of (eGFR) ≥ 60 mL/ min/1.73 m2 (high eGFR, n=65) and eGFR < 60 mL/min/1.73 m2 (low eGFR, n=68).

Results: The low eGFR group presented a significantly high MDRO ratio (p<0.01). With the decrease in eGFR, the frequency of gram-positive MDRO did not change (p=0.63), while the increase in gram-negative MDRO was statistically significant (p<0.01). Low eGFR was found to be an independent predictor for antimicrobial resistance. (Odds ratio, (OR): 2.821).

Conclusion: Among VAP patients, chronic renal failure is associated with increased MDRO infection. The eGFR may be used to identify mechanically ventilated patients with a high risk of MDR pneumonia.

Keywords: Chronic kidney disease, Glomerular filtration rate, Multiple drug resistance, Risk factors, Ventilator-associated pneumonia

1. INTRODUCTION

Patients receiving invasive mechanical ventilation (MV) suffer most commonly from ventilator-associated pneumonia (VAP) as a nosocomial infection. Approximately half of the antibiotic expenditure in the intensive care unit (ICU) is due to VAP treatment [1]. Antibiotic resistance poses an increasing threat due to the rise of infections caused by multi-drug resistant organisms (MDRO)s [2]. Even mild and moderate reductions in the estimated glomerular filtration rate (eGFR) (e.g., 30-59 mL/min/1.73 m^2) are a significant risk factor for infection [3]. Patients suffering from chronic renal failure (CRF) experience a more frequent use of antibiotics [4] and hospitalisations due to the high infection rates [5], which leads to an increase in exposure to MDROs [6]. In addition, in patients with CRF, the risk of hospitalisation and mortality due to pneumonia is increased [7]. Although, MDRO infection is more frequently detected in dialysis patients [8], it is not known enough whether the risk of multi-drug resistant (MDR) pneumonia increases in mild-to-severe chronic kidney disease (CKD) (eGFR < 60 mL/ min/1.73 m^2) patients not receiving dialysis. In this study, we aimed to assess the relationship between renal functions and MDR VAP risk and the specific microbial pattern in pneumonia patients.

2. PATIENTS and METHODS

Study population

This prospective observational study was conducted on adult patients intubated and receiving MV for at least 48 hours in the 42-bed surgical and medical ICU in Konya Numune Hospital. The study was performed with the approval of the Ethics Committee of Necmettin Erbakan University Medical School (no. 2019/1901) between August 2019 and January 2021. Also, the study has been included in the index of the National Clinical

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Trial website (www.clinicaltrials.gov: NCT04833231). Informed consents were obtained from the patients participating in the study or their relatives.

Inclusion and exclusion criteria

Patients whose age was 18 years or above, clinically suspected of VAP as defined in the American Thoracic Society (ATS) guidelines [9], with a Clinical Pulmonary Infection Score (CPIS) > 6 [10], and with no clinical evidence of any infection when admitted to the ICU, were included in the study.

Patients suffering from acute kidney injury, renal replacement treatment (RRT), dialysis, renal transplantation, active tuberculosis, malnutrition, immunosuppression (neutropenia, HIV positivity, transplantation, prednisone treatment of ≥ 20 mg/day, etc.), presenting any extrapulmonary infection other than VAP at the time of inclusion in the study, as well as respiratory cultures with fungal agents, normal flora or no growth, were not included in the study.

Definitions

Pneumonia developing at least 48 hours after the onset of endotracheal intubation and MV was defined as VAP. The formula determined by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) was used to calculate the eGFR values based on the measured serum creatinine concentrations [11]. The renal functions of the patients were recorded at the time of admission to the ICU and the diagnosis of VAP. The eGFR values on the day of diagnosis were used to divide the patients with pneumonia into two groups, ≥ 60 mL/ min/1.73 m^2 and < 60 mL/ min/1.73 m^2. An eGFR below 60 mL/ min/1.73 m^2 (low eGFR) was defined as renal dysfunction [3]. Comorbidities and the severity of underlying diseases were assessed according to the Charlson comorbidity index [12]. In contrast, the Acute Physiology and Chronic Health Evaluation score (APACHE II) was used in patients at the beginning of the infection to assess the illness severity [13].

Pan-drug resistant (PDR), extensive-drug resistant (XDR) and MDR pathogens were identified based on the study of Magiorakos et al [14]. XDR and PDR organisms were evaluated as MDR. Detecting more than one potentially pathogenic microorganism was defined as polymicrobial pneumonia [15]. In patients with polymicrobial VAP, finding at least 1 MDR organism was classified as MDR. Death from any cause occurring within 28 days since the VAP onset was defined as 28-day ICU mortality.

Study protocol

One hundred and thirty-three VAP patients without nosocomial infection were prospectively and consecutively included in the study. The diagnosis of VAP was achieved based on clinical and microbiological criteria. Confirmation of VAP diagnosis in patients clinically suspected of VAP and CPIS > 6 was accomplished by observing > 10^4 colony forming units (cfu)/ml in the conventional culture of tracheobronchial aspirate [16].

Also, blood and urine cultures were obtained to discard other possible nosocomial infections. VITEK 2 healthcare system (bioMérieux SA, Marcy-l'Étoile, France) was utilised for antimicrobial susceptibility tests. The guidelines provided by the European Committee on Antimicrobial Susceptibility Testing (EUCAST 2019) were used in the interpretation of the results of the Minimal inhibitory concentration (MIC) test [17]. Organisms with intermediate susceptibility were considered antimicrobial-resistant.

Sample size

In order to define the minimum sample size, a power analysis by a Fisher's exact test was performed using the publicly available statistical software G*Power, version 3.1. Prior to the beginning of the study, no published information about the effect of renal functions on MDR pneumonia in critically ill patients was available; as such, a sample size estimation based on previous studies was not possible. Therefore, we first conducted a pilot study on 30 patients with the same methods used in the main study. In our preliminary study, MDROs were observed in 60% of the individuals in the high eGFR group and 87% of the low eGFR group. In order to detect a significantly different rate of MDROs among the two groups, a power analysis was carried out using a 2-sided confidence level of 95% ($p<0.05$) and a power of 90%. Considering the 10% dropout rate, the sample size was finally calculated as at least 132 patients.

Statistical Analysis

SPSS statistics package version 21.0 was used to analyse the data. Number and percentage (%) were used in the definition of categorical variables, while mean ± standard deviation was applied for continuous variables. A comparison of the categorical variables was made according to their suitability with Pearson's chi-square or Fisher's exact tests. The Independent Samples t-test was used to analyse continuous variables presenting a normal distribution, while the nonparametric Mann-Whitney U test was used for those that did not. The evaluation of risk factors in the acquisition by different eGFR groups of MDR bacteria independently associated with the tracheobronchial culture result was made by multivariate logistic regression. The appropriateness of the models was assessed by the Hosmer Lemeshow goodness of fit test [18]. The significance of the association was expressed with the 95% confidence interval as the odds ratio. The Receiver Operating Characteristic (ROC) analysis was run to measure the predictive capacity of eGFR levels in the differentiation of antimicrobial resistance status. The area under the curve (AUC) and a 95% confidence interval (CI) were recorded. Youden's indices were used to determine the best discriminatory cut-off values [19]. In all tests $p<0.05$ values were regarded as significant.

3. RESULTS

In the observed period, of 248 patients clinically suspected of VAP, 133 were confirmed microbiologically and included in the study. Table I shows the demographic data. Hypertension was the most common underlying systemic disease, occurring in 38% of the patients. In the eGFR < 60 mL/min/1.73 m^2 group,

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the frequency of diabetes and hypertension and the length of the stay in the ICU were significantly higher than in the eGFR ≥ 60 mL/min/1.73 m^2 group. A significant difference in terms of other data was not detected among the groups (Table I).

Table I. *Baseline characteristics of patients with ventilator-associated pneumonia.*

Variables	eGFR, mL/min/1.73 m^2		p value
	≥ 60 (n = 65)	< 60 (n = 68)	
Age in years	62 ± 11.9	65 ± 12.6	0.48
Gender, male, n (%)	34 (52)	34 (50)	0.71
Charlson index	2.3 ± 0.31	2.4 ± 0.37	0.36
Co-morbidities, n (%)			
Hypertension	18 (28)	32 (47)	< 0.01*
Diabetes mellitus	10 (15)	17 (25)	0.04*
Malignancy	5 (8)	6 (9)	0.7
Cardiovascular diseases	15 (23)	21 (31)	0.15
Serebrovascular diseases	11 (17)	16 (24)	0.14
Hepatic disease	3 (5)	4 (6)	0.6
COPD	13 (20)	14 (21)	0.84
Rheumatical diseases	3 (5)	5 (7)	0.38
Peptic ulcus	4 (6)	6 (9)	0.35
Main causes of ICU admission, n (%)			
Medical	50 (77)	49 (72)	0.35
Surgery/Trauma	15 (23)	19 (28)	
APACHE II	21.6 ± 7.7	23.5 ± 7.2	0.15
CPIS	7.3 ± 0.4	7.4 ± 0.3	0.22
Positive blood culture, n (%)	4 (6)	6 (9)	0.35
Vazopressor use, n (%)	12 (18)	15(22)	0.47
Previous antibiotic use within 14 days			
prior to VAP onset, n (%)	46 (71)	54 (79)	0.15
ICU length of stay(days)	16 ± 6.8	20 ± 7.2	0.04*
28-day ICU mortality, n (%)	25 (38)	30 (44)	0.37

*Data shown as mean ± standard deviation or n (%). * eGFR ≥ 60 vs eGFR < 60 group, (p<0.05). eGFR: estimated glomerular filtration rate, COPD: chronic obstructive pulmonary disease, ICU: intensive care unit, APACHE II: Acute Physiological and Chronic Health Evaluation, CPIS: Clinical Pulmonary Infection Score, VAP: ventilator-associated pneumonia.*

Based on previous discoveries, the probable factors for acquiring MDR bacteria were tested using logistic regression. According to univariate logistic regression, the predictors with p<0.2, including age, gender, Charlson comorbidity index [12], hypertension, diabetes mellitus, cardiovascular disease, APACHE II and previous use of antibiotics, were included in the multivariate analyses [13]. As described in Figure 1, in the multivariate logistic regression model, low eGFR was found to be an independent predictor for antimicrobial resistance. The odds of infections by MDROs were independently associated with low eGFR (Odds ratio, OR: 2.821, 95% CI: 1.290-6.165) and negatively associated with high eGFR (OR: 0.355, 95% CI: 0.162-0.775, p = 0.009). In addition, the interaction between age and MDRO risk by low eGFR was also positively associated (OR: 1.122, 95% CI: 1.026-1.429, p=0.012). Male patients presented a lower risk of MDROs, while patients with diabetes mellitus and previous use of antibiotics had a higher risk of MDROs in both high and low eGFR categories. Although, the OR of MDROs were higher across comorbidities in patients with low eGFR, they were not statistically significant except for diabetes mellitus.

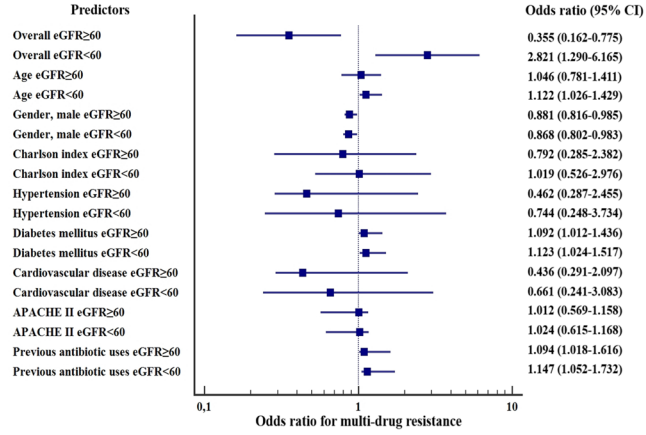

Figure 1. *Risk factors for antimicrobial resistance in patients with ventilator-associated pneumonia. eGFR: estimated glomerular filtration rate; APACHE II: Acute Physiology and Chronic Health Evaluation II; CI: confidence interval*

When pathogenic microorganisms were evaluated, 94 (71%) MDRO isolated from tracheobronchial aspirate culture showed a different microbiological pattern varying according to eGFR groups. Compared to the eGFR ≥ 60 group, there was a significant increase in the MDRO rate in the eGFR < 60 group (p<0.01). While with the decrease in eGFR, the frequency of gram-positive (Gr +) MDRO did not change (p=0.63), the increase in gram-negative (Gr –) MDRO was statistically significant (p<0.01). Predominantly, MDROs as *Enterobacteriaceae spp.* and non-fermenting Gr (-) bacilli were found.

The frequency of polymicrobial MDR pneumonia was similar in both groups. When the cases of monomicrobial pneumonia were evaluated, although there was an overall increase in the incidence of MDR monomicrobial pathogens with a decrease in eGFR, this increase was not statistically significant except for *Enterococcus spp.* (p=0.04) (Table II, Figure 2).

Since it was shown that eGFR has a significant relationship with MDR pathogens in VAP patients, ROC analysis was used to evaluate the predictive capacity of eGFR levels in the differentiation of antimicrobial resistance status. The area under curve (AUC) value of the ROC curve had a fair diagnostic value of 0.795 (95% CI, 0.704-0.886; p < 0.001). With the Youden criterion, the 61.5 mL/ min/1.73 m^2 cut-off value of eGFR was found to have 75% sensitivity, 74% specificity, 88% positive predictive value (PPV), 55% negative predictive value of (NPV) and 74% of overall accuracy in the diagnosis of MDR infection (Table III, Figure 3).

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Table II. *Pathogens associated with ventilator-associated pneumonia*

Pathogen	eGFR, mL/min/1.73 m²		p value
	≥ 60 (n = 65)	< 60 (n = 68)	
MDRO, n (%)	39 (65)	55 (81)	< 0.01*
Polimicrobial	7 (11)	8 (12)	0.75
Monomicrobial			
NFGNB	12 (18)	17 (25)	0.18
Pseudomonas aeruginosa	7 (11)	9 (13)	0.33
Acinetobacter baumannii	5 (8)	8 (12)	0.2
Enterobacteriaceae spp	9 (14)	13 (19)	0.22
Staphylococcus aureus	4 (6)	7 (10)	0.21
Enterococcus spp.	3 (5)	7 (10)	0.04*
Others	4 (6)	3 (4)	0.48
Gram (+)	16 (25)	15 (22)	0.63
Gram (-)	23 (35)	40 (59)	< 0.01*

*Data shown as mean ± standard deviation or n (%). * eGFR ≥ 60 vs eGFR < 60 group, (p<0.05). eGFR: estimated glomerular filtration rate, MDRO: multi-drug resistance organism, Gram (+): gram-positive bacteria, Gram (-): gram-negative bacteria, NFGNB: nonfermenting gram-negative bacilli*

Table III. *Predictive value of different cut-offs of eGFR in differential diagnosis between MDR and Non-MDR ventilator-associated pneumonia*

eGFR cut-off value (mL/min/1.73m²)	Sensitivity	Specificity	PPV	NPV	LR+	LR-
50	56	82	88.5	44.4	3.14	0.53
54.5	63	77	86.7	46.1	2.72	0.48
61.5	**75**	**74**	**87.5**	**54.7**	**2.9**	**0.34**
68	81	67	85.3	59.1	2.43	0.29
72	83	62	83.8	60	2.16	0.28

Data are presented as %, unless otherwise stated. MDR: multi-drug resistant, eGFR: estimated glomerular filtration rate, PPV: positive predictive value, NPV: negative predictive value, LR+: positive likelihood ratio, LR-: negative likelihood ratio.

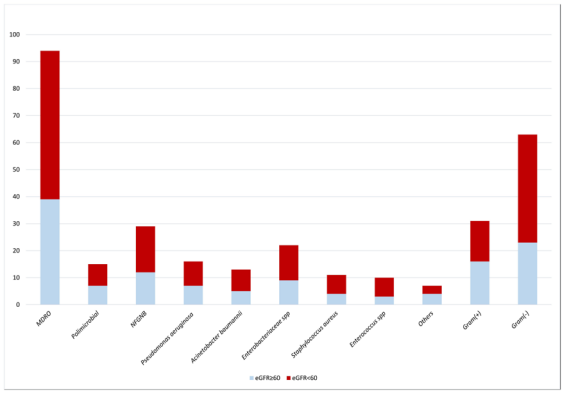

Figure 2. *Frequency distribution of specific bacterial in the culture of tracheobronchial aspirate by eGFR categories. MDRO: multi-drug resistant organism; NFGNB: nonfermenting gram-negative bacilli*

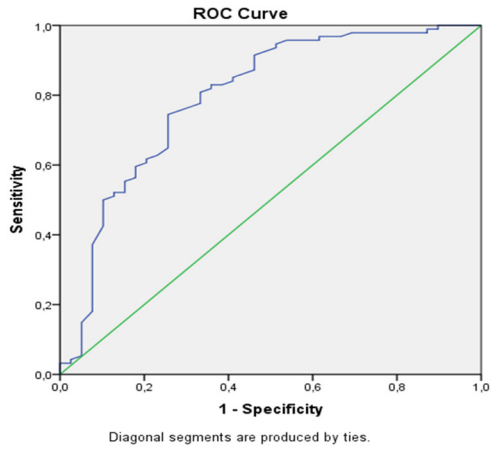

Figure 3. *Receiver Operating Characteristic (ROC) curve for eGFR (AUC: 0.795, 95% CI, 0.704-0.886) for discrimination between MDR and Non-MDR pneumonia*

4. DISCUSSION

In the present study, the characteristics of VAP patients suffering from MDRO infections associated with different eGFR categories were identified. Based on the multivariate analysis, decreased eGFR, advanced age, diabetes mellitus and previous use of antibiotics were independently associated with an actual resistant infection, while the male gender was independently associated with protection against MDRO infection. In addition, the eGFR cut-off > 61.5 mL/min/1.73 m² could assist in excluding MDR VAP. Therefore, in patients clinically suspected of VAP and with microorganisms found in the direct microscopic examination of respiratory specimens, eGFR levels may help determine an appropriate empirical antimicrobial therapy that includes MDROs. Also, mechanically ventilated patients with impaired renal function should be carefully monitored as a high-risk group for MDR VAP.

There is an increased prevalence of MDROs in end-stage renal disease (ESRD)-patients or Stage 5 CKD [20] due to accompanying immune system deficiencies [8,21]. In addition, hemodialysis (HD) patients become more prone to nosocomial infections, especially those with ESRD-producing pathogens [22]. Shorr et al., showed that in patients with healthcare-associated pneumonia, long-term hemodialysis is an independently associated risk factor for antibiotic-resistant pathogen infections [23]. However, according to our knowledge, data on whether the risk of MDR infection increases in patients with kidney dysfunction and mechanically ventilated without ESRD is limited. Su et al., showed that, in hospitalised patients with infections, the risk of MDR infection is increased even in patients with mild and moderate renal failure [24]. Our results showed that decreased eGFR levels entail independent risk factors for MDR VAP.

In the present research, the diagnostic ability of eGFR to differentiate infections caused by MDR or non-MDROs was

evaluated. The best eGFR cut-off value was measured at the onset of suspected VAP, 61.5 mL/min/1.73 m^2, demonstrated a PPV of 88% and designated as positive 75% of the patients with MDR infection. Also, the eGFR had adequate predictive power for MDROs in VAP patients (AUC: 0.795). These results indicated that eGFR has clinical value for recognising MDR infection. Therefore, it can be useful to adjust the treatment by excluding false-negative diagnoses of MDR VAP. However, the risk of unnecessary antibiotic treatment must be considered.

Gram-Negative Bacilli (GNB) were the predominant pathogens in VAP. Nonfermenting GNB, *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Acinetobacter baumannii*, were commonly isolated and carried a potential for MDR VAP. However, the risk of colonisation by antimicrobial-resistant Gr (-) organisms increased due to the frequent exposure of CKD patients to healthcare settings and antibiotics. Vicas et al., showed that 28% of hemodialysis patients were colonised with Gr (-) pathogens. Furthermore, 20% of the patients had one of these MDR Gr (-) pathogens during the 6-month follow-up period [25]. Jamil et al., studied the MDR pattern of Gr (-) pathogens isolated from patients immunocompromised for CKD and renal transplant. It was demonstrated that there was a significant increase in antibiotic resistance in patients with chronic renal failure [26]. In the study conducted by Su et al., in non-hemodialysis CKD patients, the frequency of Gr (-) MDROs increased with the decrease in renal functions [24]. The significantly increased frequency of Gr (-) MDRO in kidney dysfunction patients observed in our study was consistent with previous results.

The risk factors for MDR pathogens in pneumonia patients are recognised by the guidelines of the American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) [9]. In addition, studies conducted with heterogeneous patient populations have demonstrated various risk factors, including age, diabetes mellitus, immunosuppression and severe underlying disease [27,28]. However, a few studies reported conflicting results on the association of age with the risk of MDR infections. Micek et al., in an international multicenter study, examined the risk factors for MDR strains in *P. aeruginosa* nosocomial pneumonia. A lower age was found to be independently associated with pneumonia by MDR *P. aeruginosa* [29]. However, in studies conducted with Gr (-) bacteremia and VAP patients, advanced age was identified as a risk factor for MDR organisms [30-32]. Similar to previous studies, in our study, advanced age, diabetes mellitus and the prior use of antibiotics were determined as independently associated MDR risk factors for VAP in patients with reduced renal functions.

The main characteristic of acute kidney injury (AKI, formerly acute renal failure) is the rapid decline in the excretory function of the kidney, often with oliguria, which usually develops between hours and days. The prevalence of this complication, which is 10%-15% in hospitalised patients [33], exceeds 50% in ICU patients [34]. Discerning among AKI and/or CKD _ must be made in patients with an abnormal kidney function of unspecified duration. AKI before admission may be indicated by reduced serum creatinine after hospitalisation [35]. In the present study, the medical history and baseline renal functions were recorded at the time of admission to the ICU, and then, the renal functions at the time of VAP diagnosis were compared with the previous results. In this way, the decrease in eGFR levels due to acute kidney dysfunction in patients suffering from pneumonia was limited as much as possible.

Studies have shown that values of 105 mL/min/1.73 m^2 eGFR and above may be associated with malnutrition and increase the risk of infection. Malnutrition leads to susceptibility to infection, particularly by affecting cell-mediated immune function, and, in return, the infection affects the nutritional status [36]. The loss of muscle mass in older patients contributes to high eGFR by causing low serum creatinine levels. Concurrently, in elderly patients, malnutrition, chronic inflammation and cachexia tend to occur [37]. James et al., showed an association between mortality risk and hospitalisation due to pneumonia and an eGFR ≥ 105 mL/ min/1.73 m^2. He suggested that the reduction of muscle mass associated with chronic diseases would reduce creatinine formation and, therefore, eGFR would be overestimated [38]. Xu et al., found that elderly patients in the highest eGFR category had an increased risk of community-acquired infections [3]. However, Su et al., detected no significant difference in the MDR infection risk in individuals with eGFR ≥ 105 mL/min/1.73 m^2 [24]. In our research, due to chronic diseases, malnutrition risk and the advanced average age in mechanically ventilated patients with severe disease, the real eGFR could be potentially incorrectly calculated. Therefore, the high eGFR category included levels of 105 mL/min/1.73 m^2 and above.

The current study has several strengths. First, the evaluation of renal functions in the admission to the ICU is an important indicator of the acuteness or chronicity of the eGFR decrease at the time of pneumonia diagnosis. Excluding AKI patients from the study provided a more objective evaluation of CKD. Second, the use of eGFR as a renal function indicator. Our results are compatible with routine practice, as clinical guidelines suggest using eGFR in the evaluation of renal functions. Our study also has several limitations. First, we used serum creatinine to evaluate renal function. Since, serum creatinine depends on muscle mass, calculated eGFR values may be higher in patients with severe disease [39]. Second, the deterioration in renal functions of patients in the ICU could be caused by different reasons, including other chronic diseases. These unmeasured factors and comorbidities may have affected the relationship between eGFR and MDR VAP. Third, despite the VAP confirmation by quantitative microbiology, false positive and negative results are possible, and therefore, this may affect the results associated with kidney failure.

Conclusion

In this study, an association of renal dysfunction with an increased MDR pneumonia risk was found. In patients suffering from renal dysfunction and clinically suspected of VAP, microbiological culture results should be closely monitored, and the potential of possible factors resistant to empirical antibiotherapy should be considered.

Compliance with the Ethical Standards

Ethical Approval: The study was performed with the approval of the Ethics Committee of Necmettin Erbakan University Medical School (no. 2019/1901) between August 2019 and January 2021. Informed consents were obtained from the patients participating in the study or their relatives.

Financial Support: The authors have no relevant financial information to disclose.

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

Authors' contributions

OI: Conceptualization, writing-original draft, formal analysis, investigation, supervision, data curation, AI: project administration, methodology, validation, visualization, software, writing-review and editing.

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The effects of mean platelet volume and red cell distribution width on prognosis in patients with myelodysplastic syndrome

Ihsan AYHAN[1], Sehmus ERTOP[2]

[1] Internal Medicine Clinic, Zonguldak Ataturk State Hospital, Zonguldak, Turkey.
[2] Division of Hematology, Department of Internal Medicine, Health Application and Research Center, Zonguldak Bulent Ecevit University, Zonguldak, Turkey.

Corresponding Author: Ihsan AYHAN
E-mail: ihsanayhan@hotmail.com

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ABSTRACT

Objective: In this study, the relationship between changes in mean platelet volume (MPV) and erythrocyte distribution width (RDW) with hematological response and survival in patients with myelodysplastic syndrome was investigated.

Patients and Methods: Between 1 January 2011 and 31 December 2018, patient characteristics and hemogram results were evaluated during the treatment process among 158 patients diagnosed with myelodysplastic syndrome.

Results: The mean age of the patients who were included in the study was 71.53±12.6 years. The MPV percentage change in the 2-year follow-up of the patients with and without hematological response was significant, at 0.022±0.11 (2.2%) in those who responded and at 0.069±0.15 (6.9%) in those who did not (p=0.049). Throughout the same period, the degree of RDW changes in the patients who died was 13.23±22.97, the degree in those who survived was 2.86±21.42, and the difference between the two groups was statistically significant (p=0.006).

Conclusion: In patients diagnosed with myelodysplastic syndrome, MPV and RDW values can be considered inexpensive and simple laboratory markers that can be used in follow-ups and promising tests to predict both treatment response and survival in the early period and change treatment modalities.

Keywords: Myelodysplastic syndrome, Mean platelet volume, Red cell distribution width, Treatment response

1. INTRODUCTION

Myelodysplastic syndrome (MDS) is a heterogeneous group of myeloid diseases characterized by cytopenia(s) in the peripheral blood and dysplastic changes in the bone marrow. This disease has genetic instability and carries the risk of transforming into acute myeloid leukemia (AML). For the diagnosis of MDS, one or more of the diagnostic parameters of dysplasia by more than 10 percent in at least one hematopoietic series, an increase in the blast rate, and/or cytogenetic anomaly compatible with MDS should accompany one cytopenia [1].

The mean platelet volume (MPV) is a marker that is used to measure platelet size and provides an idea about platelet reactivity. Large platelets are metabolically and enzymatically more active and may predispose the patient to prothrombotic events. In this context, high MPV values were associated with hypertension, hypercholesterolemia, obesity, and cardiovascular diseases [2]. Additionally, MPV is often high in diseases like ITP, disseminated intravascular coagulation, congenital thrombocytopenia with giant platelets, and MDS [3]. The red blood cell distribution width (RDW) is a unit that measures the heterogeneity of the size distribution of erythrocytes. The heterogeneity of red blood cells is described by a peripheral smear test as a result of qualitative observation in medical history. If this heterogeneity is above the degree that is accepted as normal, it is defined as anisocytosis. In previous studies, the diameters of erythrocytes have been measured, and the coefficients of variation between the results have been calculated. Histograms have been obtained from these measurements with a bell-shaped distribution using the mean particle volume and coefficients of variation (similar to RDW). Later, it was understood that these data changed numerically in some diseases like pernicious anemia or bleeding. Unfortunately, it is not easy to see the sizes of red blood cells and anisocytosis on a peripheral smear. However, thanks to particle sizing technologies, the heterogeneity of erythrocytes can be measured quantitatively, quickly, and precisely [4]. When these measurements were examined in various patients, it was

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observed that they were high in chronic heart failure, peripheral artery disease, and kidney transplant patients, and they were associated with mortality in diseases like acute coronary infarction, acute pulmonary embolism, acute heart failure, pneumonia, and acute kidney injury [5]. As evidenced by many different studies, an increase in the RDW value (typically over 14.6%) is recognized as a significant marker for morbidity and mortality in the general population [6,7].

In some studies, an increase in MPV values was found between the control group and the group with MDS, but no relationship was established with mortality [8]. According to the recent studies investigated the relationship between MPV to platelet ratio and survival in MDS patients, and this index was found to be higher in patients over 70 years of age, and a positive correlation was observed with the IPSS scores [9]. Similarly, in a study conducted with RDW, the association of RDW with chromosomal anomalies in patients with MDS was examined and a significant result was tried to be obtained in MDS due to ineffective erythropoiesis. However, a relationship between RDW and chromosomal abnormalities in MDS could not be established [10].

In this study, we aimed to investigate the relationship between MPV and RDW values in MDS patients with hematological response and survival.

2. PATIENTS and METHODS

This study was conducted with the participation of 158 patients diagnosed with MDS who presented to Zonguldak Bulent Ecevit University (ZBEU) Hematology Department Outpatient Clinic between 1 January 2011 and 31 December 2018. Written informed consent was obtained from all participants. Ethical approval for the study was obtained from Zonguldak Bulent Ecevit University Non-Interventional Clinical Research Ethics Committee (08/01/2019 – 33479383). The files of the patients who were included in the study were retrospectively scanned from the hospital database in accordance with the permissions of the relevant university ethics committee and the institution. The variables to be evaluated within the scope of the study were recorded for analysis at the time of presentation, and at the 6th month, the 12th month, the 18th month, and the 24th month in follow-ups. Complete blood count tests were performed using a Beckman Coulter LH 780 brand device on approximately 3 milliliters of venous blood samples each taken into an EDTA tube in the biochemistry laboratory of our hospital.

The diagnosis of MDS is based on one or more cytopenias, ≥10 percent morphological dysplastic change in at least one series of nucleated cells, the presence of <20 percent blasts in bone marrow and peripheral blood, and/or characteristic cytogenetic or molecular findings.

Patients were scored according to the percentage of blasts in the bone marrow, karyotype, hemoglobin, platelets and absolute neutrophil counts according to the IPSS-R classification and categorized as very low, low, medium risk level-1, medium risk level-2 and high risk [11].

In the complete blood counts obtained during the follow-ups of the patients, an absolute neutrophil count equal to or greater than 1000/mm^3, a hemoglobin count equal to or greater than 11 g/dL, a platelet count equal to or greater than 100,000/mm^3, and the absence of blasts in the peripheral blood were accepted as hematological response for MDS.

Statistical Analysis

The statistical analyses of the study were conducted using the SPSS 19.0 package program. The continuous variables in the study are presented with mean, standard deviation, median, minimum, and maximum values as descriptive statistics. The categorical variables are presented with frequencies and percentages. The normality of the distributions of the continuous variables was examined using the Shapiro-Wilk test. The Mann-Whitney U test was used in the comparisons of two groups of variables that were not normally distributed. The Friedman test was used for the intragroup comparisons of the dependent variables. Pearson's, Yates, and Fisher's exact chi-squared tests were used for the intergroup comparisons of the categorical variables. In all statistical analyses in the study, results with a p-value below 0.05 were considered statistically significant.

3. RESULTS

One-hundred and fifty-eight patients with the diagnosis of MDS were included in the study. Eighty-nine (56.3%) of the cases were female, and 69 (43.7%) were male. The mean age of the patients was 71.53 ± 12.6 years, and the majority of the group was female. In the follow-ups, 83 (47.5%) patients had a hematological response, and 75 (52.5%) patients did not have such a response. It was observed that 87 (55.1%) patients survived, and 71 (44.9%) patients died. In their follow-up periods, 24 patients used Azacytidine, whereas 13 patients used decitabine, 16 patients used drugs other than hypomethylating agents, 80 patients used EPO, and 58 patients received G-CSF support (Table I). In the classification according to the IPSS scores of the patients, 12 (22.2%) patients were classified as low-risk, 31 (57.4%) were classified as medium-risk level 1, 7 (13%) were classified as medium-risk level 2, and 4 (7.5%) were classified as high-risk.

In the comparison of the hematological response statuses of the patients based on their IPSS scores, hematological response was observed in 9 (75%) of the low-risk patient group, and no response was observed in the remaining 3 (25%) patients. Hematological response was observed in 12 (38.7%) patients in the medium-risk level 1 group, and no response was observed in the remaining 19 (61.3%) patients. Response was observed in 3 (42.9%) patients in the medium-risk level 2 group, and no response was observed in the remaining 4 (57.1%) patients. In the high-risk patient group, none of the 4 (100%) patients showed hematological response. A statistically significant (p=0.021) difference was found among the risk groups in terms of their hematological response statuses.

In the comparison of the MPV values of the patients according to their hematological response statuses through time, no significant change was found in the patients who responded (p=0.077), and a highly significant change (p<0.001) was found in the patients who did not respond. In the comparison of the RDW values of the patients according to their hematological response statuses through time, no significant change was found in the patients who responded (p=0.905) or the patients who did not respond (p=0.146) (Table II).

In the comparison of the MPV values of the patients according to their survival statuses through time, a significant change was found in the patients who died (p=0.008), while a significant change was found in the patients who survived (p=0.025). According to the results of the comparison of the RDW values of the patients, there was no significant change in the values of the patients who survived (p=0.798), whereas a significant change was found in the deceased patients (p<0.001) (Table II).

The mean percentage change in the MPV values of the patients who showed hematological response between the 0th and 24th months was 0.022±0.11, while this value was 0.069±0.15 in the non-responders, and the difference between these groups was statistically significant (p=0.049). The mean percentage change in the MPV values of the deceased patients between the 0th and 24th months was 0.072±0.16, while the mean percentage change in the patients who survived was 0.023±0.11, and no significant difference was found between the two groups (p=0.098). The mean percentage change in the RDW values of the patients with hematological response between the 0th and 24th months was 0.066±0.21, the mean percentage change in those who did not respond was 0.078±0.23, and the difference between the two groups was not statistically significant (p=0.375). The mean percentage change in the RDW values of the patients who died between their measurements at the 0th and 24th months was 13.23±22.97, the mean percentage change in the patients who survived was 2.86±21.42, and a highly significant difference was found between the two groups (p=0.006) (Table III).

Table I. *Descriptive characteristics of patients diagnosed with myelodysplastic syndrome*

Characteristics (n=158)	Number (n)	Percentage (%)
Gender		
Male	69	43.7
Female	89	56.3
Age (year)	Mean±SD: 71.53±12.6	
Hematological Response		
Responders	83	52.5
Non-responders	75	47.5
Survival		
Alive	87	55.1
Deceased	71	44.9
Treatment		
Azacitidine	24	15.2
Decitabine	13	8.2
Other medications	16	10.1
Erythropoietin	80	50.6
G-CSF	58	36.7

G-CSF: Granulocyte stimulating factor, SD: Standard deviation

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Table II. *Comparison of MPV and RDW values with hematological response and survival*

		MPV			RDW		
Hematological Response (month)	**n**	**Mean±SD**	**Median (min – max)**	**p**	**Mean±SD**	**Median (min – max)**	**p**
Responder							
0th Month	83	8.43±1.15	8.40(6.4-10.6)		16.81±2.94	16.10(12.6-27.8)	
6th Month	83	8.48±1.02	8.40(5.9-12.5)	0.077	17.49±3.82	16.20(12.4-29.9)	0.905
12th Month	82	8.51±1.02	8.45(6.6-10.6)		17.05±3.20	16.25(12.9-27.4)	
18th Month	80	8.67±1.13	8.60(6.5-12.6)		17.09±3.28	16.40(12.8-28.1)	
24th Month	73	8.59±1.05	8.50(5.8-10.9)		17.77±3.77	16.90(12.8-31.6)	
Non-Responder							
0th Month	75	8.89±1.42	8.90(6.4-13.6)		18.62±4.15	17.70(12.9-34.1)	
6th Month	73	8.96±1.44	8.70(6.4-13.3)		19.4±5.16	17.80(13.6-35.1)	
12th Month	67	9.01±1.44	8.90(5.4-13.7)	**<0.001**	19.47±4.56	18.20(13.6-32.2)	0.146
18th Month	64	9.15±1.55	8.70(5.6-13.0)		19.82±4.78	18.25(13.6-31.1)	
24th Month	61	9.43±1.40	9.10(6.5-13.1)		19.70±4.23	18.90(13.1-31.3)	
Survival (month)							
Alive							
0th Month	87	8.76±1.29	8.80(6.4-13.6)		17.41±3.76	16.40(12.6-34.1)	
6th Month	85	8.67±1.28	8.50(6.5-13.3)		18.73±5.06	17.50(12.4-35.1)	
12th Month	83	8.77±1.14	8.90(6.4-11.7)	**0.025**	17.97±4.26	16.50(12.9-32.2)	0.798
18th Month	82	8.94±1.19	8.80(6.7-13.0)		17.81±4.47	16.30(12.8-31.1)	
24th Month	78	8.95±1.07	8.90(7.0-12.3)		17.73±3.84	16.70(12.8-31.6)	
Deceased							
0th Month	71	8.51±1.26	8.50(6.4-13.6)		17.99±3.55	17.70(13.1-31.9)	
6th Month	71	8.75±1.36	8.50(5.9-13.3)		17.98±3.94	16.90(13.3-30.3)	
12th Month	66	8.68±1.39	8.70(5.4-13.7)	**0.008**	18.35±3.77	17.70(13.6-30.1)	**<0.001**
18th Month	62	8.81±1.55	8.60(5.6-13.0)		18.96±3.81	18.35(13.6-30.8)	
24th Month	56	8.99±1.55	8.85(5.8-13.1)		19.92±4.12	18.55(14.2-31.3)	

SD: Standard deviation, MPV: Mean platelet volume, RDW: Red cell distribution width. Friedman test was applied to compare the mean value of parameters between all groups.

Table III. *Comparison of percentage changes in MPVs and RDWs of patients with hematological response and survival*

	n	**MPV**			**RDW**		
Hematological Response		**Mean±SD**	**Median (min – max)**	**p**	**Mean±SD**	**Median (min – max)**	**p**
Responder	73	0.022±0.11	0.021(-0.22 – 0.28)	**0.049**	0.066±0.21	0.028(-0.30 – 0.95)	0.375
Non-Responder	61	0.069±0.15	0.39(-0.039 – 0.85)		0.078±0.23	0.09(-0.53 – 0.76)	
Survival							
Alive	78	0.023±0.11	0.01 (-0.33 – 0.31)	0.098	2.86±21.42	0.45(-53.37 – 94.89)	**0.006**
Deceased	56	0.072±0.16	0.05 (-0.22 – 0.85)		13.23±22.97	10.58(-43.73 – 75.66)	

SD: Standard deviation. Mann-Whitney test was applied to compare the mean value of parameters between all groups.

4. DISCUSSION

Although, classification systems like those of WHO and FAB are used for diagnosis in MDS, these systems do not provide clues prognostically. Classification systems like IPSS and IPSS-R are used for current prognostic data. According to the statistics in these classification systems, there is a higher risk of mortality and morbidity in some IPSS (medium-risk level 2, high-risk) and IPSS-R (medium-risk, high-risk, very high-risk) patient groups than in some other IPSS (low-risk, medium-risk level 1) and IPSS-R (very low-risk, low-risk, medium-risk) patient groups, considering the duration of transformation to AML and the average life expectancy. The incidence and prevalence of MDS were higher in male patients compared to female patients in previous studies [9,12]. In our study, the number of female patients (56.3%) was higher than the number of male patients (43.7%). This result may have occurred due to the fact

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that some diagnosed patients who presented to the outpatient clinic once and continued their follow-ups in other centers were excluded from the study. In this study, the frequency of MDS was determined to increase with age, the incidence of MDS increased up to 40-50 cases per 100,000 people in the population over 70 years of age, and the mean age of the patients included in our study, which was 71.53±12.6 [median 74.5 (minimum: 29, maximum: 94)], was found to be consistent with ages reported in previous studies [12].

In the study by Wijermans et al., the mean hematological response rate was found 49%, and the rate of cases with hematological response in our study was 52.5%, which was similar [13].

According to the evaluations of the patients according to their IPSS scores, hematological response was observed in 9 (75%) patients in the low-risk group, but no response was observed in 3 (25%) patients in the same group. In the high-risk patient group, none of the 4 (100%) patients showed hematological response. A significant difference was observed among the hematological response rates of the groups that were assigned based on their IPSS values (p=0.021), and response rates decreased significantly as risk levels increased. Similarly, Greenberg et al., reported that markers such as hemoglobin and neutrophil counts are important in prognosis, and as the risk classification increases, the response decreases, and the mean survival time is shorter [14].

Masutani et al., evaluated the usability of MPV and mean platelet component values (platelet count x MPV) for screening purposes in cases with MDS. MPV and mean platelet component (MPC) values at the time of first diagnosis in MDS, aplastic anemia, ITP, and myeloproliferative neoplasms were compared to reference values. In the samples collected from 1304 healthy individuals, the mean MPV was 8.1±1.5 fL, while the mean MPV value was 8.9±2.9 fL in patients with aplastic anemia, 9.7±4.4 fL in ITP patients, and 9.0±2.7 fL in patients with myeloproliferative neoplasms, whereas it was relatively high at 12.0±5.0 fL in MDS patients (p<0.001) [8]. In our study, the MPV values of the patients who showed hematological response did not significantly change through the measurement times (p=0.077). The MPV values of the patients without hematological response, on the other hand, showed changes with a high degree of significance (p<0.001). In the analyses of the mean MPV values of the treatment-independent patients who died and those who survived, statistically significant changes were observed through time in both the deceased group (p=0.008) and the group that survived (p=0.025). This was thought to be due to the advanced age of the patients and their causes of death other than MDS. In the comparison of the MPV percentage changes of the patients from the initial measurements to the twenty-fourth month measurements, a statistically significant difference was found (p<0.05) between the patients with hematological response (2.2%) and those with no hematological response (6.9%). This gives us a clue for monitoring patients who show an increase in MPV values more closely during their follow-ups and perhaps preparing them for early stem cell transplantation. The percentage increase through time in the MPV values of the patients regardless of treatment was 2.3% in those who survived and 7.2% in those who died, while the difference between the two groups was not statistically significant [8].

Baba et al., associated RDW with clinical outcomes in MDS patients, and no significant correlation was found between RDW and prognosis in patients with increased blast counts, while a significant relationship was found between increased RDW (≥15.0%) and poor prognosis (p=0.0086) in MDS patients with refractory anemia [10]. In our study, the RDW values of the patients did not change significantly over time in the group with hematological response (p=0.905) or the group without hematological response (p=0.14). While the RDW values of the patients who survived regardless of the treatment did not show a statistically significant change through the measurement times (p=0.798), the values of those who died increased to a statistically highly significant extent (p<0.001). Thereupon, the percentage changes in RDW values were compared based on hematological response status and survival status, and no significant relationship was identified between RDW changes and hematological response statuses (p=0.375), while these values significantly differed based on survival status (p=0.006) [10]. Furthermore, in a similar study, it was reported that RDW has a potential as a prognostic marker in MDS patients [15]. For these reasons, while the RDW value could not be associated with response to treatment in this study, it was found to be significantly associated with mortality.

Limitations

There were two major factors limiting the analyses in this study. First, some patients did not comply with the two-year follow-up period. Second, there was relatively little cytogenetic information available compared to the entire patient population (cytogenetic analysis could be performed for only 54 of the 158 patients, which corresponded to a rate of 34.1%).

Conclusion

We investigated the effects of MPV and RDW values on prognosis in myelodysplastic syndrome cases. MPV value changes were found to be significantly associated with hematological response status (p=0.049). While no relationship could be established between survival status and MPV changes, a highly significant relationship was found between RDW changes and survival status (p=0.006). In this study, it was aimed to reveal prognostic markers that can be used practically in the hematology clinic to change prognosis and treatment modalities in MDS and facilitate follow-ups. Without highly invasive procedures like bone marrow examination, MPV is a simple, inexpensive hemogram parameter that can be used daily in the follow-up of MDS patients, and it is a promising predictor of prognosis. When there is an increase in MPV values that is determined by only looking at routine blood tests in the follow-up of patients, this increase can be considered an indicator of poor diagnosis.

Compliance with Ethical Standards

Ethical Approval: This study was approved by the Zonguldak Bulent Ecevit University Non-Interventional Clinical Research Ethics Committee (08/01/2019 – 33479383).

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Authors' contributions: IA and SE**:** Concept and design, IA: Data acquisition, IA and SE**:** Data analysis and interpretation, IA and SE**:** Drafting manuscript, SE: Critical revision of the manuscript, IA and SE: Technical or material support, IA and SE: Supervision, IA and SE: Final approval and accountability.

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The effect of preemptive use of plerixafor on stem cell mobilization in patients with lymphoma and multiple myeloma

Ayse UYSAL[1], Mehmet Ali ERKURT[2], Irfan KURU[2], Emin KAYA[2], Ilhami BERBER[2], Ahmet SARICI[3], Soykan BICIM[2], Ahmet KAYA[2], Emine HIDAYET[2]

[1] Division of Hematology, Department of Internal Medicine, School of Medicine, Firat University, Elazig, Turkey.
[2] Division of Hematology, Department of Internal Medicine, Turgut Ozal Medical Center, Inonu University, Malatya, Turkey.
[3] Hematology Clinic, Malatya Training and Research Hospital, Malatya, Turkey.

Corresponding Author: Mehmet Ali ERKURT
E-mail: erkurtali@hotmail.com

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ABSTRACT

Objective: The aim of this study is to investigate the effect of the preemptive use of plerixafor in patients with lymphoma and multiple myeloma which was administered as a preemptive single dose to the patients who were determined to have a CD34+ cell count of <15/μL in the peripheral blood (PB) on the 4th day of mobilization.

Patients and Methods: Thirty-five patients who were administered plerixafor on the 4th day after granulocyte colony-stimulating factor (G-CSF) alone for stem cell mobilization between January 2020 and November 2021 were included. CD34+ stem cell counts in PB before and after plerixafor, the amount of CD34+ stem cells collected, and the outcome of transplantation was examined.

Results: The median CD34+ cell count in PB on the 4th day was 5.2/μL (0.1-13.4), which was determined to increase 206.6-fold (31.57-49347) to 924.80 /μL (295.00-5056) following the administration of plerixafor on the 5th day (Z=-5.160; r= – 872.2; p<0.0001). The number of apheresis sessions was 1 in all patients. The median collected CD34+ cell count was $5.90x10^6$/kg ($2.70x10^6$-$14.4x10^6$).

Conclusion: The use of preemptive plerixafor shows that it is an effective mobilization method by increasing the rate of stem cell collection at an effective dose and reducing the mobilization time/apheresis sessions.

Keywords: Apheresis, Stem cell, Mobilization, Plerixafor, Preemptive

1. INTRODUCTION

Autologous stem cell transplantation (ASCT) is the main treatment option in multiple myeloma (MM) and lymphoma both as first-line therapy and in the treatment of relapsed/refractory disease, providing high overall survival and disease-free survival outcomes[1, 2]. The amount of infused stem cells is of great importance in ASCT in terms of ensuring repeat recovery in the bone marrow. Although, the minimum recommended CD34+ stem cell dose for infusion is $2x10^6$/kg, the targeted effective dose for rapid recovery is $5x10^6$/kg[3-5].

Peripheral blood is used for stem cell collection in ASCT, given that it leads to rapid neutrophil and platelet engraftment and is a convenient method for patients. Various methods are used for the mobilization of stem cells into the peripheral blood. Granulocyte-colony stimulating factor (G-CSF) alone, one of these mobilization methods, or its combined use with chemotherapy, may lead to mobilization failures in rates of 15% to 30% [5-8]. Among the risk factors that cause mobilization failure have been cited as advanced age, agents used in previously administered treatments such as alkylating agents, fludarabine, lenalidomide, exposure to multiple lines of chemotherapy, radiotherapy history, lymphoma diagnosis, and bone marrow involvement [9, 10].

The CD34+ stem cells count in the peripheral blood before the apheresis procedure is crucial as an indicator of the effective dose of cell collection and mobilization failure. The targeted CD34+ cell count for an effective cell collection in the peripheral blood is >20/μL on the 5th day of mobilization. CD34+ cell counts below this threshold level result in lesser amounts of stem cells to be collected by apheresis, increase the number of apheresis steps, prolong the stem cell collection time,

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and increase the overall cost [11]. For this reason, plerixafor is applied preemptively if CD34+ stem cell count is determined to be below <10-15/μL in the peripheral blood count performed on the 4th day of mobilization, in order to reduce the mobilization failure associated with the use of G-CSF [12]. Both European Group for Blood and Marrow Transplantation (EBMT) and British guidelines support the use of preemptive application of plerixafor in cases where the peripheral blood CD34+ cell count is below 20/μL [4, 13].

Plerixafor is a selective, reversible CXCR4 (chemokine receptor type 4) antagonist, blocking the interaction of CXCR4 with SDF-1α (stromal cell-derived factor 1) in stem cells and facilitating the passage of CD34+ stem cells into peripheral blood with the synergistic effect of G-CSF[14]. The combined use of plerixafor and G-CSF was reported to be superior to the use of alone G-CSF in mobilizing hematopoietic stem cells without significant toxicity[15, 16].

In this study, we aimed to investigate the effect of plerixafor, which was administered as a preemptive single dose to the patients with both MM and lymphoma [non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma (HL)] who were determined to have a CD34+ cell count of <15/μL in the peripheral blood count on the 4th day of mobilization.

2. PATIENTS and METHODS

Thirty-five patients who were administered a single dose of plerixafor preemptively on the 4th day after G-CSF alone for stem cell mobilization for ASCT between January 2020 and November 2021 were included in this study.

This study was conducted in accordance with the principles of the Declaration of Helsinki. The ethics committee approval of the study was obtained from the Clinical Research Ethics Committee of Inonu University (approval date: 14.12.2021, and approval number: 2021/2844).

Patients' diagnoses, lines of treatments before mobilization, lenalidomide treatment, number of the lenalidomide cycles, CD34+ stem cell counts in the peripheral blood per microliter before and after administration of plerixafor, number of apheresis performed for stem cell collection, the amount of CD34+ stem cells collected after apheresis (x106/kg), the conditioning regimens, the duration of neutrophil and platelet engraftment, the status of febrile neutropenia, and durations of hospitalization were examined.

Duration of neutrophil engraftment was defined as the time from the day of stem cell infusion to the first of three consecutive days with an absolute neutrophil count ≥ 0.5×10^9/L. Duration of platelet engraftment was accepted as the time from the day of stem cell infusion to the first of three consecutive days with a platelet count greater than 20×10^9/L without transfusion. Additionally, febrile neutropenia was deemed to be present in the event of fever above 38 °C with a neutrophil count below 500/μl.

Duration of hospitalization was defined as the time from the first day of mobilization to discharge or death from any cause.

Transplant-related mortality was defined as the death that occurred in association with transplant complications and not in relation to any disease in the first 30 days after transplantation.

Mobilization Protocol

Granulocyte colony-stimulating factor was administered to the patients via subcutaneous injection at a dose of 10 μg/kg/day for 4 days. CD34+ cell count was measured by flow cytometry in the peripheral blood on the 4th day of mobilization. Plerixafor was administered subcutaneously at a dose of 0.24 mg/kg/day at 11.00 pm on the 4th day of mobilization to patients whose CD34+ cell counts in the peripheral blood were found to be <15/μL. The Navios EX flow cytometer (Beckman Coulter Inc, California, USA) device was used to measure the CD34+ cell counts. GCSF was applied at 7 am on the 5th day of mobilization, and stem cell collection was performed by apheresis 11 hours after the administration of plerixafor. Patients' CD34+ cell counts were re-measured via flow cytometry from peripheral blood before apheresis. It was aimed to collect a minimum of $2x10^6$/kg CD34+ cells with apheresis. Amicus (Fresenius-Kabi, Istanbul, Turkey) device was used for all stem cell collection procedures. Mobilization failure was defined as a CD34+ stem cell count of $<2x10^6$/kg after apheresis.

The stem cells collected were cryopreserved in lymphoma patients preserved via the addition of dimethyl sulfoxide (DMSO) at a concentration of 10% followed by freezing at a mechanical freezer at – 80°C. The bags containing the cryopreserved cells were thawed in a 37 °C water bath immediately before infusion and re-infused on day 0 following the application of the conditioning regimen.

The stem cells collected were infused without freezing in MM patients. For this reason, the collected CD34+ cells were stored in blood bags without DMSO at 4 °C for up to 72 hours and re-infused on day 0 following the application of the conditioning regimen.

Transplantation Protocol

A single dose of melphalan was administered to MM patients on day – 2 as the conditioning regimen. The melphalan dosage to be administered to the fit and young patients and to fragile patients or patients with a glomerular filtration rate of less than 60 mL/min/1.73 m^2 were determined as 200 mg/m^2 and 140 mg/m^2, respectively. Stem cells were infused on day 0.

BEAM (carmustine, etoposide, cytarabine, and melphalan) regimen was used in 13 lymphoma patients, whereas Bu/Cy/E (busulfan, cyclophosphamide, etoposide) regimen was used in two lymphoma patients and in one acute lymphoblastic leukemia (ALL) patient as a conditioning regimen. BEAM regimen was applied as carmustine on day –6 (300 mg/m2/day), etoposide from day–6 to –3 (100 mg/m2 every 12 h), cytarabine from day –6 to –3 (200 mg/m2 every 12 h), and melphalan on day –2 (140 mg/m2/day). Bu/Cy/E regimen was applied as busulfan (16mg/kg/day) from day – 7 to – 4, carmustine (200 mg/m2/day) on day – 7 and cyclophosphamide (120 mg/kg/day) on day-3 and

– 2, etoposide (400 mg/m2/day) on day – 3 and – 2. Stem cells were infused on day 0.

All patients received premedication comprising chlorpheniramine, methylprednisolone, and acetaminophen before stem cell infusion. G-CSF was started to be given on day +1 and continued to be given until neutrophil engraftment was achieved. All patients received acyclovir, levofloxacin, and fluconazole as prophylactic therapy.

Statistical Analysis

In descriptive analyses, numbers and percentages were used to express the categorical data, whereas mean ± standard deviation values, percentages, and histograms were used to express parametric and median (range, minimum-maximum) values were used to express non-parametric continuous data. Skewness-Kurtosis and Kolmogorov–Smirnov tests were used for parametric and non-parametric classification of the continuous data. Mann-Whitney U test or Wilcoxon Signed-Rank test was used for the comparisons of nonparametric continuous variables. Pearson's chi-squared test or the Fisher's Exact Test was used for the comparisons of categorical data. Wilcoxon Signed-Rank test was used to analyze the difference between the CD34+ count measurements performed on the 4th and 5th days of mobilization. The difference of the marginal means between the CD34+ count measurements performed on the 4th and 5th days of mobilization in respect of MM and other cases was compared via split-plot in time-repeated measures analysis of variance (ANOVA). Probability (p) values of <0.05 were deemed to indicate statistical significance.

3. RESULTS

A total of 35 patients who were administered preemptive plerixafor were included in the study. Twenty of them (57.1%) were diagnosed with MM, 15 (42,9%) with lymphoma [NHL: 8 (53,3%), HL: 7 (46,7%)]. The median age was 53 (range, 19-74) years. Only 7 (20%) patients were over 60 years. Sixteen (45.7%) patients were female, and 19 (54.3%) patients were male. There was no significant difference between the patient groups in terms of gender (p=0.807). The median number of treatments before mobilization was 2 (range, 1-6). Twenty-three (65.7%) patients received more than one line of treatment. It was determined that 9 (25.7%) patients, who were all MM patients, received lenalidomide treatment before mobilization, and that 5 of these patients received lenalidomide treatment for 4 cycles or more. All patients had a partial or better response to treatment at the time of mobilization. None of the patients had refractory disease. Demographic and clinical characteristics of the patients according to the diagnosis subtypes are shown in Table I. Analysis by the subtypes revealed that 93.3% of the lymphoma patients as compared to 45% of the MM patients received intense pre-mobilization treatment, that is more than one line of treatment, and that there was a significant difference between the patient groups in that respect.

Table I. *The demographic and clinical characteristics of the patients*

	Multiple Myeloma N: 20	Lymphoma N: 15	Total N: 35
The median age, years (range)	57 (40-74)	39 (19-66)	53 (19-74)
>60 years, at the time of mobilization (%)	6 (30%)	1 (6,7%)	7 (20%)
Gender			
Female (%)	10 (50%)	6 (40%)	16 (45,7%)
Male (%)	10 (50%)	9 (60%)	19 (54,3%)
The median prior therapy line (range)	1 (1-3)	2 (1-6)	2 (1-6)
>1 line therapy	9 (45%)	14 (93,3%)	23 (65,7%)
Prior lenalidomide therapy (%)			
Yes	9 (45%)	-	9 (25,7%)
No	11 (55%)	-	26 (74,3%)
< 4 lines	4 (44,4%)	-	4 (11,4%)
≥ 4 lines	5 (55,6%)	-	5 (14,3%)
Disease status at the time of mobilization (%)			
CR	11 (55%)	11 (73,3%)	22 (62,8%)
VGPR	5 (25%)	-	5 (14,3%)
PR	4 (20%)	4 (26,7%)	8 (22,9%)
Refractory disease	-	-	-
The median time from diagnosis to mobilization (months)	5 (2-185)	20 (4-104)	10 (2-185)
Mobilization regimen (%)			
Melphalan 200mg/m2	14 (70%)	-	14 (40%)
Melphalan 140mg/m2	6 (30%)	-	6 (17,1%)
BEAM	-	13 (86,7%)	13 (37,1%)
Bu/Cy/E	-	2 (13,3%)	2 (5,8%)
Transplantation (%)			
First	13 (65%)	13 (86,7%)	26 (74,3%)
Second or more	7 (35%)	2 (13,3%)	9 (25,7%)

CR: Complete response, PR: Partial response, VGPR: Very good partial response BEAM: Carmustine, etoposide, cytarabine, and melphalan Bu/Cy/E: Busulfan, cyclophosphamide, and etoposide

The median CD34+ cell count in the peripheral blood on the 4th day was determined as 5.2/μL (range, 0.1-13.4), which was determined to increase 206.6-fold (range, 31.57-49347) to 924.80 /μL (range, 295.00-5056) in the peripheral blood following the administration of plerixafor on the 5th day (Z=-5.160; r= – 872.2; p<0.0001) (Figure 1). No side effects were observed in any of the patients after the administration of plerixafor.

The effects of plerixafor in the lymphoma and MM groups is shown in Table II. There was no statistical difference between the two groups in terms of CD34+ count or the increase in the CD34+ count measured on the 4th day of mobilization, that is, before the administration of plerixafor, and on the 5th day of mobilization, that is, after the administration of plerixafor (p=0.840). In addition, there was also no significant difference between the lymphoma subgroups in terms of CD34+ count or the increase in the CD34+ count measured in the peripheral blood on the 5th day of mobilization (Figure 2).

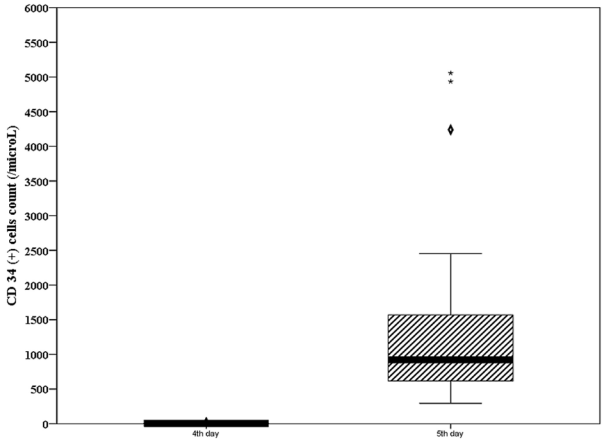

Figure 1. *CD34 (+) cell count of all patients were elavated on day 4 and on day 5 in peripheral blood. (Wilcoxon Signed rank Test; Z=-5.160; r= – 872,2; p<0.0001)*

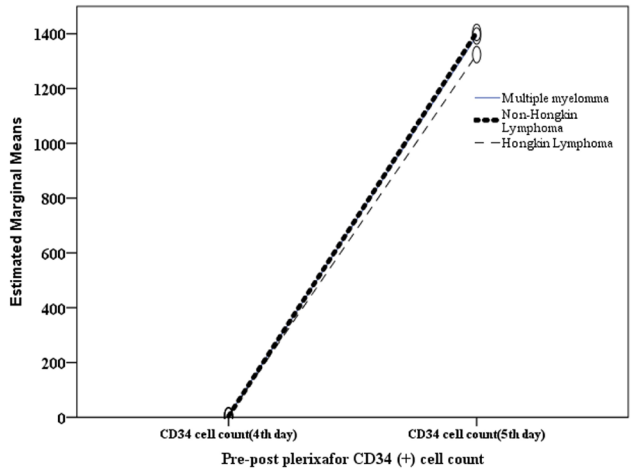

Figure 2. *Pre-post plerixafor CD34 count difference in the lymphoma and myeloma patients. Pre-post plerixafor CD34 count difference of the MM and lymphoma were not to be statistically different (two repeated measures of two groups ANOVA; p=0.840)*

Table II. *The effects of plerixafor in the lymphoma and multiple myeloma groups*

	Multiple Myeloma N: 20 (100%)	Lymphoma N: 15 (100%)	Total N: 35 (100%)	p-value
The median count of CD34/mL on +4th day (range)	5.1 (1.1-13.4)	5.2 (0.1-7.8)	5.2 (0.1-13.4)	0.317*
The median count of CD34/mL on +5th day (range)	1347.25 (295-5056)	803.6 (377.1-4934.7)	924.8 (295-5056)	0.208*
The mean difference of CD34/mL count between the 4th and 5th day	1385.9±1077.1	1302±1368.3	1349.9±1192.1	0.840**
The median fold increase with plerixafor	281.64 (31.57-884.76)	18.41 (98.81-49347)	206.6 (31.57-49347)	0.594*
The median collection CD34x10⁶/kg (range)	7,.26 (2.70-14.4)	5.09 (3.6-14.3)	5.90 (2.70-14.4)	0.314*
Collection yield, x10⁶/kg (%) <2 2-5 >5	 - 7 (35%) 13 (65%)	 - 6 (40%) 9 (60%)	 - 13 (37.1%) 22 (62.9%)	>0999***

** Mann-Whitney-U ** A split-plot in time-repeated measures ANOVA *** Chi-square test*

Table III. *Transplantation outcome of lymphoma and multiple myeloma groups*

	Multiple Myeloma N: 20 (100%)	Lymphoma N: 15 (100%)	Total N: 35 (100%)
The median duration of neutrophil engraftment, day (range)	14 (9-19)	12 (9-17)	12 (9-19)
The median duration of platelet engraftment, day (range)	14 (11-43)	15 (11-21)	15 (11-43)
The rates of febrile neutrophile (%) Yes No	 11 (55%) 9 (45%)	 13 (86.7%) 2 (13.3%)	 24 (68.6%) 11 (31.4%)
The median duration of hospitalization, day (%)	21 (15-52)	27 (16-62)	24 (15-62)
Transplant-related mortality (%)	-	2 (13,3%)	2 (5,7%)

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In all patients, CD34+ stem cells were collected using apheresis only once. Mobilization failure was not observed in any patient. The median collected CD34+ cell count was 5.90x10^6/kg (range, 2.70x10^6-14.4x10^6). In 22 (62.9%) of the patients, the collected CD34+ stem cell count was above 5x10^6/kg. The difference in the count of cells collected between MM patients who received lenalidomide or not could not be given due to the small sample size.

Transplantation outcomes were shown in Table III. Neutrophil engraftment did not occur in two patients (1; NHL and 1; HL patient), whereas platelet engraftment did not occur in 3 patients (1; HL, and 2; NHL patient). There were two transplantation-related mortalities in the lymphoma group and none in the MM group. Two patients died due to coronavirus disease 2019 (COVID-19) infection. Accordingly, the transplantation-related mortality (TRM) rate of the study group was found as 5.7%.

4. DISCUSSION

CD34+ stem cell count measured in peripheral blood is an indicator of the efficiency of the stem cell collection by apheresis. The strong correlation between the two parameters is well established in the literature [11, 17]. In one of these studies, which was carried out with a view to investigating the use of CD34+ stem cell count in the peripheral blood on the day before stem cell collection by apheresis to predict poor mobilization, Szwajcer et al., determined 10/μL as the cut-off value to predict poor mobilization. Accordingly, CD34+ stem cell counts of less than 10/μL in the peripheral blood on the day before stem cell collection by apheresis predict poor mobilization. Additionally, it was determined that setting the cut-off value to a higher cell count of 15/μL reduced the possibility of poor mobilization. Therefore, it is recommended in patients at high risk for inadequate mobilization to add plerixafor to GCSF preemptively, that is, at the first step, not to prolong the apheresis procedure or to prevent remobilization[18]. In another study conducted with 397 patients undergoing ASCT, Sancho et al., investigated the cut-off value of CD34+ count in peripheral blood in respect of the preemptive or emergency use of plerixafor. Consequentially, they found with 90% sensitivity and 91% specificity that CD34+ cells count cut-off value of 13.8/μL indicates that the stem cells are collected at an effective dose (≥2x10^6 CD34 cells/kg). In the same study, 3% of the patients with a CD34+ cell count >20/μL in the peripheral blood before apheresis were found to have poor mobilization, as compared to 22% of the patients with a CD34+ cell count between 10/μL and 20/μL in the peripheral blood before apheresis, who were found to have poor mobilization[19]. Costa et al., determined that low CD34+ stem cell counts (<12/μL) in the peripheral blood on the 4th day of mobilization constituted a high risk for mobilization failure. In comparison, in our study, median CD34+ stem cell count in the peripheral blood on the 4th day of mobilization in patients treated with preemptive plerixafor was found as 5.1/μL (range, 1.1-13.4)[20].

In ASCT, the minimum CD34+ stem cell dose that should be infused is 2x10^6/kg in order not to prolong the duration of neutrophil and platelet engraftments and to reduce the risk of transplantation-related complications. Engraftment times are prolonged in transplantations performed with stem cell doses less than the said threshold value, which increases the duration of hospital stay, the risk of infection, and the amount of transfusion. The superiority of mobilization using G-CSF and plerixafor combined to mobilization using stand-alone G-CSF has been demonstrated in the literature, in terms of the stem cell count both in the peripheral blood and collected by apheresis [5, 21, 22]. In a study by Worel et al., in which the preemptive administration of plerixafor in MM and lymphoma patients undergoing ASCT has been investigated, the CD34+ stem cell count was found to be <20/μL in the peripheral blood of the patient group in which only GCSF was used for mobilization on the 4th day of mobilization. Based on this result, they used GCSF and plerixafor combination for mobilization attempts for a median number of one time (range, 1-4 times). Consequentially, the median collected CD34+ stem cell count was increased to 4.1 (range, 0.4-11.3) x10^6/kg with a median 5.9-fold (range, 1.2-26) increase after using plerixafor along with G-CSF for mobilization. The median number of apheresis performed for cell collection was one (range, 1-3) [22]. In another study, Micallef et al., used plerixafor upfront in patients whose CD34+ stem cell counts were found to be <10/μL (<20/μL, for those who were planning to have 2 transplants) in the peripheral blood on the 4th or the 5th day of mobilization. Consequentially, the median CD34+ stem cells collected after the use of plerixafor for mobilization on the 4th day of mobilization was found to be statistically significantly higher compared to the median CD34+ stem cells collected on the 5th day of mobilization after the use of plerixafor (6.1x10^6/kg and 7.8x10^6/kg, respectively, p<0.001). In the same study, the ratios of the collected stem cell count>2x10^6/kg and >4x10^6/kg were found to be statistically significantly higher in the patient group which was administered plerixafor on the 4th day of mobilization as compared to the patient group which was administered plerixafor on the 5th day of mobilization (93% vs. 84%, respectively, p<0.001; 99% vs. 95%, respectively, p<0.001)[23]. In our study, the median CD34+ stem cell count in the peripheral blood on the 5th day of mobilization following the preemptive administration of a single dose of plerixafor was determined to increase 206.6-fold (range, 31.57-49347) to 924.80 /μL (range, 295.00-5056). Additionally, the median collected CD34+ stem cells after a single apheresis session was found as 5.90 (range, 2.70-14.4) x10^6/kg. The ratios of the collected stem cell count>2x10^6 and >5x10^6/kg were found to be 100% and 62.9%, respectively, after the administration of a single dose of preemptive plerixafor.

In a study by Vishnu et al., conducted with 42 patients with MM and lymphoma undergoing ASCT, 18 (43%) patients were mobilized with alone GCSF, whereas 24 (57%) patients were mobilized with GCSF and plerixafor combined after the collected CD34+ stem cell counts in the peripheral blood on the 4th day of mobilization was found to be <10/μL. However, there was no statistically significant difference between the patient group that was administered alone GCSF and the patient group that was administered G-CSF and plerixafor combined in terms

of the duration neutrophil and platelet engraftment [neutrophil engraftment; 11 (range, 10-14) days vs. 11 (range, 8-13) days, p=0.93; platelet engraftment; 13 (range, 10-23) days vs. 13 (range, 10-47) days, p=0.47][24]. Similarly, Micallef et al., as well did not find any statistically significant difference between the patient group that was administered stand-alone GCSF and the patient group that was administered GCSF and plerixafor combined in terms of neutrophil and platelet engraftment times [23]. In our study, median neutrophil and platelet engraftment times were found as 12 (range, 9-19) days and 15 (range, 11-43) days, respectively, which are comparable to the respective findings reported in the literature.

The results of this study, in parallel with the relevant results reported in the literature, indicate that the preemptive use of plerixafor in stem cell mobilization is an effective and reliable mobilization method in increasing the rate of stem cell collection at an effective dose, decreasing the mobilization times and the number of apheresis sessions, and saving the patients from the risk of remobilization.

Compliance with Ethical Standards

Ethical Approval: This study was conducted in accordance with the principles of the Declaration of Helsinki. The ethics committee approval of the study was obtained from the Clinical Research Ethics Committee of Inonu University (approval date: 14.12.2021, and approval number: 2021/2844).

Financial disclosure: The authors have nothing to disclose.

Conflict of interest statement: There are no conflicts of interest to declare.

Authors' contributions: AU and MAE: Conceptualization, AU, AS, SB, EH, EK and IK: Data curation and formal analysis, AU, AS, IB, SB, EK and IK: Investigation and resources, MAE, IB; EK and IK: Project administration, AU, SB, EH and AK: Software, MAE, EK, IK, IB and AU: Supervision, validation, visualization, AU and MAE: Writing – review and editing. All authors accepted the final version of the article.

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Anger and aggression in children aged 6-12 in lockdowns during the COVID-19 pandemic in Turkey

Hilal KURT SEZER[1], Nilay BEKTAS AKPINAR[2], Merve ASKIN CERAN[3], Gozdenur TANRIKULU[4]

[1] Department of Nursing, Zubeyde Hanim Faculty of Health Sciences, Nigde Omer Halisdemir University, Nigde, Turkey.
[2] Department of Nursing, Faculty of Health Sciences, Ankara Medipol University, Ankara, Turkey.
[3] Vocational School of Healthcare Services, KTO Karatay University, Konya, Turkey.
[4] Vocational School of Healthcare Services, Lokman Hekim University, Ankara, Turkey.

Corresponding Author: Merve ASKIN CERAN
E-mail: ms.cerancer3642@gmail.com

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ABSTRACT

Objective: This study investigated the relationship between parents' emotional states and the anger and aggression levels of their children between the ages of 6-12 who were in social isolation during the coronavirus disease 2019 (COVID-19) pandemic in Turkey.

Materials and Methods: The study adopted a cross-sectional and descriptive design. The sample consisted of 261 parents. Data were collected online during lockdowns between May and September 2020 due to the COVID-19 pandemic.

Results: More than half of the participants reported that they were unhappy (53.2%), sad (63.2%), and worried about the future due to the COVID-19 pandemic (70.8%). Less than a quarter of the participants were angry (17.6%). Children living in districts had significantly higher verbal aggression scores than those living in city centers ($p < 0.05$). Children with relatives who tested positive for COVID-19 had significantly higher verbal aggression scores than those without ($p < 0.05$).

Conclusion: Although, preventive measures in response to the COVID-19 pandemic prevent the spread of the virus and allow parents and children to spend time together, they also adversely affect mental health.

Keywords: COVID-19, Pandemic, Social isolation, Parent-child relationship

1. INTRODUCTION

A pandemic is a common phenomenon that spreads in a wide range of areas and affects more than one country or continent [1]. The coronavirus disease 2019 (COVID-19) quickly spread all over the world, killing millions of people. Governments took preventive measures (school closures, online learning, lockdowns, social isolation, etc.) to prevent the spread of the virus. However, these measures affected people physically, psychologically, socially, and economically [1-3]. It is considered normal for children to experience concern, anxiety, and fear for themselves and their families and friends due to the COVID-19 pandemic because they cannot understand the process on their own [4, 5]. Children who cannot go out and are kept away from their teachers and friends are alienated from their social circles in crises like COVID-19. They need to see, play, and communicate with their friends. Children who are not socially supported feel anger, worthlessness, fear, sadness, guilt, and anxiety during the COVID-19 pandemic and project these feelings onto other people [5,6]. Children may act out anger by crying, blaming, and resenting their parents, hitting siblings and other family members, or throwing their toys because they are not yet fully developed cognitively, socially, and emotionally [7]. Anger and aggression affect many areas of life and impair functioning [8]. Anger is a negative emotional state which triggers aggression [1,9]. Anger is defined as a hidden or disguised emotion. However, it is also defined as an expression of aggression, which can occur in the form of passive aggression when it is suppressed, and that occurs as even more severe violence [1,7,9].

Although, everyone knows what aggression is, it is not easy to define. Aggression can also be indirect, such as physical violence, abusive or threatening words and behavior, or intentional social exclusion [10]. Even if anger and aggression among children are the product of a developmental process, they can turn into permanent behavior [8,9]. Since, children cannot manage their emotions in such situations; it is difficult to deal

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with anger [10]. Although, children of developmental age often express anger and frustration as tantrums, the intensity and number of tantrums may tend to decrease with age [9]. Many biological, psychological, and social factors affect aggression in children [11]. Social factors triggering aggressive behaviors are poor peer relationships, exposure to violence or bullying, low school success, poor living conditions, alcohol and substance abuse, domestic violence, and economic problems [11-13]. The significant risk factors for aggression are family, intelligence, personality traits, school, peer group, economic status, cultural environment, learning experiences, and communication and interaction [11-13].

Turkey underwent a process of social isolation due to the COVID-19 pandemic. It caused serious problems, which were hard for children to cope with. Inhibitions lead to aggressive behavior in children. Social isolation also prevents children from meeting some of their needs [13,15]. Parents are also adversely affected by social isolation, which may aggravate their children's moods. Therefore, it can be challenging for both parents and children to manage the process and cope with the situation. Parents in panic can cause their children to experience anxiety. Although, it is necessary to control our anxiety, it may not always be easy. Research shows that adults/parents have different emotional situations caused by many factors during the pandemic.

Brooks et al., found that people experienced post-traumatic stress disorder (PTSD), anxiety, anger, depression, anxiety-related insomnia, and frustration during the COVID-19 pandemic [15]. Kundu and Bhowmik also reported that adults had stress, anger, and fear during the COVID-19 pandemic [16].

Parents who spend the whole day at home during the pandemic will likely project their feelings on their children [17]. Therefore, parents' attitudes affect their children's social behavior [18]. In other words, children can suffer more permanent damage due to the pandemic and their parents' reactions [19].

Parents help their children go through the pandemic more easily. However, this process positively or negatively affects both parents and children. In addition, school closures, lockdowns, and changes in daily routines cause sudden emotional changes and disturb sleep patterns in children [1,17,19]. Therefore, this study had two objectives: (1) investigating the relationship between parents' emotional states and their children's aggression and (2) determining the anger and aggression levels of children between the ages of 6-12 who were in social isolation during the COVID-19 pandemic in Turkey.

2. MATERIALS and METHODS

Design

The study adopted a cross-sectional and descriptive design.

Participants and Methods

The study was carried out during the curfew in the early days of the pandemic. For this reason, data were collected through social media using haphazard sampling and snowball sampling method. Some form of connection is made to one of the units in the universe to make a snowball sampling. Then, with the help of the contact person, another person is contacted, and then another in the same way. Thus, the sample is enlarged in a chain manner, in the form of a sample snowball effect [20]. Data were collected online. Parents who have children between 6 and 12 years of age were included in the study. The evaluation was conducted by a single parent. While determining the sample, the results of the previous studies in the literature and the generally accepted "effect sizes" in the related field were used [21]. Cohen's effect size was taken into account to determine the number of samples, and a sample size of 0.80 test power was calculated using G.Power – 3.1.6 program [21]. As a result of power analysis, it was determined that at the α=0.05 level, 95% trust and 80% test power should be reached to at least 235 parents. The data were collected during the COVID-19 pandemic. Therefore, the researchers aimed to recruit 260 participants to avoid missing data.

Inclusion Criteria

The study was conducted during social isolation and restrictions due to the COVID-19 pandemic. The study investigated the relationship between parents' emotions and their children's aggression and anger during social isolation. The sample consisted of 261 parents with children between 6-12. Participation was voluntary.

Exclusion Criteria

The exclusion criteria were not having children between the ages of 6-12 and not having an internet connection.

Data Collection Tools

The data were collected using an information form and the Children's Aggression Scale-Parent Version (CAS-P) [23].

Information Form

The information form was based on a literature review conducted by the researchers. The form consisted of 24 items on social demographic characteristics (n=10), social isolation (n=10), and the effect of social isolation on children and parents (n=4) [3-5, 8, 17, 26].

Children's Aggression Scale-Parent Version (CAS-P)

The Children's Aggression Scale-Parent Version (CAS-P) was developed by Halperin et al. and adapted to Turkish by Ercan et al. [22, 23]. The scale measures the severity, frequency, prevalence, and diversity of children's aggressive behavior. The Turkish version consists of 33 items and five subscales: verbal aggression (α=0.89), aggression toward objects and animals (α=0.55), provoked physical aggression (α=0.75), unprovoked physical aggression (α=0.74), and total family aggression (α=0.93).

Data Collection

Participants filled out the forms to evaluate their children's changing behaviors during social isolation. Each participant used her active e-mail address to log into the system and filled out the form only once. It took each participant 8-10 minutes to fill out the form.

Ethical Considerations

The study was approved by the ethics committee of Ankara Medipol University (Date: 29.04.2020 and No: 74791132-109/322). Authorization was obtained from the authors who developed the CAS-P. All mothers were briefed about the research purpose and procedure. Those who agreed to participate in the study clicked the approval button.

Statistical Analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS, v. 25) at a significance level of .05. Frequency, percentage, mean, and standard error were used for descriptive data. The Shapiro-Wilk test was used for normality testing. Independent groups t-test was used for normally distributed data, while the Mann-Whitney U test was used for nonnormally distributed data. The Kruskal-Wallis test was used to compare more than two independent variables that were nonnormally distributed, while the One-Way ANOVA test was used for variables that were normally distributed.

3. RESULTS

Tables I and II show all participants' sociodemographic characteristics. More than half of the participants stated that their children's sleep patterns changed during social isolation (64.7%). Less than half of the participants reported that their children had difficulty falling asleep (42.9%). Most participants noted that their children had no problem maintaining sleep when they fell asleep (97.8%) (Table II). More than a quarter of the participants remarked that their children felt very nervous and anxious some days (37.9%). Less than half of the participants stated that their children never felt uneasy (38.3%). Less than half of the participants noted that their children got angry easily some days (34.8%). More than a quarter of the participants reported that their children were less interested in doing activities (32.1%).

Children living in districts had a significantly higher verbal aggression score than those living in city centers ($p < 0.05$). Children with relatives who tested positive for COVID-19 had a significantly higher mean verbal aggression score than those without ($p < 0.05$). Children who were always angry about staying at home due to social isolation had significantly higher mean verbal aggression ($p < 0.001$) and general aggression scores ($p < 0.05$). Children who experienced fear due to social isolation had significantly higher mean general aggression and verbal aggression scores than those who did not experience fear due to social isolation ($p < 0.05$). Children who experienced sadness due to social isolation had a significantly higher mean verbal aggression score than those who did not ($p < 0.05$). Finally, children who were concerned about the future due to social isolation had significantly higher mean verbal aggression and general aggression scores than those who were not ($p < 0.05$; Table 1).

Table I. *Relationship between characteristics of parents and aggression scale score means*

Variables (n=261; 100%)	The parent form of aggression scale for children (α=0.92)			
	Verbal Aggression Sub Dimension (α=0.87) (Mean ± SEM)	P value*	General (Mean ± SEM)	P value*
Number of children 1 (n=68; 26.1%) 2 (n=124; 47.5%) ≥3 (n=69; 26.4%)	 0.57 ± 0.09 0.61 ± 0.06 0.54 ± 0.05	0.604	 0.29 ± 0.05 0.33 ± 0.03 0.28 ± 0.03	0.373
Age (Child) 6-8 (n=49; 18.7%) 9-12 (n=212; 81.3%)	 0.72 ± 0.1 0.55 ± 0.04	0.159	 0.38 ± 0.06 0.29 ± 0.02	0.235
Gender (Child) Female (n=128; 49%) Male (n=133; 51%)	 0.53 ± 0.05 0.63 ± 0.06	0.270	 0.28 ± 0.03 0.33 ± 0.03	0.895
Place of residence City (n=191; 73.1%) Town (n=70; 26.9%)	 0.54 ± 0.04 0.70 ± 0.08	0.010*	 0.29 ± 0.02 0.35 ± 0.05	0.081
COVID in familiy member Yes (n=23; 8.8%) No (n=238; 91.2%)	 1.03 ± 0.23 0.54 ± 0.03	0.033*	 0.55 ± 0.14 0.28 ± 0.02	0.107
Parent's unhappiness Yes (n=139; 53,2%) No (n=122 46,8%)	 0.66 ± 0.05 0.50 ± 0.05	0.026*	 0.36 ± 0.03 0.25 ± 0.03	0.005*
Parent's anger Yes (n=46; 17.6%) No(n=215 82.4%)	 0.89 ± 0.12 0.52 ± 0.03	<0.001*	 0.49 ± 0.08 0.27 ± 0.02	0.001*
Parent's fear Yes (n=109; 41.7%) No (n=152 58.3%)	 0.73 ± 0.07 0.48 ± 0.04	0.004*	 0.39 ± 0.04 0.25 ± 0.02	0.03*
Parent's sadness Yes (n=165; 63,2%) No (n=96 36,8%)	 0.61 ± 0.04 0.53 ± 0.07	0.030*	 0.33 ± 0.03 0.27 ± 0.03	0.08
Parent's concern for the future Yes (n=185; 70.8%) No (n=76 29.2%)	 0.66 ± 0.05 0.40 ± 0.04	0.008*	 0.35 ± 0.03 0.19 ± 0.02	0.01*

**Student t test, Mann-Whitney U test, One Way ANOVA and Kruskal-Wallis test, SEM; Standard error*

Children who cried more during lockdowns had significantly higher mean verbal aggression and general aggression scores than those who did not. Children who threw their belongings more during lockdowns had significantly higher mean verbal

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aggression and general aggression scores than those who did not. Children whose sleep patterns changed negatively during lockdowns had significantly higher mean verbal aggression and general aggression scores than those with the same sleep patterns during lockdowns. Children who were restless and nervous most of the day had significantly higher mean verbal aggression and general aggression scores than those who were not. Children who were uninterested in their daily routines had significantly higher mean verbal aggression and general aggression scores than those who were not (p<0.001, Table II).

Table II. *Relationship between children's emotional states and aggression scale score means*

Variables (n=261; 100%)	The parent form of aggression scale for children (α=0.92)			
	Verbal Aggression Sub Dimension (α=0.87) (Mean ± SEM)	P value*	General (Mean ± SEM)	P value*
Symptoms of anger (Child) Yes (n=106; 40.6%) No (n=155; 59.4%)	 0.91 ± 0.07 0.36 ± 0.03	 <0.001*	 0.51 ± 0.05 0.17 ± 0.02	 <0.001*
Frequent crying (Child) Yes (n=47; 18%) No (n=284; 82%)	 0.94 ± 0.13 0.51 ± 0.03	 <0.001*	 0.51 ± 0.08 0.26 ± 0.02	 <0.001*
Throwing items (Child) Yes (n=47; 18%) No (n=214; 82%)	 1.22 ± 0.13 0.44 ± 0.03	 <0.001*	 0.69 ± 0.08 0.22 ± 0.01	 <0.001*
Change in sleep pattern (Child) Yes (n=169; 64.7%) No (n=92; 35.3%)	 0.65 ± 0.04 0.46 ± 0.06	 <0.001*	 0.34 ± 0.03 0.25 ± 0.03	 <0.001*
Difficulty in falling asleep (Child) Yes (n=112; 42.9%) No (n=149; 57.1%)	 0.63 ± 0.06 0.55 ± 0.05	 0.032*	 0.32 ± 0.03 0.30 ± 0.03	 0.12
Difficulty in maintaining sleep (Child) Yes (n=6; 2.2%) No (n=255 97.8%)	 1.23 ± 0.28 0.57 ± 0.04	 0.010*	 0.83 ± 0.26 0.30 ± 0.03	 0.068
The child feels very angry and nervous Never1 (n=78; 29.8%) Sometimes2 (n=99; 37.9%) More than half of the days3 (n=52; 19.9%) Almost everyday4 (n=32; 12.2%)	 0.31 ± 0.05 0.47 ± 0.04 0.88 ± 0.10 1.13 ± 0.17	 <0,001* 1<2<3.4	 0.14 ± 0.02 0.23 ± 0.02 0.48 ± 0.06 0.67 ± 0.12	 <0.001* 1<2<3.4
The child feels cranky Never1 (n=100; 38.3%) Sometimes2 (n=88; 33.7%) More than half of the days3 (n=48; 18.3%) Almost everyday4 (n=25; 9.5%)	 0.39 ± 0.04 0.52 ± 0.06 0.90 ± 0.10 0.96 ± 0.19	 <0.001* 1.2<3.4	 0.20 ± 0.02 0.26 ± 0.03 0.53 ± 0.07 0.48 ± 0.12	 <0.001* 1.2<3.4
Easily angered (Child) Never1 (n=63; 24.1%) Sometimes2 (n=91; 34.8%) More than half of the days3 (n=57; 21.8%) Almost everyday4 (n=50; 19.1%)	 0.24 ± 0.05 0.38 ± 0.03 0.93 ± 0.09 0.99 ± 0.11	 <0.001* 1<2<3.4	 0.10 ± 0.01 0.21 ± 0.02 0.49 ± 0.06 0.53 ± 0.06	 <0.001* 1<2<3.4
Decrease in interest and pleasure (Child) Never1 (n=57; 21.8%) Sometimes2 (n=77; 29.5%) More than the half of the days3 (n=84; 32.1%) Almost everyday4 (n=43; 16.4%)	 0.28 ± 0.04 0.45 ± 0.06 0.69 ± 0.07 1.01 ± 0.13	 <0.001* 1.2<3.4	 0.15 ± 0.02 0.20 ± 0.03 0.38 ± 0.04 0.58 ± 0.09	 <0.001* 1.2<3.4

**Student t test, Mann-Whitney U test, One Way ANOVA and Kruskal-Wallis test, SEM; Standard error, 1,2,3,4: Tukey's Honest Significant Difference test*

4. DISCUSSION

This study investigated the relationship between parents' emotional states and their children's aggression during the COVID-19 pandemic in Turkey. Our results showed that children had high verbal and general aggression scores, suggesting that they suffered from insufficient physical activity, irregular sleep patterns, frustration, separation anxiety, anger, and aggression [3, 13,15,19,23-25]. Although, there is limited research on the effects of COVID-19 and social isolation on children and parents [2,19,24,25], the OECD 2020 report argues that economic status, home conditions, parental education, and sociocultural factors can affect children psychosocially during the pandemic [13]. The pandemic affects all children. However, how much it affects them depends on risk and protective factors. Studies before the pandemic showed that age, parental education, and economic status did not affect children's aggression and anger levels [25-27], which is consistent with our results.

However, some researchers have reported that gender and age affect aggression [28,29]. They state that boys are more aggressive than girls [28-31]. Card et al. argue that gender is an essential factor in aggression [32]. On the other hand, some other researchers maintain that aggression in boys may be related to gender roles, high muscle strength, and hormonal changes [26,31].

Children living in districts has a significantly higher mean verbal aggression score than those living in city centers ($p < 0.05$). Research also shows that children living in the countryside (villages, rural areas, regions with limited access to health services, etc.) have higher aggression levels than those living in cities [13, 19, 32, 34]. Aggressive children are more likely to experience anxiety, stress, and anger due to limited access to education and inadequate technological, health, and social facilities in districts.

Children with relatives who tested positive for COVID-19 had a significantly higher mean verbal aggression score than those without ($p < 0.05$). Jiao et al. also reported that children living in regions where the pandemic was more severe had more anger, anxiety, fear, and other mood disorders than those living in regions where the pandemic was less severe [3]. Children experienced anxiety during the COVID-19 pandemic because they could not grasp the gravity of the situation and lacked the coping mechanisms that could have helped them cope with the challenges of the process. In the study of Demirbaş et al., parents reported that their children experienced stress, anxiety, and mood swings due to constant homestay during the pandemic [2]. Our results support the results that suggest that the higher the aggression, the higher the stress levels in children [19,35-37].

Our results pointed to a significant relationship between parents' emotional states and their children's verbal aggression levels. Research also shows that parents experienced fear, anxiety, sadness, and panic during the COVID-19 pandemic [2, 3,16,24]. Parents had to spend a lot of time with their children at home during the pandemic. Therefore, it is no surprise that their emotional states directly affect their children [1-3, 12,19,24]. Regarding developmental theories, parents play an important role in how their children make sense of and manage this process. In other words, how parents cope with the challenges of the COVID-19 pandemic is likely to determine how their children handle the situation because the latter take the former as role models. Therefore, parents are responsible for preventing secondary trauma by spending quality time with their children, coming up with new daily routines, and explaining the whole situation carefully [15, 38, 39]. From another perspective, social isolation can give parents and children the opportunity to interact more, which can help children get involved in family activities and develop self-sufficiency skills. With the right approaches, parents can form stronger ties with their children and meet their needs. Therefore, we can state that children who are supported, cared for, and loved by their parents can better cope with the pandemic's challenges, resulting in reduced anger and aggression levels [15, 38-40].

Children who cried more during lockdowns had significantly higher mean verbal aggression and general aggression scores than those who did not. Children who threw their belongings more during lockdowns had significantly higher mean verbal aggression and general aggression scores than those who did not. Children whose sleep patterns changed negatively during lockdowns had significantly higher mean verbal aggression and general aggression scores than those with the same sleep patterns during lockdowns. Children who were restless and nervous most of the day had significantly higher mean verbal aggression and general aggression scores than those who were not. Children who were uninterested in their daily routines had significantly higher mean verbal aggression and general aggression scores than those who were not. Children unable to complete their cognitive, social, and emotional development can show their reactions during isolation by crying, accusing their parents, shouting at them, and acting aggressively toward their toys [12,14]. Research also shows that children and adolescents who experience house arrest due to COVID-19 experience stress and anger and exhibit violent behaviors [1,3,12,19]. Demirbaş et al., determined that children experienced emotional changes due to lockdowns and changes in daily routines. They suffered from anxiety because they got sick or missed their peers [2]. Jiao et al., also documented that children experienced sudden emotional changes more frequently due to the COVID-19 pandemic and house arrest [3]. Pisano et al. (2020) determined that one in two children was more irritable and experienced mood swings and agitation due to isolation [12].

Sometimes, children become aggressive due to anxiety and loss of control. However, aggression is considered normal unless it reaches a pathological level [6, 7, 9]. Children with a tendency to anger are more likely to exhibit physical, verbal, and indirect aggression [24,25,30]. Our results showed that aggressive children were angrier. Therefore, preventive measures (lockdowns, school closures, etc.) paved the way for a sense of disability, especially in school-age children. In addition, little to no interaction with peers and limited play and sports activities lead to aggression.

Children whose sleep patterns changed negatively during lockdowns had significantly higher mean verbal aggression and general aggression scores than those with the same sleep patterns during lockdowns. Although, many environmental, physiological, and psychological factors affect sleep quality, it is generally accepted that this triggering factor should be a source of stress for sleep problems [3,12]. Research has also shown that children who experience house arrest due to COVID-19 experience a change in sleep patterns [2,12,19,40]. Liu and colleagues found that two in five students experienced sleep problems during the COVID pandemic [42]. Ghosh et al., determined that children had significantly more sleep problems after school closures. These results suggest that social isolation, uncertainty, and limited physical activity lead to changes in sleep patterns, resulting in fear and anxiety [19]

Children who were restless and nervous most of the day had significantly higher mean verbal aggression and general aggression scores than those who were not. Children who were uninterested in their daily routines had significantly higher mean verbal aggression and general aggression scores than those who were not. Studies suggest that angry and nervous children are more likely to attack others [1,3,15]. For example, Sprang and Silman found that quarantined children were four times more likely to have PTSD than those not quarantined. Prolonged social isolation and lifestyle changes cause psychosocial problems in children [41-43].

This study had three limitations. First, the data were collected during the pandemic. However, we had no data on children's mental and physical health before the pandemic. Second, we excluded parents who could not use smart devices and had no Internet connection. Therefore, the sample consisted only of 261 parents. Third, the results are sample-specific and cannot be generalized to the whole population.

However, our results provide an overview of the psychological impact of COVID-19 on parents and children. Therefore, we think that they will pave the way for further research. Researchers should conduct more studies with larger samples to better understand the impact of COVID-19 on parents and children. We also think our results will help authorities formulate interventions to help parents and children cope with outbreaks.

Conclusion

Although, the pandemic and related preventive measures allow parents to spend more quality time with their children, they have adverse effects on their mental health. Authorities should take various measures to protect the mental health of children and parents. For example, educators should provide parents with training on how to protect their mental and physical health during outbreaks. Moreover, healthcare professionals should provide psychosocial support and encourage parents and children to engage in physical activities to help them cope with the challenges of outbreaks. They should train them in healthy lifestyle behaviors, balanced diets, sleep patterns, and personal hygiene. In addition, researchers should determine the risk factors associated with outbreaks and focus on ways to strengthen social support resources and help parents and children develop functional coping and problem-solving skills.

Compliance with Ethical Standards

Ethical Approval: The study was approved by the ethics committee of Ankara Medipol University (Date: 29.04.2020 and No: 74791132-109/322). Authorization was obtained from the authors who developed the CAS-P. All procedures adhered to the ethical standards of the institutional and/or national research committee. The study was carried out according to the World Medical Association (WMA) Declaration of Helsinki.

Conflicts of interest: The authors declare no conflicts of interest.

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Can plasma fibrinogen level predict bone marrow fibrosis?

Yildiz IPEK, Ayse Nilgun KUL

Department of Hematology, Kartal Lutfi Kirdar City Hospital, Istanbul, Turkey

Corresponding Author: Yildiz IPEK
E-mail: dryildizipek@hotmail.com

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ABSTRACT

Objective: We aimed to assess the possible relationship between plasma fibrinogen level and bone marrow fibrosis (BMF) grades in patients who had undergone bone marrow (BM) biopsy for any reason.

Patients and Method: This retrospective cohort study included 106 participants aged 18 years and over who had undergone simultaneous BM biopsy and circulatory fibrinogen level measurement during 2020 and 2021 at our center. BMF grade was measured by the modified Bauermeister grading system (MBGS). Participants were divided into two groups according to MBGS as those without BMF and those with BMF.

Results: Fifty-eight male were included in our study, and the median age of the patients was 63 (range: 19-97) years. Fibrinogen (p=0.004) and lactate dehydrogenase (LDH) (p=0.030) levels were significantly higher in the fibrosis group. Multiple regression revealed that high fibrinogen (≥359) and high LDH (≥238) were independently associated with a higher likelihood of fibrosis presence (adjusted for age and sex); however, diagnostic analyses revealed low accuracy.

Conclusion: High plasma fibrinogen and LDH levels were found to be independently associated with the presence of BMF. However, it was also evident that neither of these parameters could be used for diagnostic purposes.

Keywords: Bone marrow fibrosis, Plasma fibrinogen, Lactate dehydrogenase, Modified Bauermeister grading system

1. INTRODUCTION

Bone marrow fibrosis (BMF) is a histopathological process known for abnormal excess deposition of reticulin or collagen fibers in the BM. A number of malignant and non-malignant conditions and diseases can cause BMF [1, 2]. Although, studies have examined whether the severity and type of fibrosis are associated with disease prognosis, the results are inconsistent [2]. The presence of reticulin fibers alone, which is characteristic of mild fibrosis, does not appear to be associated with disease severity or comorbidities. However, an increase in collagen fibers, which indicates worse BMF, has been associated with the severity of primary disorders [2, 3]. BMF level and likelihood has been associated with disease severity or treatment response in various diseases, including chronic myeloid leukemia (CML) [4], myelodysplastic syndrome (MDS) [5].

Fibrinogen is a hexameric plasma glycoprotein produced in hepatocytes that contributes to various processes, including inflammation, atherogenesis, and thrombogenesis [6]. The coagulation system not only carries out blood clotting, but also contributes to various processes, including inflammation and tissue repair [7]. One of the most important functions of fibrinogen is its contribution to wound healing, particularly since fibrinogen is suggested to influence the development of healthy wound healing or fibrotic scarring [8]. Imbalances in wound healing mechanisms can lead to excessive scar formation and organ fibrosis [7]. Studies have shown that fibrinogen plays diverse roles in the fibrosis of various organs such as the kidney [6], liver [9], pancreas [10], skin [8], muscle [11], lung [12] and oral submucosa [13]. Even, fibrinogen is considered to be a useful biomarker for diseases in which fibrosis plays an important role in the pathogenesis, such as idiopathic pulmonary fibrosis and liver fibrosis [14, 15]. Therefore, fibrinogen may also contribute to BMF development in various diseases. However, to our knowledge, there is no study examining the role of fibrinogen in BMF and the relationship between plasma fibrinogen level and BMF grade.

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Since fibrinogen measurement can be performed from blood samples, it is evident that possible associations with BMF presence or its severity could yield a method for diagnosing BMF without the need for BM biopsy (BMB), which is an invasive technique. Therefore, we aimed to assess the possible relationships between plasma fibrinogen level and BMF grades in patients who had undergone BM biopsy (BMB) due to any cause.

2. PATIENTS and METHODS

Study Design and Ethics

This was a single-centered, retrospective cohort study carried out at Kartal Dr. Lutfi Kirdar City Hospital. The study was started after ethics committee approval was obtained from Clinical Research Ethics Committee of Kartal Dr. Lutfi Kirdar City Hospital. Since the study was retrospective, obtaining written informed consent from the participants was not required. All information were recorded anonymously.

Study Population and Power Analysis

The study included 106 participants aged 18 years or older who had undergone BMB for any indication and simultaneous fibrinogen level measurement in our hospital during 2020 and 2021. Exclusion criteria were as follows: being younger than 18 years old, pregnancy, having additional malignancy other than the primary hematological disease, and history of rheumatological or autoimmune disease, liver or kidney failure, organ transplantation, acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL). Patients with a history of infection within the two weeks before BMB and those with any history of fibrosis in any other organ were also excluded from the study. Severe BMF is usually seen in cases of primary myelofibrosis. Therefore, in order to define the relationship between fibrosis and fibrinogen, patients with other bone marrow diseases showing different degrees of BMF were also included in this study.

According to descriptive statistics from the study by Yu et al. [16], which demonstrated an effect size of 0.549, we performed power analysis and found that a sample size of 106 patients achieved 81% power according to the two-sided 0.05 significance level (Hintze J., 2011, PASS 11. NCSS, LLC. Kaysville, Utah, USA. www.ncss.com).

Data Collection

The demographic features including age and gender, indications for BMB, laboratory results, and the histopathological findings of patients were collected retrospectively from the electronic database of our hospital.

Laboratory Analysis

Patients' blood samples were obtained from the antecubital vein after 8 hours of fasting immediately before BMB. Fibrinogen (mg/dL), D-dimer (ng/mL), lactate dehydrogenase (LDH; U/L), C-reactive protein (CRP) (mg/L), white blood cell (WBC) count ($x10^3$), hemoglobin (g/dL), platelet count ($x10^3$), prothrombin time (PT) (sec), activated partial thromboplastin time (aPTT) (sec), and international normalized ratio (INR) were measured in the clinical chemistry department of our hospital via use of routine devices and routine techniques.

Pathological Assessment-BMB Grading

Iliac BMB samples were sent to the pathology unit of XXX for examination. BMF grade was determined according to the modified Bauermeister grading system (MBGS) [17] by qualified pathologists. MBGS is a system that classified the degree of BMF according to reticulin staining and collagen fibrosis in BMB specimens. Grade 0: lack of reticulin fibers, Grade 1: sporadic areas of fine individual fibers and presence of fine fiber network, Grade 2: fine fiber network present throughout the section without any coarse fibers, Grade 3: presence of diffuse fiber network with scattered coarse fibers without mature collagen (negative trichrome staining), Grade 4: presence of diffuse coarse fiber networks with areas of collagenization (positive trichrome staining) [17]. Participants were divided into two groups according to MBGS class; those without BMF (Grade 0 and 1, non-fibrosis group) and those with BMF (Grade 2 and 3 and 4, fibrosis group).

Statistical Analysis

All analyses were performed on SPSS v25 (IBM, Armonk, NY, USA) with a significance threshold of <0.05 (p value). For the normality check, the Kolmogorov-Smirnov test was used. Data are given as mean ± standard deviation or median (1st quartile – 3rd quartile) for continuous variables according to normality of distribution, and as frequency (percentage) for categorical variables. Normally distributed variables were compared with the independent samples *t*-test; whereas the Mann-Whitney *U* test was used for non-normally distributed variables. Chi-squared tests were used to compare distributions of categorical variables. Prediction performances were assessed by using Receiver Operating Characteristic (ROC) curve analysis. Optimal cut-off points were determined by using Youden index. Multiple logistic regression analysis was performed to evaluate the BMF prediction performance of variables by adjusting for age and sex.

3. RESULTS

Fifty-eight male and 48 female patients were included in our study, and the median age of the patients was 63 (IQR: 48 – 70) (range: 19 – 97) years. The indications for BMB, laboratory results, and distribution of fibrosis grades according to MBGS are summarized in Table I.

There were 53 patients in the non-fibrosis group and 53 patients in the fibrosis group. Median age was 61 (IQR: 46 – 70) years in the non-fibrosis group, and 64 (IQR: 55 – 70) years in the fibrosis group (p = 0.200). Sex distribution was also similar in the two groups (p = 0.329). The percentage of patients without hematological malignancy in the non-fibrosis group was significantly higher (p=0.001), while the number of patients with chronic myeloproliferative disorders (CMPDs) was significantly higher in the fibrosis group (p=0.001). Fibrinogen (p = 0.004) and LDH (p = 0.030) levels were significantly higher in the fibrosis group, but there was no significant difference between the two groups in terms of other laboratory parameters (Table II, Figure 1, and Figure 2).

Table I. *Summary of patients' characteristics and laboratory measurements*

Age	63 (48 – 70)
Sex	
Male	58 (54.7%)
Female	48 (45.3%)
Diagnosis	
Normal	22 (20.8%)
Lymphoma	10 (9.4%)
MM	33 (31.1%)
CML	3 (2.8%)
Chronic MPDs	17 (16.0%)
CLL	2 (1.9%)
MDS	15 (14.2%)
AA	4 (3.8%)
Fibrinogen	349 (289 – 392)
D-dimer	1190 (485 – 3010)
LDH	217 (178 – 335)
CRP	3.17 (2.06 – 6.26)
WBC (x10^3)	5.52 (3.88 – 8.20)
Hemoglobin	11.00 ± 2.39
Platelet (x10^3)	177 (73 – 259)
PT	14.05 (13.1 – 14.9)
aPTT	30.62 ± 4.33
INR	1.08 (0.98 – 1.21)
Modified Bauermeister Grading System	
0	3 (2.8%)
1	50 (47.2%)
2	27 (25.5%)
3	24 (22.6%)
4	2 (1.9%)

Data are given as mean ± standard deviation or median (1st quartile – 3rd quartile) for continuous variables according to normality of distribution and as frequency (percentage) for categorical variables. MM: Multiple myelom, CML: Chronic myeloid leucaemia, MPDs: Myelodysplastic syndromes, CLL: Chronic lymphocytic leukemia, MDS: Myelodysplastic syndrome, AA: AA amyloidosis, LDH: Lactate dehydrogenase, CRP: C-reactive protein, WBC: White blood cell, PT: Prothrombin time, aPTT: Activated partial thromboplastin time, INR: International normalized ratio

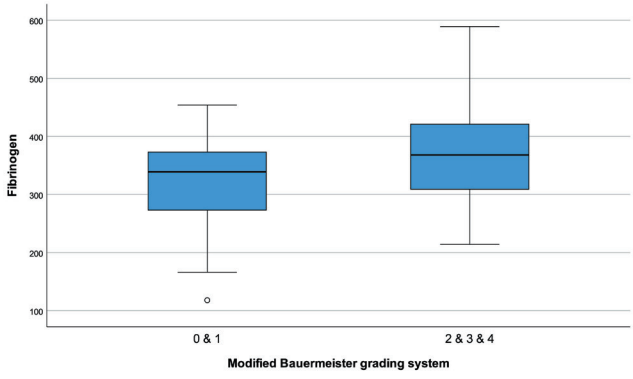

Figure 1. *Fibrinogen levels with regard to bone marrow reticulin*

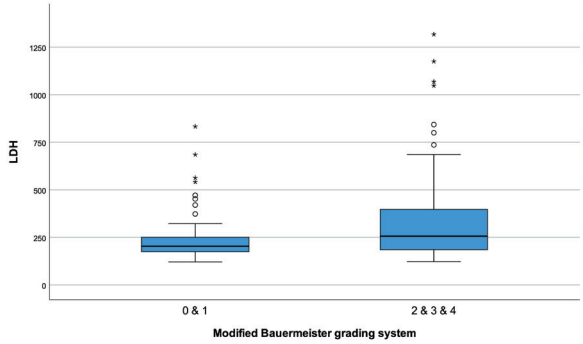

Figure 2. *LDH levels with regard to bone marrow reticulin*

Table II. *Summary of patients' characteristics and laboratory measurements with regard to bone marrow reticulin*

	Modified Bauermeister Grading System		
	0 & 1 (n=53)	2 & 3 & 4 (n=53)	p
Age	61 (46 – 70)	64 (55 – 70)	0.200
Sex			
Male	26 (49.1%)	32 (60.4%)	0.329
Female	27 (50.9%)	21 (39.6%)	
Diagnosis			
Normal	20 (37.7%)	2 (3.8%)	**0.001**
Lymphoma	5 (9.4%)	5 (9.4%)	
MM	17 (32.1%)	16 (30.2%)	
CML	1 (1.9%)	2 (3.8%)	
Chronic MPDs	3 (5.7%)	14 (26.4%)	
CLL	0 (0.0%)	2 (3.8%)	
MDS	5 (9.4%)	10 (18.9%)	
AA	2 (3.8%)	2 (3.8%)	
Fibrinogen	339 (273 – 373)	368 (309 – 421)	**0.004**
D-dimer	1145 (485 – 2685)	1295 (660 – 3010)	0.830
LDH	203 (174 – 251)	256 (185 – 397)	**0.030**
CRP	3.00 (1.37 – 6.70)	3.40 (2.50 – 5.73)	0.197
WBC (x10^3)	5.61 (3.88 – 7.96)	5.41 (3.90 – 9.10)	0.665
Hemoglobin	11.43 ± 2.52	10.56 ± 2.19	0.060
Platelet (x10^3)	178 (70 – 243)	160 (78 – 271)	0.924
PT	13.8 (13 – 14.7)	14.2 (13.1 – 16.1)	0.219
aPTT	30.32 ± 3.94	30.93 ± 4.71	0.472
INR	1.09 (0.98 – 1.19)	1.08 (0.98 – 1.28)	0.497

Data are given as mean ± standard deviation or median (1st quartile – 3rd quartile) for continuous variables according to normality of distribution and as frequency (percentage) for categorical variables. MM: Multiple myeloma, CML: Chronic myeloid leucaemia, MPDs: Myelodysplastic syndromes, CLL: Chronic lymphocytic leukemia, MDS: Myelodysplastic syndrome, AA: AA amyloidosis, LDH: Lactate dehydrogenase, CRP: C-reactive protein, WBC: White blood cell, PT: Prothrombin time, aPTT: Activated partial thromboplastin time, INR: International normalized ratio

In ROC curve analysis, a cut-off value of ≥359 for fibrinogen revealed a sensitivity of 56.6% and a specificity of 73.6% for the identification of patients in the fibrosis group (AUC: 0.662, 95% CI: 0.559 – 0.765). For LDH level, a cut-off value of ≥238 had a sensitivity of 56.6% and a specificity of 71.7% in predicting patients with fibrosis (AUC: 0.622, 95% CI: 0.515 – 0.730) (Table III, Figure 3).

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Table III. *Performance of the variables to predict patients with mild/severe fibrosis (Modified Bauermeister grading system 2-4)*

	Fibrinogen	LDH
Cut-off	≥ 359	≥ 238
Sensitivity	56.6%	56.6%
Specificity	73.6%	71.7%
Accuracy	65.1%	64.2%
PPV	68.2%	66.7%
NPV	62.9%	62.3%
AUC (95.0% CI)	0.662 (0.559 – 0.765)	0.622 (0.515 – 0.730)
p	**0.004**	**0.030**

PPV: Positive predictive value, NPV: Negative predictive value, AUC: Area under ROC curve, CI: Confidence intervals, LDH: Lactate dehydrogenase

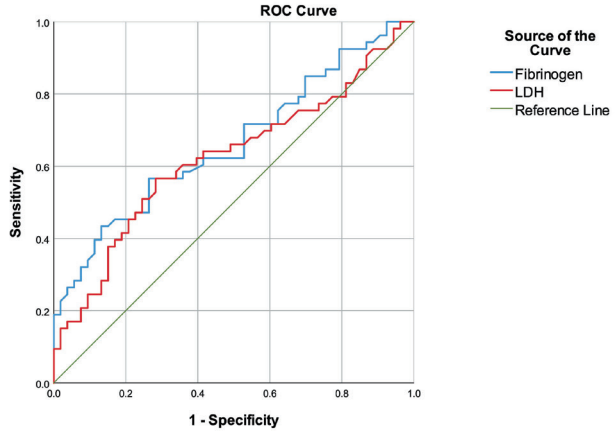

Figure 3. *ROC curve of the variables to predict bone marrow fibrosis*

We performed multiple logistic regression analysis to evaluate the BMF prediction performance of the variables by adjusting for age and sex. Patients with high fibrinogen (≥ 359) had 3.084-fold higher likelihood to have BMF compared to those with low fibrinogen after adjusting for age and sex (OR: 3.084, 95% CI: 1.303 – 7.299; p = 0.010). Patients with high LDH (≥ 238) had a 2.865-fold higher likelihood for BMF compared to other patients after adjusting for age and sex (OR: 2.865, 95% CI: 1.214 – 6.761; p = 0.016) (Table IV).

Table IV. *Significant predictive factors of the bone marrow fibrosis, multiple logistic regression analysis*

	β coefficient	Standard Error	p	Exp(β)	95.0% CI for Exp(β)	
Age	0.015	0.015	0.324	1.015	0.986	1.045
Sex, female	-0.332	0.433	0.443	0.717	0.307	1.676
Fibrinogen (≥ 359)	1.126	0.440	**0.010**	3.084	1.303	7.299
LDH (≥ 238)	1.053	0.438	**0.016**	2.865	1.214	6.761
Constant	-1.617	0.951	0.089	0.198		
Dependent variable: Modified Bauermeister GS 2-4; Nagelkerke R^2=0.213						

CI: Confidence interval, GS: Grading system, LDH: Lactate dehydrogenase

4. DISCUSSION

A large number of benign and malignant diseases may cause an increase in reticulin or collagen fibers in the BM. The reason for this abnormal fiber increase in the BM stroma has not been clarified. The clinical consequences of increased fibers (either reticulin or collagen) may differ [18]. The literature reports that the amount of reticulin increase in the BM rarely correlates with the severity of causative disease; however, increased collagen fiber levels often correlate strongly with abnormal blood counts and the severity of the causative disease [19-22]. Reticulin fibers accumulate more commonly in BMF; however, it is difficult to detect the presence and severity of fibrosis by measuring reticulin level via a noninvasive method. In this study, we aimed to assess whether plasma fibrinogen level could be a diagnostic tool that could predict BMF. Univariate analyses showed that high fibrinogen and LDH levels were significantly associated with the presence of BMF. Multiple logistic regression also supported the association of fibrinogen and LDH elevation with BMF, and demonstrated these variables to be independent risk factors associated with BMF after adjusting for age.

BMF can occur in many hematological and non-hematological, benign or malignant diseases. However, it can be seen at different degrees even in the absence of diseases or conditions directly associated with BMF. While collagen fibrosis is exceedingly rare in the BM of healthy individuals, reticulin staining of various grades can be observed in around 70% of BM samples [17]. The most common malignant diseases in which generalized BMF is seen are chronic idiopathic myelofibrosis (MF), CML, AML, ALL, MDS, lymphomas and metastatic tumors [23]. Non-malignant causes of BMF include endocrine disorders, autoimmune diseases, vitamin D deficiency and infections (HIV, tuberculosis) [1, 2, 23]. The identification of BMF is vital in some diseases. For example, in CML, BMF is an important factor associated with post-transplant therapeutic efficacy and prognosis [20]. Additionally, BMF grade is an indicator of treatment failure in CML [24], and BMF development during the course of MDS has been associated with worse outcomes [5]. In a study of 301 patients with MDS, patients with grade 2 and 3 BMF were shown to have shorter overall and leukemia-free survival than those with grade 0 or 1 BMF [5]. One study evaluating CLL patients showed that those with high-grade BMF (grades 2 and 3) had worse 5-year overall survival rate than those with grade 0-1 BMF [25]. However, identifying the presence of BMF necessitates BM aspiration and pathological examination with special dyes. Although BMB is known to be a safe procedure, pain at the biopsy site, discomfort, bleeding, hematoma, gluteal artery pseudoaneurysm, and infection are the most common complications [26, 27].

Various studies have explored possible parameters that can replace the need for biopsy for the detection of BMF. It has been shown that latent and active transforming growth factor-β (TGF-β) is increased in BM plasma as well as in the serum of patients with hairy cell leukemia who have BMF, and the concentration of active TGF-β is associated with the degree of reticulin fibrosis. That is, serum TGF-β may allow for noninvasive assessment of BMF in general [28]. Also,

measurement of serum procollagen III peptide (PIIINP) is another recommended biochemical marker for the diagnosis of reticulin fibrosis. One study showed that 35 participants with CMPD had higher PIIINP levels than 35 healthy volunteers, and that PIIINP levels correlated with the degree of reticulin fibrosis; however, PIIINP levels were not associated with collagen fibrosis [29]. Interleukin (IL)-8, IL-2R and lipocalin-2 are other cytokines that have been suggested to be involved in the pathophysiology of BMF, especially among patients with MF [30]. Genetic studies assessing JAK2 V617, MPL and CALR gene mutations have suggested possible associations with BMF [1]. However, their routine diagnostic value has not been consistent [30]. In the present study, we examined the relationship between plasma fibrinogen level and BMF. Our results were promising, as demonstrated by elevated fibrinogen and LDH levels in patients with BMF. In multiple regression, we found that high fibrinogen and LDH were independent risk factors for BMF after adjusting for age and sex. However, ROC analyses revealed relatively low sensitivity, specificity, PPV and NPV values in predicting BMF.

Reticulin and/or collagen production are associated with adventitial reticular cells, perisinusoidal adventitial cells, periarterial adventitial cells, adipocytes, and endosteal cells, which are fibroblastic BM cells in the stroma [31]. Fibroblasts are in close association with collagen fibers and respond to many fibrogenic factors such as interleukin (IL)-6, IL-12, IL-8, TNFα, IFNy, TGFβ, bFGF, VEGF and TGFβ1 [32]. Most of these profibrotic, angiogenic, and proinflammatory factors are stored and released by the α-granules of megakaryocytes [33]. Abnormal deposition of reticulin and collagen in the BM stroma is associated with abnormalities in the number and/or function of megakaryocytes and platelets, and thus the cytokines released from these cells [18]. The reason for the close relationship between fibrosis and neoplastic proliferation of abnormal megakaryocytes has not been explained. Few studies have examined the mechanism by which abnormal megakaryocytes exert their effects on collagen-producing cells [34, 35]. Also, it was shown that fibrinogen may participate in the development of fibrosis by triggering the expression of TGF-β1 and by activating cellular signaling pathways [6]. This complex relationships between fibrinogen, megakaryocytes, platelets, and fibroblasts make it difficult to clarify the role of each cell. While the contribution of fibrinogen to other organ fibrosis is established, its role in BMF is unclear. In a genetic study, it was determined that many extracellular matrix components, including increased expression of fibrinogen, are altered during the development of MF [36]. In a case report, the fibrinogen level of a patient with TAFRO syndrome (a rare systemic inflammatory disease characterized by thrombocytopenia, pleural effusion, fever, renal dysfunction, reticulin fibrosis of the BM, and organomegaly) was reported to be high (fibrinogen: 434 mg/dL) together with somewhat elevated D-dimer level (22.5 μg/mL) [37]. However, prior studies do not demonstrate the relationship between BMF and fibrinogen.

Fibrinogen is a 340 kDa glycoprotein that is mainly synthesized by hepatocytes and has many biological functions [38, 39]. During tissue maintenance, fibrinogen and fibrin matrix elements are associated with re-epithelialization, vascularization and collagen deposition, possibly explaining relationships with BMF [40]. Fibrinogen is also a main acute phase protein and its concentration in plasma is often used as a marker for systemic inflammation [9]. It has been observed that different components of the coagulation system, including fibrinogen, play an important role in the development of tissue fibrosis. In a mice study, in vitro experiments showed that fibrinogen was a potent mitogen that promoted renal fibroblast expansion [7]. In this study, it was showed that fibrinogen deficiency confers significant protection from interstitial damage, attenuated collagen deposition, and limited interstitial cell proliferation, a hallmark of fibrosis. It was concluded that fibrinogen increases renal fibrosis by triggering resident fibroblast proliferation [7]. Another study demonstrated that nephropathy patients with high serum fibrinogen had higher renal tubular atrophy and interstitial fibrosis [6]. Additionally, studies have shown a possible role for fibrinogen in the early phase of acute inflammation associated with pulmonary fibrosis [41, 42]. A study investigating the role of fibrinogen in dystrophic muscle fibrosis demonstrated that fibrinogen stimulated collagen production in fibroblasts, while TGFβ produced due to fibrinogen potentiated collagen accumulation [11]. Such results provide evidence for the profibrotic effects of fibrinogen deposition which have been demonstrated in other disease states [10]. Therefore, fibrinogen, whose role is evident in the fibrosis of various organs, is likely to play a role in BMF as well. By investigating the mechanisms involving fibrinogen in the fibrosis of these organs, its role can be clarified and new parameters can be obtained to facilitate easier diagnosis of BMF.

There are some limitations in our study. The fact that the study was retrospective and single-center made it difficult to add new data and limited the assessment of results from other perspectives. Although potential mechanisms by which fibrinogen could be associated with BMF were examined in the light of the literature, this study could not evaluate the mechanisms of this effect, but aimed to elucidate a possible relationship between circulatory fibrinogen level and BMF. Since the number of patients with Grade 0 and Grade 4 BMF were very low, we had to categorize patients into two groups with respect to the results of MBGS (fibrosis versus non-fibrosis). However, it is evident that this dichotomization may have led to inaccuracy. We believe studies that can assess fibrinogen and BMF levels in a higher number of patients are warranted.

Conclusion

In conclusion, high plasma fibrinogen and LDH levels were found to be associated with the presence of BMF. It was shown that the elevation of these each of these two parameters were independent risk factors for the presence of BMF (adjusted for age and sex). Clarifying the role of fibrinogen in the development of BMF and demonstration of higher levels of accuracy with respect to BMF grades may yield a new and non-invasive approach to the identification of BMF development. Possible advantages of such an approach include early diagnosis and better management.

Compliance with Ethical Standards

Ethical approval: The study was started after ethics committee approval was obtained from Clinical Research Ethics Committee of Kartal Dr. Lutfi Kirdar City Hospital (Date: 10.11.2021, No: 2021/514/213/2).

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

Author Contributions: YI and ANK: Concept and design of the study, YI: Data acquisition, ANK: Statistical analysis, YI and ANK: Literature YI and ANK: Drafting and Writing. Both authors critically revised the manuscript, approved the final version to be published, and agreed to be accountable for all aspects of the work.

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Evaluation of autonomic nervous system functions by using tilt table test and heart rate variability in epileptic children

Azad REDJEPOV[1], Sinem ALTUNYUVA USTA[4], Yuksel YILMAZ[3], Figen AKALIN[2]

[1] Department of Pediatrics, School of Medicine, Marmara University, Istanbul, Turkey
[2] Divison of Pediatric Cardiology, Department of Child Health and Pediatrics, School of Medicine, Marmara University, Istanbul, Turkey
[3] Department of Pediatric Neurology, Academic Hospital, Istanbul, Turkey
[4] Department of Cardiology, University of Health Sciences Kartal Kosuyolu Research and Training Hospital, Istanbul, Turkey

Corresponding Author: Sinem ALTUNYUVA USTA
E-mail: sa.usta2007@gmail.com

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ABSTRACT

Objective: The value of head-up tilt test (HUTT) for differential diagnosis of epilepsy and the autonomic nervous system functions in epileptic children using heart rate variability (HRV) are studied.

Patients and Methods: The study group consisted of 16 children with idiopatic/criptogenic epilepsy and 12 controls. Heart rate, PR interval, corrected QT (QTc) interval, QT and QTc dispersion were calculated using 12-lead electrocardiogram (ECG), HRV analysis was performed using the Holter recordings obtained both during HUTT and throughout the day. Time domain parameters, standard deviation of all RR intervals (SDNN), the standard deviation of mean NN intervals in five-minutes recording (SDANN), mean standard deviation of NN intervals in five-minutes recordings (SDNNi), root mean square of successive differences (RMSSD), count divided by the total number of all NN intervals (pNN50) and frequency domain parameters low frequency (LF), high frequency (HF), low-frequency/high-frequency ratio (LF/HF) were calculated in both and compared between the two groups.

Results: Head-up tilt test was positive in 4 epileptic children (25%), none of controls were positive. The heart rate of the patients were higher than the controls (p=0.015). LF/HF ratio in 24-hour Holter recordings, were significantly lower (1.13±0.6, 1.83±0.7 respectively, p=0.002); the SDANN during HUTT (28.7±20.2, 18.2 ± 19.9 respectively, p=0.024) were significantly higher in the patients than the controls.

Conclusion: Head-up tilt test positivity is frequent in epileptic children, and cannot be used in differential diagnosis. HRV calculated both from 24 hour Holter recordings and Holter recordings under orthostatic stress were impaired in favour of parasympathetic system in epileptic children.

Keywords: Epilepsy, Syncope, Head-up tilt test, Heart rate variability, Autonomous nervous system, Arrhythmia.

1. INTRODUCTION

Epilepsy is a common paroxysmal childhood disease that is characterized by abnormal cortical electrical activity [1-3]. Epilepsy has a complex relationship with cardiac and autonomic functions. Various rhythm abnormalities both tachyarrhythmia, and bradyarrhythmia can be seen in epileptic patients during seizures [4-7]. Some studies also show that epileptic patients has an increased risk of sudden cardiac death, mostly related to arrhythmia [8,9]. It may be difficult to distinguish seizures of patients with atonic, hypotonic epileptic seizures from neurocardiogenic syncope attacks. Head-up tilt test (HUTT) used in diagnosis of neurocardiogenic syncope may give false positive results in the presence of autonomic dysfunction [10].

Heart rate variability is a beneficial method for evaluating autonomic functions. Time-based and frequency-based parameters can be used to investigate sympathetic or parasympathetic system dominance. Reduction of heart rate variability suggests sympathetic system dominance and an increased risk of arrhythmia and sudden death.

In our study, the utility of HUTT has been investigated in patients with idiopathic and cryptogenic epilepsy and the autonomic function changes both under orthostatic stress and throughout the day have been evaluated using heart rate variability parameters obtained via Holter monitorization.

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2. PATIENTS and METHODS

Patient Population

The study group included the patients who were diagnosed to have idiopathic or cryptogenic epilepsy at the Marmara University Pediatric Neurology Department. Patients with systemic or local diseases effecting cardiovascular and central nervous system, psychiatric diseases, genetic syndromes, symptomatic epilepsy, chronic or progressive neurological diseases, and those on cardiotoxic or neurotoxic drug treatment other than antiepileptic treatment were excluded from the study.

Control group consisted of healthy children with similar age and gender characteristics, without any cardiac or neurologic abnormality. Patients between 7 to 18 years who can cooperate for HUTT and Holter monitorization were included in the study. All the patients and the control group underwent echocardiographic examination for exclusion of structural heart disease.

In addition to a detailed history of seizures, general medical and family history, a detailed neurologic, cardiac, and systemic physical examination was performed. The antiepileptic medications taken and electroencephalography (EEG) data were recorded.

All the children were evaluated at Marmara University Paediatric Cardiology Department by using electrocardiography, transthoracic echocardiography, 24-hour Holter monitorisation and HUTT.

Standard 12-lead surface electrocardiogram was obtained from all subjects. Heart rate, PR interval, P wave duration, P wave height, P axis, QRS duration, QRS axis, QT interval, maximum and minimum QT intervals were measured and the corrected QT interval (QTc), QT dispersion, and QTc dispersion were calculated using Bazett's formula [11].

Head-up tilt test protocol: All patients were studied during the afternoon in a quiet room with low lightning after 6 hours of fasting. An intravenous line is inserted as a precaution for possible emergency interventions. Children were monitorised by using a standard three lead cardiac monitor and a sphygmomanometer. During the tilt period, vital signs and ECG were continuously monitored and reported at 3-minute interval. After 15 minutes in the supine position, the table was tilted to 60°. Whenever a syncope or presyncope developed and if associated with a blood pressure or heart rate drop of at least 30% of baseline levels, children were returned to supine position immediately. The test was terminated, and the HUTT was considered positive [12]. If hypotension, bradycardia, syncope, or presyncope did not occur, the patient was returned to the supine position after 45 minutes, and HUTT was considered negative.

Participants in both groups underwent a 30-minute 60° HUTT. Holter recordings were obtained during the HUTT. Additionally, 24-hour Holter recordings were obtained. Del Mar Reynolds Holter System (Spacelabs Medical Inc, West Sussex, UK) was used for Holter recordings. The groups were compared in terms of the time-based (SDNN, SDANN, SDNNi, RMSSD, pNN50) and frequency-based (LF, HF, LF / HF) HRV parameters calculated both from HUTT and Holter recordings and 24-hour Holter recordings.

All the patients and the parents were informed about the study, consent forms were signed and Local Ethics Committee has approved the study. (Ethics Committee of Marmara University Medical School, date: 07.04.2011, number: B.30.2.MAR.0.01.02/AEK/58)

Statistical Evaluation

Statistical Package for Social Sciences (SPSS) 17.0 software package was used for statistical analyses to evaluate the study findings. Kolmogorov-Smirnov distribution test was used to investigate normal distribution. Qualitative data between the groups were compared using Pearson's chi-squared and Fisher's exact tests. McNemar's test was used for comparing qualitative data between the groups. parameters with quantitative data were compared with Mann Whitney U test. Quantitative parameters within the group were compared using Wilcoxon signed rank test. The results were evaluated within a 95% confidence interval at $p<0.05$ significance level, and at $p<0.01$ and $p<0.001$ high significance levels.

3. RESULTS

The study group consisted of 16 epileptic children (8 male and 8 female), between 7 and 16 years of age (10.5±2.1). The control group consisted of 12 healthy children (7 male and 5 female) between 7 and 15 years of age (9.6± 3). The diagnosis was rolandic epilepsy in 7, occipital lobe epilepsy in 3 and absence epilepsy in 6 children. Twelve patients were using antiepileptic medication (11 of them Valproic acid, 1 Carbamazepine). EEG records of all of the patients revealed epileptic activity.

Height and weight of all children in the study and the control groups were within the normal range for their age. No statistically significant difference was observed between the groups in terms of height or weight (Table I).

Table I. *Demographic comparison*

DEMOGRAPHIC DATA				
		STUDY GROUP	CONTROL GROUP	p-value
Age (years) (Mean±SD)		10.56±2.19	9.67±3.09	0.437
Gender (n (%))	♀	8 (5%)	5 (42%)	0.445
	♂	8 (50%)	7 (58%)	
Height (cm) (Mean±SD)		143.18±12.40	138.83±15.58	0.390
Weight (kg) (Mean±SD)		38.75±10.67	33.50±12.21	0.113
On drug therapy (n (%))		12 (75%)	0	<0.001
Not on drug therapy (n (%))		4 (25%)	12 (100%)	

SD: Standard deviation

The average heart rate, measured during physical examination, was 83.56±11.25 beats/min in the study group and 79.00±7.25 beats/min in the control group. No statistically significant difference was observed between the groups.

The average systolic blood pressure of the cases in the study group (110.44±9.36) was significantly higher than the control group (102.17±11.79) (p<0.05). There was no significant difference between the study (63.63±5.69) and the control (63.17±9.50) groups in terms of their diastolic blood pressure.

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Twelve-lead electrocardiogram

Heart rate, measured in the surface ECG, was significantly higher in the study group (93.7±11.3 beats/minute) compared to the control group (80±14.5 beats/minute) (***p*<0.05**). There was no statistically significant difference in terms of PR interval, P wave duration, P wave height, P wave axis, QRS axis, and QRS duration between the groups. The QT interval in the study group (325±26.3 msec) was significantly lower than the QT interval the control group (360±38.2 msec) (p<0.05). The longest QT interval was 360.7±25 msec in the study group and 384.4±36.6 msec in the control group; there was no significant difference between the groups. The shortest QT interval was 291.5±30.7 msec in the study group and 337.2±41 msec in the control group, and this difference was statistically significant (p<0.01). While the QT dispersion and QTc values were higher in the study group than in the control group, no statistically significant difference was observed in terms of these parameters. The longest QTc was 449.5±23.5 msec in the study group and 428±30.4 msec in the control group and the difference between the groups was statistically significant (p<0.05) (Table II).

Table II. *Comparison of the ECG results*

	Study Group (n=16)		Control Group (n=12)		P value
	Mean	SD	Mean	SD	
Heart rate	93.750	11.375	80.000	14.510	**0.015**
PR distance	0.121	0.014	0.130	0.013	0.089
P wave duration	0.074	0.016	0.080	0.009	0.156
P wave height	1.388	0.492	1.267	0.401	0.669
P wave axis	40.625	21.747	31.250	17.726	0.315
QRS duration	0.058	0.010	0.065	0.015	0.153
QRS axis	47.500	22,.876	47.083	24.445	0.963
QT distance	325.000	26.331	360.417	38.209	**0.013**
Maximum QT distance	360.750	25.093	384.417	36.600	0.088
Minimum QT distance	291.500	30.783	337.250	41.083	**0.003**
QT dispersion	58.063	16.060	47.167	11.839	0.085
QTc	404.375	16.354	396.750	28.010	0.724
Maximum QTc	449,500	23.509	428.000	30.466	**0.049**
Minimum QTc	356.250	31.323	367.750	28.062	0.245
QTc dispersion	73.375	22.387	60.250	16.961	0.125

SD: standard deviation

Head-up Tilt Test

Four patients in the study group had positive HUTT results. All of the patients with positive HUTT results had a mixed type positive response. None of the control group participants had a positive Tilt response. No significant difference was observed between the groups in terms of Tilt test positivity (p=0.089). HUTT positivity occurred in one patient at 15th minute, in two patients at 18th minute, and in another patient at 21st minute (Table III).

Table III. *Comparison of head-up tilt test results*

HEAD-UP TILT TEST RESULTS				
		STUDY GROUP (Mean±SD)	CONTROL GROUP (Mean±SD)	p-value
Tilt positive patients (N (%))		4 / 25%	0 / 0%	0.089
Tilt negative patients (N (%))		12/ 75%	20/ 100%	
Average Heart Rate (beats/min)		98.25±9.97	96.75±11.88	0.693
Minimum Heart Rate (beats/min)		70.81±11.2	71.92±10.77	0.727
Maximum Heart Rate (beats/min)		123.56±17.24	120.33±12.21	0.963
SDNN		61.13±28.47	57.92±22.92	0.940
SDANN		28.69±20.23	18.25±19.91	0.024
SDNNi		49.00±15.82	53.33±16.04	0.429
rMSSD		26.25±11.13	29.08±12.8	0.625
PNN50		7.25±8.15	9.67±11.36	0.575
LF		669.63±397.45	976.62±568.6	0.144
HF		271.44±242.07	359.83±229.97	0.164
LF/HF		2.75±2.11	3.13±1.5	0.400
ARRHYTHMIA (N/%)	Present	1/ 6%	0/ 0%	0.378
	Absent	15/ 94%	12/ 100%	

SD: Standard deviation, SDNN: standard deviation of all RR intervals, SDNNi: mean standard deviation of NN intervals in 5-minutes recordings, SDANN: the standard deviation of mean NN intervals in 5-minutes recording, RMSSD: root mean square of successive differences, pNN50: NN50 count divided by the total number of all NN intervals, LF: low frequency, HF: high frequency, LF/HF,: low-frequency/high-frequency ratio

Twenty-four-hour Holter Monitorization

There was no significant difference between the study and the control groups in terms of lowest heart rate (study group: 52.94±4.8; control group: 54.67±12.4 beats/minute), highest heart rate (study group: 164.06±16.9; control group: 155.2±16.6 beats/minute), and average heart rate (study group: 88.1±8.3; control group: 88.08±10.4 beats/minute), observed in the 24-hour Holter monitorization. Supraventricular and ventricular premature beats (varying between 1 or 2 observances per case) were observed in 6 children in the study group and 2 children in the control group. Atrioventricular block, sinus pause, RR interval longer than 2.5 seconds, supraventricular tachycardia (SVT), or ventricular tachycardia (VT) attack was not observed in either groups. No significant difference was present according to the arrhythmia rates between the groups (p>0.05).

Heart Rate Variability

Time based heart rate variability parameters SDNN, SDANN, SDNNi, RMSSD, and pNN50 obtained from the 24-hour Holter recordings did not differ between the two groups. However, LF/HF ratio was significantly lower in the study group (study group: 1.13±0.62; control group: 1.83±0.70, p<0.01) while LF and HF values were comparable, indicating parasympathetic system dominance in epileptic children.

In comparison of heart rate variability during HUTT; SDANN values were higher in the study group (28.6±20.2 msec) than in the control group (18.2±19.9 msec) (p=0.024), while other time domain parameters SDNN, SDNNi, RMSSD, and pNN50 values did not differ significantly, indicating the parasympathetic system dominance was also present during HUTT.

The study and the control groups did not have statistically significant differences in terms of their LF, HF, LF/HF values obtained from Holter recordings obtained during the HUTT (Table IV).

Table IV. *Comparison of the 24-hour Holter monitorization results*

24-HOUR HOLTER MONITORIZATION RESULTS				
		STUDY GROUP (Mean±SD)	CONTROL GROUP :(Mean±SD)	p-value
Minimum Heart Rate (beats/min)		52.94±4.88	54.67±12.42	0.675
Maximum Heart Rate (beats/min)		164.06±16.93	155.25±16.60	0.15
Average Heart Rate (beats/min)		88.19±8.37	88.08±10.40	0.963
SDNN		124.69±22.62	131.33±48.55	0.693
SDANN		106.81±22.68	112.42±53.90	0.693
SDNNi		59.69±16.32	67.17±22.29	0.285
RMSSD		41.25±15.02	44.83±19.53	0.816
PNN50		17.88±10.35	18.67±11.74	0.944
LF		726.31±304.69	1009.67±509.03	0.210
HF		498.56±257.53	645.25±420.79	0.577
LF/HF		1.13±0.62	1.83±0.70	0.002
ARRHYTHMIA	Present	(6 / 40%)	(2 / 17%)	0.187
	Absent	(10 / 60%)	(10 / 83%)	

SD: Standard deviation, SDNN: standard deviation of all RR intervals, SDNNi: mean standard deviation of NN intervals in 5-minutes recording, SDANN: the standard deviation of mean NN intervals in 5-minutes recordings, RMSSD: root mean square of successive differences, pNN50, NN50: count divided by the total number of all NN intervals, LF: low frequency, HF: high frequency, LF/HF: low-frequency/high-frequency ratio

4. DISCUSSION

Epilepsy is a paroxysmal chronic disease group that occurs frequently during childhood. It has an incidence between 0.4 to 1.9%. Etiology can be identified in only 1/4 or 1/3 of the cases. About 2/3 of the cases are idiopathic or cryptogenic and believed to occur due to genetic causes [2, 13, 14]. The remaining causes are congenital abnormalities, trauma, infections, vascular, neoplastic, and degenerative diseases [2, 15].

It may be difficult to differentiate atonic or hypotonic epileptic seizures from syncopal attacks. HUTT is used for diagnosis of neurocardiogenic syncope, however in our study 25% of the epileptic patients were tilt positive while none of the healthy control group had positive tilt table test result. Although, this difference was not statistically significant, it is much higher than the expected tilt table test positivity for the normal population (7-10%) [16,17]. HUTT positivity among the patients with neurocardiogenic syncope range between 27-75% [18]. In the study conducted by Topcu et al., within our department, 54% of the patients with neurocardiogenic syncope had positive HUTT [10]. Therefore, HUTT may provide direct observation of the syncopal attacks and the vital changes during syncope but is not suitable for differentiation of epileptic seizures from vasovagal syncope. Sabri et al. [19], also found 30% HUTT positivity rate within a proven epileptic group in accordance to our study. High HUTT positivity rate in epileptic patients compared to the normal population can be an indicator of the impaired autonomic functions among these patients.

Our study showed increased resting heart rate and systolic blood pressure in epileptic children comparing the healthy control group, which may be due to the higher emotional pressure experienced in patients with a chronic illness, during routine tests. Absence of any difference in terms of the heart rates obtained in the Holter recordings between the groups also validates this.

Maximum QTc value was higher among epileptic children compared to healthy children and QTc dispersion was higher in the epileptic patients compared to the control group in this study, however the difference was not statistically significant. However, a prior study conducted in our clinic by Akalin et al., found that QT dispersion in epileptic patients is significantly higher than that in healthy children [20]. The lack of a significant QTc dispersion difference in this study may be due to the small sample size and that most of the patients were receiving antiepileptic drug-therapy. These findings may be significant in terms of arrhythmia risk. Both prolonged QT interval and increased QTc dispertion may suggest increased risk of rhythm abnormalities and sudden cardiac death. Arrhythmia can be observed in epileptic children during ictal and interictal periods. Many studies have been conducted to investigate the causes, frequency, and the dysrhythmia-based risks of arrhythmia observed in epileptic children [8,9] . The reason for this effort is that the unexplained sudden death in epilepsy (SUDEP) risk, as demonstrated by autopsy and clinical studies, is 0.7-1.3/1000, which is 24 times higher than the normal population, especially in case of idiopathic and cryptogenic epilepsy [21]. Electrical stimulation of different locations within the brain was found to induce dysrhythmia such as tachyarrhythmia, bradyarrhythmia (time to time), asystole (rarely), AV block, and ventricular fibrillation [9,22,23]. Seizures affecting the temporal lobe, insular cortex, and amygdala tend to cause dysrhythmia more frequently [24]. The rate of dysrhythmia during seizures vary between 10-60% and about 5-20% of them are serious dysrhythmias [25]. It is known that the heart rate and heart rate variability increase during the ictal period [9,25]. However, the ECG data of epileptic patients obtained during the interictal period are not different than that of the normal population [16,26,27]. Common genetic basis affecting electrical activity both in the central nervous system and the conduction system of the heart is still remains to be clarified.

Heart rate variability is a measure of cardiac autonomic function, indicating the cyclic variations of heartbeat intervals [28]. It reflects the complex relationship between the parasympathetic and sympathetic innervation of the heart. Decreased HRV is known to be associated with susceptibility to cardiac arrhythmias [29,30]. This study investigated HRV based on the Holter recordings obtained both during the 24-hour recordings and during the HUTT. Among the time domain measurements, SDNN, SDANN, and SDNNi indicate the sympathetic and parasympathetic effects; and the vagal control is indicated by RMSSD and pNN50. Numerically lower values in all these indexes indicate reduced HRV [31-37]. Decreased HRV means a heart that has relatively faster pulse and lost its diurnal rhythm. This suggests lost parasympathetic tone and sympathetic tone dominance [28]. While SDNN is informative of the whole autonomic structure of the heart, it fails to provide significant information regarding the sympathetic or the parasympathetic

activities separately [29]. On the other hand, RMSSD and PNN50 generally accompany vagal activity. The frequency domain measurement LF is associated mainly with the sympathetic activity and HF with vagal activity [30]. LF/HF ratio on the other hand, reflects the balance between the sympathetic and the parasympathetic autonomic nervous systems. Increases in the ratio suggest sympathetic activity dominance and decreases suggest parasympathetic activity dominance [31,32].

In this study, the 24-hour recordings yielded higher LF/HF ratios and increased SDANN values during HUTT in epileptic children compared to healthy children; this suggests that both under orthostatic stress and throughout the day, the heart rate variability of these patients is increased compared to the control group and that their autonomic functions are impaired in favor of parasympathetic system. On the other hand, our prior study demonstrated increased sympathetic system activity in children with neurocardiogenic syncope during orthostatic stress [10]. Thus, while both epilepsy and neurocardiogenic syncope may cause HUTT positivity and autonomic dysfunction is present in both groups, the underlying mechanism and autonomic factors are different.

El-Sayed et al. has evaluated autonomic functions of children with idiopathic epilepsy during the interictal period. In all age groups, SDNN was decreased, and in the older children, pNN50 and RMSSD were decreased compared to the control group [33]. Similarly, the SDNN values were found to be decreased in patients with temporal lobe epilepsy in other studies [33-35]. Ferri et al., reported a decline in both the time-based and frequency based HRV parameters of epileptic children throughout their sleep [35]. Massetani et al., reported significantly reduced R-R variability among adult epilepsy patients [36]. Increased heart rate variability in this study is not consistent with these studies. However, the consistency between the data collected during the HUTT and the 24-hour Holter monitorization suggest that these findings are reliable. The difference between the findings of this study and the literature may be due to the differences in the epilepsy type and localization or the drug-therapies received. In addition, similar to our findings, Yang TF et al,, have also reported increased heart rate variability in epileptic patients compared to healthy children, though not significant [38]. Raju et al.'s study reported reduced pNN50 values in children with refractory epilepsy, and in well-controlled patients HRV parameters were similar to those in healthy children [39]. The study group in this study had a very specific type of epilepsy, all were well-controlled, did not have a seizure within the past 6 months, and were receiving antiepileptic drug therapy. Additionally, physiologically the sympathetic system is dominant within the first 10 years of life; parasympathetic system dominance is observed starting from adolescence and throughout adulthood [40, 41]. The children in both the study and the control group being at this transition period may also be the reason for obtaining inconsistent results.

This is the only study where heart rate variability is examined over a 24-hour period and under stress via 24-hour Holter monitorization and the HUTT is applied.

The head-up tilt test had higher positivity among epileptic patients compared to healthy children in this study and their autonomic nervous system functions were impaired in favor of parasympathetic system both under orthostatic stress and during rest. These findings suggest that the HUTT is not suitable for use in syncope-epilepsy differentiation. More comprehensive studies are needed to investigate the effects of different types of epilepsy and antiepileptic therapies on the autonomic nervous system.

Compliance with Ethical Standards

Ethical Approval: This study was approved by the Marmara University, School of Medicine Clinical Research Ethics Committee (approval date: 07.04.2011, number: B.30.2.MAR.0.01.02/AEK/58). All the patients and the parents were informed about the study, consent forms were signed.

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Reliability of antibody tests for COVID-19 diagnosis

Nilay COPLU[1], Cetin KILINC[2], Aysegul GOZALAN[3], Busra CALISIR[2], Cemile SONMEZ[4], Mustafa Muhammet GUL[5], Zeynep AYGUN AHLATCIOGLU[6]

[1] Medical Microbiology, Ankara City Hospital, Ankara, Turkey
[2] Medical Microbiology, Kastamonu Training and Research Hospital, Kastamonu, Turkey
[3] Medical Microbiology, Alanya Alaaddin Keykubat University, Antalya, Turkey
[4] Microbiology Reference Laboratories and Biological Products Department, Sexually Transmitted Diseases Reference Laboratory, Public Health General Directorate, Ministry of Health, Ankara, Turkey
[5] Infectious Disease Clinics, Kastamonu Training and Research Horpital, Kastamonu, Turkey
[6] Pathology Laboratory, Kastamonu Training and Research Horpital, Kastamonu, Turkey

Corresponding Author: Nilay COPLU
E-mail: nilaycoplu@gmail.com

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ABSTRACT

Objective: The reverse transcription–polymerase chain reaction test (RT-PCR) is the gold standard for the diagnosis of coronavirus disease 2019 (COVID-19), and antibody tests are useful as supplemental tools for diagnosis, for measuring the population's immunity levels, and for checking infection in asymptomatic contacts. This study aimed to evaluate the reliability of five commercial antibody detection test kits.

Materials and Methods: The reliability of the Colloidal Gold COVID-19 IgG/IgM Rapid Test Kit, Antibody Rapid Test Hotgen, Beijing Hotgen Biotech Co., Ltd., China), Abbott Chemiluminescent Microparticle Immunoassay (Illinois, USA), Roche Electrochemiluminescence Immunoassay (Roche Diagnostics, Switzerland), Siemens Chemiluminescence (Munich, Germany), and Euroimmun ELISA (Lübeck, Germany) for COVID-19 diagnosis was studied. The antibody-negative group included 50 sera from 2018, and the antibody-positive group included 98 patients with positive RT-PCR results from whom blood samples had been collected 3–9 weeks after hospital discharge. Statistical analysis was performed using SPSS version 23.0 (IBM Corporation, Armonk, NY, USA). The antibody tests' validity and intra-assay reproducibility were examined, and the Cohen's kappa coefficients were obtained. The disease prevalence was pegged at 10%.

Results: The antibody tests' sensitivity (69.12–72.46%) and positive predictive values (42.44–100.0%) were low, and their specificity (89.58–100%) and negative predictive values (96.31–97.03%) were high. Their accuracy rates varied from 87.54% to 97.25%, and their intra-assay coefficients of variation varied from 1% to 10%.

Conclusion: The agreement between the results of the antibody detection test kits was higher when the kits were classified according to the targeted antigens. The time of blood sample collection, targeted antigens, and antibody types affected the results. Serological tests were found to be useful, and the commercial kits were found to be largely reliable, although, some parameters need to be improved.

Keywords: COVID-19, Antibody, Validity, Chemiluminescent, Electrochemiluminescence, ELISA

1. INTRODUCTION

Coronavirus disease 2019 (COVID-19) appeared in December 2019 for the first time. The World Health Organization declared a pandemic in March 2020, and the first case in Turkey was diagnosed on March 19, 2020. The new virus is different from the other coronaviruses that have caused diseases, and its infection course and identification methods also differ from those of the other coronaviruses [1, 2].

The reverse transcription–polymerase chain reaction (RT-PCR) test is the gold standard for laboratory diagnosis, but it requires the proper equipment and skilled staff, and biosafety risks play an important role while performing it [3]. On the other hand, antibody tests are easier to perform and use blood or sera, which are less risky in terms of biosafety, but antibody synthesis takes time, and the tests' reliability still needs to be proven [2-4]. However, antibodytests are not only supplemental tools for disease diagnosis but are also necessary for measuring the immunity levels in surveillance and vaccine efficacy studies on the population, and for checking if the asymptomatic contacts

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have been infected [1, 5, 6]. Antibody tests can target different antigens, but the anti-nucleocapsid (anti-NCP) and anti-spike (anti-S) antibodies are the most studied antibodies because they are highly immunogenic and are thus widely used in serologic assays [7]. Antibodies' synthesis timing, concentration decline, and infection protection efficacy are variable [5, 6]. In addition, whether the antibodies are immunoglobin M (IgM), immunoglobin G (IgG), or immunoglobin A (IgA) may affect the test results due to their rise and disappearance at different times throughout the course of the disease [6]. The serological tests include enzyme-linked immunosorbent assay (ELISA), chemiluminescent microparticle immunoassay (CMIA), and lateral-flow immunoassays [3].

The aim of the present study was to evaluate the reliability of five commercial antibody detection test kits used in Turkey.

2. MATERIALS and METHODS

Serological tests

The Colloidal Gold COVID-19 IgG/IgM Rapid Test Kit (Beijing Hotgen Biotech Co. Ltd.), a rapid immunochromatographic test, was used according to the manufacturer's instructions. The results were evaluated qualitatively, but for the figures in this paper, the positive results are presented as 10, and the negative results are presented as 0, for comparability. Abbott Chemiluminescent Microparticle Immunoassay (CMIA), (Illinois, USA), Roche Electrochemiluminescence Immunoassay (ECLIA) (Roche Diagnostics, Switzerland), Siemens Chemiluminescence (Munich, Germany), and Euroimmun ELISA (Lübeck, Germany) had been studied by the staff of their respective manufacturing companies, and the semi-quantitative results of the studies were sent to our laboratory. Siemens used > 10.0 and < 0.05 for the unmeasurable values, and these values were fixed at 12.5 and 0, respectively, to be apparent in the figures herein. Similarly, Euroimmun's results were fixed at 12.5 for unmeasurably high antibody levels.

The distribution of the antigens targeted, and immunoglobulins detected by the tests are presented in Table I. As Euroimmun recommended combining the results of anti-S antibodies (IgG and IgA) and reporting the result as positive if the result of either test was found to be positive, the results were combined. For the anti-NCP antibodies, the IgG and IgM results were also combined. The results were evaluated according to the target antigens in the statistical analysis.

Serum samples

In this study, 50 sera known to be antibody negative were used. The negative sera were those that were sent to the Turkish Ministry of Health, General Directorate of Public Health, Microbiology Reference Laboratories and Biological Products Department, and Sexually Transmitted Diseases Reference Laboratory in 2018 for syphilis confirmation.

Positive sera were obtained from blood samples collected 3–9 weeks after hospital discharge from 98 patients with positive COVID-19 RT-PCR results within the period from March 18 to July 31, 2020. The numbers of sera according to the time of blood sample collection in weeks were 5, 20, 19, 13, 26, 14, and 1 at 3, 4, 5, 6, 7, 8, and 9 weeks, respectively.

All the sera were kept at – 20°C until they were studied.

Real-time quantitative reverse transcription–polymerase chain reaction test for coronavirus disease 2019 diagnosis

Quantitative RT-PCR (RT-qPCR) Test Kits (Bio-Eksen, Istanbul, Turkey) for COVID-19 were provided by Turkey's Public Health Directorate General. For viral nucleic acid isolation, 100 µl of the viral transport medium, including swab samples from the patients, was taken and added to a tube containing 100 µl of the Bio-Speedy Viral Nucleic Acid Isolation Kit (Bio-Eksen, Istanbul, Turkey). The tube was vortexed for 15 s at the highest speed, incubated for 5 min at room temperature, and used as a template. The reagents included in the Bio-Speedy COVID-19 RT-qPCR Detection Kit were prepared according to the manufacturer's recommendations and were distributed to PCR plates at 15 µL per well, with a 5 µL template added. The plates were incubated at 45°C for 15 min, at 95°C for 3 min, and then 50 times (at 95°C for 5 s and at 55°C for 35 s in consecutive cycles) at the C1000 Touch Thermal Cycler CFX96 Real-Time System (BioRad, Watford, UK). Interpretation was done by evaluating the shape of the replication curves obtained in the FAM/HEX channels. Non-sigmoidal curves were considered negative. The threshold value was set to 200; if the number of threshold cycles calculated was up to 38–40 ≤ Cq (according to the lot studied), it was evaluated as positive, and when it exceeded this value, the test was repeated.

Statistical Analysis

Statistical analyses were performed using SPSS version 23.0 (IBM Corporation, Armonk, NY, USA). The antibody titers were investigated using histograms and the Kolmogorov–Smirnov test to determine whether they were normally distributed. Descriptive analyses were presented using medians, and the interquartile ranges of the antibody titers were not normally distributed. The Siemens test kit was excluded from the Kolmogorov–Smirnov test because the maximum value of its anti-S IgM/IgG test was > 10.0 and the minimum value was < 0.05, accounting for 66% of the total sera. The Hotgen test kit was also excluded from the Kolmogorov–Smirnov test because its results were qualitative.

The validity (sensitivity, specificity, positive predictive value [PPV], negative predictive value [NPV], and accuracy) values of the antibody detection test kits that were investigated in the present study were calculated. The reference test was PCR; positive cases were defined as true positive [3].

The agreement between the tests in determining positive and negative sera was investigated using the kappa test. Statistical significance was set at $p< .05$. The disease prevalence was accepted as 10%.

The intra-assay reproducibility was examined through assays of one positive and one negative sera, which were tested 10 times on the same day by different staff. A < 10% intra-assay

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coefficient of variation (CV) indicated acceptable reliability. For the Siemens anti-S IgM/IgG test kit, the positive serum had a > 10.0 level, and the negative serum had a < 0.05 level in every 10 tests; thus, the reproducibility CVs could not be calculated. Likewise, Euroimmun's anti-S IgA test kit had a "no calculation" level other than for two serum samples, and the CVs could not be calculated either.

This reaserch was approved by the Kastamonu Clinical Research Ethics Committee (Approval number: 2020-KAEK-143-05 and date: 14 December 2020).

3. RESULTS

Of the 98 patient sera with positive PCR results, 29 (29.6%) had no antibody in any of the tests. When the distribution of these sera by week was examined, no clustering was observed. In the antibody-positive group, when IgM was excluded, a one-to-one agreement between the tests was observed in only 24 (24.4%) sera, and the results of the remaining 45 (45.9%) sera differed by test kit or targeted antigen. The differences were observed mostly for anti-NCP IgM, and positive results were obtained for 14 sera, which were positive in at least one other test. The distribution of the numbers of anti-NCP IgM-positive sera by week was 4, 5, 1, 2, and 2 at 4, 5, 6, 7, and 8 weeks, respectively.

In Table I, the validity results (sensitivity, specificity, accuracy, PPV, and NPV) of the five commercial antibody detection test kits are presented by a 95% confidence interval. It was observed that the test kits' sensitivity (48.5–51.0%) and PPV (45.2–100.0%) results were low but their specificity (93.5–100%) and NPV (94.2–94.8%) results were high. The accuracy values varied from 88.9% to 95.1%. The intra-assay CVs for the positive and negative sera are also presented in Table I. The positive-sera CVs varied from 1% to 8%, with the lowest values obtained by the Abbott and Roche test kits and the highest value obtained by Euroimmun's anti-NCP IgM test, but the CVs of all the positive sera were acceptable. For the Euroimmun test kit, the negative-sera CV was 48% in the anti-S IgA test and 21% in the anti-NCP IgM test. The other results were 10% or lower, which were within the acceptable range.

The validity test scores were recalculated by excluding the 29 sera with no antibody found in any of the tests (Table II). The new sensitivity values were found to be much higher, varying by 69.12–72.46%. Other validity parameters were influenced slightly, and most of them increased, but the specificity, PPV, and accuracy results of Euroimmun's anti-NCP total test and the specificity and PPV results of the Siemens test kit decreased.

Table I. *Validity test results (sensitivity, specificity, accuracy, and positive and negative predictive values)*

Firm	Antibody detected	Sensitivity		Specificity		PPV		NPV		Accuracy		Inter-assay Repeatbility	
		(%)	95% CI	(%)	95% CI	(%)	95% CI	(%)	95% CI	(%)	95% CI	Negative	Positive
Siemens	Anti spike IgG /IgM total	51.04	40.63 to 61.39	96.00	86.29 to 99.51	58.64	26.45 to 84.83	94.64	93.45 to 95.62	91.50	85.75 to 95.48	-	-
Euroimmun	Anti spike IgA/Anti spike IgG	49.48	39.17 to 59.83	97.92	88.93 to 99.95	72.52	27.30 to 94.88	94.58	93.45 to 95.52	93.07	87.65 to 96.62	48/1	-/5
	Anti NCP IgG/Anti NCP IgM	48.45	38.18 to 58.82	93.48	82.10 to 98.63	45.22	21.33 to 71.53	94.23	92.99 to 95.26	88.98	82.66 to 93.60	9/21	6/8
Abbott	Anti NCP IgG	48.98	38.74 to 59.28	100.00	92.89 to 100.00	100.00	-	94.64	93.56 to 95.54	94.90	90.02 to 97.84	5	1
Roche	Anti NCP IgG /IgM total	50.00	39.73 to 60.27	100.00	92.89 to 100.00	100.00	-	94.74	93.66 to 95.64	95.00	90.15 to 97.90	10	1
Hotgen	Anti NCP IgG / IgM total	51.02	40.72 to 61.26	100.00	92.89 to 100.00	100.00	-	94.84	93.76 to 95.74	95.10	90.28 to 97.97	-	-

PPV: positive predictive value, NPV: negative predictive value

Table II. *Recalculated validity test results recalculated by excluding 29 sera with no antibody found in any of the tests*

Firm	Antibody detected	Sensitivity		Specificity		PPV		NPV		Accuracy	
		(%)	95% CI	(%)	95% CI	(%)	95% CI	(%)	95% CI	(%)	95% CI
Siemens	Anti spike IgG /IgM total	71.01	58.84-81.31	95.83	85.75-99.49	65.44	32.60-88.12	96.75	95.34-97.74	93.35	87.21-97.13
Euroimmun	Anti spike IgA/Anti spike IgG	70.59	58.29-81.02	97.92	88.93-99.95	79.01	34.98-96.34	96.77	95.39-97.75	95.18	89.55-98.29
	Anti NCP IgG/Anti NCP IgM	69.12	56.74-79.76	89.58	77.34-96.53	42.44	24.06-63.18	96.31	94.75-97.42	87.54	80.12-92.94
Abbott	Anti NCP IgG	69.57	57.31-80.08	100.00	92.89-100.00	100.00	-	96.73	95.39-97.69	96.69	92.06-99.24
Roche	Anti NCP IgG /IgM total	71.01	58.84-81.31	100.00	92.89-100.00	100.00	-	96.88	95.55-97.82	97.10	92.27-99.31
Hotgen	Anti NCP IgG / IgM total	72.46	60.38-82.54	100.00	92.89-100.00	100.00	-	97.03	95.71-97.96	97.25	92.47-99.37

PPV: positive predictive value, NPV: negative predictive value

In Table III, the agreement between the different tests' results is presented. The agreement between the anti-NCP antibody results of the Hotgen Biotech, Abbott, and Roche test kits was 94–99% (p = .001), but the results of the Euroimmun anti-NCP total antibody test had lower agreement with those of the other tests, only moderate or fair agreement, even only slight agreement for IgM. On the other hand, the anti-S antibody results of the Siemens test kit were in perfect agreement with the anti-S IgA and IgG results of the Euroimmun test kit (83% and 91%, respectively; p = .001). The anti-NCP IgM results of the Euroimmun test kit had the lowest agreement levels with the anti-NCP IgM results of all the other test kits.

Table III. *Agreement levels between the results of different commercial antibody detection test kits (%; p = 0.001)*

Firms	Hotgen Anti-NCP IgM/IgG	Abbott Anti-NCP IgG	Roche Anti-NCP IgM/IgG	Siemens Anti-Spike IgM/IgG
Abbott Anti-NCP IgG	94*	-	-	-
Roche Anti-NCP IgM/IgG	95*	99*	-	-
Siemens Anti-Spike IgM/IgG	47**	44**	45**	-
Euroimmune Anti-Spike total	41**	38***	40***	89*
Euroimmune Anti-NCP total	38***	34***	36***	82*

* Perfect, ** Moderate, *** Fair

The distributions of anti-NCP and anti-S antibody levels (Figures 1 and 2, respectively) are presented according to the day of blood sample collection. In eight tests from the five firms in the present study, there were 98 patient sera with positive PCR results; thus, there are five results for each patient in Figure 1 and three results for each patient in Figure 2, according to the patients' blood sample collection days. It was found that the anti-NCP antibody results obtained by the Hotgen Biotech, Abbott, and Roche test kits agreed with each other (Figure 1), but those obtained by the Euroimmun test kit (especially for IgM) did not agree with those obtained by the other firms' test kits. As for the anti-S antibody results, those obtained by the Siemens and Euroimmun test kits agreed with each other (Figure 2).

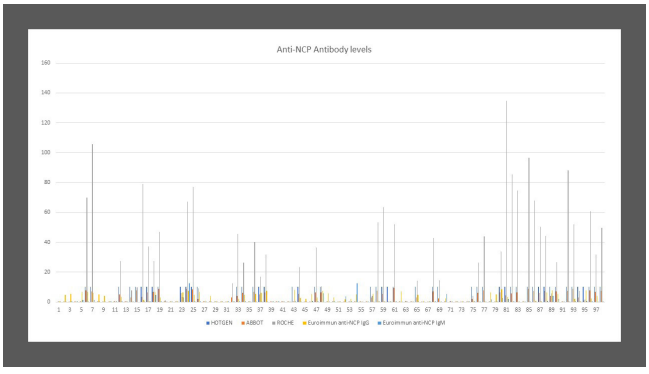

Figure 1. *Comparison of anti-nucleocapsid (NCP) antibody levels obtained from different commercial antibody detection test kits (Hotgen Biotech, Abbott, Roche, and Euroimmun anti-NCP IgG and anti-NCP IgM tests) from the blood samples of patients with positive polymerase chain reaction results (the antibody levels are presented on the y-axis, and the patients are presented on the x-axis).*

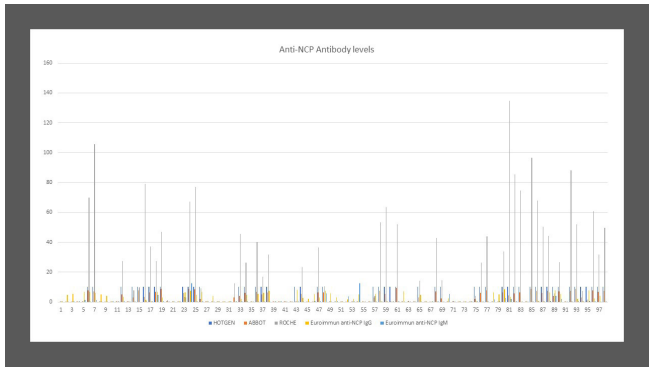

Figure 2. *Comparison of anti-spike (S) antibody levels obtained from Siemens and Euroimmun anti-S IgA and anti-S IgG tests from the blood samples of patients with positive polymerase chain reaction results (the antibody levels are presented on the y-axis, and the patients are presented on the x-axis).*

4. DISCUSSION

Neutralization tests are considered the gold standard for antibody detection for COVID-19, but they are not widely used because they require specialized expertise and laboratory containment [8]. On the other hand, the gold standard the diagnosis of COVID-19 is PCR, and in the present study, the sera of patients with positive PCR results were evaluated as falling under the "disease present" category in the statistical analysis. There were other studies with the same category [3, 6]. The statistical validity tests were performed according to this acception, and the results are presented in Table I. According to Table I, the specificity, NPV, and accuracy results of the tests were higher than 93.48%, 95.26%, and 88.98%, respectively; thus, the tests were found to be reliable. On the other hand, the tests' sensitivity and PPV results were low due to the antibody-negative sera of the patients with positive PCR results. However, 29 sera with no antibody in any of the tests were found, which suggests that in the cases with positive PCR results, the antibody might not have been synthesized. Alternatively, there might have been seroreversion, and the antibody might have waned, which could explain why no detectable antibody was present in these sera. For this reason, it was thought that the results of the validity tests would have been different if the 29 sera were excluded and if recalculation were done (Table II). When we compared Tables I and II, we found that the new sensitivity and PPV results were reasonable.

Regarding the validity of the anti-NCP antibody test results, it was found that patients with positive results in such tests have a very high probability of having COVID-19. Similarly, when a patient's antibody test result is negative, the possibility of ruling out the disease is high. On the other hand, with the anti-S antibody tests, there is no certainty that the patient has COVID-19 when the antibody test result is positive, but it is possible to rule out COVID-19 when the test result is negative.

The tests' accuracy results were high enough for the tests to be considered reliable. The inter-assay repeatability values were

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also reliable, except for the Euroimmun anti-S IgA and anti-NCP IgM tests, indicating a need to improve these two tests.

On the other hand, the sera that were found to be positive in only one test accounted for 46% of the sample. This shows that some patients might have been positive for some antibodies, while others might have been negative. As can be seen in Figures 1 and 2 and Table III, the tests that detected both anti-NCP and anti-S antibodies obtained more compatible results.

The Euroimmun anti-NCP IgM test had positive results in only 14 sera, and the agreement between its results and those of the other tests was the lowest. This may be due to the timing of the blood sample collection, which was mostly clustered in 4–5 weeks and up to the 9th week after the PCR test, and the PCR test was performed possibly later than symptom onset.

As mentioned earlier, 29 sera in the present study were found not to have an antibody in any of the tests. Other studies have found undetectable neutralizing antibodies in asymptomatic COVID-19 patients with positive PCR results [2, 5, 8-10]. Some studies reported that the titers fell below the detection threshold in more than 20% of the mild cases [6, 11].

When the sensitivity results of other studies and the present study were compared, it was found that the results of other studies varied from those of the present study by 87.8–95% for the Euroimmun anti-S IgA test, by 70.7–95.5% for the Euroimmun anti-S IgG test, by 76% for the Euroimmun anti-NCP IgG test, by 89.1–100% for the Siemens anti-S IgM/IgG test, by 73–95.7% for the Abbott anti-NCP IgG test, by 34.2% for the Hotgen anti-NCP IgM/IgG test, and by 75.6–99.5% for the Roche anti-NCP IgM/IgG total test; most of the sensitivity results of other studies were found to be higher than ours [3, 9, 12–21]. In one of these studies, the sampling time median was 12 days after symptom onset, which was earlier than ours, and it was mentioned that both the blood sample collection timing and the disease severity could potentially affect the sensitivity of the assays [3].

Another study reported that they had obtained very variable performance values, which highlights the need for laboratories to carefully consider their testing processes to optimize the overall performance of their serodiagnostics [9].

According to one study, the low specificity value of the anti-S antibody could be due to its cross-reactivity with other human coronaviruses [3]. In that study, it was found that Euroimmun anti-S IgA and IgG had 93.7% and 99.7% specificity, respectively, which were close to our findings. In another study, Euroimmun anti-S IgA reacted in samples retrieved from patients with autoantibodies in a negative panel of samples, which might explain our results [9]. When the data of other studies were examined for specificity, it was found that the results varied by 68.3–93.7% for Euroimmun anti-S IgA, by 86.6–100% for Euroimmun anti-S IgG, by 98% for Euroimmun anti-NCP IgG, by 99.8–100% for Siemens anti-S IgM/IgG, by 92.2–100% for Abbott anti-NCP IgG, by 93.2% for Hotgen anti-NCP IgM/IgG, and by 97–100% for Roche anti-NCP IgM/IgG total, mostly similar to our results, except for Euroimmun anti-S IgA and Hotgen, whose results were higher than ours, and for Euroimmun anti-NCP IgG and Siemens anti-S IgM/IgG, whose results were higher than ours but were very close to the values mentioned in the literature [3, 9, 12–21].

In our study, 46% of the sera were positive in some of the tests and negative in others. In another study conducted in Turkey, the results for four of the five firms that we studied were compared, and differences were observed between the firms' results in 30% of the sample [13].

When compared with our study, other studies reported that anti-NCP antibodies were more sensitive than anti-S antibodies in the early phase of the infection and became significantly less sensitive in the late phase [3, 6, 22]. In addition, the IgA and IgM antibody levels have been reported to decrease significantly over time [1, 6]. A study found that IgG was more frequently positive than IgM and that anti-S antibodies were more frequently positive than anti-NCP antibodies 14 days or longer after symptom onset [23]. In another study, either an S and/or an N protein was detected in the follow-up samples of the same patients, indicating different individual immune responses to SARS-CoV-2 and the influence of the assay used for the detection of IgG antibodies [18]. These facts might explain the differences in the validity test results of the test kits investigated in the present study for immunoglobulins and the targeted antibodies.

Previous studies have reported that the median day of seroconversion for IgM was 13 days after symptom onset, and a slight decrease was shown after 3 weeks [1, 6]. The blood sample collection timing in these studies was earlier than that in the present study, which might explain our Euroimmun anti-NCP IgM results. Another study reported that the IgM antibodies showed the lowest sensitivity in all the assays; they had many IgG-positive and IgM-negative cases, and IgM antibodies were not detected substantially earlier than IgG antibodies [3].

It is thus concluded that serological tests are useful as supplemental tools for diagnosing disease and measuring the immunity level, and that most of the commercial antibody detection test kits investigated in the present study are reliable, although improvement is needed in some parameters.

Compliance with Ethical Standards

Ethical Approval: This reaserch was approved by the Kastamonu Clinical Research Ethics Committee (Approval number: 2020-KAEK-143-05 and date: 14 December 2020).

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Authors' contribution: NC: Planning the study, collecting data, editing the article, CK: Patient follow-up and blood collection, data collection, article writing, AG: Statistical analysis, editing the article, BC, MMG AND ZAA: Patient follow-up and blood collection, data collection, article writing, CS: Planning the study, providing negative serums, writing the article, All authors approved the final mauscript.

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Effect of angiotensin receptor-neprilysin inhibitor treatment on erectile dysfunction in heart failure with a reduced ejection fraction

Sena SERT[1], Emre KARABAY[2], Baris GUNGOR[1], Ozlem YILDIRIMTURK[1]

[1] Department of Cardiology, Dr. Siyami Ersek Thoracic and Cardiovascular Surgery Training and Research Hospital, Istanbul, Turkey.
[2] Department of Urology, Acıbadem Kadıkoy Hospital, Istanbul, Turkey.

Corresponding Author: Sena SERT
E-mail: senasert@live.com

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ABSTRACT

Objective: Erectile dysfunction (ED) is a common condition in patients with heart failure (HF), which impairs quality of life. Our study aimed to compare those patients, who received traditional treatment with a diagnosis of HF and those who received angiotensin receptor-neprilysin inhibitor (ARNI) treatment in addition to the current treatment, in terms of ED at the end of 6 months.

Patients and Methods: The study was planned as a single-center, prospective study. The study included 200 patients with heart failure. The patients' demographic, clinical, and echocardiographic characteristics were recorded, and an international ED scoring questionnaire was applied. The participants in the study were divided into two groups: those who received ARNI treatment and those who did not. After 6 months, the ED questionnaire was applied to the patients again and the groups were compared.

Results: The median age of the patients was 53 (years). The median ejection fraction (EF) value was calculated to be 30% and no significant difference was found between the groups (p: 0.122). It was found that N-terminal pro-brain natriuretic peptide (NT-pro-BNP) levels measured at the end of the 6th month were significantly lower in patients who had received ARNI treatment than in those who had not (respectively, 245 pg/ml, 200 pg/ml; p: 0.003). In the analysis performed to detect the presence of ED, it was discovered that the ED score change was significantly higher in the group that had received 6 months of ARNI treatment (p: 0.031) compared to that in the group that had not (p: 0.031). When the ED sub-parameters were compared in terms of the 6-month change rate, it was found that the ARNI group had a significant increase in terms of ED and sexual satisfaction scores, but no significant difference was found in the other parameters (p: 0.001, p: 0.029).

Conclusion: Erectile dysfunction is more common in patients with heart failure compared to the rest of society and impairs quality of life. In our study, it was determined that ED complaints decreased significantly in HF patients, who had received ARNI treatment for 6 months than in patients who had not.

Keywords: Erectile dysfunction, Heart failure, Angiotensin receptor-neprilysin inhibitor

1. INTRODUCTION

Heart failure (HF) is a complicated disease that develops due to structural or functional problems of the heart. Its prevalence varies between 1-2% in society and its incidence is increasing [1]. In everyday life, HF appears a difficult disease, and HF patients' quality of life and comfort is low.

Erectile dysfunction (ED) is one of the reasons for the reduction in quality of life in patients with HF. ED is defined as the inability to achieve a sufficient level of penile erection for sexual performance [2]. Its incidence in the general population ranges from 19 to 52% [3-5]. Several studies have found a strong link between ED and cardiovascular disease [6,7]. The frequency of ED in HF patients is more common when compared to that in those who are healthy within the general population. In publications, the frequency of ED in patients with HF has been reported at rates ranging from 30-80% [8,9]. Endothelial dysfunction, atherosclerosis, decreased effort capacity, additional drugs used, and psychological effects are prominent causes of ED in HF patients [10-14].

Heart failure treatment is constantly updated with the addition of new drugs to the treatment regimen. When real-world data from

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these drugs with different mechanisms of action were examined, it was discovered that there may be positive pleiotropic effects in the target effect structure. Angiotensin receptor-neprilysin inhibitor (ARNI) is a molecule that has recently been used in HF treatment. It improves the quality of life while decreasing hospitalization. This drug, which acts as a neprilysin inhibitor, has been shown to have numerous pleiotropic effects in patients with HF. In our study, the possible pleiotropic effect on ED was investigated in HF patients receiving ARNI treatment.

2. PATIENTS and METHODS

The study included 200 patients with a HF diagnosis, who applied to the cardiology unit of the Training and Research Hospital. Male patients over the age of 18, who were newly diagnosed with HF and for whom HF treatment was initiated in accordance with current guidelines, taking into account the patients' blood pressure and pulse rate, etc., basal kidney and liver functions, and concomitant diseases, and who agreed to fill out the ED-related questionnaire, were included. Patients under the age of 18, who had had a heart transplant, or a left ventricular assist device implanted, as well as those who were unable to receive conventional and/or ARNI treatment due to chronic renal failure (CRF), drug allergy, drug intolerance, or other reasons, were excluded from the study.

In addition to recording age, sex, height, and weight, questions were asked about patients' smoking habits, comorbidities, (diabetes mellitus (DM), coronary artery disease (CAD), hyperlipidemia (HL), CRF, hypertension (HF), atrial fibrillation (AF)), and HF etiologies. Among the biochemical markers, creatine, glomerular filtration rate (GFR), and N-terminal pro-brain natriuretic peptide (NT-pro-BNP) values were examined.

Using echocardiography (ECHO), ejection fraction (EF), end-diastolic diameter (EDD), end-systolic diameter (ESD), right ventricular basal diameter, tricuspid annular plane systolic excursion (TAPSE), and systolic pulmonary artery pressure (SPAB) were measured, with insufficiencies and stenosis of the heart valves included in the assessment.

In our study, the patients completed The International Index of Erectile Function Questionnaire (IIEF) on the day they were included in the study before the initiation of the medication after diagnosis and 6 months after the initiation of the medication. IIEF is a widely used, multi-dimensional self-report instrument for the evaluation of male sexual function. It is has been recommended as a primary endpoint for clinical trials of erectile dysfunction (ED) and for diagnostic evaluation of ED severity. In this questionnaire developed by Rosen et al., patients' erectile function, orgasmic function, sexual desire, sexual satisfaction and general satisfaction are analyzed [15]. In many studies of ED, classification has been made based on the IIEF questionnaire score [16]. It has been linguistically validated in 32 languages and used as a primary endpoint in more than 50 clinical trials [15]. This questionnaire is ranked from 1 to 5 on the Likert scale (1: very low or not at all/ very dissatisfied, 5: very high/ very satisfied). 5 has the most positive results, and 1 shows the weakest answer, and each field is added up to reach the scores. High scores support a better quality of erectile functions. The questionnaire's question number 1-5 and 15 were evaluated in terms of erectile functions. Patients with scores of 0 to 10 were considered to have severe ED, those with 11-16 were considered to have moderate ED, those with 17-21 were considered to have mild to moderate ED, those with 22-25 were considered to have mild ED, and those with 26-30 were considered to have no ED. Answers to questions 6, 7 and 8 were evaluated for sexual satisfaction, answers to questions 9 and 10 for orgasmic function, answers to questions 11 and 12 for sexual desire and questions 13 and 14 for general satisfaction. Patients completed the questionnaire alone and in a calm environment.

The participants of this study provided written informed consent of the utilization of their medical records. All subjects gave informed consent, the study complied with the Declaration of Helsinki, and the trial was approved by the institutional local ethics committee (The protocol code: HNEAH-KAEK 2022/KK 16.).

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) 25.0 program was used for data analysis in the study. It was planned to give descriptive data on the sociodemographic information of the participants as N and %, and data on continuous variables as Mean±SD and Median (min-max).

When the study data were analyzed in terms of normality assumptions, Kolmogorov-Smirnov values were determined as $p<0.05$. Therefore, Spearman correlation analysis, one of the nonparametric tests, was performed to determine the relationship between erectile function total and subscale scores and body mass index (BMI), age, GFR, and EF variables. In addition, the nonparametric Mann-Whitney U test and Kruskal-Wallis H test were used to determine whether there was a significant difference between various categorical variables and the 0^{th} and 6^{th}-month erectile function total and subscale scores. If there was a significant difference between the groups, the posthoc test was used to answer the following question: Among which groups was the significance? Performing the Games-Howell posthoc test was planned because the variance was not homogeneously distributed, and the number of samples was not equal (Sparks, 1963). Finally, Fisher's exact test was used to compare categorical variables. A value of $p<0.05$ was considered statistically significant.

3. RESULTS

The median age of the patients, who participated in the study, was 53 (years). Median height was 174 cm and median weight was 80 kg, and no significant difference was found between the groups (p: 0.431, p: 0.303, respectively). The mean BMI was found to be 26.2 in the BMI analysis, while the BMI of the patients, who did not receive ARNI, was significantly lower than that of the patients who did receive ARNI (p: 0.017) (Table I).

Table I. Demographic and clinical parameters of patients

Parameters	Total	Group without ARNI	Group with ARNI	p
	Median (Q1/Q3)	Median (Q1/Q3)	Median (Q1/Q3)	
Age (year)	53 (47 / 57.5)	51.5 (47 / 57)	54 (48 / 58.5)	0.108 [u]
Height (cm)	174 (170 / 178)	173.5 (170 / 177.5)	175 (169.5 / 178.5)	0.491 [u]
Weight (kg)	80 (74 / 89)	80 (75 / 90)	79 (73 / 87.5)	0.303 [u]
BMI (kg/m2)	26.27 (24.91 / 28.95)	27.67 (25.01 / 29.36)	25.83 (24.69 / 27.75)	**0.017** [u]
	n (%)	n (%)	n (%)	
CAD	82 (41)	38 (38)	44 (44)	0.472 [c]
CRF	155 (77.5)	81 (81)	74 (74)	0.310 [c]
DM	116 (58)	61 (61)	55 (55)	0.474 [c]
AF	165 (82.5)	80 (80)	85 (85)	0.457 [c]
HT	94 (47)	50 (50)	44 (44)	0.479 [c]
HL	112 (56)	55 (55)	57 (57)	0.887 [c]
IHF Etiology	118 (59)	62 (62)	56 (56)	0.472 [c]

[u] *Mann Whitney U test (Monte Carlo),* [c] *Pearson Chi-Square Test (Monte Carlo), Q1: 1st Quartile, Q3: 3rd Quartile*

BMI: body mass index, CAD: Coronary artery disease, CRF: Chronic renal failure, DM: Diabetes mellitus, AF: Atrial Fibrillation, HT: Hypertension, HL: Hyperlipidemia, IHF: Ischemic heart failure

Coronary artery disease was found in 41% of the patients (n: 82), CRF in 22.5% of the patients (n: 45), DM in 58% (n: 116), AF in 82.5% (n: 165), HT in 47% (n: 94), HL in 56% (n: 112) and ischemic HF etiology in 59% (n: 118). In terms of chronic diseases, no significant difference was found between the groups (Table I). The patients' median creatinine value was 1.01 mg/dl, and the median GFR value was 86 ml/min. Between the groups, no significant difference was found regarding these parameters (p: 0.221, and p: 0290, respectively) (Table II). While the mean NT-pro-BNP value in the non-ARNI group was 750 pg/ml, the initial NT-pro-BNP value in the ARNI group was 670 pg/ml. No significant difference was found between the groups in terms of baseline values (p: 0.051) (Table IV).

When the patients' ECHO parameters were examined, the median EF value was discovered to be 30%, with no significant difference between the groups (p: 0.122). When the patients were examined in terms of EDD and ESD, the median values were 64 mm and 53 mm, respectively, and no difference was observed between the groups (p: 0.520, p: 0174, respectively). In terms of valve pathologies, right-heart size and functions, no statistically significant difference was found between the groups of patients (Table II).

Sixty-three (63%) of patients, who did not receive ARNI had ED, while 66 (66%) of patients, who were initially scheduled to receive ARNI treatment, had ED. After 6 months, one patient in the non-ARNI group developed novel ED, while 2 patients recovered. The changes were not statistically significant (p: 0.999). In the ARNI group, 6 patients recovered after 6 months, while no change was observed in terms of ED in the other patients. The rate of change in patients, who recovered from ED after 6 months compared to the initial ED status, was statistically significant in the ARNI treatment group (p: 0.031) (Table III and IV). When the patients' NT-pro-BNP levels were compared after 6 months of treatment, those who received ARNI had significantly lower levels than those who did not. After 6 months, no statistically significant difference was found in the groups' NT-pro-BNP change rates (respectively, p: 0.03, p: 0.800) (Table IV).

Table II. Echocardiographic and biochemical parameters of patients

Parameters	Total (n=200) n (%)	Group without ARNI (n=100) n (%)	Group with ARNI (n=100) n (%)	p
GFR				0.155 [ff]
Severely decreased	2 (1)	1 (1)	1 (1)	
Moderately to severely	8 (4)	6 (6)	2 (2)	
Mildly to moderate decreased	35 (17.5)	12 (12)	23 (23)	
Mildly decreased	69 (34.5)	34 (34)	35 (35)	
Normal	86 (43)	47 (47)	39 (39)	
Mitral				0.168 [ff]
Normal valve	15 (7.5)	8 (8)	7 (7)	
Mild MR	80 (40)	39 (39)	41 (41)	
Moderate MR	61 (30.5)	37 (37)	24 (24)	
Severe MR	41 (20.5)	15 (15)	26 (26)	
MVR	3 (1.5)	1 (1)	2 (2)	
Aorta				0.401 [ff]
Normal valve	139 (69.5)	73 (73)	66 (66)	
Mild AR	42 (21)	21 (21)	21 (21)	
Moderete AR	13 (6.5)	4 (4)	9 (9)	
Severe AS	2 (1)	0 (0)	2 (2)	
AVR	4 (2)	2 (2)	2 (2)	
Tricuspid				0.399 [c]
Normal valve	39 (19.5)	22 (22)	17 (17)	
Mild TR	115 (57.5)	60 (60)	55 (55)	
Moderate TR	34 (17)	13 (13)	21 (21)	
Severe TR	12 (6)	5 (5)	7 (7)	
Right Heart Dilatation	72 (36)	36 (36)	36 (36)	0.999 [c]
Left Heart Dilatation	172 (86)	85 (85)	87 (87)	0.839 [c]
TAPSE (>16)	141 (70.5)	69 (69)	72 (72)	0.757 [c]
RVS (>9)	140 (70)	70 (70)	70 (70)	0.999 [c]
	mean(SD.)	mean(SD.)	mean(SD.)	
Tapse (mm)	19.05 (4.55)	19.05 (4.64)	19.06 (4.49)	0.987 [t]
	Median (Q1/Q3)	Median (Q1/Q3)	Median (Q1/Q3)	
EDD (mm)	64 (58.5 / 69)	64 (58 / 69)	64 (60 / 69)	0.520 [u]
ESD (mm)	53 (47 / 60)	53 (46 / 59.5)	54.5 (49.5 / 60)	0.174 [u]
Creatinine (mg/dl)	1.01 (0.9 / 1.22)	1 (0.89 / 1.17)	1.015 (0.94 / 1.26)	0.221 [u]
RV Diameter (mm)	38 (34 / 43)	38 (34.5 / 43)	38 (34 / 42.5)	0.885 [u]
SPAB (mmhg)	28 (20 / 40)	25.5 (20 / 35)	30 (20 / 41)	0.302 [u]
GFR (ml/dk)	86 (67 / 99)	87 (72 / 100)	86 (59 / 96)	0.290 [u]
LVEF (%)	30 (25 / 35)	30 (25 / 35)	30 (25 / 32.5)	0.122 [u]

[u] *Mann Whitney U test (Monte Carlo),* [t] *Independent samples t test (Boostrap),* [c] *Pearson Chi-Square Test (Monte Carlo),*

[ff] *Fisher Freeman Halton test (Monte Carlo), Q1: 1st Quartile, Q3: 3rd Quartile, SD.: Standard Deviation*

GFR: Glomerular filtration rate, AS: Aortic stenosis, AR: aortic regurgitation, MR: mitral regurgitation, TAPSE: tricuspid annulus plane systolic excursion,RVS: Right ventricle systolic excursion velocity, LVEF: Left ventricle ejection fraction, EDD: End-diastolic diameter, ESD: End-systolic diameter, SPAB: Systolic pulmonary artery pressure

Table III. *Erectile function analysis of patients*

Variables	Without ARNI Group (n=100) n (%)	With ARNI Group (n=100) n (%)	p
Erectile Function Presence			
0. months	63 (63)	66 (66)	0.768 [c]
6. months	62 (62)	60 (60)	0.885 [c]
Changes			0.434 [ff]
Negative Change rates (absent → presence)	1 (1)	0 (0)	
No Change (absent → absent)	36 (36)	34 (34)	
Positive Change rates (presence → absent)	2 (2)	6 (6)	
No Change (presence → presence)	61 (61)	60 (60)	
p value for 0. vs. 6. month	0.999 [m]	**0.031** [m]	

[c] *Pearson Chi-Square Test (Monte Carlo),* [ff] *Fisher Freeman Halton test(Monte Carlo),*

[m] *McNemar test (Monte Carlo)*

Table IV. *Analysis of patients' erectile function sub-parameters*

	Group without ARNI (n=100) Median (Q1/ Q3)	Group with ARNI (n=100) Median (Q1/ Q3)	p
Erectile Function Level			
0. months	2 (0 / 3)	1 (0 / 3)	0.819 [u]
6. months	1 (0 / 2)	1 (0 / 2)	0.220 [u]
Change rates (0-6 months)	0 (0 / 0)	-0.5 (-1 / 0)	**0.001** [u]
p value for 0. vs. 6. month	**0.002** [w]	**<0.001** [w]	
Erectile Function Score			
0. months	21 (16 / 26)	22 (16 / 26)	0.600 [u]
6. months	22 (17 / 26)	25 (21 / 27)	**0.018** [u]
Change rates (0-6 months)	1 (0 / 1)	2 (0 / 4)	**<0.001** [u]
p value for 0. vs. 6. month	**<0.001** [w]	**<0.001** [w]	
Sexual Desire Score			
0. months	6 (5 / 7)	6 (4 / 7)	0.855 [u]
6. months	7 (6 / 8)	7 (6 / 8)	0.379 [u]
Change rates (0-6 months)	1 (0 / 2)	1 (0 / 1)	0.220 [u]
p value for 0. vs. 6. month	**<0.001** [w]	**<0.001** [w]	
Orgasmic Function Score			
0. months	6.5 (5 / 8)	6 (4 / 7)	**0.046** [u]
6. months	7 (6 / 9)	7 (6 / 8)	0.076 [u]
Change rates (0-6 months)	1 (0 / 1)	1 (0 / 1)	0.892 [u]
p value for 0. vs. 6. month	**<0.001** [w]	**<0.001** [w]	
Sexual Satisfaction Score			
0. months	9 (7 / 11)	8 (6 / 10)	**0.031** [u]
6. months	11 (9 / 12)	10 (9 / 12)	0.598 [u]
Change rates (0-6 months)	1 (0 / 3)	2 (1 / 3)	**0.029** [u]
p value for 0. vs. 6. month	**<0.001** [w]	**<0.001** [w]	
General Satisfaction score			
0. months	6 (5 / 7)	6 (4 / 7)	0.165 [u]
6. months	7 (6 / 8)	7 (6 / 8)	0.431 [u]
Change rates (0-6 months)	0 (0 / 1)	1 (0 / 1)	0.423 [u]
p value for 0. vs. 6. month	**<0.001** [w]	**<0.001** [w]	
NYHA functional class score			
0. months	3 (3 / 4)	3 (3 / 3)	0.904 [u]
6. months	2 (2 / 2)	2 (2 / 2)	0.999 [u]
Change rates (0-6 months)	-1 (-2 / – 1)	-1 (-1.5 / – 1)	0.791 [u]
p value for 0. vs. 6. month	**<0.001** [w]	**<0.001** [w]	
NT-Pro-BNP(pg/ml)			
0. months	750 (590 / 890)	670 (480 / 800)	0.051 [u]
6. months	245 (200 / 340)	200 (112 / 255)	**0.003** [u]
Change rates (0-6 months)	-420 (-570 / – 245.5)	-430 (-580 / – 230)	0.800 [u]
p value for 0. vs. 6. month	**<0.001** [w]	**<0.001** [w]	

[u] *Mann Whitney U test(Monte Carlo),* [w] *Wilcoxon signed-rank test(Monte Carlo), Q1: 1st Quartile, Q3: 3rd Quartile*

When the score change rates for the ED sub-parameters were compared between the groups after 6 months of treatment, a significant difference in the ED and sexual satisfaction scores in the ARNI group was found, and recovery was observed (p: 0.001, p: 0.029). No statistically significant difference was found in post-treatment change rates in other parameters. When the groups were compared in terms of ED parameters at the baseline and after 6 months, a significant improvement was observed in all parameters (Table IV).

4. DISCUSSION

In our study, patients with heart failure diagnosis who received ARNI treatment for 6 months had significantly lower ED complaints than patients who did not, and their NT-pro-BNP levels also decreased significantly.

Heart failure is a cardiovascular disease, whose rates of occurrence have increased in recent years. New agents are being developed to improve life quality and lower mortality in HF patients. In addition to their basic effects, these drugs have a positive pleiotropic effect on the daily lives of HF patients by increasing the mediators. ARNI is one of the new agents used in HF treatment with an angiotensin-neprilysin inhibitor effect. The PARADIGM-HF study found that using ARNI in patients with HF diagnosis reduced symptoms of failure and slowed HF progression compared to results recorded in enalapril treatment [17]. In the PARADIGM-HF study, it was found that NT-pro-BNP levels decreased significantly in ARNI areas after treatment. Similarly, in our study, it was observed that NT-pro-BNP levels decreased statistically significantly in the group receiving ARNI treatment after 6 months. Ceyhun et al., investigated the relationship between ED and NT-pro-BNP in patients with chronic HF. In this study, an increase in NT-pro-BNP associated with an increase in ED severity was found [16]. Similarly, in our

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study, ED was observed more frequently in the non-ARNI group that had a high NT-pro-BNP level compared to the ARNI group that had received ARNI for 6 months and had a low NT-pro-BNP level. It was thought that this increase caused endothelial healing by increasing the possible NO bioavailability of ARNI treatment or in a way that has not been determined yet.

Angiotensin receptor-neprilysin inhibitor includes valsartan, an angiotensin receptor blocker, and sacubitril, a neprilysin inhibitor. Neutral endopeptidase neprilysin reduces many endogenous vasoactive peptides such as a natriuretic peptide, bradykinin, and adrenomedullin [18,19]. Vasoconstriction, neurohormonal overstimulation and inappropriate remodeling are prevented by the double effect of the angiotensin receptor blocking effect and neprilysin inhibitor provided by sacubitril thanks to valsartan, which is present in the molecule [20,21]. According to some published studies, ARNI treatment caused partial left ventricular EF elevation in all mildly reduced EF HF patients, regardless of etiology [22]. In the study of Canale et al., it was shown that ARNI treatment caused recovery of the chemotherapy-related advanced mild EF in 4 patients, with a possible antioxidant mechanism [23]. In our study, after 6 months of ARNI treatment, no significant positive change was observed in EF values according to the initial measurements.

Nitric oxide is one of the most important molecules involved in the healing of the vascular barrier and vasodilation. It was reported that ARNI treatment particularly increased NO bioavailability and more favorable results were obtained in vascular functions in HF than valsartan alone. It is thought that the increase in NO bioavailability contributes positively to the treatment of diseases caused by underlying endothelial problems [24]. In the study of Mario et al., it was stated that in vitro ARNI treatment in rats could improve the endothelial barrier, increasing the contractility of the detrusor muscles and thus helping the recovery of EF [25]. After this study, ARNI treatment continued to be examined in terms of increased sexual function, and in the study of Zhuang et al., it was found that ARNI administration increased sexual function in female hypertensive rats. They discovered that in addition to this increase in sexual function, there was an improvement in clitoral and vaginal fibrosis. They determined that the molecule's effect on the PTEN/PI3K/AKT pathway was effective at the biochemical level [26]. In our study, it was observed that in HF patients with frequent ED complaints, patients who had received ARNI treatment had a significant increase in their ED score (ED recovery) at the end of 6 months compared to that in non-ARNI patients. Since there was no significant difference between the groups in the comparison of EF, chronic diseases, and age, and there was no change in the doses of other drugs used in the process, it was thought that a positive and significant effect in terms of ED was achieved with ARNI treatment. This is consistent with other studies that show ARNI treatment improves sexual functions. A recent study called PARADOR aimed to show the difference between ARNI and enalapril treatment in the recovery of ED in patients with low EF HF [27]. Results of randomized controlled and double-blind studies with an active-control group are awaited. Recovery of ED with ARNI treatment is important in terms of increasing the quality of life in patients with HF.

Limitation

Our study's limitations are the small number of samples and the lack of comparison of other medications used between groups.

Conclusion

It has been determined that new drugs used in HF have many pleiotropic effects apart from the target effect. In our study, it was found that there was a statistically significant decrease in ED complaints in patients with HF, who had received 6 months of ARNI treatment, compared to that those who had not.

Compliance with Ethical Standards

Ethical Approval: This study was approved by the Haydarpasa Numune Training and Research Hospital Clinical Research Ethics Committee (approval number: HNEAH-KAEK 2022/KK 16.)). The study adhered to the principles of the Helsinki Declaration.

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Healthy life-style behaviors and related factors among Turkish primary health care professionals

Belgin ORAL[1], Nergiz SEVINC[2], Burcu KORKUT[3]

[1] Occupational Diseases Clinic, Kayseri City Hospital, Ministry of Health, Kayseri, Turkey
[2] Department of Public Health, Faculty of Medicine, Karabuk University, Karabuk, Turkey
[3] Department of Family Medicine, Faculty of Medicine, Karabuk University, Karabuk, Turkey

Corresponding Author: Belgin ORAL
E-mail: belgin.zeybek@hotmail.com

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ABSTRACT

Objective: The role of health professionals working in primary care in providing services to all segments of society and in protecting and improving the health of all individuals is extremely important. The aim of this study is to determine the healthy lifestyle behaviors of primary health care workers in Karabük city center and to examine some factors that may affect the choice of a healthy lifestyle.

Materials and Methods: This cross-sectional study was conducted between October-December 2019 with the participation of 334 healthcare professionals in Karabük. The questionnaire with 27 questions and the Healthy Lifestyle Behaviors Scale II (HLBS-II) were administered.

Results: The median age of the health care workers participating in the study was 38.2 ± 10.6. Of the participants 53.8% were women and approximately three-quarters of the participants (70.8%) were married. In terms of occupational groups, 10.9% of the participants were physicians, 25.0% were nurses and midwives, 16.7% were health officers/technicians and 47.4% were composed of other employees. The HLBS-II total score was found to be 126.8±21.3. The HLBS-II total score were higher in women, those who defined their health status as good, university graduates those who did not smoke, had good nutrition, exercised, and were satisfied with their body appearance.

Conclusion: In our study, it was found that the healthy life behavior scores of health workers were at a good level. Especially men, smokers, high school graduates, obese, those who do not pay attention to their nutrition and physical activity should be informed and be supported by healthy living behavior programs.

Keywords: Primary health care, Health care professionals, Healthy life behaviors

1. INTRODUCTION

As in the definition of the World Health Organization (WHO), health; is "not only the absence of illness or disability but a state of complete physical, social and mental well-being" [1]. In the light of this comprehensive definition of health, health is a phenomenon that has social characteristics as well as individual. Healthy life behaviors, which are a model for health promotion, have been developed as a multidimensional concept that includes self-actualization, health responsibility, exercise, nutrition, interpersonal support, and stress management [2]. In this concept, a healthy lifestyle is possible by reinforcing positive behaviors in order to protect the health, and by having individuals control their own life in avoiding negative behaviors [3].

According to WHO 2022, approximately 74% of all deaths each year are due to non-communicable diseases such as cardiovascular diseases, cancers, chronic obstructive pulmonary disease (COPD) and diabetes mellitus (DM), which are responsible for the majority of these deaths, and it has been stated that these deaths are also associated with an unhealthy lifestyle [4]. In the 'Global Burden of Disease Study 2017' a study reported that, high blood pressure and blood sugar, high body mass index, and smoking and alcohol use were among the causes of early deaths and disabilities [5]. In the burden of disease studies (Disability-adjusted life years – DALY), physical inactivity and obesity have been shown to play an active role in addition to high blood pressure, blood sugar, blood cholesterol levels, smoking, and alcohol use [6]. It means that when healthy behaviors are adopted and harmful behaviors are abandoned,

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deaths, disabilities and diseases can be prevented, health costs can be reduced and healthier societies can be reached.

Primary healthcare services are an important step in the management of chronic diseases and the protection of health. When people get sick, their first application is especially to primary health care institutions. In these institutions, in addition to special services such as immunization, maternal and child health, healthy nutrition, prevention of obesity, tobacco control and cancer screening, which play the biggest role in preventive health services, diagnosis and treatment of diseases are also provided. In this context, it can be said that primary health care workers are primarily in contact with all segments of the society. Although, it is a very easy field for community-based studies and interventions, the population it can affect is quite large.

It is noteworthy that healthcare professionals are role models as well as informative roles for society. In a study conducted in Australia, the importance of primary care workers in terms of their protective and preventive roles in patient behavior change is emphasized, and in another study, it was stated that physicians are role models for their patients and should adopt healthy behaviors [7,8]. In an example based on nurses in Iran , it was found that there are dimensions that should be supported in terms of healthy behavior such as stress management and physical activity, in another study involving nurses in Saudi Arabia, it was shown that nurses adopt healthy behaviors at a moderate level and they should be supported in this regard [3,9]. All of these studies pointed that it is important to determine the healthy behaviors of primary health care professionals who play an active role in the protection and improvement of public health.

In this study, we aim to determine the healthy lifestyle behaviors (eg, physical activity, nutrition, stress management, health responsibility) of primary health care professionals consisting of physicians, nurses, midwives, officers/technicians and other workers.

2. MATERIALS and METHODS

This cross-sectional study was conducted in the city center of Karabük to examine some variables that may be associated with the healthy lifestyle behaviors of healthcare professionals providing primary health care services. It was aimed to reach all 334 healthcare workers employed in the city center of Karabük and no sample was selected. Our research, in which data were collected in October-December 2019, was completed with 312 individuals (93% participation rate was achieved) whose consent was obtained. The study was approved by the Karabük University, Non-interventional Clinical Research Ethics Committee (approval number: 77192459-050.99-E.41206 with decision number: 6/28). The study was conducted according to the principles of Declaration of Helsinki.

Research data collected with the help of the questionnaire form consisting of 27 questions, five of which are open-ended, and which questions the socio-demographic characteristics of the participants such as age, marital status, educational status, profession, economic status, family structure, and Healthy Lifestyle Behaviors Scale-II (HLBS-II) was used. The questionnaire form was administered by a single researcher using a face-to-face interview method and it took approximately 15 minutes for each participant. Participants were specifically asked not to provide any identifying information and it was stated that the data would not be used outside the research and they could withdraw from the study at any time.

Healthy Lifestyle Behaviors Scale was modeled after Pender's health promotion model, and in 1987 Walker et al. It was developed first as a 48-question scale [2]. The scale was revised and reworked by Walker, Sechrist, and Pender in 1996 and the second scale consisting of 52 questions was named HLBS-II [2,10]. In our country, the first version's validity and reliability of the scale were made by Esin in 1999, and the second version by Bahar in 2008 [11,12]. HLBS-II is a quartered likert type as 'never, sometimes, often, regularly' and the scores range from 52 to 208. The scale has six sub-dimensions as self-actualization of scale (range of points: 9-36), health responsibility (range of points: 9-36), exercise (range of points: 8-32), nutrition (range of points: 9-36), interpersonal support (range of points: 9-36) and stress management (range of points: 8-32). The higher scores on the scale indicate that the individual applies the specified health behaviors at a good level. In our study, the smoking status was regulated according to the WHO's Tobacco Use Monitoring and Control Directive, and individuals were classified as "smoking" and "not smoking" according to their smoking status. Those who smoked regularly and occasionally were included in the group of smoking, and those who quit and those who never smoke were included in the not-smoking group [13]. Body Mass Index (BMI) (weight (kg) / height (m^2)) was calculated using the height and weight values stated by the participants according to their own expressions [14].

Statistical Analysis

At the end of the research, the data obtained through the questionnaire form were entered into the statistics package program (SPSS 21). Controls and analysis of the data were done in the same program. Frequency and percentage, mean value, standard deviation, highest and lowest values were used for descriptive statistics in statistical analysis. Shapiro Wilk test was used to check the compatibility of the data to normal distribution. Chi-square test was used for statistical analysis of categorical data, Unpaired t-test and One-Way ANOVA test (post hoc Tukey test) were used for statistical analysis of quantitative data. Statistical significance of the difference was accepted as $p < 0.05$.

The study was deemed ethically appropriate by the Karabük University Clinical Research Ethics Committee (Date and Number: 07.10.2019 – E.41206), and all participants participating in the study were informed about the study before the study and their verbal consent was obtained.

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3. RESULTS

The mean age of 312 participants participating in the study was 38.2±10.6 (min-max: 18-62) years and 53.8% of them were women. 56.7% of the participants were 40 years or younger and 70.8% were married. According to their education, 25% of the participants were a high school, 63.8% pre-license, and undergraduate, 11.2% of them were graduate. In terms of occupational groups, 10.9% of the participants were physicians, 25.0% were nurses and midwives, 16.7% were health officers/technicians and 47.4% are composed of other employees (driver, cleaning worker, auxiliary staff, etc.). Some sociodemographic characteristics of the participants are given in Table I.

Table I. *Sociodemographic Characteristics of the Participants*

Characteristic		Number	%*
Gender	Female	168	53.8
	Male	144	46.2
Age groups	40 and below	177	56.7
	41 and above	135	43.3
Marital status	Single	91	29.2
	Married	221	70.8
Education status	High school and equivalent	78	25.0
	Pre-license and undergraduate	199	63.8
	Graduate	35	11.2
Occupation	Physician	34	10.9
	Nurses/Midwive	78	25.0
	Health Officers/Technicians	52	16.7
	Others	148	47.4
Longest living place to date	Province	238	76.3
	District	66	21.2
	Village/town	8	2.5
Economical status	Good	144	46.2
	Moderate	153	49.0
	Bad	15	4.8
Family type	Nuclear	270	86.5
	Large	30	9.6
	Broken	12	3.8
Total		312	100.0

** Column percentage*

The healthcare professionals participating in our study according to their own statements, 68.6% of them were in good health, 20.2% of them had at least one chronic disease, 23.7% of them regularly used drugs. 21.5% of them stated that they received psychological support at any time until the research date and 17.6% of them had sleep problems (Table II). 37.2% of the participants stated that they smoke (32.7% of the women, 42.4% of the men smoke, there is no difference between the groups $p > 0.05$, $\chi 2 = 3.074$), 10.3% were exercising and 55.1% had nutrition regularly. The BMI mean of the participants is 25.4±4.3, this value is 24.3±4.3 for women and 26.7±4.1 for men (Table II). Participants' BMI groups and weight perceptions and their satisfaction with their body view are given in Table II.

Table II. *Findings of the participants regarding their health according to total and gender*

Some variables		Total participants		Gender		
		Number	Percentage	Female%*	Male *%	p
Health status according to their own statements	Good	214	68.6	69.0	68.1	p>0.05
	Moderate	92	29.5	28.6	30.6	
	Bad	6	1.9	2.4	1.4	
Existance of chronic illness	Yes	63	20.2	25.0	14.6	**p=0.015**
	No	249	79.8	75.0	85.4	
Using regular drug	Yes	74	23.7	29.2	17.4	**p=0.010**
	No	238	76.3	70.8	82.6	
Taking psychological support	Yes	67	21.5	26.8	15.3	**p=0.009**
	No	245	78.5	73.2	84.7	
Having trouble with sleeping	Yes	55	17.6	21.4	13.2	**p=0.039**
	No	257	82.4	78.6	86.8	
Smoking status	Yes	116	37.2	32.7	42.4	**p=0.051**
	No	196	62.8	67.3	57.6	
Exercise regularly	Yes	32	10.3	9.5	11.1	p=0.391
	No	280	89.7	90.5	88.9	
Regular nutrition	Yes	172	55.1	58.3	51.4	p=0.132
	No	140	44.9	41.7	48.6	
BMI groups	Underweight	5	1.6	1.6	1.4	**p<0.001**
	Normal	148	47.4	47.4	29.2	
	Overweight	117	37.5	37.5	52.1	
	Obese	42	13.5	10,1	17.4	
Perception of weight	Underweight	27	8.7	11.9	4.9	p=0.068
	Normal	160	51.3	51.8	50.7	
	Overweight	92	29.6	25.0	34.7	
	Obese	33	10.6	11.3	9.7	
Satisfaction with body appearance	Satisfied	208	66.7	66.1	67.4	p=0.452
	Not Satisfied	104	33.3	33.9	32.6	

** Column percentage, Chi-square test, (p<0.05 significant), BMI: Body Mass Index*

Healthy Lifestyle Behaviors Scale-II total score of the participants in the study was found as 126.8±21.3, and it is given in Table III with its sub-dimensions (Table III). Total scale score, health responsibility, and nutrition dimensions were found to be significantly higher in women. There is no difference between the groups in terms of scale scores for age groups, marital status, and family type. According to the education level, the total scale score, self-realization, health responsibility, and nutrition dimensions were found to be significantly lower in high school and equivalent graduates. While, there was no difference in scale scores between physicians, nurses, and technicians in terms of occupational groups, health responsibility, nutrition, stress management, and total scale scores were found to be significantly lower in other professions. Scale scores were generally low in those who lived in villages/towns most of their lives and significantly higher in groups who defined their economic status as good (Table IV).

Table III. *HLBS-II scores of healthcare workers and range of points*

HLBS	Mean	Median (min-max)	Range of points
Total score	126.8±21.3	125 (57-187)	52-208
Self realization	25.7±5.1	26 (9-36)	9-36
Health responsibility	21.2±4.8	21 (9-36)	9-36
Exercise	15.5±5.2	15 (8-31)	8-32
Nutrition	22.0±4.6	22 (9-35)	9-36
Interpersonal support	24.3±4.3	24 (9-35)	9-36
Stress management	18.1±3.9	18 (8-29)	8-32

HLBS: Healthy Lifestyle Behaviors Scale

Table IV. *HLBS-II scores according to the sociodemographic characteristics of healthcare professionals*

HLBS Variables		Self realization	Health responsibility	Exercise	Nutrition	Interpersonal support	Stress management	Total scale score
Gender	Female	26.1±5.2	22.0±4.7	15.6±5.2	22.8±4.6	24.7±4.5	18.5±3.8	129.7±21.1
	Male	25.2±4.9	20.3±4.8	15.4±5.3	21.0±4.3	23.9±4.1	17.7±3.9	123.4±21.1
	p*	0.107	**0.002**	0.693	**<0.001**	0.099	0.062	**0.009**
Education status	High school and equivalent	24.7±5.8[a]	20.5±5.0[a]	14.9±4.8	20.7±4.3[a]	23.7±4.6	17.6±3.9	122.0±22.8[a]
	Pre-license, and undergraduate	25.9±4.8[a,b]	21.0±4.7[a]	15.8±5.5	22.2±4.5[b]	24.5±4.2	18.2±3.9	127.7±20.8[b]
	Graduate	27.1±4.9[b]	23.8±3.9[b]	14.8±5.2	23.6±4.9[b]	24.6±4.5	18.9±3.6	132.9±19.3[b]
	p**	**0.046**	**0.002**	0.296	**0.004**	0.334	0.225	**0.029**
Occupation	Physician	26.6±5.5	23.3±4.0[a]	15.7±5.2	23.2±5.0[a]	24.1±5.2	18.9±4.5[a,b]	131.9±22.5[a]
	Nurse / midwife	26.2±4.9	22.0±4.6[a]	15.8±5.8	23.2±4.8[a]	24.6±4.0	18.5±4.0[a,b]	130.3±20.7[a]
	Health officer / technician	26.2±5.1	22.1±5.4[a]	16.8±6.0	22.5±4.6[a]	25.1±4.3	19.3±4.0[b]	132.2±20.3[a]
	Other	25.2±5.1	20.3±4.6[b]	14.5±5.2	21.1±1.6[b]	24.0±4.4	17.5±3.7[a]	122.8±20.3[b]
	p**	0.301	**0.006**	0.090	**0.002**	0.393	**0.010**	**0.007**
Longest living place to date	Province	25.9±4.9[a]	21.4±4.8[a]	15.5±5.4	22.2±4.4[a]	24.5±4.3[a]	18.3±3.8[a]	127.6±20.9[a]
	District	26.0±5.2[a]	21.5±4.7[a]	15.7±4.8	21.8±4.9[a]	24.4±3.9[a]	17.7±4.1[a]	127.2±20.8[a]
	Village / town	18.5±6.1[b]	16.8±3.8[b]	13.4±3.0	18.4±5.6[b]	18.5±5.0[b]	14.8±3.5[b]	100.3±24.9[b]
	p**	**<0.001**	**0.026**	0.503	**0.064**	**<0.001**	**0.031**	**0.002**
Economical status	Good	26.8±5.1[a]	22.0±4.8[a]	16.1±5.8	23.0±4.9[a]	25.2±4.4[a]	18.7±4.1[a]	131.7±22.5[a]
	Moderate	25.0±4.9[b]	20.8±4.6[b]	15.1±4.6	21.3±4.2[b]	23.5±4.1[b]	17.8±3.6[a,b]	123.5±19.3[b]
	Bad	22.3±3.7[b]	17.9±4.8[c]	13.5±5.1	19.6±3.6[b]	24.6±4.4[a,b]	16.3±4.3[b]	114.2±18.4[b]
	p**	**<0.001**	**0.002**	0.073	**0.001**	**0.003**	**0.030**	**<0.001**
Family Type	Nuclear	25.9±5.2	21.5±4.9	15.6±5.3	22.1±4.6	24.4±4.3	18.3±3.9	127.8±21.6
	Large	24.5±4.5	19.4±3.7	15.0±4.1	21.1±4.2	23.5±4.4	16.7±3.3	120.2±17.8
	Broken	24.8±5.4	19.3±4.0	14.6±6.0	20.6±4.9	25.3±5.4	17.8±4.4	122.4±21.0
	p**	0.334	0.075	0.723	0.273	0.406	0.097	0.142

** Unpaired t-test, ** One Way ANOVA (post hoc Tukey), a,b,c: The difference between groups that do not have the same letter in each column is significant. (p<0.05 significant), HLBS: Healthy Lifestyle Behaviors Scale*

According to the participants' own statements, in the group that defined the health status as good, the total scale score and self-realization, interpersonal support, and stress management sub-dimensions were found to be significantly higher (Table V). The health responsibility and nutritional dimensions of the participants with a chronic disease and regularly using a drug were found to be significantly higher, and the health responsibility was found to be significantly higher in individuals who received psychological support (p<0.05). On the other hand, the total score and the sub-dimensions of self-actualization and stress management were found to be significantly lower in participants with sleep problems (p<0.05). All scores were found to be significantly lower in the participants who stated that they did not exercise regularly and did not have regularly nutrition, except for the inter-personal support dimension. According to BMI groups, the total score and the dimension of interpersonal support were found to be significantly lower in obese participants. Although, there is no statistically significant difference, exercise and nutrition dimensions scores are also low in underweight individuals. According to the perception of weight, the self-actualization dimension was found to be high in participants who perceived their weight as underweight, the exercise dimension was higher in those who perceived their weight as normal, and stress management was found to be low in those who perceived their weight as overweight and obese. In those who were satisfied with their body appearance, all scores were found to be significantly higher except for the interpersonal support dimension (Table V).

Table V. HLBS-II scores of healthcare professionals according to some health status

HLBS Variables		Self realization	Health responsibility	Exercise	Nutrition	Interpersonal support	Stress management	Total scale score
Health status according to their own statements	Good	26.4±5.1[a]	21.3±4.9	15.7±5.4	22.2±4.7	24.8±4.4[a]	18.5±4.0[a]	129.1±21.8[a]
	Moderate	24.1±4.8[a,b]	20.8±4.5	15.0±4.9	21.4±4.2	23.5±4.1[b]	17.3±3.6[a,b]	122.5±19.8[b]
	Bad	19.7±3.7[b]	21.3±5.4	14.0±4.7	21.3±5.0	20.2±2.4[b]	16.0±3.5[b]	112.5±12.4[c]
	p*	**<0.001**	0.699	0.421	0.357	**0.003**	**0.015**	**0.011**
Chronic illness	Yes	25.9±5.3	22.3±4.8	14.7±4.8	23.0±4.6	24.0±4.3	18.0±3.6	127.9±20.4
	No	25.7±5.1	20.9±4.7	15.7±5.3	21.7±4.5	24.4±4.3	18.2±4.0	126.6±21.6
	p**	0.741	**0.031**	0.159	**0.045**	0.520	0.737	0.652
Using regular drug	Yes	25.8±5.0	22.3±4.5	15.0±4.8	23.0±4.4	24.2±4.2	18.1±3.4	128.4±19.1
	No	25.7±5.1	20.8±4.8	15.6±5.4	21.7±4.6	24.4±4.4	18.2±4.0	126.4±22.0
	p**	0,850	**0.021**	0.408	**0.025**	0.768	0.845	0.458
Taking psychological support	Yes	25.8±5.3	22.9±4.9	15.2±5.5	22.8±4.6	24.5±4.4	18.1±4.0	129.4±21.8
	No	25.7±5.0	20.7±4.6	15.5±5.2	21.8±4.5	24.3±4.3	18.1±3.9	126.1±21.2
	p**	0.868	**0.001**	0.670	0.086	0.731	0.994	0.261
Having trouble with sleeping	Yes	24.2±4.6	20.5±4.5	15.0±5.5	21.5±4.4	23.6±4.3	16.3±3.8	120.9±19.4
	No	26.0±5.1	21.3±4.8	15.6±5.2	22.1±4.6	24.5±4.3	18.5±3.8	128.1±21.5
	p**	**0,014**	0.231	0.420	0.341	0.140	**<0.001**	**0.023**
Smoking status	Yes	24.7±5.0	19.6±4.7	14.3±4.8	20.6±4.2	23.9±4.2	17.5±3.8	120.7±18.5
	No	26.3±5.1	22.1±4.6	16.2±5.4	22.8±4.6	24.6±4.4	18.5±3.9	130.5±22.1
	p**	**0.007**	**<0.001**	**0.002**	**<0.001**	0.167	**0.040**	**<0.001**
Exercise regularly	Yes	28.3±5.0	22.9±4.6	24.5±5.2	24.4±5.3	25.1±4.4	21.6±4.3	146.8±23.5
	No	25.4±5.0	21.0±4.8	14.4±4.1	21.7±4.4	24.3±4.3	17.7±3.6	124.6±19.9
	p**	**0.002**	**0.035**	**<0.001**	**0.001**	0.324	**<0.001**	**<0.001**
Regular nutrition	Yes	26.7±5.1	22.4±4.3	16.5±5.4	23.5±4.3	24.7±4.3	19.3±3.8	133.2±20.3
	No	24.5±4.9	19.7±4.9	14.2±4.7	20.1±4.1	23.9±4.3	16.7±3.4	119.1±19.9
	p**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.138	**<0.001**	**<0.001**
BMI	Underweight	27.2±5.5	19.6±3.4	11.8±3.4	19.0±2.2	26.8±4.8[a]	17.8±5.4	122.2±13.7[a,b]
	Normal	26.3±5.1	21.2±4.6	16.0±5.5	22.6±4.5	24.8±4.1[a]	18.6±3.7	130.1±20.2[a]
	Overweight	25.4±5.0	20.8±5.2	15.5±5.3	21.7±4.8	24.3±4.2[a]	18.0±3.9	125.6±22.4[a,b]
	Obese	24.3±5.3	20.4±4.1	14.1±3.7	21.0±4.0	22.5±5.0[b]	16.9±4.2	119.3±20.9[b]
	p*	0.096	0.204	0.091	0.071	**0.011**	0.084	**0,024**
Perception of weight	Underweight	28.4±5.6[a]	23.2±5.7	14.1±5.3[a]	22.1±3.4	25.2±4.5	18.5±4.5[a]	131.6±21.6
	Normal	25.9±5.1[a,b]	21.2±4.7	16.5±5.6[b]	22.4±4.9	24.4±3.9	18.7±3.8[a]	129.0±21.6
	Overweight	24.8±5.1[b]	20.7±5.0	14.5±4.5[a]	21.5±4.6	24.4±5.2	17.4±4.0[b]	123.3±21.9
	Obese	25.2±3.7[a,b]	21.0±3.2	14.5±4.3[a]	21.1±4.6	23.2±3.4	17.3±2.9[b]	122.2±15.2
	p*	**0.011**	0.132	**0.006**	0.313	0.300	**0.035**	0.068
Satisfaction with body appearance	Satisfied	26.1±5.5	21.6±5.1	16.1±5.5	22.6±4.7	24.4±4.4	18.6±4.1	129.4±22.9
	Not Satisfied	24.9±4.2	20.4±4.1	14.2±4.3	20.8±4.1	24.2±4.2	17.2±3.2	121.6±16.7
	p**	**0.039**	**0.042**	**0.002**	**0.001**	0.580	**0.002**	**0.002**

** One Way ANOVA (post hoc Tukey), a,b,c: The difference between groups that do not have the same letter in each column is significant ** Unpaired t-test, (p<0.05 significant), HLBS: Healthy Lifestyle Behaviors Scale, BMI: Body Mass Index*

4. DISCUSSION

Primary health care services are usually the first place that individuals go when they get sick. These health facilities also carry out preventive health services and play a key role in gaining behaviors that protect and improve the health of the society. For this reason, it is thought that the impact of these institutions that provide health services can be reflected to the whole society. In the literature, it was observed that healthcare workers showed higher healthy lifestyle behaviors in studies compared with non-healthcare workers [15,16]. For this reason, it is very useful to determine whether primary health care workers adopt healthy lifestyle behaviors because they have educational and guiding counselor roles.

Healthy Lifestyle Behaviors Scale-II total score of the participants in the study is 126.8±21.3, which can be considered at a good level. HLBS-II score was found 121.9±18.1 in healthcare workers by Yalçınkaya et al., it was found 127.9±18.2 in medical

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students in a multicenter study by Nacar et al., Oral and Çetinkaya found this score as 125.4±19.5 in university students and it was also found 130.7±21.9 in factory workers by Kolaç et al. and 86.7±7.3 in traveling seasonal agricultural workers by Göçer et al. and 122.4±44.2 in nurses by Abadi and Rezaei [3,17-21]. The existence of such different values in the literature can be attributed to the fact that those included in the study were selected from different fields and that their educational level, living conditions, and socio-economic differences affected healthy living behaviors.

In the present study, the total score, health responsibility, and nutrition dimensions were found to be high in women. Similarly, Yalçınkaya et al. found the responsibility of health and nutrition scores higher in women, Chen et al., found the scale scores higher in women but in family health center employees Koruk et al., in Taiwan in healthcare workers Tsai and Liu found no difference between genders [17,22-24]. Bhuiyan et al., in medical students, and Oral and Çetinkaya in university students found exercise dimension higher in males [21,25]. The location of this study is in the north-central region of Turkey. Due to the traditional structure of Turkish society and gender roles, women are mothers at the same time, they have responsibilities such as child, elderly and patient care, and food preparation at home. These reasons may explain the higher health responsibility, nutrition, and total scale scores in women in our study.

The total scale score, self-realization, health responsibility, and nutrition dimensions of participants in the study were found to be low in high school and equivalent graduates. Similarly, Chen et al., showed increasing healthy lifestyle behaviors with increasing education level [22]. Increasing the education level is expected to positively affect healthy living behaviors. While there was no significant difference between the scale scores of physicians, nurses, and technicians, health responsibility, nutrition, stress management, and total scale scores were lower in the other occupational groups (driver, cleaner, assistant staff). Tsai and Liu's study found that physicians' self-realization, exercise, nutrition, and stress management were lower than nurses, technicians, and administrative staff [24]. Other healthcare professionals often do not communicate directly with patients. Due to its role in informing patients about health issues, physicians, nurses, and health officer/technician may have adopted healthy living behaviors. For this reason, such differences between occupational groups may have emerged in our study.

Psychological stress and anxiety caused by economic problems may have distracted individuals from healthy lifestyle behaviors. In the study, scale scores were higher in those with good economic status. Similarly, Nacar et al., found HLBS-II score low in those who defined their economic situation as bad. Oral and Çetinkaya, on the other hand, found high healthy lifestyle behaviors in students with good economic status. Abadi and Rezaei, showed that monthly income is positively correlated with healthy lifestyle behaviors [3,18,21].

The high health responsibility of individuals who have received psychological support indicates that self-awareness and health awareness are also high. However, poor sleep quality and sleep problems can cause individuals to feel tired during the day, and may also reveal situations such as attention deficit, headache, and anxiety [8]. This may lead them away from healthy lifestyle behaviors and cause them to be inadequate in the fight against stress. In the study, the total scale score, self-realization, interpersonal support and stress management were found to be significantly higher in those who defined their health as good according to their own statements. Besides, health responsibility and nutrition dimensions were significantly higher in patients with chronic disease and regular drug use, and health responsibility was significantly higher in those who received psychological support. However, those with sleep problems had low total scale scores, self-realization, and stress management. In Taiwan Huang et al., showed the effect of high health perceptions of workers on healthy living behaviors and also Yılmazel et al., found the scale scores high who defined their own health as good in nursing students, on the other hand, Oral and Çetinkaya found the scale scores high in students who defined their own health as good in their studies at four different universities [21,26,27]. People who define their health as good can be expected to have a high perception of health. In the presence of chronic disease and the obligation to use medication regularly, individuals may have responsibilities such as taking an active role in the management of the disease. Both these studies and the results of the present study support idea that people who define their health as good can be expected to have a high perception of health.

All scores were found to be significantly lower in smokers except for the interpersonal support dimension. Similarly, Guler et al., found significantly lower scale scores in smokers among university staff, and similar results were obtained in some studies with different student groups [28-30]. Smoking can also mean that the individual does not care about his/her own health and cannot take responsibility for his/her health. The fact that smokers create a social environment together during smoking breaks may explain why the interpersonal support score is high in this group.

According to BMI groups, the total score and the dimension of interpersonal support were significantly lower in the overweight/obese participants. Similarly, Alzahrani et al. found a negative relationship between BMI values and interpersonal support scores in Medical Faculty students [31]. According to weight perception the self-realization dimension was found to be higher in participants who perceived their weight as underweight, exercise dimension was found to be high in those who perceived their weight as normal, and stress management was found to be high in those who perceived their weight as underweight and normal. In those who were satisfied with their body appearance, all scores were found to be significantly higher except for the interpersonal support dimension. The fact that individuals who perceive themselves as underweight were at peace with themselves, their higher self-confidence may mean that they were more successful in stress management. The low level of healthy lifestyle behaviors in those who were not satisfied with their body appearance indicates this.

Limitations

The main limitation of the study is that it cannot be generalized to the whole country since the study was conducted in a single province and its only about primary health care workers and does not include other healthcare workers in secondary or tertiary institutions.

Conclusion

In conclusion, Primary Healthcare professionals serve everyone in the community and keep their communication alive throughout the year. Although, healthcare workers' scores on healthy lifestyle behaviors were found to be at a good level, some points needed to be improved. So that, the exercise dimension had the lowest score, all employees should be supported with awareness raising activities on physical activities. In addition, the findings of the study showed that it is necessary to raise awareness about healthy behaviors among other health workers (driver, cleaning staff, auxiliary personnel), high school and equivalent graduates, males, smokers, obeses and those who did not pay attention to their nutrition and physical activities. It is necessary to support healthy lifestyle behaviors with different work activities, both by preparing posters and brochures and by in-service training. Social activities related to the importance of physical activity, the benefits of regular and balanced nutrition, weight control, and the importance of sleep quality might raise awareness. It may be beneficial to make tobacco control programs widespread. Providing free counseling services in obtaining healthy living behaviors can be highly motivating, especially for employees who experience economic difficulties. Thus, the awareness of gaining healthy behaviors can help improve the health of the society.

Compliance with Ethical Standards

Ethical Approval: The study was approved by the Karabük University, Non-interventional Clinical Research Ethics Committee (approval number: 77192459-050.99-E.41206 with decision number: 6/28). The study was conducted according to the principles of Declaration of Helsinki.

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Authors' contributions: BO: Study design, NS and BK: Data collection, BO and NS: Data analysis, BO, NS and BK: Manuscript writing. All authors approved the final manuscript.

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Health behavior and health needs of first-year medical and health sciences students

Kamer GUR[1], Saime EROL[1], F. Esra GUNES[2], Serap CIFCILI[3], K. Burcu CALIK[4], Aysel Yildiz OZER[5], Ilksan DEMIRBUKEN[5], M. Gulden POLAT[5], Cigdem APAYDIN KAYA[3]

[1] Department of Public Health Nursing, Faculty of Health Sciences, Marmara University, Istanbul, Turkey
[2] Department of Nutrition and Dietetics, Faculty of Health Sciences, Marmara University, Istanbul, Turkey
[3] Department of Family Medicine, School of Medicine, Marmara University, Istanbul, Turkey
[4] Department of Health Management, Faculty of Health Sciences, Marmara University, Istanbul, Turkey
[5] Department of Physiotherapy and Rehabilitation, Faculty of Health Sciences, Marmara University, Istanbul, Turkey

Corresponding Author: Kamer GUR
E-mail: kamergur@gmail.com

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ABSTRACT

Objective: The aim of this study is to evaluate the health behavior and health needs of medical and health sciences students in order to identify areas that need intervention.

Materials and Methods: This descriptive study was conducted with 770 first-year university students. The data were collected with a sociodemographic questionnaire, the Youth Risk Behavior Survey and the Eating Attitudes Test. Body mass index was calculated.

Results: Although, only 12.7% of the students were overweight or obese, 25.6% of the students perceived themselves to be overweight or obese. The students said that in the last week, 20.9% had breakfast 1 or 3 times, 5.7% ate no fruits at all, and 11.6% ate no vegetables at all. 48.1% of the students did not engage in any physical activity, and 83.2% spent more than 2 hours inactively in front of a screen. It was observed that 22.3% were current smokers. A propensity for eating behaviors disorder was found in 9.0%. Drinking energy-boosting sports drinks 1-6 times in the last week (β: 3.286), smoking (β: 1.875) and eating few vegetable dishes in the last week (β: 0.484) were identified as factors that could be associated with a "tendency for eating behavior disorder."

Conclusion: We can conclude that nutritional issues, negative body weight perception, use of tobacco, and sedentary lifestyle are the main intervention and counselling areas for our study group.

Keywords: Health behavior, Health needs, Medical students, Health sciences

1. INTRODUCTION

University life is of critical importance in developing health behavior since in this period people act more independent and with more initiative. This is the time of life in which health behaviors tend to develop [1]. Unhealthy and risky behaviors adopted in this period might lead to many diseases in adulthood and as a result cause disability or mortality in the future [2, 3]. Various health risk behaviors among university students have been identified such as smoking, use of alcohol and illicit drugs, risky sexual behaviors, unhealthy eating, poor weight control, and the lack of physical exercise [4-6]. Detecting such risky behaviors among university students and providing appropriate counseling might contribute to the protection and promotion of students' health. Knowing the health risks of university students, who are destined to be the competent adults of the future, is important in terms of planning initiatives to protect and improve health. The health science students will be teaching and performing the duties of health promotion and prevention, involved in counseling patients about appropriate health-related behavior. Moreover, they may act as a role model for other students by lead in the future [7]. Therefore, it is crucially important for health science students to develop adequate healthy behaviors that are compatible with their profession. In 2020, 44553 people were qualified for admission to the health sciences schools [Medicine 16488; Dentistry 7752; Midwifery/Nursing 4074 and others (Nutrition and Dietetic, Physiotherapy, Audiology, Orthotics Prosthetics, Ergotherapy, etc) 16239] in Turkey [8].

The first year of university, in which students are 18-19 years old, might be the best time to implement appropriate interventions just the students are about to begin their independent adult lives. In order to contribute to both their own health and that they will be a

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role model in their further working life, the freshman students in Health Sciences Campus, a project has been planned by Marmara University Family Medicine Education Application and Research Center. The aim of this project is to help the students of the Faculty of Medicine, Faculty of Dentistry and Faculty of Health Sciences, who are in the occupational group that will take an active role in the provision of health services, to gain self-awareness of healthy lifestyle behaviors from the first year of their education life. Therefore, the first step of the project was to assess the health needs of the health sciences students, make a baseline assessment to provide information for further intervention studies. The aim of this study is to evaluate the health behavior and health needs of first-year medical and health sciences students and to identify necessary interventions, as part of the project, "Starting off on a Healthy Life on the Health Sciences Campus".

2. MATERIALS and METHODS

The population of the study comprises first-year students (n=881) newly enrolled in the Faculties of Health Sciences, Dentistry and Medicine located on the Marmara University Health Sciences Campus in Istanbul. No sample was selected, the aim being to reach the whole study population. This descriptive study was conducted with 770 first-year university students

(87.4% of the study population). Students of Faculty of Health Sciences (n=537), Faculty of Medicine (n=119) and Faculty of Dentistry (n=114), who volunteered to participate were included. Those who failed to complete the questionnaires and those who had been registered prior to 2019 were excluded from the study. Following the approval of the Marmara University Faculty of Health Sciences Non-interventional Clinical Practices Ethics Committee (Approval No. 29.08.2019/90) and of school unit administrators, all newly registered students were informed about the study by brochures, invitations and pre-class announcements during the first two weeks of the academic year. The students who have given verbal and written consent were enrolled in the study.

The data were collected in a supervised classroom setting via an electronic form sent over mobile phones. The data collected was based on a nine-item sociodemographic questionnaire, Youth Risk Behavior Survey and the Eating Attitudes Test (EAT).

The Sociodemographic Questionnaire queried the students' age, sex, most commonly used health institution, chronic illness, department, regular regimen of medications.The form also contained questions on the students' BMI (calculated by the researchers) and their body perception. At the same time, some questions on the Youth Risk Behavior Survey were used. The Youth Risk Behavior Survey is a questionnaire consisting of 89 items developed by the U.S. Centers for Disease Control and Prevention in 1990 [9]. Youth Risk Behavior Surveillance System is a questionnaire system that is used in school-based studies that seeks to assess the change and tendencies in risky health-related behavior over time. In this study, fifteen questions on the young people's eating behavior, nine questions on their behaviors in the context of smoking and drinking, six questions on their physical activity behaviors, and nine questions on their sexual activity, totaling 38 closed-ended, multiple-choice questions were used. The items were translated into Turkish by two of the authors. The translators were fluent in both languages and familiar with the cultures under study. After translation, the Turkish questions were pilot tested. Since, the questionnaire did not include cultural expressions backtranslate technique was not used. According to the developers' report, the questionnaires may be translated to any language. No specific permission is required. The participant marks the most suitable one [9].

***Eating Attitudes Test*:** The Eating Attitudes Test is a 6-point (Always, Very frequently, Often, Sometimes, Rarely, Never) Likert-type of measure based on self-reporting consisting of 40 items developed by Garner and Garfinkel [10] and adapted into Turkish by Savasır and Erol [11]. It can be applied to individuals over the age of eleven to identify adolescents with an eating disorder. The cut-off point for the scale is a score of 30. Scores of ≥30 indicate a "tendency for eating behavior disorder".

***Height Measurements*:** Height was measured using a stadiometer with the student's feet together and their head on the Frankfurt plane (where the lower margins of the eyes and the upper margins of the ear canals all lie in the same horizontal plane and parallel to the floor) [12].

***Weight Measurements*:** Weight was measured using a bioelectrical impedance analyzer (BIA) (Tanita BC-418, Tanita Corp., Tokyo, Japan). Bioelectrical impedance analysis is a method for estimating body composition, in particular body fat and muscle mass, where a weak electric current flow through the body and the voltage is measured in order to calculate impedance (resistance) of the body. In this study, the BIA was used for weight measurements only [13]. Measurements of female students who were in the menstrual period were measured at the end of menstruation.

Body mass index was calculated by dividing body weight (kg) by height in meters squared (cm). The classifications of the World Health Organization were used in the assessment of the BMI results. Accordingly, the students' BMI classification was considered as follows: <18.5 = Underweight, 18.5-24.9 = Normal, 25.0-29.9 = Pre-obese, 30.0-34.9 = 1st degree obese, 35.0-39.9 = 2nd degree obese [14] .

Statistical Analyses

The data were analyzed using SPSS version 20.0 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corporation). Age and BMI score were summarized using the mean and standard deviation. Other variables were presented by category as numbers and percentages. First, univariate relationships between the dependent and independent variables were explored using the chi-squared test. Then, multiple logistic regression was performed on each dependent variable by using the independent variables that had a p-value of less than 0.1 in the corresponding univariate analysis.[15] Goodness of fit was examined using the Hosmer-Lemeshow test. For the interpretation of the findings from the multiple logistic regression analyses, statistical significance was taken to be $p<0.05$.

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3. RESULTS

A total of 770 students starting their freshman year in the 2019-2020 academic year participated in the study. The mean (standard deviation) age of the students was 19.06 (1.73), (min=17, max=42); 76.6% (n=590) were female. Table I displays the sociodemographic characteristics of the students, the department in which they were enrolled, and data on the state of their health.

Table I. *Sociodemographic characteristics, departments and health status*

Variables		n	%
Gender	Females	590	76.6
	Males	180	23.4
Age Mean (Standard deviation): 19.06 ±1.73 (min=17, max=42)	17-21	733	96.1
	22-26	24	3.1
	27 and above	6	.8
Departments (n=748)	Nutrition	98	12.7
	PTR	100	13.0
	Midwifery	78	10.1
	Nursing	191	24.8
	Health Management	70	9.1
	Dentistry	114	14.8
	Medicine	119	15.5
Health insurance (n=727)	Social Security	600	82.5
	Private Insurance	39	5.4
	No health insurance	88	12.1
Health institutions most frequently used (n=764)	Family Health Center	176	23.0
	State hospital	373	48.8
	Private healthcare institution (polyclinic, hospital, etc.)	101	13.2
	Training and Research Hospital	104	13.6
	City Hospital	10	1.4
When the most recent checkup, physical exam, teeth cleaning or any other dental procedure took place (n=769)	In the last 12 months	429	55.8
	Between 12-24 months ago	95	12.4
	Longer than 24 months ago	69	9.0
	I am not sure	94	12.2
	I never had any procedure done	82	10.7

PTR: Physical Therapy and Rehabilitation

A large majority (82.5%; n=600) of the students had social security, 9.7% (n=130) had a chronic illness, and 9.7% (n=74) had a regular regimen of medications. One striking finding was that almost half of the students (48.8%; n=373) went to state hospitals for medical services instead of primary healthcare.

According to the BMI categories, 14.7% (n=87) of the participants were underweight, 72.6% (n=430) were normal, 10.5% (n=62) were overweight, and 2.2% (n=13) were obese. However, 55.0% of the students described themselves as normal, 8.3% (n=64) as fat, 19.4% (n=149) as slender. There was no difference between both sexes in terms of being overweight or obesity (12.8% F; 12.1% M; p=0.830). Among the students, 26.3% (n=202) said they ate one or two meals a day, 71% (n=546) ate three-four meals, 74.9% (n=576) said they skipped meals, for which the most common reason given (40.7%; n=246) was "not having enough time." While most of the students (76.6%; n=589) said they had breakfast on 4-7 days of the last week, 20.9% (n=161) said they only ate breakfast on 1-3 days, 2.5% (n=19) said they did not eat breakfast at all (Table II).

The students whose scores on the Eating Attitudes Test were above the cut-off point of 30 and thus tend to develop eating behavior disorders constituted 9.0% (n=69). The prevalence of tendency toward eating behavior disorder among the students who consumed fewer vegetable dishes and glasses of milk a day, drank energy-boosting sports drinks and smoke was higher (p<0.05) (Table III).

Table IV presents the results of multiple logistic regression analysis, which was performed using a model incorporating the factors that were found to cause significant differences with tendency for eating behavior disorder were selected as independent variables in the logistic model: smoking status, frequency of eating vegetable dishes and amount of drinking glasses of milk with frequency of drinking energy-boosting sports drinks. Smoking was found to be a significant associated factor for "tendency for eating behavior disorder" (β: 1.875; 95% CI = 1.070–3.286). The other associated factor was "eating fewer vegetable dishes in the last week" (β:0.484; 95% CI = 0.251-0.936).

Of the students, 3.4% (n=26) said that they never buckle up their safety belts when sitting in the passenger seat and 27.9% (n=214) said they buckled up all the time. A group of 8.0% (n=18) said they sent out email or texted while driving at least one day in the last 30 days. Among the students who stated they had tried smoking (50.3%; n=386), 28.9% (n=208) were between the ages of 8-16 and 25.5% (n=183) said they tried smoking after the age of 17. Smoking habits of the students are shown in Table V.

The assessment of the students' physical activity showed that their activity levels were markedly low. Students who had not engaged in physical activity for at least 60 minutes in each of the last seven days constituted 48.1% (n=370); 73.7% (n=566) of the students reported that they did not do resistance and 84.2% (n=645) said that they had not played in any sports team in the last 12 months. Of the students, 21.9% (n=168) said they spent one to four hours watching television on school days. In addition, the students who spent one – or two-hours watching videos or played computer games as leisure activity on school days constituted 69.4% (n=534) while those who spent four or more hours on this made up 13.8% (n=106) of the participants (Table IV).

Among the students, 7.4% (n=54) reported they had been involved in sexual activity at least once. Among the students answered the question of "Did you or your partner use any type of birth control method the last time you had sexual relations?" (n=51), 68.6 % reported using condom, 17.7% withdrawal, 11.8% birth control pill and 3.9% reported using no method. The frequency of the students who had HPV vaccine is 1.1% (n=727).

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Table II. *Weight status, body perception and eating behaviors*

		n	%
Body Mass Index (n=592)	Underweight	87	14.7
Mean ±Standard Deviation: 21.55 ±3.28	Normal	430	72.6
	Overweight	62	10.5
	Obese	13	2.2
Body perception (n=769)	Slender	149	19.4
	Normal	423	55.0
	Overweight	133	17.3
	Fat	64	8.3
Number of meals (n=769)	One-two meals	202	26.3
	Three-four meals	546	71.0
	Five or more	21	2.7
Skipping meals (n=769)	Yes	576	74.9
	No	193	25.1
Reason for skipping meals (n=605)	I do not feel like eating	210	34.7
	I think that I cannot find healthy food that I like	102	16.9
	Dieting, want to be thin, skipping meals will lead to weight loss.	47	7.7
	I do not have time	246	40.7
Number of days you had breakfast last week (n=769)	One-three days	161	20.9
	Four-seven days	589	76.6
	Never had	19	2.5
How many times did you drink 100% fruit juice like orange, apple or grape juice in the last 7 days? (n=761)	100% fruit juice in the last 7 days	493	64.8
	I drank 1-6 times in the last 7 days	268	35.2
How many times did you eat fruit in the last 7 days? (Fruit juice does not count), (n=768)	No fruit in the last 7 days	44	5.7
	I ate 1-6 times in the last 7 days	464	60.4
	I had 4 or more a day	260	33.9
How many times did you eat green salad in the last 7 days? (n=769)	No green salad in the last 7 days	124	16.1
	I ate 1-6 times in the last 7 days	511	66.5
	I had 4 or more times a day	134	17.4
How many times did you eat a vegetable dish in the last 7 days? (Green salad, potatoes or carrots do not count) (n=768)	No vegetables in the last 7 days	89	11.6
	I ate 1-6 times in the last 7 days	576	75.0
	I had 4 or more times a day	103	13.4
How many times did you drink energy or sports drinks in the last 7 days? (n=765)	I did not drink any energy or sports drinks in the last 7 days	728	95.2
	I drank 1-6 times in the last 7 days	37	4.8
How many glasses of water did you drink in the last 7 days? (n=766)	I drank a few glasses of water in the last 7 days	11	1.4
	I drank 1-6 glasses in the last 7 days	67	8.8
	I drank 4 glasses or more a day	688	89.8
How many glasses of milk did you drink in the last 7 days? (you can count box milk or milk you had with corn flakes), (n=768)	I did not have any milk in the last 7 days	354	46.1
	I drank 1-6 glasses in the last 7 days	332	43.2
	I drank 4 glasses or more a day	82	10.7

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Table III. *Comparison of independent variables according to the Eating Attitudes Test cut-off point*

	Eating Attitudes Test scores				x^2	p
	< 30		≥ 30 (n=69; 9%)			
Sex (n=770)	n	%	n	%		
Females	535	90.7	55	9.3	0.403	0.52
Males	166	92.2	14	7.8		
BMI (n=534)						
Underweight	77	88.5	10	11.5	4.12	0.39
Normal	393	91.4	37	8.6		
Overweight	52	83.9	10	16.1		
1st degree Obesity	9	90.0	1	10.0		
2nd degree Obesity	3	100.0	0	0.0		
Frequency of eating vegetable dishes in the last 7 days (n=768)						
I never ate any	79	88.8	10	11.2	7.58	0.02
I had 1-6 times in the last week	533	92.52	43	7.5		
I had 1-4 times daily in the last week	87	84.5	16	15.5		
How many glasses of milk did you drink in the last 7 days? (n=768)						
I never drank any	322	91.0	32	9.0		
I drank 1-6 times in the last week	309	93.1	23	6.9	8.28	0.01
I had more than 1-4 glasses daily in the last week	68	82.9	14	17.1		
How many times did you drink energy-boosting sports drinks in the last 7 days? (n=765)						
I never drank any	670	92.0	58	8.0	22.12	0.00
1-6 times in the last 7 days	26	76.5	8	23.5		
1-3 times a day	1	33.3	2	66.7		
Smoker (n=749)						
Yes (n=167; **%22.3)**	144	86.2	23	13.8	5.76	0.01
No (n:582; %77.7)	537	92.3	45	7.7		
Chronic disease (n=763)						
Yes (n=74; % 9.7)	66	89.2	8	10.8	0.311	0.57
No (n=689; %90.3)	628	91.1	61	8.9		

Table IV. *Factors associated with "tendency for eating behavior disorder"Eating Attutitudes Test (EAT≥30)*

	B	SE	Exp (B)	95% Confidence Interval	p value
Smoking status					
No (Ref)	0.628	0.286	1.875	1.070–3.286	0.028
Yes					
How much milk did you drink in the last 7 days?					
More than 1-4 glasses daily in the last week (Ref)					
Never	-0.438	0.390	0.645	0.300-1.386	0.261
1-6 glasses in the last week	-0.747	0.405	0.065	0.214-1.047	0.065
Frequency of eating vegetable dishes in the last 7 days					
1-4 times daily in the last week (Ref)		0.452			
Never	-0.239	0.336	0.737	0.324-1.911	0.597
1-6 times in the last week	-0.725	0.446	0.484	0.251-0.936	0.031
Frequency of drinking energy-boosting sports drinks in the last 7 days					
Never (Ref)					
1-6 times in the last week	1.190	1.285	3.286	1.371-7.876	0.008
1-3 times daily in the last week	2.539		12.66	1.021-157.216	0.048
Hosmer & Lemeshow test: x^2 =8.406, p=0.135, Cox & Snell's R^2 =0.036, Nagelkerke's R^2 =0.080					

The model includes: smoking status, frequency of eating vegetable dishes, amount of milk consumed, frequency of drinking energy-boosting sports drinks, in the last 7 days.
Logistic regression coefficient (B), the standard error (SE)
This is the exponentiation of the B coefficient, which is an odds ratio: Exp (B)

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Table V. *Driving, smoking and drinking behaviors*

Variables		n	%
Frequency of buckling a seatbelt in the passenger seat (n=768)	Never	26	3.4
	Rarely	119	15.5
	Sometimes	127	16.5
	Usually	282	36.7
	Always	214	27.9
Frequency of texting on a cell phone while driving in the last 30 days (n=225)	Never	99	44.0
	I texted at least one day within the last 1-30 days	18	8.0
	I did not drive in the last 30 days	108	48.0
Tried out smoking? (n=768)	Yes	386	50.3
	No	382	49.7
Age of trying out (n=719)	I never tried to smoke	328	45.6
	I tried it out between the ages of 8-16	208	28.9
	At the age of 17 or older	183	25.5
Tried to quit using any kind of tobacco product in last 12 months? (n=581)	Yes	89	15.3
	No	137	23.6
	I did not use any kind of tobacco product in the last 12 months	355	61.1
Smoking status in the past 30 days (n=765)	Never	615	80.4
	1-9 days	82	10.7
	10-19 days	12	1.6
	20 days or more	56	7.3

Table VI. *Physical activity behaviors*

Variables		n	%
How many days in the last 7 days did you spend at least 60 minutes on physical activity? (n=769)	Never did	370	48.1
	1-4 days	331	43.0
	5 days or more	68	8.8
How many days in the last 7 days did you perform any exercises such as push-ups, sit-ups or weightlifting that challenged or strengthened your muscles? (n=768)	Never	566	73.7
	1-4 days	180	23.4
	5 days or more	22	2.9
How many hours do you watch television on school days? (n=768)	Never	457	59.5
	Less than 1 hour/1 hour	141	18.4
	2-4 hours	168	21.9
	5 hours or more	2	.3
How many hours do you spend watching videos that are not related to your school work or playing computer games on school days? (n=769)	I do not watch videos or play computer	65	8.5
	games or I do not use the computer for	64	8.3
	anything besides school work	534	69.4
	Less than 1 hour/ 1 hour	106	13.8
	2-3 hours		
	4 hours or more		
How many times in the last 12 months did you play in a sports team? (School or extracurricular teams can be counted) (n=766)	Never	645	84.2
	1-2 times	55	7.2
	3 times or more	66	8.6

4. DISCUSSION

In this study, the health behaviors of freshman health sciences students were studied and found that these individuals engaged in many risky health behaviors, leading to the conclusion that there was a need for intervention. Risky behaviors with the highest frequency were nutritional issues such as skipping meals and insufficient consumption of fruits and vegetables, as well as perceiving oneself to be overweight despite being of normal weight, the use of tobacco, and physical inactivity. As adopting risky health behaviors in university years is common, an important health challenge is to instill obesity awareness and encourage the implementation of preventive approaches at an early stage [16]. In a multi-site study with university students from developing countries in different continents, it was reported that prevalence of being overweight or obese was quite high (24.7% in male and 20.3% in male students) [17]. In this study, prevalence of being overweight or obese was reported to be 23.9% in male and 13.6% in female students. Another study from Turkey in 2016 reported the prevalence of being overweight or obese as 27.5% in male and 21% in female students [18]. However, in more recent studies the prevalence

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of being overweight or obese in Turkey was much higher (51.7%) suggesting an increase in prevalence of obesity [19]. The percentage of students of normal weight (72.6%) in our study is similar to the results of Dülger and Mayda's study but considerably higher than those of Özkan et al.[19].On the other hand, the percentage of our overweight or obese students is 12.7% (obese 2.2%; overweight 10.5%). The 2019 Turkey Health Survey reported that 21.1% of individuals over the age of 15 were in the obese category, also stated that 30.4% of women and 39.7% of men were overweight [20]. The fact that the obese and overweight percentages of our participating students were lower than the average of Turkey might be explained by their freshman status and that they have left their homes and families to live by themselves for the first time. Young people living with their parents may be consuming more of the home-cooked meals that are so much a part of our country's healthy eating culture. In fact, 75% of the participants reported that they had eaten a vegetable dish only 1-6 times in the last seven days. Although, students tend to prefer menus that simulate home-cooked cuisine, many of them live in dormitories and student residences, which provide easy and faster access to the various choices of the fast foods available in the city culture. Additionally, this may be the first time these students oversee their own nutrition. In this respect, we noted another important problem, which was that 9.0% of the students in our study appeared to be prone to developing eating disorders (Table III). Drinking energy-boosting sports drinks 1-6 times in the last week was 3.286 more likely to have "tendency for eating behavior disorder", and this for smokers were 1.875; for those eating fewer vegetable dishes were 0.484. Due to the nature of the cross-sectional study, a cause-effect relationship cannot be established, but it is known that smoking for weight control is prevalent among adolescents and energy drink consumption is associated with weight loss attempts, poor body image, therefore, there may be a possibility that smoking and energy drink consumption have risks for an eating disorder [21, 22]. Researches about interventions on smoking cessation, encouraging non-smoking behavior and healthy eating to prevent eating disorders may be of interest.

When these factors are considered, it can be surmised that the risk of gaining weight or developing eating disorders may increase among university students and it is important that students are monitored in this context. Another issue is the matter of body image. Although, 72.6% of the students were of normal weight, only 55% perceived themselves to be so. In addition, only 12.5% could be categorized as overweight or obese however 25.6% of the students perceived themselves to be overweight or obese. In the Health Behavior in School aged Children (HBSC) a World Health Organization collaborative cross-national study (2017/2018), 21% of the students were overweight or obese but 27% of them perceived themselves to be overweight or obese [23]. In today's society, being thin is associated with being beautiful and in some cases, this belief can lead to various pathological conditions such as eating disorders [24]. A study in which university students were evaluated in the context of the relationship between their body image and BMI indicated that 29.6% and 15.8% of underweight and normal women, respectively, perceived themselves to be overweight; these percentages were 7.7% and 1.3%, respectively, in men. Similarly, in an international study conducted in 22 countries with 18,512 university students, it was reported that women more than men perceived themselves to be overweight [25]. It was seen that the counselling would be necessary to help participants gain awareness of their body perception.

Majority of the participants displayed unhealthy nutritional habits that included skipping meals, eating insufficient fruits, and drinking sodas. Similar to our study, Dülger and Mayda reported that 20% of university students were not in the habit of eating breakfast and that 5.2%-35.5% skipped at least one meal during the day and the reasons for skipping meals were given as being late (36.2%), not wanting to eat (27.5%) and having the habit of eating regular meals (18.5%) [18]. In the present study, consistent with the literature, the most common reasons for skipping meals was not having time to eat and not feeling like eating. It can be said that our participants were deficient in their habits of nutrition, with skipping meals being a common practice, and were thus at risk (Table II). Interventions such as providing healthy breakfast options at school might be a solution and need to be studied.

The students in the present study were eating daily portions of fruits and vegetables but it was noted that the degree of their consumption was not sufficient. Approximately one third of the students had eaten four or more fruits in the last seven days, and only 13.4% ate vegetables Similar results have been reported in other studies in the literature, where it has been noted that only 10%-20% of students eat five or more portions of fruit a day [26, 27]. According to the Youth Risk Behavior Survey of the Centers for Disease Control and Prevention, 41.8% of students had eaten fruit, 40.7% had eaten vegetables <1 time/day [28]. In a study, the authors reported that 33.7% of the participants had five portions or more fruit and vegetables daily in the last one month [29]. The participants of our study however were eating more fruit than in other studies, however their consumption of green salad and vegetables was comparable to what was reported in the same studies. The higher consumption of fruits may be attributed to the fact that fruits and vegetables are abundantly produced in Turkey, fruits are relatively less expensive and more readily available to the students at dormitories and school cafeterias. On the other hand, even this data shows that a substantial portion of young people are not filling their recommended quota of consuming 5 portions a day of fruit and vegetables. Similar to the results of other studies, in our study even these students studying health-related education are not sufficiently nourished as recommended by nutritional guidelines [30-32] .

Traffic accidents make a 2% contribution to the global burden of disease. The frequency of driving increases when students start the university education, mainly a result of factors such as having to travel long distances, troubles with finding a parking place, the diversity of options available for transport, and the desire to be in close proximity to peers [31, 33]. Wearing seat belts significantly reduces the mortality in traffic accidents. [34] According to Road Traffic Regulations in Turkey (Article 78 of the Highway Traffic Law No. 2918 and Article 150 of the

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relevant Regulation), it is obligatory for drivers and passengers to use seat belts. Therefore, there are penalties for not using a seat belt. However, a concerning finding of our study was that approximately one-fifth of the students reported that they never or rarely used a seat belt [35]. In addition, 8.0% of the students said that they texted while driving at least once in the last month. In the light of the fact that using cell phones while driving is one of the most alarming causes of accidents, it was less alarming enlightening to find that participants exhibited a low frequency in this respect. There are no any arrangements about texting while driving in Turkish laws.

Smoking remains to be a major health problem in Turkey. We found that only 77.7% of our students had not smoke free in the last month. In contrast to our findings, using some form of tobacco was reported to be 88.2% as the result of a study conducted with university students of different disciplines [36]. A look into studies with students on health sciences campuses such as ours revealed that the frequency of using some form of tobacco was reported as 65.7% by Çifçi, et al. [37], 95.3% by Kendir, et al. [38], and 80.9% by Nacar, et al. [39]. A study reports a non-smoking frequency of 93.6% among students at Health Sciences Departments in the U.S.[40]. Our finding is somewhat greater than what Çiftçi et al., report, but less than the results of other studies [37]. Almost half of the students in our study (49.7%) had never smoked. Students in our country who never tried smoking were found to be 35.9% by Değer, et al. [37], 54.9% by Şahiner, et al.[41] and 75.2% by Tin Arslan, et al. [42]. On the other hand, 23.0% of our participants were current smokers. Turkey Health Survey [20], reported 28% smoking for individuals over the age of 15. The frequency reported for university students in Turkey varies between 20.6% by Oğuz, et al. [43] and 11.4% by Aksoy Kartci, et al. [6]. In the U.K., this frequency of smoking was reported as 14% [44], 17% among university students in the US. [45]. While our young participants' smoking behavior was similar to the results of other Turkish studies, it is at a higher level than the U.K. and U.S. studies but less than what was reported in Egypt. In Turkey, a comprehensive tobacco control policy has been implemented. Smoking at some indoor public places, all kinds of advertisement of tobacco products and selling tobacco products to youth less than 18 years of age were banned by the Law on Preventing Harms of Tobacco Products, No. 4207 [46]. However, despite comprehensive tobacco control policies, it has been reported that there has been an increase in the consumption of tobacco products in Turkey in recent years [20]. Nevertheless, although the percentage of individuals who have tried to quit smoking is already as low as 15.3%, young people's efforts to achieve smoking cessation should be valued and supported (Table V). At the same time, more effort should be undertaken to prevent tobacco use.

An association between physical inactivity and not only various chronic illnesses including depression and anxiety but also mortality rates has been well established. To ensure the maintenance of a healthy lifestyle, the World Health Organization recommends at least 150 minutes of moderate exercise or 75 minutes of vigorous physical activity weekly. [47]. In this context, the results of our study show that 48.1% of the students had remained insufficiently inactive in the last week, meaning that levels of physical activity are not in line with the WHO recommendation [47]. The majority of the participants did not perform any resistance exercises for muscle power and participation in physical activity and exercising was markedly low. We investigated another risk factor, the sedentary lifestyle, in connection with the physical activity in our study to find how much time the students spent for watching television or using computer for non-classwork-related content. The majority of the students reported that they never watched television at all or less than 1 hour on schooldays (77.9%), and spent less than 4 hours with the computer or played video games (86.2%) on those days (Table IV). In a cross-sectional study conducted in 24 countries, including Turkey, with 12,492 university students; 77.5% of the participants reported spending at least four hours of sedentary activity on schooldays [5]. So, we can conclude that the level of sedentary activity among the students in our study was not quite as high. Nevertheless, the students did not engage in physical activity at the desired level and that most lived a sedentary life. As a result, their risk of developing significant health problems early on in their adult life was high and therefore it would be imperative to raise their awareness and motivate them to be physically active (Table VI).

Fifty-four individuals (7.4%) in our study revealed that they engage in sexual activity. In other studies, conducted in Turkey; the frequency of having sexual experience was reported to higher among university students studying in health sciences or other than health sciences faculties [48-50]. The prevalence in our study was relatively low. A possible reason for the low prevalence might be that our participants were freshmen. In the study (2017/2018), at age 15, one in four boys and one in seven girls report having had sexual intercourse and less than two thirds (61%) of sexually active adolescents used a condom at last intercourse (Table VII). The participants of the HBSC were selected by cluster sampling a proportion of young people aged 11, 13 and 15 year. But Turkey was not included HBSC 2017/2018 study. Comparing the data, we can conclude that although high ages of the study population, prevalence of sexual intercourse (9.7%) and condom use (17.4%) was lower in our study population than the HBSC 207/208 study [23]. One of the most concerning results of our study was that only 1.1% of the young people had been vaccinated for HPV. Probable explanation of low vaccination rates might be the fact that HPV vaccine is not included in the expanded immunization program of Ministry of Health, thus not provided free of charge thereby forcing families to meet the expense with their own resources, which is deterrent since the cost of the vaccine is indeed considerably expensive. Another reason might be that there are only scant recommendations about the benefits of the vaccine. In a study in Turkey conducted with the participation of 348 family health physicians and 317 pediatricians, it was reported that this vaccine was recommended by only 15.8% and 12.7% of these professionals respectively [51].

According to our findings, there is a need to improving the students' healthy lifestyle. Physical activity, recreation and

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time spent outdoors are vital parts of a healthy lifestyle. But there are not enough areas in the Campus that the students might use for recreational activities yet. The Health Sciences Campus of our university that the study performed on has been designed as the most comprehensive health campus in Turkey with a land area of 182.000 m^2. Currently, the construction of spaces for recreational activities continues. Apart from student canteens, libraries and walking areas where they can make sports and spend their leisure activities on campus, there are no social areas yet. There are several schools in the campus each of them different times for recreational activities. There are elective courses, generally two hours a week, embedded in the curriculum of the schools. In addition, there are various student clubs on a variety of topics with voluntary involvement offering opportunity for recreational activities. Therefore, we suggest that increasing recreational acrtivities in the Campus that the study performed on, might be helpful to students improve healthy lifestyle behaviors. The courses related with health prevention and promotion can be added to the curriculum of health sciences and medical faculty students.

Limitations

Due to the study had a descriptive design, the limitations of the study are difficulty make a causal inference, susceptibility to biases such as nonresponse bias and recall bias and it is not possible to analyze the factors that affect risky behaviors over time. Since the study was conducted with the students of at a state university, the results of the study cannot be generalized to students of other universities. In addition, the fact that the use of tobacco products other than cigarettes were not asked can be considered as one of the limitations of the study.

Conclusions

The percentage of those who perceive themselves to be overweight or obese is higher than the percentage of individuals who are actually overweight or obese.

There is a tendency toward eating behavior disorder in 8.9% of the participants. Factors that exacerbate this tendency are smoking, not eating vegetables, and drinking energy-boosting sports drinks.

It was found that a large majority of the students skip meals or do not eat breakfast regularly. The fruits and vegetables intake of many young people is below the daily recommendation of 5 portions; the percentage of students who did not have any fruits and vegetables in the last week is dramatically high. It was seen that the students did not engage in physical activity at the desired level and that they lived a sedentary life.

Almost half of the students had tried to smoke at some point. Of our participants, 22.3% were currently smokers.

The matter of sexuality is still a subject that is hard to discuss in this population and therefore data collection in this area is difficult.

The number of students who had taken an HPV vaccination is negligible.

Therefore, we can conclude that nutritional issues, negative body weight perception, use of tobacco, and sedentary lifestyle are the main intervention and counselling areas for our study group.

Compliance with the Ethical Standards

Ethical Approval: Ethical approval for the study was obtained from Marmara University Faculty of Health Sciences Non-interventional Research Ethics Committee (Approval No. 29.08.2019/90).

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

Authors' Contribution: KG, AC, BC, EG and MGP: Design of work, KG,BC,CA and EG: Data collection, SE and EG: Analysis of data, SE, CA, SC and KG: Interpretation of data, KG, CA, SC, SE, ID, AYO and MGP: Preparation of manuscript, KG, CA, SC and SC: Drafting the work. All authors read and approved the final manuscript.

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Being a mother as a healthcare professional in the COVID-19 pandemic: A qualitative study

Nesibe GUNAY MOLU[1], Sadiye SERT[2], Neslihan DURMUSOGLU SALTALI[3]

[1] Department of Mental Health and Nursing, Division of Nursing, Faculty of Nursing, Necmettin Erbakan University, Konya, Turkey
[2] Child Health and Pediatrics Clinic, Konya Beyhekim Education and Research Hospital, Selcuklu, Konya, Turkey
[3] Department of Child Development, Faculty of Health Sciences, Necmettin Erbakan University, Konya, Turkey

Corresponding Author: Nesibe GUNAY MOLU
E-mail: nesibegunaymolu@gmail.com

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ABSTRACT

Objective: The aim of this research was to determine the changes in relationships between healthcare professional mothers and their preschool-aged children during the coronavirus disease 2019 (COVID-19) pandemic. In addition, the second objective of this study was to propose a new phenomenon that explains "being a mother as a healthcare professional" during the COVID-19 pandemic.

Materials and Methods: The participants of the research were 16 healthcare professional mothers (8 doctors and 8 nurses) who had worked in intensive care units during the COVID-19 pandemic and had a preschool-aged child. The research was conducted in accordance with the phenomenological approach, one of the qualitative research designs. The research data were obtained through face-to-face interviews between the researchers and the participants using half-structured interview forms prepared by the researchers. Colaizzi's 7-step method was used for evaluation of the data.

Results: According to the research findings, the phenomenon of "being a mother as a healthcare professional" was gathered under four main themes: emotional reactions, new normal in life, difficulties that pandemic brought in life and coping strategies with these difficulties.

Conclusion: The findings showed that the COVID-19 pandemic caused many changes in the lives of healthcare professional mothers and their children; these mothers and children built some emotional reactions, and they developed various strategies to overcome these emotional reactions.

Keywords: Pandemic, Mother-child relation, Healthcare professional, Preschool-aged children

1. INTRODUCTION

Originating in the Chinese city of Wuhan in 2019, the coronavirus pandemic has affected the entire world by spreading rapidly. Pandemics are an important crisis period that not only affects the physical health of individuals but also has the potential to affect the psychological health and well-being of individuals. Regardless of age, race, gender and socioeconomic conditions, the COVID-19 pandemic period has also affected the lives of all people in various aspects.

The pandemic has brought about many changes in people's habits, behaviors, social relationships, daily routines and interfamily relations [1,2]. One of the factors that the pandemic has changed is business life. During this period, some people lost their jobs, some had to work flexible hours, and the workload of others increased greatly [3]. The most significant occupational group in which the pandemic has changed working conditions and increased workload is healthcare professionals. Studies have shown that healthcare professionals, especially those working on the front line with COVID-19 patients, have a greater risk of mental health problems such as anxiety, depression, and insomnia [3]. It is also thought that these risks are bound to bring about some changes in the other roles of healthcare professionals, such as husband, wife, mother and child. In fact, in his ecological systems theory, Bronfenbrenner (1992) emphasizes that changes in the business life of the parent may also have some effects on the development of the child (as cited) [4]. In an evaluation carried out by Zeynepoglu-Akbas and Dursun [5], it is emphasized that the responsibilities of working women in Turkey increased in that period, as they did not receive

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professional support or family support about some issues such as childcare, education and housework that they can perform at normal times. In a study carried out among 5566 families by Southampton University to examine the effects of the pandemic period on mother-child relations, most of the mothers spending this period together with their children at home were proven to develop positive relationships with their children [6]. In addition, in a study conducted by Evans et al., [7] among 2130 Australian families having children between 0-18 years old, it was suggested that the pandemic brought different changes in different family structures; that is, it affected some families negatively in mental aspects while it strengthened interfamily relations in the others. The variations in study results make one think that mother-child relations in the pandemic period may be affected differently depending on some factors, such as the mother's job, her working conditions after the pandemic and social gender roles. Healthcare professional mothers are the most affected group among working mother groups from this process. Our observations are that a new phenomenon called "being a mother as a healthcare professional" has emerged in this process. From this point of view, by examining the changes that being a healthcare professional mother can bring in mother-child relationships, the phenomenon of healthcare professional mothers has been explained.

2. MATERIALS and METHODS

Research Design

This research was carried out in accordance with Colaizzi's phenomenological approach, one of the qualitative study patterns. This method focuses on the experiences of participants and finds the shared patterns rather than individual tendencies in research subjects [2]. The pandemic process is also a period in which the normal flow of life changes and people's lives are heavily affected. In this study, the phenomenological approach was preferred since the changes in the lives of female healthcare professionals and their relationships with their children during the pandemic process were examined.

Participants

The participants of this research are 16 healthcare professional mothers—8 doctors and 8 nurses—who have worked in the COVID-19 intensive care unit at different hospitals in Konya province. These participants were chosen based on certain criteria and using a purposive sampling method, which is often preferred in qualitative studies [8]. The first of these criteria was working in the COVID-19 intensive care unit since the beginning of the pandemic period. In an effort to determine the exact effects of the pandemic, going on for the last 9 months in Turkey, on interfamily relations, mothers who have been working in intensive care units since the beginning of the pandemic were chosen. The second criterion was having a child between 3 and 6 years old. Preschool children were included in the research since they need more mother care than school children do [9]. Additionally, in this research, undergoing psychological treatment, exposure to a crisis, losing a relative, divorcing, and living away from husband were accepted as criteria for exclusion from the study, as these may affect the individual psychology of the healthcare professional and result in changes in mother-child relations other than the aim of this research. The study was applied with 16 participants, with equal numbers from doctors (n=8) and nurses (n=8), regarding the studies suggesting approximately 5-10 participants for the phenomenological approach [10]. Another criterion for choosing the participants was being a volunteer. The participants were named doctors D1, D2... and nurses N1, N2. Descriptive information about them is presented in Table I.

Table I. *Descriptive data of the participants*

	Age	Seniority	Is the spouse a healthcare professional	Child Gender	Child Age	Number of children	COVID transmission status
D1	42	18	Yes	Male	5.5	3	-
D2	30	6	Yes	Female	3.5	1	-
D3	38	13	Yes	Male	3.5	2	+
D4	36	12	Yes	Male	5.5	2	+
D5	35	10	Yes	Male	6	2	-
D6	38	13	Yes	Female	4.5	2	-
D7	36	10	Yes	Female	4	2	-
D8	42	15	No	Female	6	2	-
N1	42	20	No	Female	4.5	3	-
N2	33	7	No	Female	6	2	+
N3	36	12	No	Female	6	3	+
N4	40	17	Yes	Male	5	2	+
N5	35	12	No	Female	5.5	2	-
N6	32	10	No	Male	4	1	+
N7	38	17	No	Female	4.5	2	+
N8	33	11	No	Female	5.5	1	-

D: Doctor, N: Nurse

The participants' average age was 36.62±3.66 years (mean±standard deviation). Their average length of service was 12.68±3.89 years (mean±standard deviation). The children's average age was 4.96±0.9 years (mean±standard deviation). Seven participants had COVID-19 infection before. Eight participants had healthcare professional husbands as well. Six of the children were boys.

Interview Form

Interview forms prepared by the researchers were used to collect data for the study. Only those healthcare professionals who had the necessary participation requirements and those who had no exclusion criteria took part in these interviews. This form contains some questions to obtain personal information about the mother and child, and it also questions the changes in the behaviors of both of them in this period and their coping strategies. During the interviews, the following questions were asked of the participant:

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1. What kind of changes did the pandemic period bring to your and your child's routines?
2. How did the pandemic period affect your child in terms of psychological and behavioral aspects?
3. How did the pandemic period change your mother-child relationship?
4. Which strategies did you use to cope with it when you had hard times during the pandemic period?

When necessary, inquiry questions were also put into practice to deepen the answers and obtain more detailed information.

Data Collection

Qualitative research seeks to understand why people think and feel the way they do. It is the researcher's responsibility to seek to understand the feelings and thoughts of study participants through qualitative research. In this case, it is challenging since it involves asking individuals to discuss personal information. As a researcher, one of the most important responsibilities is to protect the participants and their personal information [11]. Mechanisms to protect participants during the research (e.g., by keeping their identities confidential) were carefully explained to the participants. The interviews with the participants during the data collection process were conducted face-to-face in an environment outside the hospital. Considering the pandemic conditions, interviews with officials were planned as one-on-one and single recordings. Each interview lasted between 20-30 minutes on average (mean=26.54; Sd=3.42). These interviews were recorded with a voice recorder.

Ethical Process

The ethics committee affirmation for the research was taken with Ordu University's decision number 2020-101. In addition, the participants declared their voluntary participation verbally prior to the interviews. They were also informed that they had the right not to answer the questions they thought to be ethically unsuitable or withdraw from the interview at any phase.

Data Analysis

Phenomenology is a research method intended to explore the experiences of people as they live in different phases of their life and their meanings. When evaluating the research data, Colaizzi's seven-step interpretation method was benefited from [12]. First, each voice record was listened to and recorded in written form (each interview generated ten to twelve pages of written documentation). The transcription of each interview took approximately four hours on average. Then, this written record was read carefully, and the meaningful points were underlined with colored pens with the aim of understanding them conceptionally. As the next step, the significant expressions were determined regarding deeper meanings. These expressions were made meaningful and formulized by the researchers. In the meantime, an experienced researcher in the field of qualitative studies, other than the researchers of this study, was asked to state her thoughts so that the validity of these meanings could be strengthened [13]. Soon after that, similar expressions were put together under certain themes. Finally, considering theme-content convenience, these themes and the categories under them were examined in detail by both the researchers of this study and the experienced researcher supporting it from outside to increase the validity. In qualitative studies, validity is fulfilled through the researcher's monitoring of the subject matter as objectively as possible [14,15]. One of the main principles of increasing validity is transmissibility [16]. Transmissibility in this research was attempted to be fulfilled by quoting samples of various views from the participants. The flow chart of the research is presented in Figure 1.

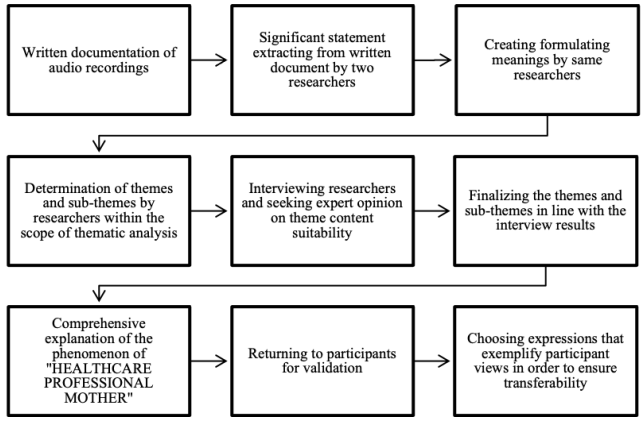

Figure 1. *The processes of qualitative analysis*

3. RESULTS

In this study, the qualitative results of children and mothers who work with COVID-19 patients in intensive care units and have children between the ages of 3-6 and how they are affected are revealed, and the phenomenon of being a healthcare professional mother is explained. As a result of the analyses, four main themes related to the phenomenon of being a healthcare professional mother were obtained. Themes, subthemes and quotations are presented in Table II.

Theme 1. Emotional Reactions

Emotional reactions theme consists of the subthemes of fear and anxiety, desperation and exhaustion, anger and emotional relationship between healthcare professional mother and child. Sample expressions for each subtheme are shown in Table II.

Subtheme 1. Fear and anxiety: All mothers working in intensive care units or joining the work during the COVID-19 pandemic period had emotions of fear and anxiety (n=16). In particular, fear of infection (n=10), fear of getting the disease (n=6) and fear of death (n=5) were seen intensely. Furthermore, getting quarantined (n=3), infecting the elderly in family (n=6) and anxiety about dissolution of family due to deaths (n=7) were experienced with high percentages. Children's fear of losing parents (n=4) and fear of getting the disease (n=5) came to exist.

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Table II. *Main Themes, Subthemes, and Quotations Regarding Views and Perceptions of Mothers and Children*

Theme	Subtheme	Quotations
Theme 1: Emotional Reactions	Fear, anxiety (both mother and child)	M-I had fears that I would get covid and die. (D4)
		M-Both being a healthcare professional and a mother is very hard in this period. There is a fear of getting the disease and infecting family members. I would have never had a child if I had known this would happen. It is terrible to worry about them all the time. (D7)
		C-As the death numbers were announced, I began to fear that anyone could die as well (N4)
		M-I had a fear of carrying the disease. More than getting the disease myself, I was concerned about infecting others. (N8)
		C – Children developed a fear of what would happen if they died. It became tedious to stay at home all the time. They are so worried. (N7)
	Desperation Exhaustion (just mother)	"We cannot find solutions as the treatment gets longer, and this resulted in desperation" (D5)
		"I can hardly stand it since the death rate is so high. I feel burnout. All my roles are half, mother, wife, doctor. I can reach none of them." (D1)
		"Seeing ex all the time affects me deeply. I am exhausted because of COVID-19." (D6)
		"Physically and psychologically, I have become fatigued." (N2)
		"The patients say they will die, and soon they are dying, which shocks me. I feel burnout. I did everything, but it was not enough. I was a healthcare professional before COVID. However, I had never felt such exhaustion." (N1)
	Anger (both mother and child)	C – The children have a great deal of anger, but they do not know exactly for whom they are angry. We are working intensively, which has caused them to become angry. (D1)
		C – It is very frustrating for my child not to be able to go outside. She is always asking when it will be over, and she will go to the park. (N2)
		M-I feel that I cannot catch up. When other children go out, I get angry at their indifference. (N7)
		M-I am so patient with children at normal times, but I am furious now. (N1)
	Mother-child relationship	"They have become more dependent on mothers. This dependence is like an obsession. They have a fear of losing. Mine even does not go out her room without me. She is always trying to hug me." (D6)
		"I stayed away children. I did not hug; I did not let them kiss me. I made them stay away." (N1)
		"I come home exhausted. It upsets them when I ask them to leave me alone after I have met their physical needs, so sometimes they cry and get angry with me. We are suffering from conflicts." (N7)
		"I used to play with them, but now I cannot as I come from work so tired. I have pricks of conscience." (N4)
Theme 2: New Normals in Life	Protective Measures	"I changed the room. I wear a mask at home. At home, I separated my personal belongings from other family members. ." (D3)
		"All the things that came from outside were cleaned. I have developed an obsession with washing hands." (D1)
		"We did not go out so often. We wandered in empty places. I did not visit my mom or anyone else. I became integrated with masks." (N4)
		"I take a shower whenever I come home. Frequently, I use a hand disinfectant to prevent. I put my clothes directly into the machine. We are careful about eating and using vitamins." (N6)
	Relational Changes	"We did not meet with anyone. We were isolated. Neither of us met the elderly in person. (D5)
		"As my husband is also a healthcare professional, one of us is always on the shift. Therefore, only one parent stays home, and we can hardly catch up with daily chores. We cannot spend enough time with our children." (D1)
		"As a healthcare professional, I feel discriminated against. Our neighbors have expressed unease toward us.." (N7)
		"We were treated by our relatives and neighbors as if we were plague." (N8)
	Changes in Child's Life	"Due to the increasing amount of time spent at home, he became accustomed to using a tablet computer. He even used it up to 7 hours a day." (D1)
		"He has been deeply affected by the absence of friends. Despite his desire to go, he knows he cannot do so. As a result, he has adopted the rules and has learned to play following the distance rules." (D4)
		"My child developed problems of pissing her pants." (N3)
		"She is bored with playing alone. She misses school and friends." (N5)
		"The pandemic changed our working conditions. My child was going to school. We have got stuck as the schools stay closed. Taking care of the child is problematic in this period. Even where she sleeps is uncertain; one day in her grandmother's house, the other at home. My child is protesting whether there is no one other than me." (N8)

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Theme 3: Difficulties that the Pandemic Brought in Life	Business Life	"Due to the increase in workload caused by colleagues' occasional exposure to COVID, I am mentally fatigued." (D4)
		"Working conditions were overwhelming. Working in overalls was hard and negative. I am so tired." (N3)
		"While the other sectors are working flexible hours, healthcare professionals are extremely working; no one is considering our motherhood, and all our efforts are almost in vain financially and spiritually, which has affected us negatively." (N8)
		"Our colleagues are dying, and we can do nothing about it. We cannot even take our annual leaves. We are suffocated." (N7)
		"Occupational injustice is making me sad. No one realizes what we are doing, which makes me sad. I love my job. Nevertheless, I would not like to be a healthcare professional during this period. I think that healthcare professional mothers are so exhausted during this period." (N6)
	Interfamily Relationships (Between partners, child)	P-A crisis breaks out even when one has a running nose a home. (D5)
		C-Either father or mother is not around. The whole family routine has been corrupted. (D1)
		P-We had some tense moments at home. The children had a temper tantrum. They questioned why they could not while their friends saw each other. (N5)
		P-I had arguments with my husband. It has become a habit for him to warn me not to enter the room without an overall. As a result, he is burdened with a great deal of conscience. (N5)
		P-She is having conflicts with her elder sister, and she has become aggressive. She was aggressive beforehand, but not as much as she is now. (N7)
	Witnessing the Patients	"Increase in death rates and intubating especially young patients, after they said that they could not breathe and begged to be saved, was too heavy. Some died after saying they did not want to die, which made me terribly sad." (D4)
		"That the patients stayed alone for a long time without being visited by anyone affected me deeply." (D2)
		"Giving bad news continuously and seeing patients unable to breathe affected me so much." (D7)
		"Giving the news is too bad. They cannot say goodbye to anyone, and they are dying alone. I am deeply impressed." (N2)
Theme 4: Coping Strategies	Social Support	"My husband cannot take a break just like me. Grandmother is tired of caring for children. My family lives far away, and thus I do not have enough support."(D7)
		"I am talking with friends on the phone." (N1)
		"I am getting relieved while talking with my elder sister, even on the phone." (N2)
		"Sharing with colleagues, who do the same job, and similar stories are causing to normalize the situation. It is working." (N3)
		"My mother is already ill, so I could not share with her as she is frightened. My husband and my friends supported me more. I also talk to my elder sister." (N4)
	Tendency Toward Religion	"I am praying as much as I can. I am trying to think positively." (N6)
		"I am more inclined to spirituality.Salaat and praying are working well." (H4)
		"Reciting Qur'an is relieving me." (N7)
		"I am trying to preach myself. Praying is doing well, too." (N6)
	Negative Coping Behaviors	"I had shifts very often and got so tired. I went into the room and cried sobbingly." (D4)
		"I constantly gave table pc to children in order to keep them busy." (D5)
		"To deal with children, I always turn on the television. It is a mistake, but I have no other option since I am so tired. (N1)
		"I have excluded many things from my life. I got isolated at home not to infect anyone. I am no longer the person I used to be." (D1)
		"I feel trapped. I cannot relieve without crying." (N8)
	Activity-based Coping Strategies	"I took up a new hobby. I started making decorative objects." (N2)
		"We planned two-day holidays. We changed our environment." (D8)
		"I am attending online personal development training." (D2)
		"Reading book usually relaxes me." (N3)
		"I go for a walk in the outdoors." (N4)

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Subtheme 2. Desperation and Exhaustion: Another subtheme is the experience of desperation (n=8) and exhaustion (n=11) emotions. In particular, the emotion of desperation was determined to result from the high percentage of deaths from the disease, patients' respiration problems, and their inability to respond to treatments. Exhaustion, on the other hand, was connected with both physical and psychological causes.

Subtheme 3. Anger: Mothers (n=6) and children (n=11) also developed the reaction of anger. It was pointed out that anger in children aroused owing to the restrictions and mothers' intense working hours. Mothers stated that they developed the emotion of anger when children carried on demanding even after their physical needs were met and when they were not able to meet these needs. The outer causes of mothers' anger were determined as the community's not obeying the protective measures and their indifference to the disease.

Subtheme 4. Mother-Child Relationship: The pandemic period brought about some changes (n=12) in mother-child relationships. The most frequent changes were an increase in their dependence on each other and a reduction in shared time and physical contact, especially after working overtime.

Theme 2. New Normals in Life

The COVID-19 pandemic brought many changes with it, and as it lasted long, it was seen as suitable to name them new normals. Under this theme, there are three subthemes: protective measures, other relational changes and changes in children's lives.

Subtheme 1. Protective Measures: Protective measures (n=14) were stated as mask, distance, obeying isolation rules, increase in hygienic behaviors and food supplements (vitamin support).

Subtheme 2. Relational Changes: Relational changes (n=14) included behaviors such as deterioration of family relations and social life, social isolation, exclusion, and discrimination.

Subtheme 3. Changes in Child's Life: The changes in child's life (n=13) were education, nutrition, sleeping, caring and particularly playing behaviors. It was found that problems such as an increase in digital technology use and child-care problems were seen to a large extent. All these new normals in life were supposed to have caused some emotional changes.

Theme 3. Difficulties that the pandemic brought to life

Healthcare professional mothers' business lives (n=9), interfamily relationships (n=8) and difficulties resulting from witnessing the patients are subthemes of Theme 3.

Subtheme 1. Business Life: Business life, working conditions (protective clothes, working duration and frequency, inability to get day off, etc.), extra overtime due to colleagues being quarantined, affliction from healthcare professional deaths, insufficient occupational satisfaction, and lack of interest and support were all included.

Subtheme 2. Interfamily Relationships: It was also stressed that these changes in life routines created some changes in some interfamily relations, and they caused interfamily conflicts, emotional outbursts and insufficient communication.

Subtheme 3. Witnessing the Patients: The healthcare professionals, witnessing what COVID-19-diagnosed patients went through in intensive care periods, experienced various emotions at the same time. In particular, factors such as patients' inability to breath, increase in death rates, young patients, frequency of giving bad news, lack of hospital attendant, loneliness, inability to farewell and empathy with the dead patients were efficient in experiencing these emotional reactions.

Theme 4. Coping Strategies

The coping strategies theme consists of subthemes of social support (n=16), tendency toward religion (n=6), negative coping behaviors (n=6) and activity-based coping behaviors (n=7). Participants were seen to have defined the perception of social support either as sufficient (n=9) or insufficient (n=7).

Subtheme 1. Social Support: They stated that they had peer and partner support, and they were relieved when they shared common experiences with their healthcare professional friends. In addition, it was clearly understood that they could not obtain enough support from family because of social restrictions and that they could not share their feelings with family members (mother, father, sibling, etc.) so as not to increase their anxiety.

Subtheme 2. Tendency Toward Religion: The participants were relieved with religious tendencies such as reciting the Qur'an and praying.

Subtheme 3. Negative Coping Behaviors: They often exhibited some negative coping behaviors, such as ignorance, continuous crying, negative attitudes toward children, withdrawal and social isolation.

Subtheme 4. Activity-based Coping Strategies: Among the activity-based coping strategies were taking up a hobby (handcraft, making decorative objects, etc.), education, changing environment, walking, breathing exercises and reading.

4. DISCUSSION

In this research, the views taken from doctors and nurses about the changes that the COVID-19 pandemic brought in the relations of healthcare professional mothers with their children were evaluated, and the themes of emotional reactions, new normals in life, the changes it brought in life and coping strategies were obtained. It was determined that healthcare professional mothers developed a number of emotional reactions, such as fear, anxiety, exhaustion, desperation and anger, during the pandemic period. Similar to this study, among the psychological effects that pandemics create, the emotions of fear and anxiety stand out in the literature [2,17-19]. Getting the disease and infecting beloved ones are pointed to be experienced more intensively. Compared to the others, healthcare professionals have a greater risk for infection, as they are in contact with the patients in person [20]. In the studies carried out by Chowell et al., during the epidemics of SARS and MERS, it was indicated that one-fourth of the cases were healthcare professionals [21]. Being aware of the risk of infection, their colleagues getting infected and close contact with the patients were thought to

be efficient in healthcare professionals' fear and anxiety. Cai et al., also expressed that healthcare professionals have anxiety about infecting their loved ones during pandemics [22]. One of these emotional changes experienced during the pandemic was detected as desperation. It was thought that since the study group was chosen among those working in intensive care units during the pandemic and the cases in intensive care units were far worse than the others, these healthcare professionals experienced desperation for feeling an inability to do anything. This kind of desperation was estimated to have a role in developing the emotion of exhaustion over time, which was another finding. This finding is compatible with that Liu et al., obtained from their study that healthcare professionals experience exhaustion intensively during the pandemic period [3]. This feeling of exhaustion was thought to result from the intensity of work conditions during the pandemic period and a lack of physical and mental conditions. The healthcare professionals also stated that they went through anger as the emotional change. Bidzan et al., pointed out anger among the emotions healthcare professionals experienced during the pandemic, which is parallel to the results of our study [23]. It was interpreted that the individuals in society did not exhibit necessary sensibility and behaved indifferently during this period, which made the healthcare professionals angry, affecting them negatively in emotional ways.

The emotional reactions developed by children between 3-6 years old whose mothers were healthcare professionals were identified as fear, anxiety and anger. Children suffer from feelings such as fear and anxiety resulting from traumatic events that limit and aggravate life, such as natural disasters and pandemics, more intensively than adults do [24]. In our study, the factors that caused them to have fear and anxiety were revealed as the risk of getting the disease, fear of death and uncertainty of their cases in the event of losing their parents. In the meantime, mother's working so hard and getting tired, inability to go outside home and play with anyone came to the front as the main sources of anger. Very active in the preschool period and keen on exploring things freely [25], children were exposed to many restrictions owing to the pandemic. These restrictions violated or limited their right to play, which has a vital role in their development and education [26]. This situation was emphasized as a risk factor for creating negative effects on developmental and educational dimensions in the long run.

Problems, such as constant dependence on mother, mothers' staying away from children to protect them and inability to meet their needs due to heavy workload, broke out within healthcare professional mothers' relations with their children. Similarly, in a study conducted by Imran, Zeshan and Pervaiz, it was found that preschool children showed reactions in the pandemic period that they did not want to leave their mothers [27]. Children were also seen to react to these relational loses (S/he is protesting like 'Isn't there anyone else other than you?'). The early childhood period is a time when their relationships with their family are at the center of children's lives [25]. In particular, affiliation with the mother in this period is of great significance in a child's identity development [28]. Either secure or insecure affiliation behaviors hold valuable tips about children's emotional status as well as their mental health and human relations [29-31]. We must be aware of the risks that children carry.

Regarding the new normals that emerged during the pandemic period, it was concluded that healthcare professional mothers took necessary protective measures, went through relational changes and caused ups and downs in children's lives. Epidemics are events that have the potential to influence society in both physical and psychological ways [1]. Furthermore, they bring along some changes (hygiene, nutrition, social distance, etc.) in people's lives [2]. As determined in the study, the feeling of being discriminated against was one of the relational changes experienced by healthcare professionals. The Canada Center for Occupational Health and Safety declared that patients, their relatives, pandemic regions and healthcare professionals may have been exposed to blacklisting or discrimination [31]. Ertem stated that this blacklisting and discrimination in the pandemic period could cause the person to feel anger and threaten his/her psychological health [32]. Regarding these risks, it was supposed that by eliminating this feeling of being discriminated against, healthcare professionals must be ensured that they are not alone through integrative studies within societies.

The research results reached the conclusion that the pandemic led to a few negative changes in some children's lives, such as an increase in digital technology use, troubles sleeping (waking up in the middle of the night, nightmares, unwillingness to sleep) and bedwetting, while others exhibited adaptable behaviors for the period, such as playing alone and obeying the distance rules. The results in the literature that indicated that children experienced troubles sleeping in pandemic periods [33] and that the use of digital technology increased [34] are compatible with this research. These findings inferred that negative changes could come out at times when traumatic effects of the pandemic period on children were not taken under control. In addition, the healthcare professionals expressed that they had difficulty taking care of their children, could not catch a stability for this caring and things got worse during the school closure time. The findings made us suppose that the pandemic was challenging not only for the healthcare professionals themselves but also for their children, and it brought about traumatic effects.

It was concluded that the pandemic brought a number of difficulties for healthcare professional mothers about business life and interfamily relations, and witnessing the patient was another challenge for them. Negative aspects of business life, such as heavy working conditions, insufficient appreciation, losing colleagues and not having flexible working hours, similar to other occupations, were emphasized. The occupational group working the most intensively and in person with the patient are healthcare professionals. The increase in intensity at hospitals and inadequate numbers of healthcare professionals for working in turn are the main causes of occupational difficulties in this period [3]. Moreover, failure in family routines, tension between partners and corruption in relationships with siblings occurred. The results also showed that the pandemic resulted in distress in the interfamily relations of healthcare professionals.

Based on the study results, we developed a number of recommendations. First, support services must be provided for the healthcare professionals themselves, their partners and children during and after the pandemic period. We must find solutions to eliminate their problems with childcare. In addition, the number of healthcare professionals should be increased, and possibilities such as flexible hours and proper relaxing conditions should be supplied. Families must make necessary adaptations and create new opportunities for physical activity and games for children, whose area of freedom and right to play were restricted during the pandemic period. Moreover, precautions must be taken regarding children's increasing use of digital technology. We must make efforts to protect children's life routines as much as possible in crises such as the pandemic. To provide occupational cooperation with the aim of creating chances of sharing ideas and experiences, platforms in which healthcare professionals come together must be built.

Limitations

Since the sampling group of this study is those who spend more time with the patient, it is limited to doctors and nurses. Nevertheless, there are also other professionals in the health system. Another restriction of the study is that personal interviews are restricted to the data obtained from face-to-face interviews due to the risk of contamination and intense working conditions. However, the study could have been richer by performing focus group interviews.

Conclusion

According to the final results of the research, the pandemic period has revealed the "healthcare professional mother" phenomenon, which includes the changes that we consider in the form of emotional reactions, coping strategies, difficulties in life, and new normals in life. It came into light that, in terms of coping strategies, they benefited from a number of positive ones such as use of social support, tendency toward religion and activity-based ones along with negative ones used by some healthcare professionals. Their partners and colleagues stood out as the main sources of social support. Similarly, in a study performed by Sun et al., it was pointed out that nurses supported one another at most, stuck to team spirit and built joint power in the pandemic period [2].

Compliance with Ethical Standards

Ethical Approval: This research was approved by the Ordu University, Social and Human Sciences Research Ethics Committee (Approval number: 2020-101-143-05 and date: 23 December, 2020).

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Conflict of Interest: The authors have stated explicitly that there are no conflicts of interest in connection with this manuscript.

Authors' contributions: NGM: Created the work design, wrote the original draft, collected data, and wrote, edited, and revised the final draft, SS: Collected data, performed the formal analysis, selected the sample, and conducted the interviews, NDS: Collected data, interpreted the data, and edited the final manuscript. All authors approved the final manuscript.

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Effect of troponin I and coagulation parameters on mortality in COVID-19 patients

Meral DAG, Nilufer BULUT, M. Cagatay TASKAPAN

Department of Medical Biochemistry, Turgut Ozal Medical Center, Inonu University, Malatya-Turkey

Corresponding Author: Meral DAG
E-mail: meraldag27@gmail.com

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ABSTRACT

Objective: Our aim is to determine the levels of troponin-I and some coagulation markers (D-dimer, fibrinogen and International Normalized Ratio (INR)) in coronavirus disease 2019 (COVID-19) patients and to investigate the effects of these markers on mortality.

Patients and Method: It is planned as a descriptive, cross-sectional and analytical study. The study was conducted by retrospectively scanning the files of COVID-19 patients who applied to Inonu University Turgut Ozal Medical Center between 01.03.2020 and 31.12.2020. Levels of cardiac troponin I markers and coagulation parameters (D-dimer, fibrinogen and INR) were detected.

Results: The results of a total of 1858 patients were obtained. One thousand, three hundred and twenty-six patients with only troponin I and D-dimer results (Group 1), 606 patients with only troponin I and fibrinogen results (Group 2), and 1308 patients with only troponin I and INR results (Group 3) were included. Troponin I levels were significantly higher in all patients who died. 96.6% of the patients with high D-dimer levels died in Group 1, 85.5% of the patients with high fibrinogen levels died in Group 2 and 77.3 % of the patients with high INR levels died in Group 3.

Conclusion: Measurements of troponin-I and coagulation markers such as D-dimer, fibrinogen and INR can help predict clinical severity and mortality in COVID-19 patients.

Keywords: COVID-19, Troponin I, Coagulation markers, D-dimer, Fibrinogen, International Normalized Ratio.

1. INTRODUCTION

Coronavirus disease 2019 (COVID-19) has become a serious health problem since it was first detected in Wuhan Province, China in December 2019; caused a global crisis in terms of economic, sociological and psychological aspects. The World Health Organization (WHO) declared this epidemic "an international public health emergency" on January 31, 2020 [1].

Complications, such as septic shock, heart, kidney and liver damage, and clotting disorders are considered to be associated with COVID-19. Clotting abnormalities and prolonged prothrombin time were reported in 6% of the patients admitted to hospital with COVID-19 diagnosis, and kidney function failure was observed in 4%. It was emphasized in previous reports that most of the patients who died because of COVID-19 had cardiovascular disease (CVD), COVID-19 infection triggered myocardial damage and cardiac dysfunction, and increased morbidity and mortality [2]. Troponin I is a sensitive cardiac indicator that can be found in the blood up to 7 days after cardiac injury [3].

Although, there is no published case series until our present time, it was reported that there may be an increased risk for venous thromboembolism in COVID-19 patients, and abnormal clotting parameters are detected in severe COVID-19 cases. It was reported in a multicenter study that was conducted in China that especially elevated D-dimer levels and fibrin destruction products were associated with mortality, and 71.4% of patients who died because of COVID-19 showed common intravenous clotting criteria during the disease [4].

Excessive inflammation, hypoxia, immobilization and widespread intravenous clotting accompany this viral respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which can cause pneumonia. These conditions can make a patient become prone to venous and arterial thromboembolism, in this way, complicating the pathological condition further, and increasing the life-threatening risk. Various studies provided evidence that coagulation is a major cause of mortality in severe COVID-19 patients [5].

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In the COVID-19 pandemic, which affected the whole world, coagulation disorders and elevations in troponin I levels were frequently observed in patients. Heart damage in COVID-19 patients is associated with high mortality and can occur at any stage of the disease. These cardiac injury mechanisms in COVID-19 are also diverse, one of which is microvascular injuries [6]. Therefore, in our study, we aimed to investigate the effects of troponin I, a cardiac biomarker, and D-dimer, fibrinogen and international normalized ratio (INR) levels, which are indicators of microvascular damage, on mortality. The reason why we chose troponin I as the cardiac marker in our study is that the detection time in blood is longer than other cardiac markers.

2. PATIENTS and METHODS

This study was planned as a descriptive, cross-sectional and analytical study. The study includes patients who applied to Inonu University Turgut Ozal Medical Center between 01.03.2020 and 31.12.2020 and were diagnosed with COVID-19 by polymerase chain reaction (PCR) test and treated in the hospital. The cardiac marker troponin I and coagulation parameters D-dimer, fibrinogen and INR levels of these patients were screened retrospectively. When the data of COVID-19 patients whose troponin I, D-dimer, fibrinogen and INR values were examined from the hospital data recording system, a total of 1858 patient results were obtained. Since, relations between troponin I and, respectively, D-dimer, fibrinogen and INR would be examined, the levels of all three categorically were classified based on troponin I. In this classification, patients were evaluated according to their survival status. According to the results obtained from the hospital data recording system; There were 1326 patients with troponin I – D-dimer results (Group 1), 606 patients with troponin I-fibrinogen results (Group 2), and 1308 patients with troponin I-INR results (Group 3). In the data obtained, among 1858 patients with troponin I results; There were 1326 patients with troponin I and D-dimer levels, 606 patients with troponin I and fibrinogen levels, and 1308 patients with troponin I and INR levels. The figure for the categorization of the data is given below (Figure 1).

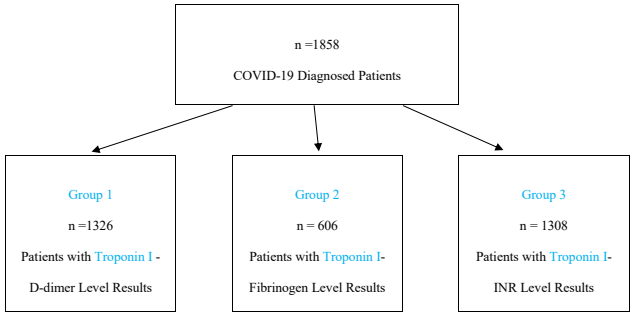

Figure 1. *Distribution of examined patients according to groups*

The test values of the patients with multiple troponin I, D-dimer, fibrinogen and INR results at first admission were included in the study. Troponin I levels were examined in Abbott brand Architect i1000 Autoanalyzer Device (Abbott Laboratories, Wiesbaden, Germany) with chemiluminescence method. The reference range for serum troponin I measurements was within the range of 0-34.2 pg/mL. D-dimer, fibrinogen and INR levels were analyzed with turbidimetric method in Sysmex CS-2500 fully automated device (Sysmex Corporation, Norderstedt, Germany). Reference ranges were accepted to be between 0-0.55 mg/L for serum D-dimer, 150-350 mg/dL for fibrinogen, and 0.8-1.2 for INR.

Statistical Analysis

Statistical Package for Social Sciences for Windows (SPSS) 20.00 (SPSS v. 22.0 software, Chicago, USA) program was used for statistical analysis. The descriptive statistics for continuous variables were summarized as mean and standard deviation values and for categorical data, values were summarized in tables with frequency and percentage values. The Pearson Chi-Square Test was used to compare categorical data. The Bonferroni Correction was made for binary comparisons in case there were differences among groups. The normality of numerical data was tested with the Kolmogorov-Smirnov Test. As not all numerical variables were distributed normally in the study, linear relations between numerical variables were determined by Spearman's rho Correlation Coefficient. The results were evaluated for a significance level of $p < 0.01$.

3. RESULTS

Troponin I – D-dimer Group

When the mortality/survival status was examined, it was determined that 15% (n=208) of 1326 patients evaluated in the 1st group died. Of the 208 patients who died in group 1, 66.8% (n=139) were male and were significantly more than women in terms of gender ($p<0.05$); 71.2% (n=148) were in the group that were aged 65 and over, and were significantly more than the 18-65 age group ($p<0.001$); 72.6% (n=151) had high troponin I levels, and were significantly higher than those with normal troponin I levels ($p<0.001$); and 96.6% (n=201) had high D-dimer levels, and were significantly higher in terms of D-dimer levels compared to those with normal D-dimer levels ($p<0.001$) (Table I).

Table I. *Distributions of Troponin I, D-Dimer Levels, Age and Gender according to Mortality/Survival Status*

	Total	Survivor	Non-Survivor	χ^2	p value
Gender					
Female	523 (39.4%)	454 (40.6%)	69 (33.2%)	4.059	0.044
Male	803 (60.6%)	664 (59.4%)	139 (66.8%)		
Age					
18-65	743 (56.0%)	683 (61.1%)	60 (28.8%)	74.015	<0.001*
≥65	583 (44.0%)	435 (38.9%)	148 (71.2%)		
Troponin I Levels					
Normal	1034 (78.0%)	977 (87.4%)	57 (27.4%)	367.469	<0.001*
High	292 (22.0%)	141 (12.6%)	151 (72.6%)		
D-Dimer Levels					
Normal	418 (31.5%)	411 (36.8%)	7 (3.4%)	90.614	<0.001*
High	908 (68.5%)	707 (63.2%)	201 (96. 6%)		
Total	**1326**	**1118**	**208**		

**p < 0.01 was considered significant*

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Since, troponin I and D-dimer values of the patients in group 1 (1326 patients) were not normal according to the Kolmogorov-Smirnov Test (p<0.001), Spearman's RHO Correlation Coefficient was calculated for the linear correlation between the variables (r=0.547; p<0.001). It was detected that there was a strong uphill linear relation between troponin I and D-dimer values (Figures II – V).

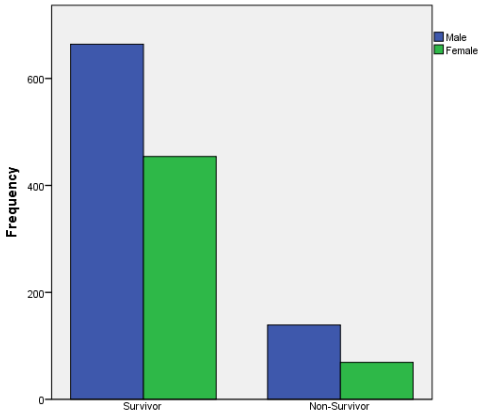

Figure II. *Mortality/Survival by gender in Group 1*

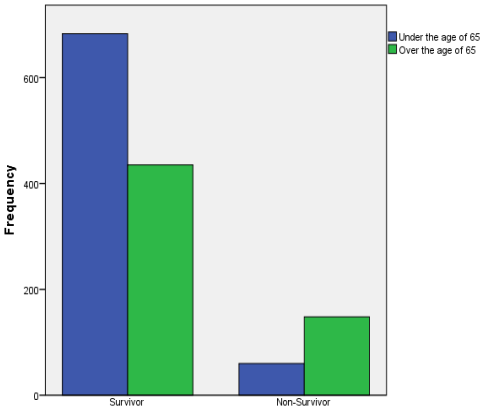

Figure III. *Mortality/Survival by age in Group 1*

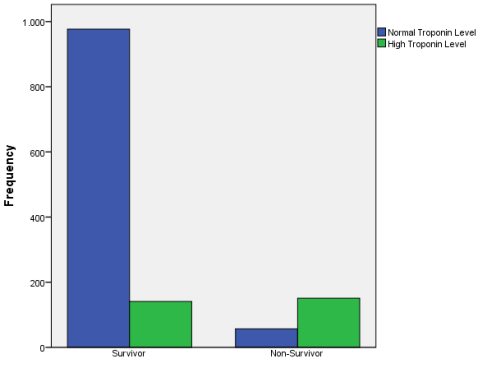

Figure IV. *Mortality/Survival by Troponin I Levels in Group 1*

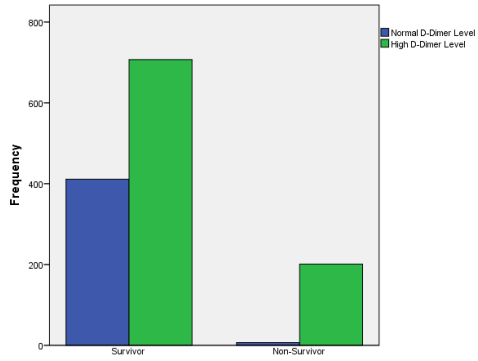

Figure V. *Mortality/Survival by D-dimer Level in Group 1*

Troponin I – Fibrinogen

When the mortality/survival status was examined, it was determined that 32% (n=193) of 606 patients evaluated in the 2st group died. Of the 193 patients who died in this group, 69.9% (n=135) were male and significantly higher than the other gender (p<0.01); 71.5% (n=138) were in the group that was aged 65 and over, and were significantly higher than the 18-65 age group (p<0.01); 73.6% (n=142) had high troponin I levels, and were significantly higher than those with normal troponin I levels (p<.001); and 85.5% (n=165) had high fibrinogen levels and were significantly higher than those with normal fibrinogen levels (p<0.001) (Table II).

Table II. *Distributions of Troponin I, Fibrinogen Levels, Age and Gender according to Mortality/Survival Status*

	Total	Survivor	Non-Survivor	χ^2	p value
Gender					
Female	224 (37.0%)	166 (40.2%)	58 (30.1%)	5.806	0.016
Male	382 (63.0%)	247 (59.8%)	135 (69.9%)		
Age					
18-65	282 (46.5%)	227 (55.0%)	55 (28.5%)	37.032	<0.001*
≥65	324 (53.5%)	186 (45.0%)	138 (71.5%)		
Troponin I Levels					
Normal	377 (62.2%)	326 (78.9%)	51 (26.4%)	154.271	<0.001*
High	229 (37.8%)	87 (21.1%)	142 (73.6%)		
Fibrinogen Levels					
Low	21 (3.5%)	10 (2.4%)	11 (5.7%)	9.882	<0.01*
Normal	85 (14. 0%)	68 (16.5%)	17 (8.8%)		
High	500 (82.5%)	335 (81.1%)	165 (85.5%)		
Total	**606**	**413**	**193**		

**p < 0.01 was considered significant*

Since, troponin I and fibrinogen values of the patients in group 2 (606 patients) were not normal distribution according to the Kolmogorov-Smirnov Test (p<0.001), Spearman's rho Correlation Coefficient was calculated for the linear relations between the variables (r=0.195; p<0.001). It was detected that there was a weak uphill linear relation between troponin I and fibrinogen values (Figures VI – IX).

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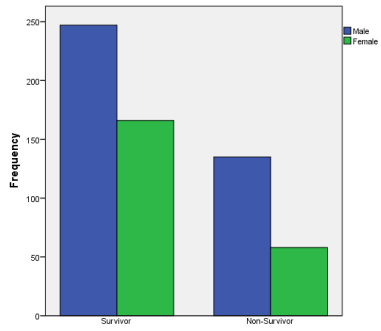

Figure VI. *Mortality/Survival by gender in Group 2*

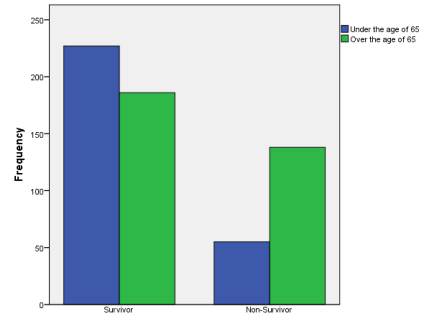

Figure VII. *Mortality/Survival by age in Group 2*

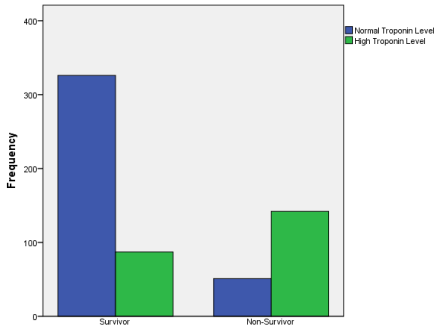

Figure VIII. *Mortality/Survival by Troponin I Levels in Group 2*

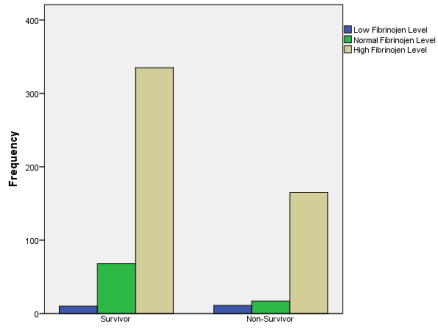

Figure IX. *Mortality/Survival by Fibrinogen Levels in Group 2*

Troponin I – INR

When the mortality/survival status was examined, it was found that 17% (n=220) of 1308 patients in the 3rd group died. Of the 220 patients who died in this group, 68.6% (n=151) were male and significantly more than the other gender (p<0.01); 71.4% (n=157) were in the group that was aged 65 and over, and were significantly more than the 18-65 age group (p<0.001); 70.5% (n=155) had high troponin I levels, and were significantly higher than those with normal troponin I levels (n=155) p<0.001); and 77.3% (n=170) had high INR levels, and were significantly higher than those with normal INR levels (p<0.001) (Table III).

Table III. *Distributions of Troponin I, INR Levels, Age, and Gender according to Mortality/Survival Status*

	Total	Survivor	Non-Survivor	χ^2	p value
Gender					
Female	511 (39.1%)	442 (40.6%)	69 (31.4%)	6.594	<0.01*
Male	797 (60.9%)	646 (59.4%)	151 (68.6%)		
Age					
18-65	725 (55.4%)	662 (60.8%)	63 (28.6%)	76.845	<0.001*
≥65	583 (44.6%)	426 (39.2%)	157 (71.4%)		
Troponin I Levels					
Normal	995 (76.1%)	930 (85.5%)	65 (29.5%)	314.500	<0.001*
High	313 (23. %9)	158 (14.5%)	155 (70.5%)		
INR Levels					
Normal	884 (67.6%)	834 (76.7%)	50 (22.7%)	242.939	<0.01*
High	424 (32.4%)	254 (23.3%)	170 (77.3%)		
Total	**1308**	**1088**	**220**		

INR: International Normalized Ratio
**p < 0.01 was considered significant*

Since, troponin I and INR values of the patients in group 3 (1308 patients) were not normally distributed according to the Kolmogorov-Smirnov Test (p<0.001), Spearman's rho Correlation Coefficient was calculated for the linear correlation between the variables (r=0.467; p<0.001). It was detected that there was a moderate uphill linear relation between troponin I and INR values (Figure X – XIII).

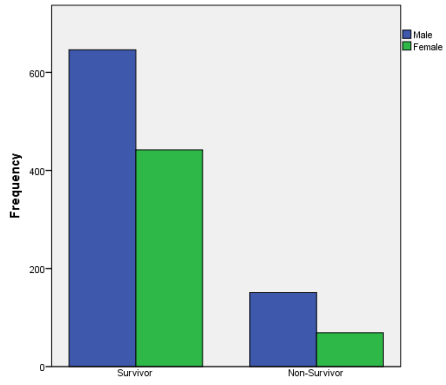

Figure X. *Mortality/Survival by gender in Group 3*

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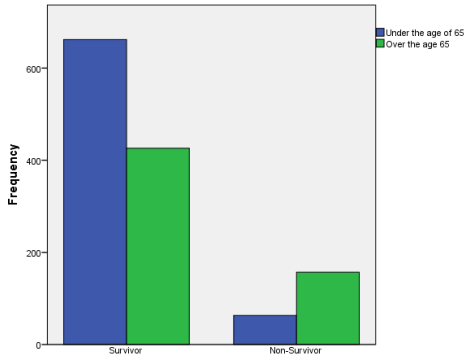

Figure XI. *Mortality/Survival by age in Group 3*

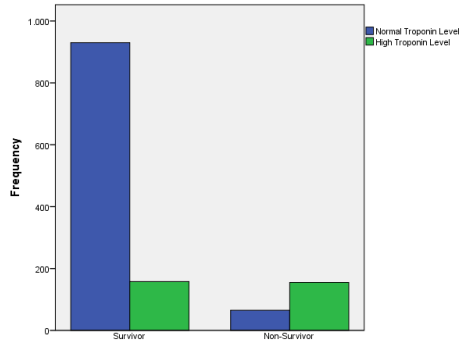

Figure XII. *Mortality/Survival by Troponin I Levels in Group 3*

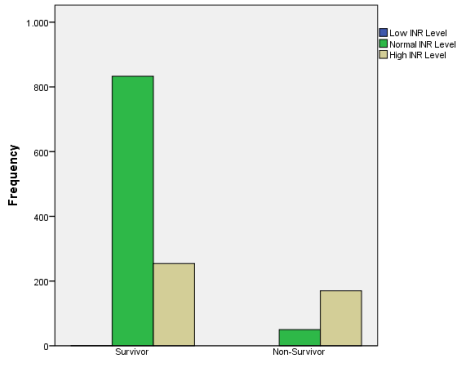

Figure XIII. *Mortality/Survival by INR Levels in Group 3*

4. DISCUSSION

In this study, we analyzed troponin-I and coagulation markers (D-dimer, fibrinogen and INR) levels in COVID-19 patients treated in our hospital. We evaluated the effect of these markers on mortality. Our study has shown that: The levels of troponin-I, D-dimer, fibrinogen and INR of patients who did not survive in all three groups (Group 1-Group 2-Group 3) were significantly higher when compared to the levels of these parameters in survivors. Therefore, elevations in these parameters may be predictive of mortality in COVID-19 patients. When evaluated according to age groups, the majority of patients who died were over 65 years of age in all three groups. This shows that advanced age is a factor that increases the risk of mortality in COVID-19 patients. In addition, there was a significant difference in gender only in the troponin I-INR Group (Group 3) in patients who died, and men had a significantly higher mortality rate than women. There was no relationship between gender and mortality in the troponin I-D-dimer group (Group 1) and troponin I-fibrinogen group (Group 2)

Although, many points about the disease caused by COVID-19 remain unclear, it is reported that the disease caused by this outbreak, which is a viral infection, may have long-term cardiovascular effects [7]. Cardiac damage, which is measured with elevated troponin I levels, was also reported among COVID-19 patients treated in hospital, and it was reported that their high levels indicated poor prognosis. The follow-up of heart damage, inflammation, and clotting markers was evaluated in relation with the severity and outcomes of the disease. Cardiac damage usually appears in critical COVID-19 patients, and elevated troponin I levels following the third day of hospitalization indicate poor prognosis. It was reported several times in previous studies that positive correlation between troponin I, IL-6, and D-dimer during hospitalization may suggest nonspecific cytokine-mediated cardiotoxicity [8].

In studies on COVID-19, high mortality rates have been associated with advanced age and male gender. Singh et al., found that among COVID-19 patients, men had a higher mortality rate than women [9]. Williamson et al., similarly reported that death associated with COVID-19 was associated with male gender and advanced age [10]. In our study, 60.6% of the patients in the group 1, 63% of the patients in group 2, and 60.9% of the patients in the group 3 were male. In our study, it is seen that men are more likely to get the disease than women in terms of being infected with COVID-19 because the percentage of male patients was higher than female patients in all three groups. There were also statistically significant differences in the gender factor among COVID-19 patients who died, and the percentage of male patients who died in all three groups was higher than the percentage of patients who survived.

In the study of Chen et al., with patients infected with COVID-19, it was found that patients aged 65 and over showed more severe symptoms and mortality rates were higher than younger patients [11]. In the study by Li et al., although, overall mortality rates in COVID-19 were low, the mortality rate was much higher in elderly patients [12]. In the cohort study of Singh et al., 93.9% of the total mortality was found in patients aged 50 and over [9]. In our study, the mortality rate of patients aged 65 and over was higher than that of the 18-65 age group in all three groups (Group 1, Group 2, Group 3). The results of our study show that advanced age is a risk factor for death from the disease in COVID-19 patients, similar to the studies in the literature.

Stefanini et al., reported that detecting high troponin I levels earlier would predict mortality in COVID-19 patients, and

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cardiac biomarkers must be evaluated systematically during hospitalization in COVID-19 patients [13]. Qin et al., conducted a retrospective study and evaluated serum cardiac biomarkers of COVID-19 patients. Laboratory results showed that troponin I had a high prognostic value for all-cause mortality [14]. Arcari et al., detected high troponin I levels in 38% of patients hospitalized with COVID-19 pneumonia, reinforcing the assumption that cardiac biomarker assessment may be useful in the follow-up of COVID-19 [15]. In their study, which was aimed at determining the cardiovascular characterization of COVID-19 patients, Rath et al., reported that elevated levels of troponin I were associated with poor prognosis in COVID-19 patients, and it was also found that D-dimer and troponin I levels were significantly higher in the mortality group compared to survivors [16]. These findings suggest that cardiac damage biomarkers are associated with an increased risk of mortality in COVID-19. Similar to previous findings [13-16], we observed that high troponin levels are a risk factor for increased mortality in COVID-19. Troponin I levels were found to be above the normal range in all three groups in patients who did not survive. This is consistent with the literature data that troponin I levels have effects on mortality in COVID-19 patients.

In a literature review of the effects of COVID-19 on coagulation, Li et al, evaluated 2,068 patients diagnosed with COVID-19 and D-dimer, which is high in coagulation markers, was observed in 58.6% of the patients and mortality was observed in 8.8% (n=183). Also, cardiac and clotting markers increased at significant levels in patients who died, except for fibrinogen levels when compared to survivors. Almost all clotting markers, including D-dimer, increased throughout the entire hospitalization of patients who were critically ill and died. D-dimer levels peaked within 1-3 days, and then decreased in survivors and non-survivors; however, median values were significantly higher in those who did not survive at any time point than in those who survived. They also found that there were significant differences between troponin I and D-dimer levels (n= 396, p <0.001) [8]. The most striking result of our work was that 96.6% (n=201) of patients who died in group 1 had elevated D-dimer levels; and there were significant differences between those with normal D-dimer levels (P<0.001). There was also a moderate and statistically significant linear relation between troponin I and D-dimer levels (r=0.547; p<0.001). However, it was reported in different studies that cardiac markers and coagulation (e.g. D-dimer) biomarkers would be useful in identifying patients at high risk of mortality, which is consistent with our results [10,11,16,17]. In the study of Miesbach and Makris, they reported that the most important change in coagulation parameters in severe COVID-19 patients was observed as an increase in D-dimer level. In addition, it was emphasized that increasing D-dimer values during the course of the disease can be used as a prognostic parameter to determine worsening prognosis [18]. Also, it was reported in a multicenter retrospective cohort study that was conducted by Zhou et al., in China that elevated D-dimer levels were significantly associated with in-hospital mortality [19]. Separate studies conducted by Terpos et al., and Wang et al., also indicated that D-dimer levels increased periodically in COVID-19 patients who did not survive compared to survivors [20, 21]. In our study, higher levels were found in the coagulation parameters in non-survivors than in survivors. This suggests that coagulopathy and especially disseminated intravascular coagulation (DIC) may contribute to mortality in COVID-19 patients.

In a protective study that evaluated the clotting profiles of COVID-19 patients, fibrinogen degradation products (FDP) and fibrinogen levels were found to be higher at significant levels among patients compared to healthy controls [22]. In the studies conducted by Connors and Levy and Thachil et al., it was recommended to monitor especially D-dimer, PZ, fibrinogen levels, and platelet counts regarding the severity of COVID-19 patients [23, 24]. In the study conducted by Terpos et al., it was stated that 71.4% of the patients who died met the clinical criteria for DIC. They noted that PT and other coagulation abnormalities, such as aPTT prolongation and fibrin degradation products, increased in patients infected with COVID-19, and severe thrombocytopenia caused life-threatening intravascular conditions. They emphasized that patients infected with COVID-19 are at high risk for venous thromboembolism [20]. It was found that D-dimer and FDP levels were significantly higher, PT(s) values were longer, and coagulation parameters were significantly associated with prognosis in patients who died from COVID-19 [4]. In a study investigating the relationship between coagulation and disease severity in COVID-19 patients, the PT, INR, APTT, and D-dimer levels of deceased patients were found to be significantly higher than those of surviving patients [25]. Li et al., also reported high INR (1.6) and prolonged PT (14.5 sec) levels in COVID-19 patients [26]. In our study, the rate of those who died and who had high D-dimer levels and those who had high INR levels was higher than in patients with normal values. Previous studies [4, 20, 25, 26] support our study results and it was observed that high INR level is associated with mortality in COVID-19 patients.

It is important to detect fatal complications early, improve patient outcomes, and reduce mortality rates in infected patients in COVID-19. The results of our study showed that measurements of troponin-I and coagulation markers such as D-dimer, fibrinogen and INR can help predict clinical severity in COVID-19 patients. These results can guide clinicians in estimating the risk of death in patients and adjusting treatment. Adjusting the treatment according to the changes in these parameters may be beneficial in reducing mortality rates by providing more effective recovery opportunities.

Since, our study was retrospective, we could not focus on patients' comorbidity data, causes of death, and could not conduct research on this subject. However, we think that the causes of death of the patients who lost their lives are COVID-19 and various complications related to it. In addition, the study needs to be confirmed by multicenter prospective studies, as control of variables cannot be achieved in retrospective studies.

Compliance with Ethical Standards

Ethical Approval: The study was approved by the Ministry of Health of the Republic of Türkiye (Reference number: 2020-11-13T11_11_42). In addition, the study was approved by the Inonu University Health Sciences Non-Interventional Clinical Research Ethics Committee (with the number of 2020/1272).

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Author Contributions: MD, NB: Equally contributed to the idea and design of the research, the acquisition, analysis and the interpretation of the data, MD, NB and MCT: Critically revised the manuscript, MD, NB: Drafted the manuscript. All authors agree to be fully accountable for ensuring the integrity and accuracy of the work and read and approved the final manuscript.

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Medical students' knowledge of the disease, frequency of depression, anxiety, stress symptoms, and related factors in the COVID-19 pandemic: A web-based questionnaire

Esra CINAR TANRIVERDI[1], Mustafa BAYRAKTAR[2], Suat SINCAN[2], Kamber KASALI[3], Yasemin CAYIR[2], Mine SAHINGOZ[4], Zulal OZKURT[5]

[1] Department of Medical Education, School of Medicine, Atatürk University, Erzurum, Turkey.
[2] Department of Family Medicine, School of Medicine, Atatürk University, Erzurum, Turkey.
[3] Department of Biostatistics, School of Medicine, Atatürk University, Erzurum, Turkey.
[4] Department of Psychiatry, Meram School of Medicine, Necmettin Erbakan University, Konya, Turkey.
[5] Department of Infectious Diseases, School of Medicine, Atatürk University, Erzurum, Turkey.

Corresponding Author: Esra CINAR TANRIVERDI
E-mail: esracinart@yahoo.com

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ABSTRACT

Objective: In this study, it was aimed to determine the level of knowledge of medical students about coronavirus disease 2019 (COVID-19), to investigate the frequency of depression, anxiety, stress symptoms and related factors.

Materials and Methods: The study is a cross-sectional study conducted with 904 volunteer medical students. Data were collected with an online questionnaire, including sociodemographic characteristics, knowledge about COVID-19, the Depression, Anxiety, and Stress Scale.

Results: Mean age was 21.3±2.2 years, and 54% of them (n=488) were female. Fifty five percent (n=497) thought that their level of knowledge about COVID-19 was sufficient, and 94.6% (n=846) were concerned about the disruption of their education. Their knowledge level was found to be 15.09±2.43 points out of 23 points. The depression, anxiety and stress symptoms were found in 64.9%, 70.4% and 34.1% of participants, respectively. The risk of anxiety (OR=0.51, 95%CI=0.94, p=0.020) and depression (OR=0.95, 95%CI=1.15, p=0.025) were higher in women. Those with a high fear of transmitting the COVID-19 infection to their relatives had higher symptoms of depression, anxiety, and stress. (p<0.001).

Conclusion: Medical students have a good knowledge level of COVID-19. However, they experience high levels of anxiety, stress and depression symptoms; and concerned about the disruption of their education.

Keywords: Medical student, COVID-19, Pandemic, Depression, Stress, Anxiety

1. INTRODUCTION

The novel coronavirus was described in China at the end of 2019, and the disease was named as coronavirus disease 2019 (COVID-19). The virus spread worldwide in few months and became a pandemic [1].

The COVID-19 pandemic has affected all areas in life. Life has been regulated according to the prevention of COVID-19 transmission. Variety of restrictions and rules were applied, leading to many changes in social life. Adaptation to the new life was different from person to person. Not only social life but also economic situations were affected negatively, and many people lost their jobs [2].

Restrictions, social distance and isolation, fear of disease, and stigma affected the mental health of people during this period. Anxiety and depression increased in both previously healthy persons and patients [3,4].

Healthcare workers (HCW) are the first-line group in defense against the COVID-19. Despite this life-threatening infectious disease, HCWs continued their services in all branches. Many healthcare professionals isolated themselves from their families by staying in a different home to prevent the spread of the disease. Face-to-face training has been transferred to digital

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platforms. This situation caused disruption in the education of professions that require skills and practice, such as medicine.

Medical students have been affected by all aspects of this pandemic, both as members of the public and as future doctors. They were concerned not only about the changes associated with life and education but also because of their professional role in public health. Since they saw the difficulties of their future profession, they may started to question their medical school preferences.

The uncertainty of the end of the pandemic, the transition to the unfamiliar distance education model, the interruption of the education, the inability to carry out practical and bedside learning, or the concerns about exam type uncertainties have created another stress and anxiety situations in students.

In this study, we aimed to investigate the knowledge levels of medical students about COVID-19 and to research depression, anxiety, stress frequencies and related factors.

2. MATERIALS and METHODS

A cross-sectional study was conducted at the Ataturk University, Faculty of Medicine. The study protocol was approved by the Turkish Ministry of Health Scientific Research Platform and the Ethics Committee of Clinical Researches, Faculty of Medicine, Atatürk University (IRB number B.30.2.ATA.0.01.00/264, Date 28.05.2020). The study was carried out in accordance with the principles of the Helsinki Declaration. Informed consent of the participants was obtained.

Setting and Participants

The eligible population of the research consisted of 1592 medical students who were 1-5th semester students at the Faculty of Medicine of Atatürk University, at the time of the study. The students were informed about the purpose of the study via e-mail. Of the students, 645 could not be reached, or they did not accept participation, and 43 were excluded due to insufficient or unreliable data. Complete data of 904 participants were included and analyzed. Thus, 60.7% of the population has been reached out. Inclusion criteria for the study were determined as studying at the Faculty of Medicine of Atatürk University, being a volunteer for participation, not having a psychiatric disease, and not using medication. Those with a previous diagnosis of psychiatric disease and/or drug use, alcohol and substance abuse were excluded from the study.

Data collection

Data were collected via an online questionnaire. The survey link was shared with all students via e-mail on 2 June 2020. Participation in the study was on a voluntary basis. The online consent of the students was obtained by having them type "I agree to participate in the study" button in the first section of the online survey. Students who did not give consent could not allowed to answer the questions. Data collection was terminated on 10 June 2020. Data collection tool consisted three parts: (1) Sociodemographic features, (2) Knowledge about COVID-19, and (3) Depression, Anxiety and Stress Scale (DASS-21).

The Knowledge Score

In the knowledge level evaluations, a literature-based form which was prepared by the researchers was used. A total of 23 questions were asked, such as the ways of transmission of COVID-19, ways of protection, personal protective equipment, isolation recommendations, hygiene measures, and lung findings of COVID-19. The options were given as "true", "false", and "I don't know". Correct answers were coded as 1, and incorrect or 'I don't know' responses as 0. The knowledge level of the students who knew more than 60% of the questions was evaluated as 'adequate.'

Depression Anxiety Stress Scales Short Form – DASS-21

DASS-21 is a scale firstly developed by Lovibond PF and Lovibond SH in 1995 as 42 items and later converted into a short form by reducing the items. There are 21 items on this short form, and it is answered according to last week's perceptions. There are three sub-dimensions showing depression, anxiety, and stress in the scale answered according to a 4-point Likert system. The score that can be obtained from each item is between 0 and 3. The lowest score that can be obtained from each sub-dimension is 0, and the highest score is 21 [5]. The Turkish adaptation of the scale were performed by Sarıçam et al. (2018), and the Cronbach's alpha coefficients for the anxiety, depression, and stress sub-domains were 0.84, 0.87, and 0.85, respectively [6]. The cut-off scores of the of the scale are given below.

The total depression subscale score was identified as normal (0-4 points), mild depression (5-6 points), moderate depression (7-10 points), severe depression (11-13 points), or extremely severe depression (14 and above). The total anxiety subscale score was defined as normal (0–3 points), mild anxiety (4-5 points), moderate anxiety (6-7 points), severe anxiety (8-9 points), and extremely severe anxiety (10 and above). The total stress subscale score was descriped as normal (0-7 points), mild stress (8-9 points), moderate stress (10-12 points), severe stress (13-16 points), and extremely severe stress (17 and above). Cut-off scores of ^{3} 5, ^{3}4 and ^{3}8 represent a positive screen of depression, anxiety and stress, respectively [6].

Statistical Analysis

Data were analyzed using the SPSS 20.0 software (SPSS Inc., Chicago, IL, USA), and presented as mean, standard deviation, median, minimum, maximum, percentage, and number. The normal distribution of continuous variables was evaluated with the Shapiro–Wilk test and the Kolmogorov Smirnov test. Categorical variables were compared with the Pearson Chi-square test, the Chi-square Yates test, the Fisher's Exact test, or the Fisher-Freeman-Halton test, depending on the expected values. The relationship between the quantitative variables was analyzed by using Pearson correlation analysis for normal distributions and Spearman correlation analysis for non-normal distributions. In multivariate analysis,

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predictive risk factors between groups were examined using the logistic regression analysis, which included possible risk factors identified in univariate analyzes. Logistic regression model results were presented with 95% confidence intervals of B±S.E, and OR. A p-value of <0.05 was considered statistically significant.

3. RESULTS

Participants

The study included 904 medical students. The mean age of the students was 21.3±2.2 years, and 488 (54%) were females. Sociodemographic variables of the students are given in Table I.

Of the students, 283 (31%) read the COVID-19 information guide published by the Ministry of Health of the Republic of Turkish, and 497 (55%) thought that their level of knowledge was sufficient. During the pandemic, 851 (94.1%) of them had increased screen time, 716 (79.2%) had concerns about the interruption of their education, 448 (49.5%) were concerned about their own transmission of COVID-19, and 727 (80.4%) were concerned about their family being infected with COVID-19. Two students individually (0.2%) and 152 relatives of them (16.8%) had COVID-19. Responses to the questions about COVID-19 are presented in Table II.

Table I. *Sociodemographic characteristics of the participants*

Variable (n=904)		n	%
Sex	Female	488	54
	Male	416	46
Grade	1st year	150	16
	2nd year	324	35.8
	3rd year	211	23.3
	4th year	136	15
	5th year	83	9.2
Nationality	Turkish	863	95.5
	Other	41	4.5
Place of accommodation	Family house	856	94.7
	Student house with friends	29	3.2
	Student house alone	9	1.0
	Dormitory	10	1.1
Smoking	Never	671	74.2
	Active smoker	93	10.3
	Ex-smoker, quit during the pandemic	48	5.3
	Ex-smoker, quit before the pandemic	71	9.93
	Tried quitting in the pandemic but could not	21	2.3

Table II. *The responses to questions about COVID-19*

		n	%
Have you read the COVID-19 guideline?	Yes	283	31.3
	No	191	21.1
	I have read part of it	221	24.4
	I browsed but did not read	209	23.1
How would you describe your level of knowledge about COVID-19?	Low	85	9.4
	Moderate	322	35.6
	Sufficient	497	55.0
Has your daily screen time increased during the pandemic?	Yes	851	94.1
	No	53	5.9
How much time do you spend on news about COVID-19? (hour/day)	I never follow the news	26	2.9
	Less than 1 hour	614	67.9
	1-3 hours	227	25.1
	3-5 hours	29	3.2
	5-7 hours	5	.6
What is your level of anxiety about the interruption of your education?	None	58	6.4
	Very low	40	4.4
	Low	90	10.0
	Moderate	292	32.3
	Severe	230	25.4
	Extremely severe	194	21.5
What is your level of anxiety about contracting yourself with COVID-19?	None	69	7.6
	Very low	167	18.5
	Low	220	24.3
	Moderate	286	31.6
	Severe	117	12.9
	Extremely severe	45	5
What is your level of anxiety about the transmission of COVID-19 to your acquaintances?	None	16	1.8
	Very low	64	7.1
	Low	94	10.7
	Moderate	341	37.7
	Severe	245	27.1
	Extremely severe	141	15.6
Have you had COVID-19?†	Yes	2	0.2
	No	902	99.8
Has anyone close to you had COVID-19?†	Yes	152	16.8
	No	752	83.2

† Results in the third month of the pandemic; n=904

The Knowledge Scores

Responses to the knowledge questions are presented in Table III. The mean knowledge scores were 15.0951±2.43. Their knowledge level was found to be 65%. The most known information was that social isolation was the most effective way to prevent transmission, and the least known was that patients with COVID-19 should use heparin for thrombosis prophylaxis. Disease stigmatization was assessed by the question "The virus is also called the Chinese virus," which was marked as 'true' by 54.2% of the students.

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Table III. *Responses to knowledge questions*

Knowledge questions	True n (%)	False n (%)	Do not know n (%)
Washing hands with soap and water for at least 20 seconds kills the coronavirus. (T)	746 (82.5)	112 (12.4)	46 (5.1)
The novel coronavirus is an enveloped DNA virus. (F)	395 (43.7)	304 (33.6)	205 (22.7)
The virus is also called the Chinese virus. (F)	490 (54.2)	300 (33.2)	114 (12.6)
The incubation period is between 2-14 days. (T)	847 (96.7)	16 (1.8)	14 (1.5)
The COVID-19 agent is also called SARS-CoV-2. (T)	590 (65.3)	123 (13.6)	191 (21.1)
COVID-19 is mainly transmitted by droplet and contact. (T)	859 (95)	31 (3.4)	14 (1.5)
The first case was seen in China on 31 December 2019. (T)	558 (61.7)	120 (13.3)	226 (25)
Asymptomatic people are not contagious. (F)	12 (1.3)	785 (86.8)	107 (11.8)
Surfaces such as door handles, faucets, and sinks should be cleaned with disinfectant or diluted bleach. (F)	875 (96.8)	11 (1.2)	18 (2)
Social isolation is the most effective method to prevent the transmission of the virus. (T)	889 (98.3)	6 (0.7)	9 (1.0)
A negative COVID-19 test does not exclude the infection. (T)	787 (87.1)	49 (5.4)	68 (7.5)
Patients who have recovered from the infection and are discharged enter the home follow-up period. (T)	658 (72.8)	100 (11.1)	146 (16.2)
COVID-19 patients monitored at home should use separate toilets and bathrooms if possible. (T)	849 (93.9)	13 (1.4)	42 (4.6)
Patients monitored at home should also apply isolation at home and stay in a separate room if possible. (T)	889 (98.3)	3 (0.3)	12 (1.3)
Patients under 50 without any additional disease and with mild clinical symptoms can be monitored at home. (T)	728 (80.5)	63 (7)	113 (12.5)
70% alcohol can be used to clean surfaces that are thought to be contaminated. (T)	635 (70.2)	104 (11.5)	135 (18.3)
1/100 diluted bleach is used on surfaces contaminated with patient excreta. (T)	339 (37.5)	76 (8.4)	489 (54.1)
All individuals with COVID-19 must be hospitalized and treated. (F)	108 (11.9)	692 (76.5)	104 (11.5)
Bilateral ground-glass opacities in the lungs are typical for COVID-19. (T)	459 (50.8)	49 (5.4)	396 (43.8)
Magnetic resonance (MR) is preferred as the imaging method of the lungs in COVID-19 patients. (F)	98 (10.8)	160 (17.7)	646 (71.5)
Heparin is administered to patients with COVID-19 for thrombosis prophylaxis. (T)	129 (14.3)	140 (15.5)	635 (70.2)
Bilateral diffuse infiltrates in the lungs are typical in the disease. (T)	684 (75.7)	17 (1.9)	203 (22.5)
Even if the test result is negative. treatment should be started for clinically positive patients. (T)	633 (70)	55 (6.1)	216 (23.9)

Table IV. *Prevalence of depression, anxiety, and stress symptoms as to the DASS-21 subscales*

DASS subscales	(n)	(%)
Stress		
Normal	596	65.9
Mild	102	11.3
Moderate	113	12.5
Severe	68	7.5
Extremely severe	25	2.8
Anxiety		
Normal	268	29.6
Mild	176	19.5
Moderate	156	17.3
Severe	108	11.9
Extremely severe	196	21.7
Depression		
Normal	317	35.1
Mild	178	19.7
Moderate	255	28.2
Severe	98	10.8
Extremely severe	56	6.2

Prevalence of Depression, Anxiety, and Stress Symptoms

Of the students, 64.9% (n=587) had depression, 70.4% (n=636) anxiety, and 34.1% (n=308) stress symptoms (Table IV).

Depression was severe or extremely severe in 17% (n=154), anxiety in 33.6% (n=304), and stress in 10.3% (n=93) of the participants. Depression, anxiety, and stress subgroups are presented in Table IV.

Evaluation of DASS and knowledge scores by sex and study period

Women's anxiety (6.68±4.38 vs. 5.65±4.24, p=0.001) and depression scores (6.80±4.17 vs. 5.96±4.09, p=0.002, respectively) were significantly higher than men's scores. There was no significant difference between the sex regarding stress scores (p=0.16) (Figure 1).

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Table V. *Comparison of DASS subgroups according to various variables*

		Stress group					Anxiety group					Depression group				
		Normal		Stressful			Normal		Anxiety			Normal		Depression		
		n	%	n	%	p	n	%	n	%	p	n	%	n	%	p
Sex (n=596)	Male	284	47.7	132	42.9	0.17	145	54.1	271	42.6	0.00	167	52.7	249	42.4	0.00
	Female	312	52.3	176	57.1		123	45.9	365	57.4		150	47.3	338	57.6	
Study period (n=596)	Preclinic	458	76.8	227	73.7	0.29	210	78.4	475	74.7	0.23	240	75.7	445	75.8	0.97
	Clinic	138	23.2	81	26.3		58	21.6	161	25.3		77	24.3	142	24.2	
Instruction (n=596)	Turkish	500	83.9	252	81.8	0.42	226	84.3	526	82.7	0.55	270	85.2	482	82.1	0.24
	English	96	16.1	56	18.2		42	15.7	110	17.3		47	14.8	105	17.9	
Nationality (n=596)	Turkish	562	94.3	301	97.7	0.01	249	92.9	614	96.5	0.01	294	92.7	569	96.9	0.00
	Other	34	5.7	7	2.3		19	7.1	22	3.5		23	7.3	18	3.1	
Had COVID-19 (n=596)	Yes	1	0.2	1	0.3	1	1	0.4	1	0.2	0.50	1	0.3	1	0.2	1
	No	595	99.8	307	99.7		267	99.6	635	99.8		316	99.7	586	99.8	
Smoking (n=596)	Current smoker	54	9.1	39	12.7	0.00	27	10.1	66	10.4	0.50	34	10.7	59	10.1	0.56
	Never smoked	464	77.9	207	67.2		207	77.2	464	73.0		236	74.4	435	74.1	
	Quit during the pandemic	27	4.5	21	6.8		14	5.2	34	5.3		19	6.0	29	4.9	
	Quit before the pandemic	42	7.0	29	9.4		16	6.0	55	8.6		24	7.6	47	8.0	
	Tried quitting in the pandemic, but couldn't	9	1.5	12	3.9		4	1.5	17	2.7		4	1.3	17	2.9	
[Level of fear about your relatives (mother, father, sibling, close relative) being infected with COVID-19] (n=596)	None	12	2.0	4	1.3	<0.001	9	3.4	7	1.1	<0.001	9	2.8	7	1.2	<0.001
	Very little	49	8.2	15	4.9		25	9.3	39	6.1		33	10.4	31	5.3	
	Little	64	10.7	33	10.7		33	12.3	64	10.1		35	11.0	62	10.6	
	Moderate	244	40.9	97	31.5		127	47.4	214	33.6		136	42.9	205	34.9	
	Severe	156	26.2	89	28.9		54	20.1	191	30.0		73	23.0	172	29.3	
	Very severe	71	11.9	70	22.7		20	7.5	121	19.0		31	9.8	110	18.7	
[Level of fear about getting infected with COVID-19] (n=596)	None	49	8.2	20	6.5	0.00	29	10.8	40	6.3	<0.001	33	10.4	36	6.1	0.02
	Very little	118	19.8	49	15.9		58	21.6	109	17.1		63	19.9	104	17.7	
	Little	135	22.7	85	27.6		73	27.2	147	23.1		81	25.6	139	23.7	
	Moderate	204	34.2	82	26.6		81	30.2	205	32.2		99	31.2	187	31.9	
	Extreme	69	11.6	48	15.6		21	7.8	96	15.1		31	9.8	86	14.7	
	Very severe	21	3.5	24	7.8		6	2.2	39	6.1		10	3.2	35	6.0	
Time spent a day on news about COVID-19 (n=596)	Never follows the news	19	3.2	7	2.3	0.05	12	4.5	14	2.2	0.14	11	3.5	15	2.6	0.54
	Less than 1 hour	422	70.8	192	62.3		190	70.9	424	66.7		222	70.0	392	66.8	
	1-3 hours	134	22.5	93	30.2		58	21.6	169	26.6		74	23.3	153	26.1	
	5-7 hours	16	2.7	13	4.2		6	2.2	23	3.6		7	2.2	22	3.7	
	≥7 hours	5	0.8	3	1.0		2	0.7	6	0.9		3	.9	5	.9	
Perceived COVID-19 knowledge (n=596)	Very little	56	9.4	29	9.4	0.27	29	10.8	56	8.8	0.41	35	11.0	50	8.5	0.34
	Moderate	223	37.4	99	32.1		88	32.8	234	36.8		116	36.6	206	35.1	
	Adequate	317	53.2	180	58.4		151	56.3	346	54.4		166	52.4	331	56.4	
	Very good	0	0	0	0		0	0	0	0		0	0.0	0	0.0	
Perceived anxiety of interruption of education (n=596)	None	41	6,9%	17	5,5%	<0,001	27	10,1%	31	4,9%	<0,001	33	10,4%	25	4,3%	<0,001
	Very little	28	4,7%	12	3,9%		16	6,0%	24	3,8%		17	5,4%	23	3,9%	
	Little	66	11,1%	24	7,8%		31	11,6%	59	9,3%		37	11,7%	53	9,0%	
	Moderate	219	36,7%	73	23,7%		94	35,1%	198	31,1%		119	37,5%	173	29,5%	
	Extreme	152	25,5%	78	25,3%		64	23,9%	166	26,1%		74	23,3%	156	26,6%	
	Very severe	90	15,1%	104	33,8%		36	13,4%	158	24,8%		37	11,7%	157	26,7%	

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Table VI. *Stress, anxiety, and depression odds ratios (OR) by selected demographic characteristics*

Variables B±SE		Stress			Anxiety			Depression		
		B±SE	OR (OR-95 CI)	p	B±SE	OR (OR-95 CI)	p	B±SE	OR (OR-95 CI)	P
	Age	0.06 ± 0.04	1.06 (0.97-1.17)	0.142	0.05 ± 0.05	1.05 (0.94-1.17)	0.323	0.04 ± 0.04	1.05 (0.95-1.15)	0.316
	Sex M	-0.14 ± 0.14	0.86 (0.65-1.16)	0.342	-0.35 ± 0.15	0.7 (0.51-0.94)	0.020	-0.32 ± 0.14	0.72 (0.54-0.96)	0.025
Study year	1st grade			0.170			0.614			0.631
	2nd grade	0.53 ± 0.36	1.7 (0.83-3.47)	0.143	-0.12 ± 0.38	0.88 (0.41-1.85)	0.739	0.28 ± 0.35	1.32 (0.66-2.65)	0.425
	3rd grade	0.41 ± 0.31	1.51 (0.81-2.81)	0.185	0.11 ± 0.33	1.12 (0.58-2.15)	0.720	0.4 ± 0.3	1.5 (0.82-2.72)	0.183
	4th grade	0.2 ± 0.31	1.22 (0.66-2.25)	0.519	-0.12 ± 0.32	0.88 (0.47-1.65)	0.699	0.27 ± 0.29	1.31 (0.73-2.35)	0.356
	5th grade	0.64 ± 0.31	1.91 (1.03-3.53)	0.037	0.19 ± 0.32	1.21 (0.63-2.31)	0.555	0.42 ± 0.3	1.53 (0.84-2.77)	0.159
Nationality	Turkish	1.13 ± 0.44	3.11 (1.3-7.44)	0.010	0.74 ± 0.35	2.11 (1.05-4.23)	0.034	0.92 ± 0.34	2.53 (1.28-4.98)	0.007
Level of fear about relatives being infected with COVID-19	None			0.032			0.001			0.021
	Very little	-0.82 ± 0.69	0.44 (0.11-1.7)	0.235	-1.49 ± 0.65	0.22 (0.06-0.8)	0.022	-1.07 ± 0.63	0.34 (0.09-1.19)	0.091
	Little	-1.07 ± 0.39	0.34 (0.15-0.74)	0.006	-0.98 ± 0.41	0.37 (0.16-0.84)	0.018	-1.15 ± 0.38	0.31 (0.14-0.67)	0.002
	Moderate	-0.68 ± 0.32	0.5 (0.27-0.95)	0.034	-0.83 ± 0.37	0.43 (0.2-0.9)	0.025	-0.56 ± 0.34	0.56 (0.28-1.11)	0.098
	Severe	-0.79 ± 0.24	0.45 (0.28-0.73)	0.001	-1.15 ± 0.31	0.31 (0.17-0.58)	0.000	-0.81 ± 0.27	0.44 (0.25-0.75)	0.003
	Very severe	-0.54 ± 0.23	0.57 (0.36-0.92)	0.022	-0.5 ± 0.31	0.6 (0.32-1.11)	0.108	-0.44 ± 0.27	0.63 (0.37-1.09)	0.102
Level about getting infected with COVID-19	None			0.297			0.581			0.934
	Very little	-0.32 ± 0.48	0.72 (0.27-1.87)	0.501	-0.6 ± 0.59	0.54 (0.16-1.75)	0.308	-0.32 ± 0.52	0.72 (0.25-2)	0.533
	Little	-0.38 ± 0.41	0.67 (0.3-1.52)	0.347	-0.41 ± 0.54	0.65 (0.22-1.9)	0.440	-0.06 ± 0.46	0.93 (0.37-2.32)	0.882
	Moderate	-0.07 ± 0.39	0.92 (0.43-1.99)	0.846	-0.48 ± 0.52	0.61 (0.21-1.73)	0.358	-0.2 ± 0.44	0.81 (0.33-1.95)	0.643
	Severe	-0.52 ± 0.37	0.59 (0.28-1.24)	0.168	-0.19 ± 0.52	0.82 (0.29-2.29)	0.710	-0.08 ± 0.43	0.92 (0.38-2.18)	0.851
	Very severe	-0.21 ± 0.38	0.8 (0.37-1.72)	0.575	-0.06 ± 0.54	0.94 (0.32-2.71)	0.909	0 ± 0.45	0.99 (0.41-2.42)	0.994
	Constant	-2.52 ± 1.33	0.07	0.057	0.37 ± 1.49	1.45	0.801	-0.61 ± 1.37	0.54	0.655

Data were regrouped according to stress, anxiety, and depression scores (normal/stressed; normal/depressed, normal/anxious).

There was no difference between depression, anxiety, and stress scores of preclinical and clinical term students (p=0.22, p=0.40, and p=0.33, respectively). Knowledge scores of; women compared to men (15.37±2.25 vs. 14.76±2.59, p<0.001), clinical students compared to preclinical students (15.60±2.19 vs. 14.93±2.49, p<0.001) (Figure 2), and students who read the COVID-19 scientific guideline compared to those who did not (15.48±2.41 vs. 14.58±2.61; p<0.001) were significantly higher.

There was no correlation between the knowledge scores and the stress, anxiety, and depression scores (p=0.49, p=0.29, and p=0.47, respectively). Anxiety, stress, and depression rates were all higher in those who had a fear of their relatives getting infected with COVID-19 than those who did not. Comparisons of DASS subgroups according to various variables are presented in Table V.

Probability of stress, anxiety, and depression by demographics

A regression model was created with the dependent variables with and without stress, anxiety, and depression, and the independent variables of age, sex, class, program, nationality, fear of COVID-19 contagion to relatives, and self. While class, nationality, and fear of contracting COVID-19 to relatives were effective on stress (p<0.05 for all); sex, nationality, and fear of contracting COVID-19 to relatives were effective on anxiety and depression (p<0.05 for all) (Table VI).

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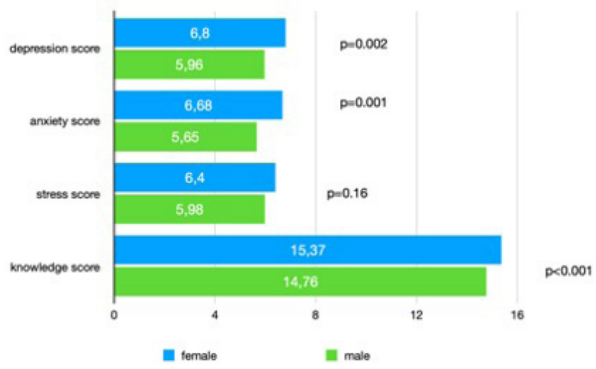

Figure 1. *Evaluation of DASS and knowledge scores by sex*

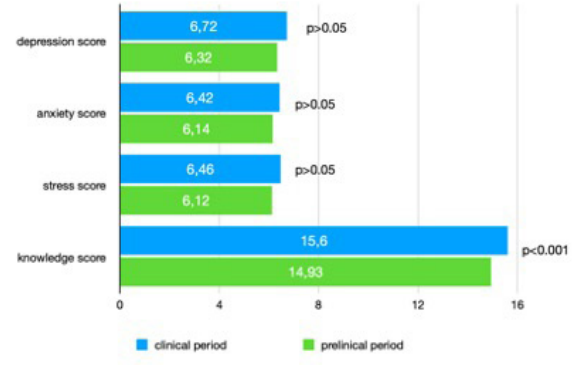

Figure 2. *Evaluation of DASS and knowledge scores by period*

4. DISCUSSION

The present study has two main results. First, around two-thirds of medical students had sufficient knowledge about COVID-19. However, only half thought they had enough knowledge. Second, approximately one in two students experienced moderate-to-severe depression and anxiety symptoms, and one in five students experienced moderate-to-severe stress symptoms at the third month of the pandemic.

The implementation of disease-related preventive measures by a large part of the general population is critical to contain the spread of epidemics. In this context, increasing the knowledge of individuals on disease-related issues will facilitate their adaptation to preventive measures. Additionally, it is known among medical students that learning and developing knowledge about a new infectious disease will help improve students' perception of disease and preventive behaviors. As a generation of future healthcare professionals, the positive knowledge, attitudes, and behaviors of medical students in the context of a pandemic not only guide to communication and mitigation strategies during the event but can also provide information to prepare for the future pandemic.

In our study, 65% of all participants had sufficient knowledge of the main clinical symptoms, transmission, and prevention associated with COVID-19. This level of knowledge is lower than that demonstrated among medical students in Iran (86.96%) [7] and in Uganda (91%) [8]. A recent study conducted on medical students in Turkey has also described a higher level of knowledge on COVID-19 (78.3%) [9]. Our findings also show that the level of knowledge of clinical-term students is higher than preclinical students, which is consistent with earlier studies [9]. While, most of the participants in our study were preclinical students, previous investigations included more clinical students than preclinical ones. This methodological difference may lead to a lower level of knowledge among medical students in the current study.

In females, higher levels of knowledge were observed, which contrasts with the previously mentioned studies. However, some other studies report no significant relationship between knowledge level and sex [7,10,11]. There are contradictory reports regarding the relationship between sex and knowledge [9]. This inconsistency between studies can be attributed to socio-cultural differences and the tools used.

In the current study, the low participation rate of 4th and 5th grade students and the fact that interns were not included in the study may have affected the results. However, in terms of the knowledge about COVID-19, medical students commonly scored highest in the items related to the transmission route of the virus and preventive behaviors. Our findings are supported by similar studies in the literature [7,8]. In our research, the lowest-scoring items were related to the treatment of COVID-19 and the imaging method used. As mentioned earlier, more than half of our participants were preclinical students; internship students were not included in our study. Therefore, it is not surprising that preclinical students had less or no contact with patients and did not have experience of working in risky and patient-contact environments, such as emergency and intensive work. Therefore, they had less knowledge of these items.

The transformation of COVID-19 into a pandemic that started from China and spread to the world has caused a stigma against China in some individuals. To evaluate the students' attitudes towards stigmatization, the proposition "The virus is also called the Chinese Virus" was included among the information questions. It was observed that more than half of the students marked this proposition as 'true'. Although we asked to evaluate stigma, this conclusion should be approached with caution as this statement does not necessarily measure stigma. The World Health Organization announced that the virus was named SAR-CoV2, and the disease of this novel coronavirus as COVID-19, to prevent such stigma at the beginning of the pandemic [12] and our study was not long after the onset of the pandemic, which means there was still confusion about the nomenclature in minds.

The current study shows high rates of depression, anxiety, and stress symptoms among medical students (64.9%, 70.4%, and

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34.1%, respectively). This finding is supported by several studies showing the negative psychological effects of the COVID-19 outbreak on medical students in many other countries. For example, a study from Brazil reported moderate-severe depressive symptoms in 64.41% of medical students [13]. Another study assessing the psychological impacts of COVID-19 in medical students in the United States demonstrated self-reported anxiety in 66.1% of participants [14]. Similarly, the result from a study by Kumar et al., who reported 38.9% of stress among students during the pandemic, is in line with our findings [15]. As expected, the prevalence of psychological distress in medical students during the COVID-19 pandemic was higher than those reported before the outbreak [16]. In a study from Turkey using the DASS scale before the epidemic, 27.1%, 47.1%, and 27% of medical students suffered from depression, anxiety, and stress, respectively [17]. Current research shows higher depression, anxiety, and stress levels in medical students compared to before the pandemic [18,19]. In previous studies, depression and anxiety symptoms were also significantly higher in students in their basic academic years (years 1–3) than those in clinical years (years 4–6) [20,21]. Similarly, a literature review reported that female and first-year medical students are at a higher risk of depression [22]. Another finding of the study was that depressive and anxiety symptoms were significantly higher among students worried about transmitting the virus to family members. This finding contradicts an earlier study that showed students who were worried about transmitting the virus to family members, which found no statistically significant difference compared to students who were not worried [23]. Another finding of the study was that students with the worry about transmitting the virus to the family members are at a higher risk of anxiety, depression, and stress, which complies with a previous study conducted in the general population [24]. However, contrary to these, one study did not find any significant relationship between the risk of suffering from depressive and anxiety symptoms and the worry about transmitting the virus to family members in medical students [23,25].

This study shows that medical students are psychologically affected by the pandemic. They are more concerned about infecting their relatives with the disease than being infected themselves and experience high levels of depression, anxiety, and stress symptoms.

Limitations

The study has some limitations. First of all, it is a cross-sectional study conducted with 1st – 5th year students of a single medical school. Thus, the results cannot be generalized to all medical students. The measurement tool used is a self-assessment instrument and is based on students' self-evaluations. Due to the pandemic, students could not be interviewed face-to-face, and data were collected online. The fact that students, who did not have a device or internet connection or did not use social media, could not be reached and therefore may have impacted the results. Finally, since the study was conducted in the third month of the pandemic, it should be noted that the findings are preliminary and may have changed over time.

Conclusion

In this study, medical students' psychological status at the beginning of the pandemic was evaluated, and high levels of depression, anxiety, and stress were revealed. On one hand, students were concerned about the COVID-19 contamination of themselves and their relatives, and on the other hand, about the interruption of their education. Their anxiety levels about their relatives who get sick were higher than their own. While female sex is a risk factor for depression and anxiety, those who had a fear of contracting COVID-19 to their relatives, and those who were concerned about the interruption of their education had significantly higher depression, anxiety, and stress levels.

Students' knowledge rate for COVID-19 was found to be good (65%) even though it was just the beginning of the pandemic. Women's knowledge scores were significantly higher than men's, and knowledge scores of clinical students were significantly higher than preclinical students.

Compliance with Ethical Standards

Ethical Approval: The study protocol was approved by the Turkish Ministry of Health Scientific Research Platform and the Ethics Committee of Clinical Researches, Faculty of Medicine, Atatürk University (IRB number B.30.2.ATA.0.01.00/264, Date 28.05.2020). The study was carried out in accordance with the principles of the Helsinki Declaration. Informed consent of the participants was obtained.

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