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3-Year Survival of Resin Restorations of Severely Damaged Permanent Molars in Children

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ABSTRACT

Objective: Aim of this retrospective study was to determine survival rates of direct composite resin (DCR) restorations of permanent first molars (PFMs) with excessive material loss at the end of 3 years. **Material and Methods:** Children between the ages of 7-14 whose PFMs had 3-surfaced DCR restoration were included. Survival rates of the restorations after 3 years were evaluated using The United States Public Health Service criteria and the presence of symptoms indicating irreversible pulp damage such as spontaneous pain, percussion, abscess in the related teeth were examined clinically and radiographically. Chi-square and Kruskal Wallis analyzes were used for the statistical analysis. **Results:** Totally 58 DCR restorations of 34 patients who agreed to come for the control after 3 years were examined. The survival rate of restorations was 70.69%. Findings indicating irreversible pulp damage were found in 15.5% of the restorations examined. **Conclusion:** Survival rate of DCR restorations applied to PFMs with excessive material loss is not satisfactory after 3 years, however it is thought that DCR applications will continue in PFMs with excessive material loss thanks to the developments in adhesive systems and composite resins. Therefore, long-term randomized controlled clinical studies on this subject should be continued.

Keywords: Composite Resins, Dental Caries, Dental Restoration, Permanent.

Çocuklarda Aşırı Madde Kayıplı Daimi Molarların Rezin Restorasyonlarının 3 Yıllık Sağ Kalımı

ÖZ

Amaç: Bu retrospektif çalışmanın amacı, aşırı madde kayıplı daimi birinci büyük azılara uygulanan direkt kompozit rezin (DKR) restorasyonların 3 yıl sonundaki sağ kalım oranlarının belirlenmesidir. **Gereç ve yöntem:** Araştırmaya aynı hekim tarafından daimi birinci büyük azılarına 3 yüzlü DKR restorasyon uygulanmış 7-14 yaş aralığındaki çocuklar dahil edilmiştir. Restorasyonların 3 yıl sonundaki sağ kalım değerlendirmesinde United States Public Health Service (USPHS) kriterleri kullanılmış ve ilgili dişlerde spontan ağrı, perküsyon hassasiyeti, apse gibi geri dönüşümsüz pulpa hasarını işaret eden semptomların varlığı incelenmiştir. Verilerin istatistiksel analizinde Ki-kare ve Kruskal Wallis analizleri kullanılmıştır. **Bulgular:** Üç yıl sonundaki kontrol randevusuna gelmeyi kabul eden toplam 34 hastanın daimi birinci büyük azı dişlerine yapılmış 58 DKR restorasyon klinik ve radyografik olarak incelenmiştir. Restorasyonların sağ kalım oranı %70.69'dur. İncelenen restorasyonların %15.5'inde geri dönüşümsüz pulpa hasarını ve kök kanal tedavisi ihtiyacını gösteren bulgular saptanmıştır. **Sonuç:** Aşırı madde kayıplı daimi birinci büyük azı dişlerine uygulanan DKR restorasyonların 3 yıl sonundaki sağ kalım oranının tatmin edici olmadığı ancak, adeziv sistemler ve kompozit rezinlerdeki gelişmeler sayesinde aşırı madde kayıplı genç daimi posterior dişlerde DKR uygulamalarının devam edeceği düşünülmektedir. Bu nedenle konuyla ilgili uzun vadeli, randomize, kontrollü klinik çalışmalar sürdürülmelidir.

Anahtar Kelimeler: Kompozit Dental Rezine, Diş Çürükleri, Diş Restorasyonu, Kalıcı.

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INTRODUCTION

Permanent first molars are the earliest erupted permanent teeth in the jaws, and they are often mistaken for primary teeth by young children and their parents, therefore it is observed that these teeth are not given enough attention because of considering that they will change with their succedaneous permanent teeth anyway. Dental caries able to occur and progress easily in these teeth due to reasons such as food and bacterial accumulation because of the inability of the young children to perform oral hygiene practices adequately, incomplete enamel maturation of the newly erupted first permanent molar tooth, and the deprivation of mechanical cleaning due to chewing during the period until the tooth comes into occlusion (Fejerskov et al., 1984; Carvalho et al., 1989).

Direct composite resin (DCR) restorations, indirect composite resin restorations and prefabricated crowns prepared with various materials are used in the treatment of carious lesions of young permanent first molars with excessive material loss in pediatric patients. Direct posterior restorations are restorative procedures that are routinely preferred in public dental health institutions today, due to the fact that they are more practical and more economical due to the possibility of finishing the treatment in a single session, and they require less preparation on the tooth (ADA, 2003). Although the common opinion in the literature was direct applications of resin-containing composite materials is ideal for use in small and medium sized cavities (ADA, 1998), afterwards with the reporting that the long-term clinical follow-up results of indirect and direct restorations do not differ significantly, the interest in direct composite resin applications has increased in large restorations including the tubercle tips (Denehy & Cobb, 2004; Deliperi & Bardwell, 2006a). With the widespread use of composite resins, the longevity of posterior composite resin restorations and the awareness of potential failure reasons have come into question (Zotti et al., 2021). In studies investigating the survival time of composite resins in posterior restorations, survival rates over a 5-year period have been reported ranging from 55% to 95% (Hickel & Manhart, 2001).

Another important issue in the restoration of permanent first molars with excessive material loss and deep dentin caries is the protection of pulp vitality. In a study investigating the 3-year follow-up results of indirect pulp capping treatments in permanent molars, the rate of cases without clinical and radiographic pulpal pathology was reported as 93% (Gruythuysen et al., 2010). It is stated that when an accurate diagnosis is made with a detailed clinical and radiographic evaluation, success rates ranging from 73% to 95% are obtained in indirect pulp treatments (Fuks, 2000).

In this retrospective study, which includes the clinical and radiographical evaluation of direct composite resin restorations of permanent first molars with excessive material loss due to deep dentin caries in pediatric patients, it was aimed to examine the survival rates of the restorations and to evaluate the possible irreversible pulp

damage symptoms of the related teeth at the end of 3 years.

MATERIALS AND METHODS

Case selection

Three-surface direct composite resin restorations applied to the permanent first molars of children aged 7-14 years, who are systemically healthy, able to provide the necessary cooperation with the dentist (with Frankl Scales 3 or 4) for a successful dental treatment and have similar oral hygiene habits (brushing their teeth with fluoride toothpaste at least once a day), were included in the study. The inclusion criteria of restorations were as follows:

- To include 3 surfaces of the tooth and to have a size that exceed 2/3 of the distance between the buccal and lingual tubercle tips in the buccolingual direction,
- No molar incisor hypomineralization in restored teeth,
- No sign of direct pulp exposure and/or irreversible pulp damage in the restored teeth,
- Absence of any radiographic pathology indicating damage to the root pulp such as loss of lamina dura, periapical radiolucency or lesion, internal or external resorption,
- Having been performed by the same physician in the year 2018.

Power analysis was not performed in the study because the sample group of the study included all the cases within the specified time interval and criteria.

Clinical and radiographical data of the included patients and restorations were obtained from hospital database and anamnesis forms, and patients were invited for clinical examination in order to investigate the survival of restorations after 3 years.

Clinical procedure

All restorations examined in the study were made by a specialist physician in the pediatric dentistry clinic of a university hospital. After the application of local anesthesia, isolation measures were taken with cotton rolls and saliva suction and all carious tissues were removed under water cooling at the enamel level by using high speed rotary diamond burs and at the dentin level using a low-speed rotary instrument with tungsten carbide rotary burs and hand excavators when it was necessary. At the bottom of the cavity closest to the pulp horns, an indirect pulp capping material containing calcium hydroxide (Dycal, Dentsply, Milford, DE, USA.) was used. Glass ionomer cement (Ionofil Molar, VOCO, Cuxhaven, Germany) as the base cement, Clearfil SE Bond (Kuraray Noritake Dental Inc., Okayama, Japan.) as the adhesive agent, and Clearfil Majesty posterior composite resin (Kuraray Noritake Dental Inc. Okayama, Japan.) as the restoration material were used. Composite resin material was placed in the cavity in layers of 2 mm thickness in accordance with the manufacturer's instructions, and each layer was polymerized with an LED light device for 20 seconds. Restorations were polished using diamond finishing burs and silicone polishing rubbers after occlusion control.

3-Year control visits

Restorations were examined clinical and radiographically by the same physician who performed the restorations at the end of 3 years. United States Public Health Service (USPHS) criteria (Cvar & Ryge, 2005) (Table 1) were used in the clinical evaluation of the restorations. Among the restorations, those got A and B scores from the USPHS color match, marginal discoloration and anatomical form criteria, A score from the marginal adaptation and secondary caries criteria were considered successful in terms of survival. The presence of clinical symptoms in the involved teeth, such as provoked or spontaneous pain, percussion tenderness, abscess, fistula which are indicating irreversible pulp damage and the need for root canal treatment, were determined by periapical radiographic examination in addition to clinical inspection.

Statistical analysis

Data were analyzed with the SPSS package program (SPSS v23.0, SPSS Inc., Chicago, IL, USA). Categorical variables were given as numbers and percentages, and the differences between categorical variables were analyzed with Chi-square analysis, and data that did not meet the prerequisites of parametric tests were examined with the Kruskal-Wallis test.

Ethical considerations

Ethical approval of this retrospective study was received from the Faculty of Medicine Clinical Research Ethics Committee (date: 10.09.2019 number: 15) and written informed consent which is stating that their data could be used for scientific purposes was obtained from the parents of all patients who participated in the study.

Table 1. USPHS Clinical evaluation criteria and scoring (Cvar & Ryge, 2005).

Criteria	A	B	C	D
Color match	No difference in color or transparency between restoration and tooth	A deviation within the natural tooth color and transparency values	A deviation other than the natural tooth color and transparency values	
Marginal discoloration	No discoloration at the restoration and tooth interface	A discoloration at the restoration and tooth interface that does not progress towards the pulp	A discoloration at the restoration and tooth interface that progress towards the pulp	
Anatomical form	Restoration in continuity with tooth anatomy	Acceptable restoration that is not identical to the anatomical form	Insufficient anatomical form with exposed dentin	
Marginal adaptation	No visible gap at the restoration and tooth interface	There is little visible gap between restoration and tooth interface, the probe is inserted	Probe progresses to dentine or restoration base	Restoration lost or mobile
Seconder caries	No caries	Caries present		

RESULTS

Totally 58 composite resin restorations made on the permanent first molars of 34 patients who agreed to

come for the control appointment at the end of 3 years were examined clinically and radiographically.

Table 2. Clinical success rates of restorations according to USPHS criteria.

Criteria	A n (%)	B n (%)	C n (%)	D n (%)
Anatomical form	26 (44.83)	26 (44.83)	6 (10.34)	-
Marginal adaptation	41 (70.69)	9 (15.52)	7 (12.07)	1 (1.72)
Seconder caries	55 (94.83)	3 (5.17)	-	-

Mean age of the patients at the control visit was 13.5 and 22 (64.7%) of them were female and 12 (35.3%) were male. Of the restorations examined, 22.4% were applied to the right upper 1st molar, 22.4% to the left upper 1st molar, 27.6% to the left lower 1st molar, and 27.6% to the right lower 1st molar tooth.

All restorations evaluated as successful according to anatomical form, marginal adaptation and secondary caries criteria received an A or B score from the color match and marginal discoloration criteria, therefore color match and marginal discoloration were not considered as decisive criteria for clinical failure and survival. The survival rate of the restorations examined with this method was determined as 70.69%.

The clinical success rates of restorations according to USPHS criteria is shown in Table 2. Symptoms indicating irreversible pulp damage and the need for root canal treatment were found in 15.5% (9 teeth) of the restorations. In two cases, it was determined that crown restorations were performed due to clinical failure of the restorations, and there was no irreversible pulp damage and need for root canal treatment in the related teeth, and these restorations were considered unsuccessful in terms of survival. As a result of the statistical analysis of the data, it was determined that there was no significant difference between the jaw (upper or lower) on which the restorations were made and the survival rates ($p=0.242$), no significant relationship between the tooth number and the clinical success of the restoration applied ($p=0.702$), and between the tooth number and the cases with irreversible pulp damage ($p=0.836$).

DISCUSSION

It is desired that the restorative materials used in the posterior teeth have advantages such as being resistant to chewing pressure and occlusal forces, long manipulation time, easy to use and reasonable application time. In addition to these properties, composite resins which have strong aesthetic properties, are the most widely used materials in the restoration of posterior teeth today (Zotti et al., 2021; Leprince et al., 2013; Jang et al., 2015).

As well as material-specific factors, clinical success and survival time of posterior restorations are also affected by dental factors such as the applied tooth, depth and size of the restoration, relationship with the pulp, and patient-specific factors such as age, chewing habits, oral hygiene, frequency of dental visits, and caries activity (Hickel & Manhart, 2001).

In addition, in a study of Sonkaya et al. (Sonkaya et al., 2021) from Turkey, it was found that factors such as the type of health institution in which the restoration was applied and the experience of the practicing physician also affect the clinical performance of posterior restorations and it has been reported that the highest success is seen in those performed by physicians working in university hospitals (Sonkaya et al., 2021). The direct composite restoration failure rate of 50.8% determined by Sonkaya et al. (Sonkaya et al., 2021) is higher than our results and the reason for this situation may be the examination of restorations made by a physician in only

one university hospital in our study, although they included samples from all health institutions in our country in their study.

The restoration survival rate of 70.69% at the end of 3 years in our study is not compatible with the annual failure rate of 1-4% found in systematic reviews of direct composite restorations (Demarco et al., 2012; Heintze & Rousson, 2012). This may be due to the evaluation of the clinical success of only 3-surface composite restorations of the teeth with excessive material loss in this study, and reporting the results of all cavity types, that is, restorations with a higher chance of success in mentioned systematic reviews.

In a study investigating the 17-year follow-up results of composite restorations, it was found that restoration fractures were the primary causes of failure in the first 5 years, and it has been reported that secondary caries emerged as the main cause of failure in the 6-17-year period. (Brunthaler et al., 2003). In the same study, it was found that none of the factors such as the isolation of the operation area with cotton rolls or rubber-dam, the skill of the dentist, the type of bonding agent or the filler properties of the composite resin material had a significant effect on the failure rates, however, restoration size (Class II restorations) has been reported to have a significant effect on clinical failure (Brunthaler et al., 2003). In another study, it was reported that restoration fractures or losses in the first 5-year observation and secondary caries in the 5-10-year observation period were the main failure factors (Gaengler et al., 2001).

As a result of the present study, similarly, the primary failure causes were adaptation problems and partial restoration losses in the way that the probe could be inserted at the tooth and restoration interface or exposed the dentin, and secondary caries was not the leading cause of failure.

Indirect composite resin restorations are one of the alternative treatment methods to direct composite resin restorations in the restorations of permanent molars with excessive material loss. In the in-vitro study of Kuijs et al., it was reported that ceramic, indirect composite and direct composite resin adhesive restorations with tubercle replacement showed comparable fracture resistance and similar reasons for failure (Kuijs et al., 2006). Some clinical studies have also supported this laboratory study (Pallesen & Qvist, 2003; Thordrup et al., 2001).

In a literature review, it was reported that no significant difference was found in the 3-year clinical performance of posterior direct and indirect composite resin restorations (Hickel & Manhart, 2001). Furthermore, in a recent study it was reported that, after 2 years indirect composite restorations created using the CAD-CAM system and DCR restorations have success rates of 90% and 93.3%, respectively and presented similar and good clinical behavior for all the properties analyzed (Rocha Gomes Torres et al., 2021). However, in the last 20 years, the opinion that indirect restorations can be a better alternative than direct composite restorations have

become widespread (Galiatsatos et al., 2022; Ravasini et al., 2018; Koyutürk et al., 2013).

In a recent study, it was stated that the marginal integrity was not impaired in indirect restorations even during the 20-year follow-up period, and therefore the incidence of secondary caries was lower than in direct restorations (Ravasini et al., 2018). In a study conducted in pediatric patients as a result of 2-year follow-up of indirect and direct composite resin restorations, no significant difference was found between the two techniques in terms of any of the USPHS criteria evaluated, however, at the end of 6 months, it was stated that there was more staining at the margins of direct composite resin restorations as a result of staining with basic fuchsin (Koyutürk et al., 2013).

In a clinical study including 3-year follow-up of restorations, it was reported that indirect composite inlays (93%) and direct composites (87%) showed similar clinical success rates, but indirect inlays showed significantly better results in anatomical form criteria (Manhart et al., 2000). Furthermore, as a result of a recent study in which indirect inlay and onlay restorations were followed for 9 years, a high success rate of 85% was reported (Galiatsatos et al., 2022).

From the research results, it is understood that the clinical success of direct composite restorations is comparable to indirect restorations, but indirect restorations show more satisfactory performances, especially in terms of anatomical form, marginal leakage and secondary caries. Another alternative treatment method to direct and indirect composite restorations of young permanent posterior teeth with extensive crown destruction is stainless steel crown (SSC) applications (Heidari et al., 2019; Sigal et al., 2020). Although there are many sources in the literature on the use of SSC on primary posterior teeth, long-term clinical studies investigating its use in young permanent teeth are few (Heidari et al., 2019; Sigal et al., 2020; Felemban et al., 2021). The reason for this may be the generally temporary use of SSCs in young permanent teeth until the individual aesthetic crown restorations are made.

The long-term success of SSC restorations applied to permanent posterior teeth of 10-15 year-old adolescents has been reported as 86.1% (Felemban et al., 2021).

As a result of a study comparing the clinical success of SSC, amalgam and direct composite resin restorations applied to permanent posterior teeth, the highest success rate was found in SSC (88.1%), then in amalgam (59.3%) and the lowest was in direct composite restorations (41.2%) (Sigal et al., 2020). Researchers stated that PCCs can be a long-term and effective treatment option for the restoration of carious lesions involving 3 or more surfaces of permanent posterior teeth and/or for the replacement of failed conventional restorations (Sigal et al., 2020). In the accessible literature, studies investigating the long-term clinical success of direct composite restorations of carious lesions causing excessive crown destruction of permanent molars in pediatric patients are rare (Zotti et al., 2021). For this reason, it is thought that our study which examines the 3-

year survival of direct composite restorations of teeth with excessive material loss in children aged 7-14 years, will contribute to the literature.

Limitation of the study

Although it is known that indirect restorations or stainless-steel crowns perform better than direct restorations especially in terms of anatomical form, marginal leakage and secondary caries for extremely damaged permanent teeth with the criteria specified in the study, direct composite resin restorations are preferred due to financial inadequacies and limited time for restoration during routine dental treatments in public university hospitals. Therefore, direct restorations have been evaluated in this study.

CONCLUSION

Survival rate of DCR restorations applied to PFMs with excessive material loss is not satisfactory after 3 years, however it is thought that the popularity of DCR among dentists will continue in permanent first molars with excessive material loss thanks to the developments in adhesive systems, composite resins and light-curing technology. Therefore, long-term randomized controlled clinical studies on this subject should be maintained.

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Conflict of Interest

The author declares no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Author Contributions

Plan, design: CÇE; **Material, methods and data collection:** CÇE; **Data analysis and comments:** CÇE; **Writing and corrections:** CÇE.

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Investigating the Image of the Profession of Nursing in the Society in the COVID-19 Pandemic Process: A Cross-Sectional Study

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ABSTRACT

Objective: This study was conducted to investigate the image of the profession of nursing in the society in the COVID-19 pandemic process. **Materials and Methods:** The study is of the cross-sectional descriptive type. The sample was determined with the snowball sampling method. The questionnaire forms were sent online to the participants between 15 August and 1 September 2020. 1020 individuals who responded to the questionnaires were included in the study. A Descriptive Information Form and the Nursing Image Scale (NIS) were used to collect the data. The data were analyzed with descriptive statistics, independent-samples t-test and ANOVA. **Results:** There were significant differences in the mean NIS general view and communication subscale scores based on gender, in the mean NIS total and subscale scores based on marital status, educational level, occupation, place of residence, having a nurse relative working during the pandemic and view towards the profession of nursing and in the mean NIS occupational and educational qualities subscale score based on hospitalization during the pandemic process ($p<0.05$). **Conclusion:** It was found in the study that the image of nursing in the society was positive in the pandemic period.

Keywords: Covid-19, Nursing Image, Pandemic, Society.

COVID-19 Salgını Sürecinde Hemşirelik Mesleğinin Toplumdaki İmajının İncelenmesi: Kesitsel Bir Çalışma

ÖZ

Amaç: Araştırma, COVID-19 salgını sürecinde hemşirelik mesleğinin toplumdaki imajının incelenmesi amacıyla yapıldı. **Gereç ve Yöntem:** Kesitsel tanımlayıcı türdedir. Araştırmanın örnekleme, kartopu örnekleme tekniği ile belirlendi. Anket formları 15 Ağustos-1 Eylül 2020 tarihleri arasında online olarak katılımcılara gönderildi. Anketlere geri dönüş sağlayan 1020 kişi araştırmaya dahil edildi. Verilerin toplanmasında Tanıtıcı Bilgi Formu ve Hemşirelik İmaj Ölçeği (HİÖ) kullanıldı. Veriler, tanımlayıcı istatistik, bağımsız gruplarda t testi ve ANOVA testleri ile değerlendirildi. **Bulgular:** Bireylerin cinsiyete göre HİÖ genel görünüm ve iletişim alt boyutu puan ortalamaları arasında, medeni durum, eğitim düzeyi, mesleği, yaşadığı yer, çalışan hemşire yakını ve hemşirelik mesleğine karşı bakış açısı göre HİÖ toplam ve alt boyut puan ortalamaları arasında ve hastaneye yatışa göre HİÖ mesleki ve eğitsel nitelikler alt boyutu puan ortalamaları arasında istatistiksel olarak anlamlı fark olduğu saptandı ($p<0.05$). **Sonuç:** Araştırmada, pandemi döneminde toplumda hemşirelik imajının olumlu yönde olduğu bulundu.

Anahtar Kelimeler: Covid-19, Hemşirelik İmajı, Pandemi, Toplum.

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INTRODUCTION

Pneumonia cases with unknown cause were reported in the city of Wuhan in China by the World Health Organization (WHO) on 31 December 2019. Chinese officials defined the cause of the cases as a novel coronavirus (COVID-19). On 20 January 2020, COVID-19 which was showing a rapid spread was declared as an emergency by WHO (*WHO Coronavirus Disease (COVID-19) Dashboard*, 2020). Following this, after stating that 118,000 cases had been encountered in 114 countries, and 4,291 people had died, WHO declared COVID-19 a pandemic on 11 March 2020. The first case in Turkey was reported on 11 March 2020, and the first virus-related death was seen on 17 March 2020 (Çevirme & Kurt, 2020). By 8 November 2020, there has been 49,578,590 positive cases in the world, and due to this virus, 1,245,717 patients have lost their lives. In Turkey, while the stated number of positive cases was 391,739, the number of deaths was reported as 10,803 (*WHO Coronavirus Disease (COVID-19) Dashboard*, 2020). As the number of cases increased, various precautions started to be taken, and still, precautions continue to be taken on a broad spectrum (Özdin & Bayrak Özdin, 2020; Yang et al., 2020).

In the risk assessment of the virus that has emerged in a broad area worldwide and affected several people by crossing international borders, it is stated that healthcare workers are in the primary risk group. In this process, nurses have been worked for weeks, in long shifts and without any leave. Many got infected with the COVID-19 virus and lost their lives due to lack of suitable personal protective equipment (Catton, 2020). According to the report by the International Council of Nurses (ICN), the number of nurses who died after getting infected with COVID-19 increase in August from 1,097 to 1,500, and 15% of the COVID-19-positive cases consisted of healthcare workers, most of whom were nurses (*International Council of Nurses (ICN)*, 2020). In this process, events experienced by nurses, who have a significant place in the health system, in the pandemic period have been widely included on social media. In the literature, it was stated that news stories about nurses increased by 3 times even in the first months of the COVID-19 pandemic (Bennett et al., 2020). In the pandemic period, the media has frequently included news stories showing the images of exhausted nurses who have bruises on their faces after wearing protective masks for hours while looking after COVID-19 patients and the personal sacrifices of nurses. In the media, nurses are shown as heroes working in the frontline in the fight against COVID-19 (Bennett et al., 2020; Catton, 2020). In addition to patient-nurse relationships, the perception of the society on nurses is also affected by mass communication tools and the image of a nurse portrayed by the media (Glerean et al., 2017; Kalisch et al., 2007; Rezaei-Adaryani et al., 2012; Rubbi et al., 2017). A study determined that inclusion of news reflecting the struggle shown by nurses in disaster periods such as epidemics and earthquakes in the media affects the image of nursing

positively (Mohammed, Peter, Killackey, & Maciver, 2021).

As in other professions, the views and expectations of the society are important in terms of the development of the profession of nursing (Çelik et al., 2013). Therefore, how the profession of nursing is perceived by the society in its development process is important. The literature review that was conducted did not reveal any study investigating the effects of the COVID-19 pandemic process on the image of nursing. In this study conducted in line with this information, it was aimed to investigate the image of the profession of nursing in the society in the process of the COVID-19 pandemic.

MATERIALS AND METHODS

Study type

This is a cross-sectional and descriptive study conducted for the purpose of investigating the image of the profession of nursing in the process of the COVID-19 pandemic.

Study group

The study was carried out in Eastern Turkey between 15 August and 1 September 2020. The population of the study consisted of individuals over the age of 18 years who were living in Eastern Turkey. The sample of the study was determined with the non-random sampling method of "snowball sampling". Based on this sampling method, the data collection forms prepared with the Google Docs platform were sent to individuals over the age of 18 online (e-mail, WhatsApp) at the data of the study, and the individuals were asked to fill out the forms and share the links with individuals over the age of 18 around them. The study was completed with 1020 individuals who responded to the data collection forms.

Based on the percentage measurement values on the methods to be studied in the conducted literature review (Özdelikara et al., 2015; Tortumluoğlu et al., 2010), with an effect size of 0.10257, power of 95% and error rate of 0.05, the sample size required for the study was calculated with the G-POWER software as $n=1020$. With the power analysis, it was determined that the collected data were sufficient.

Dependent and independent variables

The independent variables of this research are gender, age, Occupation, marital status, educational level, place of residence. The dependent variable is image of nursing in the society.

Procedures

The data of the study were collected by using a Descriptive Information Form and the Nursing Image Scale.

Descriptive information form: This questionnaire which was prepared by the researchers in line with the literature (Dinç et al., 2010; Özdelikara et al., 2015; Tortumluoğlu et al., 2010) consisted of questions on the descriptive characteristics such as the individuals' age, gender, marital status, educational level, Occupation and place of residence, as well as information on the topic such as having a nurse relative working in the pandemic process,

status of hospitalization and views towards nursing care and profession.

Nursing Image Scale (NIS): The scale was developed by Özsoy, but its psychometric properties were not assessed (Özsoy, 2000). The form was turned into a scale by conducting the necessary analyses by Çınar and Demir (Çınar & Demir, 2010). The scale consists of 28 items towards measuring the image of nursing in the society. The scale consists of three subscales as General View, Communication and Occupational and Educational Qualities. These subscales consist of 7, 6 and 15 items, respectively. The scale items are scored as Agree=3, Somehow Agree=2 and Disagree=1. This 3-point Likert-type scale has a total score range of 28-84. Higher total scores indicate a more positive nursing image (Çınar & Demir, 2010; Özsoy, 2000). The Cronbach's Alpha coefficient of the scale was reported as 0.81 (Çınar & Demir, 2010). In this study, the Cronbach's Alpha value was also found as 0.81.

Statistical analysis

The data were analyzed by using the SPSS 25.0 (the Statistical Package for the Social Sciences) statistical package software. Normal distribution was tested with Shapiro-Wilk normality test and Q-Q plots. The

descriptive statistics were analyzed by using independent-samples t-test and one-way analysis of variance (ANOVA). The level of statistical significance was accepted as $p < 0.05$.

Ethical considerations

For this study, ethical approval from the Non-Interventional Clinical Studies Ethics Board of Siirt University (E.509), written permission from the Ministry of Health and written consent from the participants who volunteered for the study were obtained.

RESULTS

In the study, it was determined that the mean age of the participants was 26.50 ± 7.49 , 68.5% were female, 69.9% were single, 55.3% had undergraduate degrees, 28.1% were working as civil servants, 71.0% were living in the city center, 64.0% had nurse relatives working in the pandemic period, 97.4% were not hospitalized in the pandemic period, 93.0% did not receive care from nurses in the pandemic period, the views of 53.3% towards the profession of nursing changed in the positive direction in the pandemic period (Table 1).

Table 1. Sociodemographic characteristics of the study group.

Descriptive Characteristics	n	%
Gender		
Female	699	68.5
Male	321	31.5
Marital Status		
Single	713	69.9
Married	307	30.1
Educational Level		
Literate-Primary-Secondary School	77	7.5
High School	145	14.2
Associate's	173	17.0
Undergraduate	564	55.3
Postgraduate	61	6.0
Occupation		
Unemployed	176	17.3
Laborer	78	7.6
Civil Servant	287	28.1
Freelancer	93	9.1
Homemaker	80	7.8
Teacher	131	12.8
Others	175	17.2
Place of Residence		
Village	100	9.8
District-town	196	19.2
City Center	724	71.0
Do you have a nurse relative working in the pandemic period?		
Yes	653	64.0
No	367	36.0
Were you hospitalized during the pandemic process?		
Yes	27	2.6
No	993	97.4
Did you receive care from a nurse in the pandemic period?		
Yes	71	7.0
No	949	93.0

Table 1. (Continue) Sociodemographic characteristics of the study group.

Descriptive Characteristics	n	%
Did your view towards the profession of nursing change in the pandemic process?		
Changed positively	544	53.3
Did not change	425	41.7
Changed negatively	51	5.0
Mean age	26.50±7.49	

*Column percentage

In the study, the participants' mean total NIS, as 68.77±7.35, 16.34±2.64, 14.85±2.87 and general view, communication and occupational and educational qualities scores were found respectively 37.57±4.34 (Table 2).

Table 2. Distributions of the NIS scores of the participants (n=1020).

NIS and Subscales	Number of items	Min. Score	Max. Score	X̄±SD	Cronbach's alpha
General View Dimension	7	7	21	16.34±2.64	0.68
Communication Dimension	6	6	18	14.85±2.87	0.84
Occupational and Educational Qualities Dimension	15	15	45	37.57±4.34	0.72
Total NIS	28	28	84	68.77±7.35	0.87

X:Mean, SD:Standard deviation.

There was a significant difference in the NIS general view and communication subscale scores of the participants based on their gender (p<0.05). There were significant differences in their NIS total and subscale scores based on marital status, educational level,

occupation, place of residence, having a working nurse relative and views towards the profession of nursing (p<0.05). There was also a significant difference in the NIS occupational and educational qualities scores based on hospitalization status (p<0.05) (Table 3).

Table 3. Comparison of the NIS mean scores based on the participants' demographic characteristics (n=1020).

Descriptive Characteristics	NIS Total and Subscale Scores (X̄±SD)			
	General View	Communication	Occupational and Educational Qualities	Total NIS
Gender				
Female	16.17±2.66	14.74±2.94	37.61±4.19	68.76±7.40
Male	16.70±2.57	15.10±2.72	37.48±4.65	68.76±7.25
Test value and p	t=-2.94 p=0.03	t=-1.89 p=0.05	t=0.45 p=0.65	t=-0.07 p=0.94
Marital status				
Single	16.55±2.58	15.21±2.72	37.94±4.32	69.68±7.16
Married	15.85±2.72	14.03±3.05	36.70±4.26	66.66±7.37
Test value and p	t=3.89 p=0.00	t=6.11 p=0.00	t=4.23 p=0.00	t=6.12 p=0.00
Educational status				
Literate-Primary-Secundary School	17.20±2.51	15.55±2.17	37.67±4.12	70.00±5.72
High School	15.94±2.60	14.73±2.76	37.01±4.43	68.02±7.56
Associate's	17.02±2.47	15.40±2.76	37.79±4.52	69.79±7.27
Undergraduate	16.21±2.66	14.75±2.96	37.78±4.26	68.79±7.40
Postgraduate	15.42±2.57	13.68±2.92	36.21±4.38	65.95±7.72
Test value and p	F=8.10 p=0.00	F=5.57 p=0.00	F=2.56 p=0.03	F=4.03 p=0.00

X:Mean, SD:Standard deviation.

Table 3. (Continue) Comparison of the NIS mean scores based on the participants' demographic characteristics (n=1020).

Descriptive Characteristics	NIS Total and Subscale Scores (X±SD)			
	General View	Communication	Occupational and Educational Qualities	Total NIS
Occupation				
Unemployed	16.48±2.55	14.95±2.84	37.23±4.64	68.62±7.65
Laborer	16.25±2.61	15.05±2.82	37.29±4.42	68.39±7.01
Civil Servant	15.82±2.80	14.21±2.96	37.03±4.18	67.28±7.03
Freelancer	17.13±2.46	15.39±2.84	38.18±3.89	70.26±6.72
Homemaker	16.30±2.60	14.66±2.81	36.86±4.04	67.92±7.16
Teacher	16.66±2.46	15.18±2.95	39.19±3.95	71.03±7.32
Others	16.44±2.60	15.28±2.61	37.72±4.58	69.44 ±7.62
Test value and p	F=3.78 p=0.01	F=4.03 p=0.00	F=4.80 p=0.00	F=5.26 p=0.00
Place of Residence				
Village	16.76±2.60	15.37±2.73	38.31±3.63	70.34±6.95
District-town	16.69 ± 2.64	15.26±2.67	37.92±4.60	69.46±7.44
City center	16.18 ± 2.63	14.67±2.93	37.37±4.34	68.37±7.35
Test value and p	F=4.28 p=0.01	F=4.94 p=0.00	F=2.85 p=0.05	F= 4.25 p=0.01
Do you have a nurse relative working in the pandemic process?				
Yes	16.60±2.59	15.19±2.75	38.07±4.08	69.82±6.95
No	15.87±2.66	14.25±2.99	36.68±4.63	66.91±7.68
Test and Significance	t=4.29 p=0.00	t=5.04 p=0.00	t=4.97 p=0.00	t=6.16 p=0.00
Were you hospitalized during the pandemic process?				
Yes	16.51±2.95	14.00±3.70	35.70±5.36	67.25±9.39
No	16.33±2.63	14.88±2.85	37.62±4.30	68.81±7.29
Test value and p	t=-0.35 p=0.72	t=-1.57 p=0.11	t=-2.27 p=0.02	t=-1.08 p=0.27
Did you receive care from a nurse in the pandemic process?				
Yes	16.54±3.05	14.42±3.34	36.91±4.72	67.97±8.27
No	16.32±2.61	14.89±2.83	37.62 ± 4.31	68.83±7.28
Test value and p	t=-0.68 p=0.49	t=-1.32 p=0.18	t=-1.32 p=0.18	t=-0.95 p=0.34
Did your view towards the profession of nursing change in the pandemic process?				
Changed positively	17.03±2.43	15.70±2.40	38.50±3.83	70.94±6.28
Did not change	15.67±2.52	14.01±2.87	36.68±4.31	66.59±6.99
Changed negatively	14.49±3.41	12.82±4.20	35.07±6.62	63.84±11.96
Test value and p	F=48.91 p=0.00	F=60.69 p=0.00	F=31.52 p=0.00	F=60.07 p=0.00

X:Mean, SD:Standard deviation.

DISCUSSION

According to the findings obtained in this study, it was determined that the participants' total NIS and the general view, communication and occupational and educational qualities subscale scores were in the positive direction (Table 2). In a similar study to ours conducted by Rubbi et al. on the nursing perceptions of the society, it was found that the image of nursing was positive (Rubbi et al., 2017). In their study on 481 individuals over the age of 18, Tortumluoğlu et al. stated that the image of the society towards nurses was positive (Tortumluoğlu et al., 2010). Similar results were obtained in studies in the literature on different populations (Bozkır et al., 2008; Ekinçi et al., 2014; Özdelikara et al., 2015; Özpancar et al., 2008). As opposed to the finding in our study, Takase et al. determined that the social image of nurses was not on a desired level as the job they did was uncertain in the eyes

of the society (Takase et al., 2006). Another study also found the image of nursing in the society to be low (Elmorshedy et al., 2020). Likewise, there are also other studies in the literature which contradicted the results of our study (Dinç et al., 2010; Tan et al., 2010). The nursing image in the society is also affected by what is read, viewed and heard from the media (Kalisch et al., 2007). As these studies have been conducted at different times and considering that this study was conducted in the pandemic period, it may be considered that showing nurses as heroes on the media and frequently including their fight against the pandemic in the media affected the image of nursing positively.

In the study, it was found that there was a significant difference in the NIS general view and communication subscale scores of the individuals based on gender, and the scores of the men were higher (Table 3). In difference to our study, the study by Çelik et al. on individuals over

the age of 18 did not find a significant relationship between gender and nursing image (Çelik et al., 2013). The study by Özkan et al. on nursing students found that gender did not affect nursing image scores (Kızılcık-Özkan et al., 2017). It is thought that the fact that men have started to be included in the profession of nursing affected the image of nursing in the men positively. Based on all these studies, it may be stated that nursing image varies based on individual characteristics.

There was a significant difference in the NIS total and subscale scores based on marital status, and the scores of the single participants were higher (Table 3). The heavy working conditions of nurses may have affected the nursing image of the married participants negatively. Additionally, the fact that most of the participants in our study (69.9%) were single may have affected this result. There was a significant difference in the NIS total and subscale scores based on educational levels (Table 3). As the educational level of the participants increased, their nursing image turned towards more negative. This result may have been affected by that the profession of nursing is not considered as an independent profession, but it is thought of as assistive healthcare personnel, whereas the fact that there are differences in the education levels of nurses, and their salaries are found to be low may have led the university graduate participants to look at the profession negatively.

It was found that there was a significant difference in the NIS total and subscale scores based on occupation, and the scores of the participants who were teachers were higher (Table 3). As opposed to the case in our study, in a study conducted with individuals over the age of 18 at a city center, it was found that the image of nursing was more positive among those who were freelancers (Çelik et al., 2013). In the study by Eskimez et al. conducted with the purpose of determining the views of female high school senior students towards the profession of nursing, it was determined that 66.7% of those whose fathers were not working considered choosing the profession of nursing, while 19.7% of those whose fathers were civil servants considered choosing the profession (Z. Eskimez et al., 2008). It may be stated that the type of profession that individuals have and their socioeconomic level affect their nursing image.

It was ascertained that there was a significant difference in the NIS total and subscale scores based on the participants' places of residence, where those living in villages had higher scores (Table 3). It may be stated that the increase in the need for nursing services among those living in regions where access to healthcare services is limited affects the image of the profession positively.

It was found that there was a significant difference in the NIS total and subscale scores based on the participants' status of having a nurse relative working in the pandemic process, and the scores of those with nurse relatives were higher (Table 3). The study by Çelik et al. also found that the nursing image of those who had a nurse relative in their family was more positive (Çelik et al., 2013). In the study by Tortumluoğlu et al., 79.4% of the participants stated that there were nurses among their close relatives

(Tortumluoğlu et al., 2010). Eskimez et al. determined that students who had nurse relatives had higher rates of preferring nursing as a profession (Z. Eskimez et al., 2008). The results of these studies were in parallel with the results of ours. However, there are also studies which reported that the presence of a family member working as a nurse affected nursing image negatively (Degazon et al., 2015; Kay M, 2015). The differences among studies' findings may be related to the time periods in which these studies were conducted. Considering especially that this study was conducted in the pandemic period, the close witnessing of the individual sacrifices of nurses in this process by their relatives may have affected the nursing image of the relatives positively.

There was a significant difference in the NIS occupational and educational qualities scores based on hospitalization status, and those that were hospitalized in this process had higher scores (Table 3). Tortumluoğlu et al. determined that 58% of the society had positive experiences in their communication with nurses (Tortumluoğlu et al., 2010). It may be thought that, especially due to the isolation of patients hospitalized in the pandemic period, the fact that no family members were accepted as visitors or attendants, and all necessities were met by nurses affected nursing image positively.

A significant difference was found in the NIS total and subscale scores based on views towards the profession of nursing in the pandemic process (Table 3). It may be stated that highly frequent inclusion of the events experienced by nurses in the pandemic process on the media and nurses' heroic fight against the pandemic by risking themselves despite poor working conditions changed the society's views towards the profession in the positive direction.

Limitations of Study

The first limitation of this study is that only individuals who could be reached online participated in the study. Moreover, instead of a random sampling method, a snowball sampling method was used, which might have caused sampling bias.

CONCLUSION

As a result of this study, it was found that the mean scores of nursing image in the society in the pandemic process were positively higher in comparison to those in previous studies. In this pandemic process, it is thought that it was effective for nurses to serve their duties 24/7 by working in the frontlines and making compromises from their families and for this to be included frequently on social media.

It was determined that the number of studies on the social image of nurses conducted in different populations was very low. Therefore, it may be recommended to conduct more studies towards the image perceptions of the society on the profession of nursing and increase awareness regarding the importance of image in line with the obtained results.

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Conflict of Interest

The author declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Author Contributions

Plan, design: ZB, MA; **Material and methods:** ZB, MA; **Data analysis and comments:** ZB; **Writing and corrections:** ZB, MA

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Results of Cementless and Cemented Bipolar Hemiarthroplasty in Proximal Femur Fractures with Dorr Type B Morphology

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ABSTRACT

Objective: Surgical outcomes of patients with Dorr type B femoral morphology operated for hip fracture with bipolar hemiarthroplasty were compared. **Materials and Methods:** This retrospective study included 255 patients with a follow-up of at least five years. Patients were divided into two groups: cementless (group 1; n=87) and cemented (group 2; n=168). The study focused on the following parameters: Admission cost, fracture type, gender, need for blood transfusion, Harris Hip Score (HHS), additional fracture, use of cement, mortality rate, revision surgery, the time between trauma and surgery, length of hospital stays, and surgery time. **Results:** The mean age of patients was similar in both groups. Group 2 had a significantly longer duration of surgery and a higher need for blood transfusions than group 1. The mean hemoglobin levels of patients were similar in both groups. Pertrochanteric femoral fractures were more common than femoral neck fractures in group 2. The two groups did not differ on the parameters of time between trauma and surgery, mortality rate, revision surgery, length of hospital stay, cost, and additional fractures. **Conclusion:** In patients aged 75 years or older who are scheduled for cemented bipolar hemiarthroplasty, erythrocyte suspension preparation should be performed before surgery if a hip fracture with Dorr type B femoral morphology is present.

Keywords: Elderly, Hemiarthroplasty, Hip, Hip Fractures.

Dorr Tip B Morfolojili Proksimal Femur Kırıklarında Çimentosuz ve Çimentolu Bipolar Hemiartroplasti Sonuçları

ÖZ

Amaç: Bipolar hemiarthroplasti ile kalça kırığı nedeniyle ameliyat edilen Dorr tip B femur morfolojisine sahip hastaların cerrahi sonuçları karşılaştırıldı. **Gereç ve Yöntem:** Bu retrospektif çalışmada, en az 5-yıllık takibi olan 255 hasta değerlendirildi. Hastalar iki gruba ayrıldı: sementsiz (Grup 1; n=87) ve sementli (Grup 2; n=168). Çalışma şu parametrelere odaklandı: yatış maliyetleri, kırık tipi, cinsiyet, kan transfüzyonu ihtiyacı, Harris hip skoru (HHS), ek kırık, çimento kullanımı, ölüm oranı, revizyon cerrahisi, travma ile cerrahi arasındaki süre, hastanede kalış süresi ve ameliyat süresi. **Bulgular:** Gruplarda yer alan hastaların yaş ortalaması benzer idi. Grup 2, Grup 1'e göre anlamlı olarak daha uzun ameliyat süresine ve daha yüksek kan transfüzyonu oranına sahipti. Ortalama hemoglobina değerleri her iki grupta benzerdi. Grup 2'de pertrokanterik femur kırıkları, femur boyun kırıklarından daha yaygındı. Travma ve ameliyat arasındaki süre, ölüm oranı, revizyon cerrahisi, hastanede kalış süresi, yatış maliyeti ve ek kırık açısından iki grup arasında anlamlı bir fark yoktu. **Sonuç:** 75 yaş veya üzeri Dorr tip B femur morfolojisine sahip kalça kırığı olan hastalar çimentolu bipolar hemiarthroplasti planlanıyorsa ameliyat öncesi mutlaka eritrosit süspansiyonu hazırlığı yapılmalıdır.

Anahtar kelimeler: Yaşlı, Hemiartroplasti, Kalça, Kalça Kırıkları.

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INTRODUCTION

Osteoporotic hip fractures are more common with increasing age. It is estimated that by 2050, a total of 63 million hip fractures will be identified worldwide (Melton III et al., 1999). These fractures are becoming increasingly critical due to high mortality and the cost of postoperative care. A patient with a hip fracture incurs three times the cost per year as a patient without a hip fracture (Haentjens et al., 2001).

The Dorr type, Singh index, cortical thickness index (CTI), and canal-to-calcar ratio (CCR) are classification systems for the quality of the proximal femur (Singh et al., 1970). The Dorr classification was developed by Dorr (1993). While Dorr type A indicates good bone quality, types B and C are more common indications of osteoporotic bone (Sah et al., 2007).

Hip fractures are treated in a variety of ways in older patients. Total hip arthroplasty (THA) and internal fixation (IF) are standard options in elderly patients or patients with osteoporotic hip fractures, but hemiarthroplasty (cemented or cementless) is still a treatment of choice. Hemiarthroplasty has been reported to be relatively superior to THA and IF (Fan et al., 2012; Kiran Kumar et al., 2013; Parker & Johansen, 2006; Sonar et al., 2014). Therefore, hemiarthroplasty is the primary procedure of choice in our clinic for the treatment of older hip fractures with low bone quality.

Some intraoperative and postoperative complications of hemiarthroplasty include periprosthetic fractures, dislocation, deep infection, venous thromboembolism, cardiopulmonary disease, and mortality (Weinrauch, 2006).

This retrospective study with a follow-up of five years compared the differences between cementless and cemented bipolar hemiarthroplasty in elderly patients with a Dorr B-type proximal femur fracture.

MATERIALS AND METHODS

Three hundred two patients aged 75 years or older with Dorr type B femoral morphology underwent bipolar hemiarthroplasty for proximal femoral fractures. Forty-seven patients were not included in the sample because we did not have access to their data. Eighty-seven (34.10%) patients underwent surgery with cementless bipolar hemiarthroplasty (group 1), whereas the remaining 168 (65.90%) underwent surgery with cemented bipolar hemiarthroplasty (group 2). Thirty-two (12.50%) patients died within the first five years. The remaining 223 patients (87.50%) were followed up for at least five years.

Parameters

The parameters were: Age (≥ 75), sex, duration of surgery, time between first admission and surgery, additional fracture, blood transfusion, hemoglobin (Hg), sodium (Na), potassium (K), creatinine,

glucose, thyroid-stimulating hormone (TSH), free thyroxine (T4), Dorr classification, fracture classification, Harris Hip Score (HHS), mortality, revision surgery, and admission cost. Blood tests were performed at initial admission.

The Dorr classification is used to determine bone quality and structure of the proximal femur (Dorr et al., 1993). Type A has a dense cortical bone beginning at the tip of the lesser trochanter, which looks like a "champagne flute" and a slender femoral canal. Type B has a wide diaphyseal canal and a thin cortex. Type C has a wide diaphyseal canal that looks like a "stovepipe."

William H. Harris developed the Harris Hip Score to assess the health consequences of hip surgery (Mahomed et al., 2001). It is a ten-item instrument used to assess postoperative recovery and compare health outcomes. The patient answers eight questions while the physician scores two items (deformity and range of motion). The highest score is 100, and the worst score is < 70 .

The total cost was the sum of the cost of all procedures performed from admission to discharge. During the 5-year follow-up, patients who underwent reoperation for infection, periprosthetic fracture, or dislocation were documented.

Statistical analysis

The calculation of the values of skewness and kurtosis is a method for analyzing the normal distribution of the data. The values for kurtosis and skewness of the measurements were between -3 and +3, so the normality test could be considered valid (DeCarlo, 1997; Groeneveld & Meeden, 1984; Hopkins & Weeks, 1990; Moors, 1986). The data and results all conformed to the range of normal distribution. Statistical analyzes were performed using parametric tools.

The independent-samples t-test for numerical data was used to examine differences between groups. We evaluated categorical data and descriptive statistical methods (mean, standard deviation) using Pearson chi square and Fisher's exact tests. SPSS® version 20.00 for Windows (SPSS, Armonk, NY, USA) was used for statistical analysis. The significance threshold was chosen at $p < 0.05$.

Ethical considerations

Informed consent was obtained from all patients who participated in the study during their first hospitalization. The study was conducted in accordance with the principles of the Declaration of Helsinki. We obtained ethical approval from the Ethics Committee of Erzurum Regional Training and Research Hospital (approval date/number: 01.03.2021/ 05-89).

RESULTS

The mean age of the patients was 83.03 years. While the mean age of patients in group 1 was 82.34 years, the mean age of patients in group 2 was 83.38 years. There was no statistically

significant difference between the groups in terms of age ($p=0.142$). The mean operation time was 92.28 min in group 1 and 109.03 min in group 2,

respectively. We found a statistically significant difference between the two groups ($p=0.002$) (Table 1).

Table 1. Demographic data of the patients.

Dorr type b	Cementless (n=87)	Cemented (n=168)	Total (n=255)	P
	X±SD (Min-Max)	X±SD (Min-Max)	X±SD (Min-Max)	
Age	82.34±5.26 (75-94)	83.38±5.36 (75-106)	83.03±5.34 (75-106)	0.142
Surgery time/minutes	92.28±43.05 (29-240)	109.03±38.51 (35-245)	103.31±40.82 (29-245)	0.002*
Total cost / USD	1769±443 (471-3025)	1783±528 (507-3227)	1778±500 (471-3227)	0.839
Hospitalization time / day	8.86±4.37 (1.70-22.90)	8.44±3.21 (2.30-19.40)	8.58±3.64 (1.70-22.90)	0.431
Time between trauma and surgery / day	2.94±1.85 (0.30-8.10)	2.95±1.76 (0.40-9.60)	2.95±1.79 (0.30-9.60)	0.970
Hemoglobin (g/dL)	12.90±1.86 (8.00-17.90)	12.55±1.96 (7.50-17.60)	12.67±1.93 (7.50-17.90)	0.173
Glucose (mg/dL)	127.26±33.33 (65-221)	131.51±31.46 (57-229)	130.06±32.11 (57-229)	0.317
Creatinine (mg/dL)	1.01±0.43 (0.30-2.40)	0.97±0.37 (0.50-2.40)	0.99±0.39 (0.30-2.40)	0.477
TSH (mU/L)	1.49±0.99 (0-5)	1.49±1.16 (0-6)	1.49±1.10 (0-6)	0.967
T4 (ng/L)	1.11±0.22 (0.60-1.80)	1.10±0.23 (0.60-1.80)	1.10±0.23 (0.60-1.80)	0.641
Na (mmol/L)	137.83±3.78 (125.00-148.00)	137.25±3.62 (121.00-148.30)	137.45±3.68 (121-148.30)	0.236
K (mmol/L)	4.35±0.63 (3.10-7.30)	4.25±0.60 (3.10-6.50)	4.28±0.61 (3.10-7.30)	0.194

TSH: Tirotropin; T4: Tiroksin; Na: Sodium; K: Potassium. * Independent Samples t-test.

The patients who received blood transfusion in the postoperative period were 53 patients in group 1 and 138 patients in group 2 ($p<0.001$) (Table 2).

Trauma caused by the fracture was low-energy in 251 (98.40%) patients. Only 4 (1.60%) patients had fractures after high-energy trauma. These four patients underwent cementless bipolar hemiarthroplasty for a pertrochanteric femoral fracture.

In group 1, 25 (28.70%) patients had pertrochanteric femur fractures, while 62 (71.30%) patients had femoral neck fractures. In group 2, 123 (73.20%) patients had pertrochanteric femoral fractures, while the remaining 45 (26.80%) patients had femoral neck fractures. There was considerable variability in the type of fractures between the two groups ($p=0.017$) (Table 2).

Patients operated on with cementless bipolar hemiarthroplasty had HHS scores of 75.06±8.65 and 84.34±7.02 at six months and 12 months after surgery, respectively, and there was a statistically significant difference ($p<0.001$). In patients operated with cemented bipolar hemiarthroplasty, the HHS scores at six months and 12 months after surgery were 77.30±8.69 and 84.41±7.00, respectively, and there was a statistically significant difference ($p<0.001$). The mean HHS score at postoperative month 12 was significantly higher than the mean HHS score at postoperative month 6 in all patients ($p<0.001$). In contrast, there was no significant difference in postoperative HHS scores at six months ($p=0.052$) and at 12 months ($p=0.943$) between the two groups (Table 3).

Table 2. Surgical data of the patients.

Dorr type b		Cementless (n=87)	Cemented (n=168)	Total (n=255)	p
		n (%)	n (%)	n (%)	
Blood transfusion	None	34 (39.10%)	30 (17.90%)	64 (25.10%)	<0.001**
	Transfusion	53 (60.90%)	138 (82.10%)	191 (74.90%)	
Death	Live	74 (85.10%)	149 (88.70%)	223 (87.50%)	0.504
	Dead	13 (14.90%)	19 (11.30%)	32 (12.50%)	
Gender	Male	46 (52.90%)	86 (51.20%)	132 (51.80%)	0.801
	Female	41 (47.10%)	82 (48.80%)	123 (48.20%)	
Revision surgery	None	80 (92.00%)	157 (93.50%)	237 (92.90%)	0.124
	Revised	7 (8.00%)	11 (6.50%)	18 (7.10%)	
Additional fractures	None	85 (97.70%)	161 (95.80%)	246 (96.50%)	0.402
	Fractures	2 (2.30%)	7 (4.20%)	9 (3.50%)	
Trauma	Low	83 (95.40%)	168 (100.00%)	251 (98.40%)	0.004*
	High	4 (4.60%)	0	4 (1.60%)	
Fracture type	Petrochanteric	25 (28.70%)	123 (73.20%)	148 (58.00%)	0.017**
	Femoral Neck	62 (71.30%)	45 (26.80%)	107 (42.00%)	

* Fisher's Exact Test (1-sided), ** Pearson Chi-Square.

Table 3. Harris Hip Score in the postoperative 6th and 12th months.

Dorr Type B	Cementless N: 87	Cemented N: 168	Total N: 255	p
	X±SD (Min-Max)	X±SD (Min-Max)	X±SD (Min-Max)	
HHS- 6 th month	75.06 ± 8.65 (63-90)	77.30 ± 8.69 (63-90)	76.53 ± 8.73 (63-90)	0.052
HHS- 12 th month	84.34 ± 7.02 (70-95)	84.41 ± 7.00 (70-95)	84.39 ± 6.99 (70-95)	0.943
p-value	<0.001*	<0.001*	<0.001*	

HHS: Harris Hip Score

* Related-Samples Test

There was no significant difference between the two groups in total cost (p=0.839), length of hospital stays (p=0.431), and time from trauma to surgery (p=0.970). There was no significant difference in hemoglobin (p=0.173), creatine (p=0.477), glucose (p=0.317), TSH (p=0.967), T4 (p=0.641), Na (p=0.236) and K (p=0.194). There was also no significant difference in death (p=0.504), sex

(p=0.801), revision surgery (p=0.124), and additional fractures (p=0.402).

DISCUSSION

One of the most striking results was that petrochanteric femoral fractures were significantly more common in group 2 than in group 1. The petrochanteric fractures are more complicated to treat than femoral neck fractures. The fact that the operative time was longer and the need for blood

transfusion was higher in group 2 also supports this contention.

Hip fractures, which increase with age, are more common in women than in men. After menopause, estrogen levels in women decrease, increasing the risk of fractures in women (Anderson et al., 1964; Ong et al., 2002; Seckin, 2003). In contrast, we found that the number was slightly higher in men.

There is an ongoing debate about the ideal treatment for hip fractures caused by osteoporosis in the elderly. The most common complications are avascular necrosis after internal fixation, nonunion and implant failure, and hip dislocation and reoperation for total hip arthroplasty (Guyen, 2019; McKinley & Robinson, 2002). Therefore, hemiarthroplasty appears to be the ideal treatment for such patients. Initially, the equipment for arthroplasty may be expensive, but given the high reoperation rates and complications from internal fixation, we think hemiarthroplasty will cost less (Lu-Yao et al., 1994; Rogmark et al., 2003).

Current research, which includes national registries of joint replacements, recommends cemented stems for femoral neck fractures because they offer immediate full weight-bearing, less postoperative discomfort in the mid-thigh, lower risk of intraoperative fractures, and fewer late fractures requiring revision (Song et al., 2019).

Complications and revision surgery can also drive up costs. Dorr et al. (1993) reported that patients with femur type C have a higher risk of intraoperative fractures than patients with femur types A and B (Dorr et al., 1993). Cement may reduce the risk of intraoperative fractures (Nash & Harris, 2014). We did not find a significant difference in revision surgery and intraoperative fracture rates between the two groups.

Some studies reported higher blood transfusion rates, longer operative time, and more cardiovascular complications in patients with cemented hemiarthroplasty than in patients with cementless hemiarthroplasty (Barenus et al., 2018; Frenken et al., 2018). According to some authors, there is no hemodynamically significant difference between cemented and cementless hemiarthroplasty (Inngul et al., 2015; Miyamoto et al., 2018). We found that group 2 required more blood transfusions in the postoperative period than group 1.

Most studies show that the mortality rate in the postoperative period is higher in patients with cemented hemiarthroplasty than in patients with cementless hemiarthroplasty (Holt et al., 1994; Muirhead-Allwood et al., 1983). However, some other studies reported similar mortality rates in both groups (Frenken et al., 2018). A delay of more than four days between admission and surgery increases mortality rates in the first year (Moran et al., 2005). We found that both groups 1 and 2 had a mean delay of 72 hours between admission and surgery and

similar mortality rates in the first five years (14.90% and 11.30%, respectively).

This study had some limitations. First, we did not include postoperative hemoglobin levels in the analysis because the blood tests were performed on different days. Second, we did not compare pain scores between the two groups. Third, the tension band technique with K-wire and cerclage was used to fix fractures in the greater trochanter in some patients. However, no differential grouping was performed for these patients.

This study had three strengths. First, the sample size was large. Second, we followed the patients over a long period of time. Third, a comparative approach was used, which is rare in the literature. Therefore, we believe our results will contribute to the literature.

CONCLUSION

Surgical time was longer in patients operated on with cemented bipolar hemiarthroplasty. The need for blood transfusion was higher in patients operated on with cemented bipolar hemiarthroplasty. While femoral neck fractures were more common in patients operated on with cementless bipolar hemiarthroplasty, petrochanteric femoral fractures were more common in patients operated on with cemented bipolar hemiarthroplasty. The postoperative recovery and health outcomes of patients operated on with cemented or cementless bipolar hemiarthroplasty were similar.

Therefore, in patients aged 75 years or older who are scheduled for cemented bipolar hemiarthroplasty, the erythrocyte suspension preparation should be performed before surgery if a hip fracture with Dorr type B femoral morphology is present.

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Conflict of Interest

The authors declare that there are no potential conflicts of interest related to the research, authorship, and/or publication of this article.

Author Contributions

Plan, design: MÇE, ST, KZ; **Material, Methods, and data collection:** KZ, MÇE, MCT; **Data analysis and comments:** ST, MCT; **Writing and corrections:** MÇE, ST, MCT, KZ.

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An Investigation of Psychological Resilience and Core Belief Levels of Women During the COVID-19 Pandemic and Affecting Factors

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ABSTRACT

Objective: The aim of the study is to determine the psychological resilience and core belief levels of women during the COVID-19 pandemic period and examine the affecting factors. **Material and Methods:** The study is in a cross-sectional design. The data were collected from 792 women using the Descriptive Information Form, the Psychological Resilience Scale, and the Core Beliefs Inventory. Descriptive statistics and the Ordinary Least Square Regression analysis were used to analyze the data. **Results:** The mean score of the “psychological resilience scale” was 66.50±13.40 and “core beliefs inventory” was 27.87±10.76. The COVID-19 fear score of women was found to be 6.31±1.98 out of 10. The psychological resilience levels were higher in working women, older women, and those with higher education levels (p<0.05). A negative significant relationship was found between the fear of COVID-19 and psychological resilience (p<0.05), and a positive correlation was found between women’s age, working status, psychological resilience, and core belief levels (p<0.05). **Conclusion:** The mean score of psychological resilience and core beliefs of women in the COVID-19 pandemic was found to be moderately good. Some socio-demographic characteristics and factors can affect women’s resilience and core beliefs.

Keywords: COVID-19, Psychological Resilience, Core Belief, Women Health.

COVID-19 Pandemi Sürecinde Kadınların Psikolojik Sağlamlık ve Temel İnanç Düzeyleri ve Etkileyen Faktörlerin İncelenmesi

ÖZ

Amaç: Çalışmada amaç; COVID-19 pandemi sürecinde kadınların psikolojik sağlamlık ve temel inanç düzeyini belirlemek ve etkileyen faktörleri incelemektir. **Gereç ve Yöntem:** Çalışma kesitsel tiptedir. Araştırmanın verileri Google anket aracılığı ile online olarak 792 kadından toplanılmıştır. Verilerin toplanılmasında tanıtıcı bilgi formu, Psikolojik Sağlamlık Ölçeği ve Temel İnançlar Ölçeği kullanılmıştır. Verilerin analizinde tanımlayıcı istatistikler (sayı, yüzde, toplam puan) ve the Ordinary Least Square Regression (OLSR) analizi kullanılmıştır. **Bulgular:** Kadınların “psikoloji sağlamlık ölçeği” puan ortalaması 66.50±13.40 “temel inançlar ölçeği” toplam puanı 27.87±10.76 bulunmuştur. Kadınların COVID-19 korku puanı 10 üzerinden 6.31±1.98 olarak saptanmıştır. Yaşı ve eğitim düzeyi yüksek olan ve çalışan kadınların psikolojik sağlamlık düzeyinin daha yüksek olduğu saptanmıştır (p<0.05). COVID-19 korkusu ile psikolojik sağlamlık arasında negatif yönde anlamlı ilişki bulunmuştur (p<0.05). Kadınların yaşı, çalışıyor olması ve psikolojik sağlamlık düzeyi ile temel inanç düzeyi arasında pozitif yönde bir ilişki bulunmuştur (p<0.05). **Sonuç:** COVID-19 pandemisinde kadınların psikolojik sağlamlık ve temel inançlar puan ortalamasının moderately good olduğu ortaya koyulmuştur. Bazı sosyo-demografik özellikler ve COVID-19 korkusu gibi durumlar kadınların psikolojik sağlamlık ve temel inançlarını etkileyebilmektedir.

Anahtar kelime: COVID-19, Psikolojik Sağlamlık, Temel İnanç, Kadın Sağlığı.

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INTRODUCTION

The COVID-19 pandemic has caused many people to become ill and die worldwide, and people still suffer from its negative impacts such as social isolation, fear, anxiety (WHO, 2020). Countries all over the world have taken various restrictions and measures (stay at home/social isolation/quarantine, work from home, online education, restriction in public transportation, closure of workplaces and social areas, etc.) to prevent and reduce the transmission of Coronavirus 19 (COVID-19) (Brooks, Webster, Smith, Woodland, Wessely, Greenberg, & Rubin, 2020).

The COVID-19 pandemic has caused individuals to change their daily life activities, experience fear of death, and lose many people from their families or immediate circles (Riehm, Brenneke, Adams, Gilan, Lieb, Kunzler, Smail, Hologue, Stuart, & Kalb, 2021). This pandemic has adversely affected the health, economy, and socio-cultural life of the 8 billion world population (Li et al., 2020) and caused an unprecedented disaster in human history and a global trauma (Chen & Bonanno, 2020).

The COVID-19 pandemic has had negative economic and health impacts on people, including job loss, financial difficulties, inability to benefit from health services adequately, high levels of stress, anxiety, fear of virus transmission and death, and mental health problems like depression and post-traumatic stress disorder (Satici et al., 2020; Wang, Pan, Wan, Tan, Xu, Ho, & Ho, 2020). Women who have a more caring role in the family and who are going through other special processes such as pregnancy, childbirth, and breastfeeding have been influenced more intensely (Aydın & Aktaş, 2021). Worldwide, women mostly have the caregiver role in the family. Two out of every three women perform house cleaning, childcare, education, and cooking within the caregiver role of the family (CDC, 2021). The economic difficulties, spouse/partner violence, the increase in workload due to staying at home and gender roles, and the problems related to women-specific periods such as pregnancy and childbirth during the pandemic have caused women's psychosocial health to deteriorate more than men (Benassi, Vallone, Camia, & Scorza., 2020; CDC, 2021; Roberto, Sellon, Cherry, Hunter-Jones, & Winslow 2020). Deterioration of psycho-social health is associated with deterioration of psychological resilience and shaking of core beliefs.

Psychological resilience is defined as an individual's ability to adapt to challenges effectively and restore balance (Yıldırım et al., 2020). It includes the concepts of growing, developing, and getting better. Many factors such as gender, age, education level, type of trauma, exposure to trauma, economic situation, social support, current, and previous life stressors affect the level of resilience (Bonanno, Galea, Bucciarelli, & Vlahov, 2007; Machisa, Christofides, & Jewkes, 2018). Various studies reported the psychological resilience of women during the COVID-19 pandemic to be moderate (Kılınç & Sis Çelik, 2021) and low (Lai, Ma, Wang,

Cai, Hu, Wei, Wu, Du, Chen, & Li, 2020). The psychological resilience levels of individuals affect their reactions to the events during the pandemic and are very effective in coping with this difficult condition, protecting their mental health, and realizing their goals (Kılınç & Sis Çelik, 2021).

A national comprehensive study conducted in the early days of the pandemic in the United States showed that individuals with higher levels of education and middle-aged or adult individuals had higher resilience levels. Besides, those with high levels of psychological resilience had a low level of pandemic-related stress (Riehm et al., 2021). Psychological resilience is a protective factor in coping with the difficulties encountered in the COVID-19 pandemic (Coulombe, Pacheco, Cox, Khalil, Doucerain, Auger, & Meunier, 2020; Luu, 2021; Riehm et al., 2021), and affects the core beliefs of the individual.

Core beliefs are the individuals' perceptions of themselves, other people, and the world (Luu, 2021). For example, the belief "the world is a safe place, no harm will ever come to me, etc." is a core belief of the individual. Pandemic, war, disaster, accidents, etc. can have a shocking effect on individuals and cause them to question their core beliefs. The core beliefs like "the world is a safe place, no harm will ever come to me, etc." can be shaken by situations like "being diagnosed with cancer", "being at war" "mother losing her baby" and questioning/struggling against core beliefs may begin (Lianchao & Tingting, 2020). As a result of this questioning, there can be a constructive and positive interpretation and change as well as a negative change about the event (such as cancer, COVID-19 infection) in the core beliefs (Luu, 2021; Riehm et al., 2021). The fact that individuals struggle with core beliefs may be a sign that they can enter the process of making a positive meaning to events, gain new alternatives and perspectives, enter a positive path in their lives, and show post-traumatic development (Eze, Ifeagwazi, & Chukwuorji, 2020). The COVID-19 pandemic can affect women's core belief levels by creating a traumatic effect (Riehm et al., 2021). Therefore, all health professionals working in the field of women's health have significant responsibilities in identifying the factors that affect women's levels of psychological resilience and core beliefs and supporting them to gain positive coping methods with traumatic experiences in challenging periods such as the COVID-19 pandemic.

The aim of this study is to determine the psychological resilience and core belief levels of women during the COVID-19 pandemic and affecting factors. Much has been written about the effects of the COVID-19 pandemic on women's mental health problems (depression, anxiety, etc.) during the pandemic; however, there is a paucity in the literature in terms of factors affecting women's resilience and core belief levels during the pandemic (Kılınç & Sis Çelik, 2021; Lai et al., 2020). Studies on the core beliefs of women in the pandemic are quite limited. This study was carried out based on such a need, and it is believed to

fill the gap in the literature and contribute to the planning of new strategies for the protection and promotion of women's psychological health during the pandemic.

The questions of this study are as follow

What is the level of the psychological resilience of women during the COVID-19 pandemic?

What is the level of the core belief of women during the COVID-19 pandemic?

What are the factors affecting the level the psychological resilience and core belief levels of women during the COVID-19 pandemic?

MATERIALS AND METHODS

Study type

The study is cross-sectional and analytical type.

Population and sample

The population of the research consists of women living in Turkey. Inclusion criteria of the study were (1) being a volunteer, (2) knowing Turkish, (3) using one of the WhatsApp or social media accounts, (4) being literate, (5) not having a comprehension problem, and (6) not having a psychiatric disorder. The participants were reached by private messages and open posts from the researchers' WhatsApp and social media accounts, and they were asked to forward the questionnaire to the individuals in their family and social circle. The sample size was determined by performing the G-Power analysis. The sample calculation is based on the difference between the two averages in the G-Power analysis. In the calculation, the type I error rate was (α)=0.05, and the power of the study ($1 - \beta$) was 95%.

The sample size was calculated based on the Karairmak (2010) study. The analysis showed that the sample size should be at least 446 people to obtain the 95% power ratio at the 95% confidence interval. In this study, 792 people were reached, considering the possibility of missing and extreme values due to online data collection.

Data collection

The data were collected using the "Descriptive Information Form", the "Psychological Resilience Scale" and the "Core Beliefs Inventory" between June 2021 and August 2021 through "Google online Survey". The survey form was sent by the researchers to the "WhatsApp" line on the mobile phones of the women, and the researchers shared the survey form from social media accounts such as "Facebook, Twitter, Instagram".

The Descriptive Information Form: Created by the researchers in line with the literature, the form consists of nine questions about the socio-demographic characteristics of women like age, gender, marital status, and working status, and the fear of experiencing COVID-19.

The Connor-Davidson Psychological Resilience Scale: The scale was developed by Connor and Davidson (2003) and adapted into Turkish culture by Karairmak (2010). It consists of 25 items in a 5-point Likert type (0 "Not true at all" - 4 "Always true"). The scores to be obtained from the scale are between 0-100. High scores indicate high levels of psychological resilience. The total cronbach alpha of the scale is 0.92. In this study, the cronbach's alpha value was found to be 0.91.

The "Core Beliefs Inventory":The inventory was developed by Cann, Calhoun, Tedeschi, Kilmer, Gil-Rivas, Vishnevsky, & Danhauer, (2010) and adapted into Turkish by Haselden (2014). The scale consists of two factors, Core Beliefs About Other People and Core Beliefs About Self, and nine questions. The scale is a six-point Likert type (0=not at all, 6=a very great degree). High scores indicate a possible deterioration in assumptions about the world. The general Cronbach's alpha coefficient of the scale is 0.87, 0.90 for the core beliefs about the "other people" sub-factor, and 0.82 for the core beliefs about the "self" sub-factor. In this study, The Cronbach's alpha value was found to be 0.90.

Data evaluation and analysis

In the analysis of the data, the SPSS package program was used. Percentage and frequency were utilized to present participants' socio-demographic information. To understand the relationship between psychological resilience scale total score (P_{RS}), core belief inventory total score (C_{BI}), and personal characteristics, the Ordinary Least Square Regression (OLSR) was conducted. P_{RS} and core belief inventory total score C_{BI} were taken as dependent variables in the proposed two regression models. Additionally, age, education level, family type, marital status, working status, the status of having children, the status of obeying the 14 rules of COVID-19 pandemic, the status of losing a family member due to COVID-19, and COVID-19 fear status were taken as independent variables of both proposed models using the OLSR. The dependent and independent variables of the study can be summarized as given in Table 1.

Table 1. Dependent and independent variables of psychological resilience scale total score (P_{RS}) and core belief inventory total score (C_{BI}) using OLS regression.

P_{RS}	Psychological resilience scale total score
C_{BI}	Core belief inventory total score
Covariates	
C_{19F}	Status of COVID-19 fear

Table 1. (Continue) Dependent and independent variables of Psychological Resilience Scale total score (P_{RS}) and Core Belief Inventory total score (C_{BI}) using OLS regression.

Dummy Variables	
Age_2	(If the participant age is between 26-35 years:1, otherwise: 0)
Age_3	(If the participant age is higher than 35 years:1, otherwise: 0)
Education_2	(If the participant education level as high school: 1, otherwise: 0)
Education_3	(If the participant education level as university (undergraduate and graduate): 1, otherwise: 0)
Family type	(If family type is nuclear family:1, otherwise:0)
Marital status	(If the participant is married:1, otherwise:0)
Working status	(If the participant is working:1, otherwise:0)
Status of having children	(If the participant has child/children:1, otherwise:0)
Status of obeying the 14 rules_2	(If the participant does not obey the 14 COVID-19 pandemic rules:1, otherwise:0)
Status of obeying the 14 rules_3	(If the participant partially obeys the 14 COVID-19 pandemic rules:1, otherwise:0)
Status for losing family member	(If the participant loss a family member caused by COVID-19:1, otherwise:0)

* P_{RS} is also used independent variable as covariates in the second model.

To determine the most effective parameters on the psychological resilience scale total score (P_{RS}) and core belief inventory total score (C_{BI}), a regression analysis was conducted, and two models were proposed for each dependent variable. The developed models include qualitative and quantitative variables and named as Analysis of Covariance (ANCOVA) model. The equation of the proposed model for P_{RS} can be summarized in Eq. 1 and for C_{BI} in Eq. 2.

$$P_{RS} = \beta_0 + \beta_1 \text{Age}_2 + \beta_2 \text{Age}_3 + \beta_3 \text{Education}_2 + \beta_4 \text{Education}_3 + \beta_5 \text{Family Type} + \beta_6 \text{Marital Status} + \beta_7 \text{Working Status} + \beta_8 \text{Status of having Children} + \beta_9 \text{Status_of_obeying_14_rules_2} + \beta_{10} \text{status_of_obeying_14_rules_3} + \beta_{11} \text{Status for losing family member} + q_1 C_{19F} + u \quad (1)$$

$$C_{BI} = \beta_0 + \beta_1 \text{Age}_2 + \beta_2 \text{Age}_3 + \beta_3 \text{Education}_2 + \beta_4 \text{Education}_3 + \beta_5 \text{Family Type} + \beta_6 \text{Marital Status} + \beta_7 \text{Working Status} + \beta_8 \text{Status of having Children} + \beta_9 \text{Status_of_obeying_14_rules_2} + \beta_{10} \text{Status_of_obeying_14_rules_3} + \beta_{11} \text{Status for losing a family member} + q_1 C_{19F} + q_2 P_{RS} + u \quad (2)$$

where:

β_0 = A constant term,

β_i = Dummy variables of the model ($i \neq 0$),

q_j = Obtained coefficients of the variables in the developed model ($j=1, \dots, 2$),

u =A disturbance term.

Ethical consideration

Approval for the research was obtained from the Rectorate of XXX University, Scientific Research and Publication Ethics Committee (No: XXX), and the COVID-19 Scientific Research Board of the Turkish Ministry of Health (No: XXX). Online informed consent was obtained from the participants in the study. The research was conducted in line with the Declaration of Helsinki.

RESULTS

The mean age of women was 30.92 ± 9.66 years, and most of them had undergraduate and post-graduate education (69%). About half of the women were married (46%), had an income-generating job (52%), and had children (42%). In Table 2, 87% of women obeyed the COVID-19 14 rules, and 26% lost a family member due to COVID-19. The COVID-19 fear score was found to be 6.31 out of 10. As seen in Table 3, the total mean score of the "psychological resilience scale" was 66.50 ± 13.40 and "core beliefs inventory" was 27.87 ± 10 .

Table 2. Descriptive characteristics of the women (n=792).

Descriptive Characteristics	n	%
Age		
18-25 years	287	36
26-35 years	267	34
36+ years	Mean: 0.92 ± 9.66	238
238		30
Education level		
Primary school	76	10
High school	164	21
Bachelors' degree and post-graduate	552	69

Table 2 (Continue) Descriptive characteristics of the women (n=792).

Marital status	n	%
Married	365	46
Single	427	54
Family type		
Nuclear	716	90
Extended	76	10
Working status		
Yes	409	52
No	383	48
Status of having children		
Yes	335	42
No	457	58
Status of obeying COVID-19 pandemic rules		
Yes	688	87
No	27	3
Partly	77	10
Losing a family member due to COVID-19		
Yes	208	26
No	584	74
The mean score of COVID-19 fear	Mean: 6.31±1.98	

Table 3 The total mean score of the women's resilience and core beliefs scale.

Scales	Min	Max	Mean	SD
Psychological Resilience Scale	17	96	66.50	13.40
Core Beliefs Inventory	0	25	27.80	10.76

SD:Standard deviation.

According to the Ordinary Least Square Regression (OLSR) model multicollinearity, heteroscedasticity and model specification error problems were examined by using diagnostic tests, and results are given in Table 4 for the proposed model. After checking multicollinearity, heteroscedasticity, and model specification error problems, it was assumed that

disturbances are distributed normally because of the big sample size (792 participants) based on the Central Limit Theorem and Shapiro-Wilk W normality test. As seen in Table 4, the proposed model was found significant for P_{RS} ($F=41.69$, $p=0.000$) and C_{BI} ($F=4.54$, $p=0.000$).

Table 4 Ordinary least square regression model results for Psychological Resilience Scale (PRS) and Core Beliefs Inventory (CBI).

Variables	P_{RS}				C_{BI}			
	β	St. E.	t	p	β	St. E.	t	p
Constant Term	88.24	2.75	32.09	0.000*	17.54	3.91	4.48	0.000*
Age_2	2.25	1.10	2.05	0.041*	-0.50	1.08	-0.46	0.645
Age_3	3.21	0.79	2.16	0.031*	3.51	1.64	2.14	0.035*
Education_2	-1.33	1.51	-0.89	0.375	0.46	1.48	0.32	0.752
Education_3	3.14	1.39	2.27	0.024*	-1.54	1.36	-1.13	0.260
Family Type	-1.78	1.29	-1.38	0.168	-0.94	1.27	-0.75	0.456
Marital Status	1.83	1.30	1.41	0.158	1.62	1.28	1.27	0.204
Working Status	-2.32	0.87	-2.69	0.007*	2.15	0.98	0.64	0.010*
Status of having children	0.36	1.38	0.27	0.791	1.94	1.35	1.43	0.153
Status of obeying 14 rules 2	1.09	2.12	0.52	0.605	0.94	2.07	0.45	0.650
Status of obeying 14 rules 3	-0.71	1.30	-0.55	0.584	-1.47	1.27	-1.16	0.248
Status of losing a family member	0.75	1.02	0.74	0.458	-0.66	1.00	-0.66	0.508

Table 4 (Continue) Ordinary least square regression model results for Psychological Resilience Scale (PRS) and Core Beliefs Inventory (CBI).

Variables	P _{RS}				C _{BI}			
	β	St. E.	t	p	β	St. E.	t	p
Status of COVID-19 fear	-3.94	0.23	-17.42	0.000*	-0.15	0.26	-0.61	0.543
P _{RS}				—	0.15	0.03	4.38	0.000*
Prerequisite analysis results for regression analysis								
Max. VIF	1.56 (no multicollinearity problem)				3.89 (no multicollinearity problem)			
White Test	p=0.156 (no heteroscedasticity problem)				p=0.112 (no heteroscedasticity problem)			
Shapiro-Wilk W Normality Test	p=0.189 (disturbances are normally distributed)				p=0.126 (disturbances are normally distributed)			
Ramsey Reset Test	p=0.356 (no model specification error problem)				p=0.287 (no model specification error problem)			

*Significant at 0.05 level.

As a result of the Ordinary Least Square Regression (OLSR) model performed to examine the predictive effect of women's characteristics on resilience in Table 4, the effects of age, education level, working status, and fear of COVID-19 on resilience were found to be statistically significant ($p=0.000 < 0.05$). However, the examination of the effect of each variable on psychological resilience revealed that age and education level had a positive ($\beta=2.25$, $p=0.041$ for age 2; $\beta=3.21$, $p=0.031$ for age 3 and $\beta=3.14$, $p=0.024$ Education 4) and working status and COVID-19 fear had a significantly negative predictive effect ($\beta=-2.32$, $p=0.007$ for working status; $\beta=-3.94$, $p=0.000$ for status of COVID-19 fear). In addition, family type ($p=0.168$), marital status ($p=0.158$), the status of having children ($p=0.791$), the status of obeying the 14 rules of COVID-19 and losing a family member due to COVID-19 ($p=0.458$) had no predictive effect on resilience ($p>0.05$).

The regression coefficients (β) in Table 4 show that among the 10 independent variables, age ($\beta=3.51$) is the strongest predictor of core belief, followed by working status ($\beta=2.15$) and psychological resilience (0.15), respectively. The β coefficients of the other independent variables ranged from -0.15 to 1.94. Age ($p=0.035$ for age3), working status ($p=0.010$), and resilience level ($p=0.000$) were found to predict core beliefs statistically significantly ($p\leq 0.05$).

DISCUSSION

This study aimed to determine the psychological resilience and core belief levels of women in the COVID-19 pandemic and the affecting factors. Resilience is vital for coping with distress, uncertainty, and change effectively. The psychological resilience levels of individuals have decreased during the pandemic (Yıldırım et al., 2020).

In the study, the mean score of women's psychological resilience was moderately good with 66.50 ± 13.40 out of 100. A study conducted with nurses during the COVID-19 pandemic found that the mean score of psychological resilience of nurses was 64.28, which was similar to our study (Kılınç & Sis Çelik, 2021). In another study

conducted by Roberto et al (2020), the level of psychological resilience of women was found to be close to high with a score of 77.94. Lai et al. (2020). reported the psychological resilience of health workers to be poor. In the study in which the short form of the resilience scale was used by Karaşar and Yaşam (2020) in the COVID-19 pandemic, it was found that the psychological resilience score of women was moderately good with 21.53 ± 3.79 out of 30 points. It is thought that the difference in the levels of resilience in the COVID-19 pandemic may be due to the different scales, samples, culture, and time of the research.

In this study, as the age of women increased, their psychological resilience tended to increase. In a study conducted in America in the early days of the pandemic in which more than 6000 people participated, it was seen that middle-aged or adult individuals experienced higher psychological resilience (Riehm et al., 2021). In a study conducted during the COVID-19 pandemic in Turkey, psychological resilience increased as age increased (Kılınç & Sis Çelik, 2021), which is consistent with the study of Kimhi, Marciano, Eshel, & Adini, (2020), and Kimter (2020). However, there are some other studies showing that psychological resilience decreases with age (Kimhi & Eshel, 2019).

In this study, as the education level of women increased, the level of psychological resilience increased. Literature has citations that there is a positive relationship between education level and psychological resilience level (Kimter, 2020; Riehm et al., 2021). The reason for this is thought to be because individuals' education levels increase, their awareness of the situation increase, and they take the necessary precautions, and their skills to cope with the traumatic event improve (Kimter, 2020). In some studies, no relationship was found between education level and psychological resilience level (Deniz, Çimen, & Yüksel, 2020; Kılınç & Sis Çelik, 2021).

According to the results, there was no significant difference between psychological resilience levels based on marital status, family type, and having children in this study, which is supported by some studies (Benassi et al., 2020; Kılınç & Sis Çelik, 2021), while

contradicted by Kimter, 2020). In a study conducted in Italy, mothers experienced higher levels of stress than others during the COVID-19 pandemic and having a child does not make a difference in terms of psychological resilience (Benassi et al., 2020).

In this study, an inverse relationship was found between the working status of women and their level of resilience. This may be because households generally stay at home during the pandemic period, adding the burden of housework to the workload of working women (Roberto et al., 2020). To our knowledge, there is no study in the literature investigating the relationship between working status and resilience level. However, the results of the studies on the relationship between the perception of economic level and the level of resilience were found to differ (Kılınç & Sis Çelik, 2021).

Psychological resilience acts as a shield in finding the meaning of life, making sense of the event, gaining a positive perspective, and coping with the undesirable situation (Roberto et al., 2020). In this study, there is an inverse relationship between fear of COVID-19 and resilience. In other words, women with high psychological resilience have a lower fear of COVID-19. Kimhi et al. (2021) have showed a negative close relationship between the COVID-19 sense of danger and psychological resilience. Various studies have demonstrated that psychological resilience is a significant resource in coping with negative feelings (worry, anxiety, depression, etc.) caused by COVID-19 (Kasapoğlu, 2020; Kimhi et al., 2020; Riehm et al., 2021; Yıldırım et al., 2020).

Shaking of the core beliefs of the individual after traumatic or undesirable situations is the leading factor for positive changes (Cann et al., 2010). After the traumatic event, the individuals' questioning about the world and their position in the world can negatively affect their lives, but it can also contribute to their attempts to make sense of life and lead them on the way to recovery. In this study, the total score of the women's core beliefs scale was moderately good with 27.87 ± 10.76 .

Limited studies have been conducted on core beliefs. Conflicting results were obtained between the struggle with core beliefs and socio-demographic (age, gender, education, etc.) variables in the relevant studies (Cann et al., 2010; Haspolat, 2019; Luu, 2021), which may be because shaking in core beliefs is a cognitive process and a personal experience. Milman, Lee, Neimeyer, Mathis, & Jobe, (2020) found a significant relationship between being diagnosed with COVID-19, losing someone due to COVID-19, and the level of shaking in core beliefs. In this study, a statistically significant difference was also found between the working status and the level of shaking in core beliefs, and it is thought that this result may be due to the higher exposure to the risks of the pandemic in working individuals.

There is a statistically significant difference in the same direction between the level of shaking in core beliefs and psychological resilience in our study, which is consistent with a study conducted in Australia (Luu,

2021). While an inverse relationship was expected between the level of resilience and shaking in core beliefs in studies, it was noteworthy that a positive relationship emerged during the pandemic period. On this result, both the ongoing pandemic/trauma and the process of making sense of the trauma and the effort to have psychological resilience and struggle to question core beliefs are believed to be effective. On the other hand, while evaluating women's core belief and psychological resilience levels, their religious and spiritual dimensions should also be taken into consideration. In a study in which 80.6% of the sample consisted of women, it was determined that there was a close correlation between spiritual cope and religiousness and also emotional distress during the COVID-19 pandemic (Margetić et al., 2022).

The limitation of this study is that the data were collected during the partial lockdown and gradual normalization process of the COVID-19 pandemic.

CONCLUSION

This study showed that the mean scores of women's psychological resilience and core beliefs during the COVID-19 pandemic were moderately good. Some socio-demographic variables like age, education level, working status, and fear of COVID-19 affect women's resilience and core beliefs. It is suggested that all health professionals, especially women's health nurses, midwives, and physicians involved in the field of women's health should carry out more quantitative and qualitative research that "reveal the psychological resilience and coping level of women" in extraordinary situations such as pandemics and wars. These studies will contribute to "making the situation/problem visible". In addition, training on "education for developing emotion regulation strategies, psycho-education, developing problem-solving skills, making sense of life" should be organized by a multidisciplinary team during extraordinary times like pandemics and wars, which will help protect and improve women's mental health.

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Author Contributions

Plan, design: RA, SA, DKB; **Material, methods and data collection:** RA, SA, DKB; **Data analysis and comments:** RA, SA; **Writing and corrections:** RA, SA, DKB.

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Somatosensory Temporal Discrimination and Neutrophil/Lymphocyte Ratio in Patients with Chronic Low Back Pain

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ABSTRACT

Objective: Central sensitization is known in the pathogenesis of chronic low back pain. We aimed at this study considering that somatosensory temporal discrimination (STD) may also be impaired in this process. We also looked at the neutrophil-lymphocyte ratio in terms of its contribution to inflammation suggested in the pathogenesis. **Materials and Methods:** A total of 52 participants have been enrolled in this study. They were divided into two groups, 27 patients with chronic low back pain (pain caused by facet, sacroiliac joint arthropathy and intervertebral disc degeneration) and 25 healthy volunteers. STDT (somatosensory temporal discrimination threshold) stimulus intensity and STD measurements were obtained in the dorsum of the hands and foot in four extremities of both groups, and neutrophil-lymphocyte ratio was evaluated. **Results:** STD thresholds in patients with chronic low back pain were found significantly prolonged in all four extremities compared to the control group. Neutrophil-lymphocyte ratio was also found to be statistically significantly higher in the low back pain group compared to the control group. **Conclusion:** Prolonged STD thresholds indicate that pain perception of patients with chronic low back pain is disrupted. In addition, the high rate of neutrophils-lymphocytes indicates that the inflammatory process continues even if low back pain becomes chronic.

Keywords: Low Back Pain, Somatosensorial Temporal Discrimination, Neutrophil-Lymphocyte Ratio.

Kronik Bel Ağrılı Hastalarda Somatosensoryel Temporal Diskriminasyon ve Nötrofil / Lenfosit Oranı

ÖZ

Amaç: Kronik bel ağrısının patogeneğinde santral duyarlılaşma bilindiğinden, somatosensoryel temporal diskriminasyonun (STD) da bu süreçte bozulabileceğini düşünerek bu çalışmayı amaçladık. Nötrofil lenfosit oranına da patogeneşte ileri sürülen inflamasyona katkısı açısından baktık. **Gereç ve Yöntem:** Bu çalışmaya toplam 52 katılımcı katılmıştır. Kronik bel ağrılı (faset, sakroiliak eklem artropatisi ve intervertebral disk dejenerasyonu kaynaklı ağrı) 27 hasta ve 25 sağlıklı gönüllü olmak üzere iki gruba ayrıldı. Her iki grubun dört ekstremitesinde el ve ayak dorsumundan somatosensoryel temporal diskriminasyon eşik (STDT) uyarı şiddeti ve STD ölçümleri yapıldı ve nötrofil/lenfosit oranı değerlendirildi. **Bulgular:** Kronik bel ağrısı olan hastalarda STD eşikleri, kontrol grubuna kıyasla dört ekstremitede önemli ölçüde uzamış bulundu. Nötrofil/lenfosit oranı da bel ağrısı grubunda kontrol grubuna göre istatistiksel olarak anlamlı derecede yüksek bulundu. **Sonuç:** Uzamış STD eşikleri, kronik bel ağrılı hastaların ağrı algısının bozulduğuna işaret etmektedir. Ayrıca nötrofil/lenfosit oranının yüksek olması da bel ağrısı kronikleşse bile inflamatuvar sürecin devam ettiğini gösterir.

Anahtar Kelimeler: Bel Ağrısı, İki Nokta Diskriminasyonu, Nötrofil/Lenfosit Oranı.

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INTRODUCTION

Low back pain is a significant public health problem due to its high prevalence, economic burden, chronicity, and associated disability. Its peak incidence occurs between 35 and 55 years of age. The reported lifetime prevalence of non-specific low back pain in developed countries is estimated to be in the range of 84%, and best estimates suggest that the prevalence of chronic low back pain is about 23% (Airaksinen et al., 2006). Low back pain is non-specific (85%) and usually caused by mechanical. Mechanical low back pain originates in the spine, intervertebral discs, or surrounding soft tissues. Specific causes are uncommon (<15% of all back pain, e.g., infection, tumor, osteoporosis, fracture, structural deformity, inflammatory disorder, radicular syndrome, or cauda equine syndrome). Clinical clues or red flags can help identify non-mechanical low back pain cases. Performing a good examination or imaging helps make an accurate diagnosis (Giesecke et al., 2004; Will et al., 2018). Somatosensory temporal discrimination (STD) is the ability to perceive as temporally separate two successive somatosensory stimuli applied to the same or different parts of the body (Hoshiyama et al., 2004). A normal STD requires the presence of intact peripheral and central pathways and the proper functioning of the primary somatosensory cortex. The threshold for the somatosensory temporal discrimination provides information on the cortical functioning of the sensory stimuli.

Individuals with chronic low back pain have been found to have several neurochemicals, structural, and functional cortical alterations in many brain areas, including the somatosensory cortex. In patients with complex regional pain syndrome and low back pain, cortical re-organization has been found to correlate with impairments in tactile discrimination, increased pain intensity and decreased tactile accuracy (Wand et al., 2011). The neutrophil/lymphocyte ratio (NLR) is an inflammation marker used for prognostic estimations regarding the systemic inflammatory responses. NLR is a widely available and inexpensive test (Aktürk and Büyükavcı 2017). Several systemic cytokines and biomarkers have been previously tested in terms of their ability to gauge the efficacy of different treatment modalities. In this regard, diagnostic biomarkers may offer a potential benefit in guiding individualized therapeutic plans in patients with low back pain. (Khan et al., 2017).

This study was primarily undertaken to determine whether the STD test may be utilized as a novel adjunctive diagnostic test allowing quantitative measurements in patients with chronic low back pain (pain caused by facet, sacroiliac joint arthropathy and intervertebral disc degeneration); to provide initial data for its validity, and to discuss its potential clinical utility. The secondary objective was to examine the role of NLR as a diagnostic and therapeutic biomarker in patients with low back pain.

MATERIALS AND METHODS

Patient Selection

This study was carried out by including 27 patients diagnosed with chronic low back pain at the algology outpatient clinic. 25 healthy volunteers were recruited. The overall study population consisted of 52 males and females aged between 18 and 65 years. The inclusion criteria were as follows: Absence of pregnancy and space-occupying cranial lesions, absence of known systemic disorders or regular use of medications; lack of hepatic or kidney dysfunction, low vitamin B12, hypothyroidism, or anemia in laboratory tests performed within the past six months; adequate patient cooperation for study tests; pain caused by facet, sacroiliac joint arthropathy and intervertebral disc degeneration with ongoing degenerative changes. The exclusion criteria were as follows: Patients with radiculopathy were not included in the study. Patients with red flags were not included in the study. Red flags include progressive motor or sensory loss, recent urinary retention and incontinence, invasive spinal procedure, significant trauma, cauda equina syndrome, malignancy, fracture, or infection. Imaging methods were requested from all patients (MRI or CT) for exclusion criteria. STDT (somatosensory temporal discrimination threshold) measurements were made from four extremities by the same investigator in the patient and control groups. Also, NLR was measured in all participants. VAS (Visual analogue scale) was measured in the patient group.

STD procedure

STDT measurements were performed by previous descriptions (Hoshiyama et al., 2004). STDT was measured at four different sites, at the dorsum of both hands and feet. Superficial Ag-AgCl electrodes of 10 mm diameter were used for this purpose. A distance of approximately 1 cm was allowed between the anode and cathode. A constant current stimulator (Medtronic, Keypoint) was used for tactile stimulation. The required current intensity for the minimum sensory threshold level was determined by stimuli of 0.2 msec. Again, to determine the minimal sensory threshold, the current intensity was increased with increments of 0.2 mA starting from 1 mA with three stimuli applied at a time. The intensity at which the patient perceived all three stimuli was accepted as the minimum sensory threshold. Initial interstimulus interval (ISI) between the paired stimuli was 5 ms and it was increased with of steps 5 ms. A paired stimuli was given every 10 s. The first of three consecutive ISIs where the subjects discriminated two discrete stimuli, was recorded as the ascending STDT (aSTDT). Then ISI was decreased with steps of 5 ms until the patient could not discriminate a paired stimuli and the first of three consecutive ISIs where the subject reported paired stimuli as one stimulus was recorded as descending STDT (dSTDT). Similarly, the procedure was repeated at 1.5 folds of the minimal threshold level. The arithmetic mean of two aSTDT and dSTDT values was calculated as the STDT value for the dorsum hand/foot. In addition, when each participant was given

a single stimulus before the test and asked whether they were pair or single, those who gave a double answer were not included in the study.

Statistical analysis

Statistical analyses were performed using SPSS for Windows, Release 22.0. The normal distribution was tested using the Skewness and Kurtosis values for each group. The significance of the difference between the groups was tested with Sample t-test. The results of the variables after normalization were taken as the mean and standard deviation. Statistical significance level was set at ≤ 0.05 .

Ethical considerations

Prior to study procedures, the study protocol was approved by the Ethics Committee for Clinical Research with the number 2019/50. Also, all participants provided written informed consent before the study.

RESULTS

The patient and control groups were comparable in terms of demographic characteristics. The mean age among patients with low back pain and controls was 45.4 ± 10.6 years (19 female, 8 male) and 29.1 ± 8.5 years (21 female, 4 male). Since STDT results are not affected under the age of 65, the difference in the average age of the control and low back pain groups does not affect our results (Hoshiyama et al., 2004).

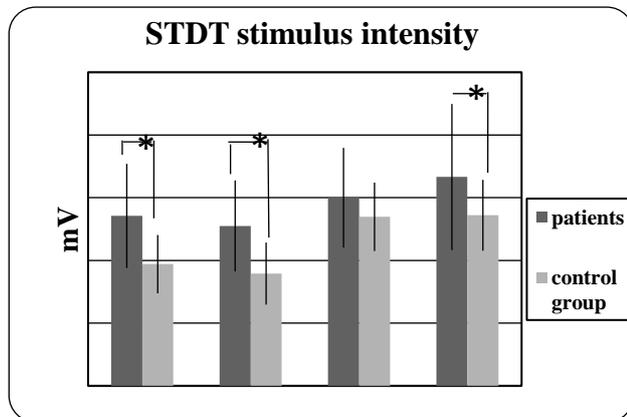


Figure 1. In the patient group, STDT stimulus intensity were higher in both hands and left foot. * $p \leq 0.05$

The stimulus intensity of STDT was significantly higher in three extremities as compared to controls shown in Figure 1 and Table 1. The STDT from four extremities were significantly prolonged among patients compared to controls, shown in Figure 2 ($p \leq 0.05$) and Table 2. Also, NLR was significantly higher in those with low back pain as compared to controls, shown in Figure 3.

Table 1. STDT stimulus intensity between patient and control groups.

	Patient group	Control group	p
	Mean± SD (mV)	Mean± SD (mV)	
Right hand	5.42±1.66	3.88±0.93	0.000**
Left hand	5.10±1.45	3.58±0.99	0.000**
Right foot	6±1.59	5.39±1.09	0.111
Left foot	6.66±2.33	5.44±1.13	0.020*

* $p < 0.05$, ** $p < 0.001$, SD: Standard deviation.

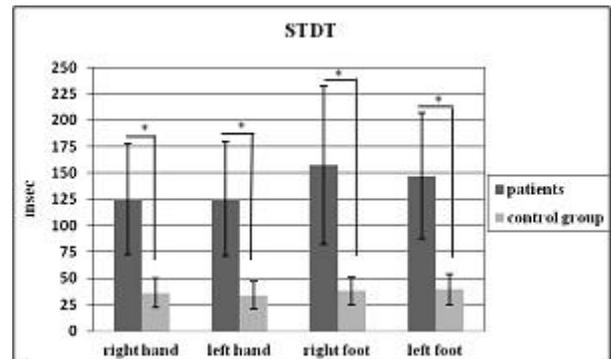


Figure 2. STDT was significantly prolonged in all extremities in the patient group compared to the control group. STDT: Somatosensory temporal discrimination thresholds. * $p \leq 0.05$

Table 2. STDT between patient and control groups.

	Patient group	control group	p
	Mean±SD(msec)	Mean±SD(msec)	
Right hand	125±52.6	36.1± 13.8	0.000*
Left hand	125.5±54.2	34.0± 12.9	0.000*
Right foot	157.3±75.0	38.0± 13.0	0.000*
Left foot	147.15 ± 59.40	39.6± 14.5	0.000*

* $p < 0.001$, SD: Standard deviation.

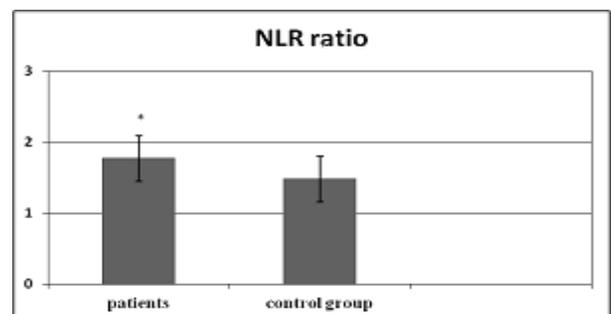


Figure 3. NLR rate was statistically significant in the patient group. NLR ratio: neutrophil-lymphocyte ratio. * $p \leq 0.05$

DISCUSSION

Chronic low back pain is a common condition associated with high economic burden and reduced quality of life. The lack of treatment options that provide long-term remission in patients with chronic low back pain indicates that there is a need for new studies on the pathogenesis. Central sensitization has recently been recognized as a potential pathophysiological mechanism underlying a group of chronic pain disorders including chronic low back pain (Nijs et al., 2015). In parallel with this information, we wanted to examine the potential contributions of STDT and NLR measurement to the etiopathogenesis of chronic low back pain in patients with chronic low back pain due to facet, sacroiliac joint arthropathy and intervertebral disc degeneration. STDT measured from four extremities was prolonged among patients with chronic low back pain than controls, suggesting an impaired perception of non-painful stimuli in chronic low back pain patients. Furthermore, higher NLR in the patient group than in controls supports ongoing inflammation in these subjects.

Low back pain usually is muscle tension or stiffness localized below the costal margin and above the inferior gluteal folds. Many spinal structures have sensory innervation, e.g., muscles, tendons, ligaments, fascia, facet joints, sacroiliac joint, vertebrae, the outer ring of the intervertebral disc, vascular tissue, dura, nerve roots, and dorsal root ganglia. However, all of these structures can cause widespread low back pain (Vlaeyen et al., 2018). Pain originating from the facet joint and sacroiliac joints can be accurately diagnosed with a combination of physical examination and image guidance (magnetic resonance (MR) or computed tomography (CT)) (Jeffrey and Walter 2009). Although most intervertebral disc lacks sensory innervation, this structure has a nociceptive contribution to back pain, especially in relation to disc degeneration (Brinjikji et al., 2015; Endea et al., 2011).

Chronic low back pain is a prevalent condition associated with a high economic burden and significantly reduced quality of life. The lack of treatment options that provide long-term remission in chronic low back pain patients indicates a need for new studies on pathogenesis. Central sensitization has recently been recognized as a potential pathophysiological mechanism underlying a group of chronic pain disorders, including chronic low back pain (Nijs et al., 2015). Moreover, patients with chronic low back pain experience generalized deep-tissue hyperalgesia when exposed to quantitative nociceptive stimuli (O'Neill et al., 2007).

Examination of both spatial and temporal aspects of the two-point discrimination test using functional magnetic resonance imaging showed activation at the cortical level in areas such as the inferior parietal lobule, middle and inferior frontal gyri, anterior part of the right insula, and right anterior cingulate gyrus. In subcortical areas, basal ganglia were found to be activated, particularly at both caudate heads, substantia nigra, and the subthalamic nucleus. In addition, significant activity

was found in the cerebellum (Pastor et al., 2004). Chronic low back pain has been associated with neurochemical, structural, and functional cortical alterations in many brain areas, including the somatosensory cortex. Cortical re-organization leads to increased pain intensity, in addition to hampering successful treatment. However, plasticity, which is the basis for cortical re-organization, is also an indication of the ability of the brain to respond to targeted therapy (Moseley and Flor, 2012). While prolonged STD values may represent an electrophysiological indicator of altered intra-cortical inhibition, they may also be associated with increased pain intensity and clinical signs of reduced functionality (Lim et al., 2015). STD measured at four extremities was significantly impaired in patients with chronic low back pain compared to controls, suggesting alterations in cognitive-sensory processing in these patients. This finding may suggest that our therapeutic and diagnostic approach may be re-evaluated in this disorder. Our literature search has not revealed any studies similar to ours, which, to the best of our knowledge, has evaluated the potential diagnostic and therapeutic role of these practical clinical measurements for the first time.

Patients with chronic low back pain suffer from pain due to various spinal disorders, including intervertebral disc degeneration, disc herniation, spinal stenosis, and arthritis of the facets. In recent years, inflammatory markers have been the subject of important studies with the thought that inflammation may play a role in the pathogenesis of disc degeneration and related pain mechanisms. Furthermore, increasingly more studies have revealed that presence of inflammatory markers in the blood may be shown systematically and may be utilized as a novel tool to guide patient management. Although surgical treatments may offer anatomical correction and pain reduction, they are also invasive and costly procedures, in addition to difficulties associated with the estimation of response and recurrence. Diagnostic biomarkers of spinal degeneration have the potential to trigger an era of personalized spine medicine in the treatment of chronic low back pain. Increased levels of inflammatory biomarkers such as TNF-alpha, IL-6, and IL-1B were found to increase inflammation and neuropathic pain (Sommer and Kress, 2004). Also, NLR has been studied in systemic disorders with increased neutrophil and decreased lymphocyte counts during inflammation. NLR (increased neutrophil and reduced lymphocyte counts) is associated with increased disease activity in many systemic, rheumatologic, neurologic, and neoplastic conditions (Proctor et al., 2012; Torun et al., 2012). Furthermore, NLR has been reported to be a prognostic marker for determining systemic inflammatory responses (Uslu et al., 2013). For example, in a study, patients with lumbar disc herniation had elevated NLR compared to controls (Yılmaz et al., 2019). It has even been suggested that role of antibiotic therapy should be re-evaluated in patients with chronic low back pain (Kjersti et al., 2017).

Similarly, our patients with chronic low back pain had increased NLR compared to control subjects, indicating persistent inflammation despite the chronicity of pain in these patients. One potential limitation of the NLR rate is the lack of sensitivity of MRI imaging modalities early in the disease process.

Our study limitations firstly, our patient and control groups were small. Secondly, STD measurements require proper attention and cooperation of the patient, they should be carried out in individuals with good cognitive functions. Therefore, lack of STD testing among patients with low level of education is also a disadvantage, indicating the need for improved test procedures. Thirdly, we could compare non-specific low back pain and specific low back pain groups. Fourthly, patients could be sub grouped, but could not be done due to the small number of patients.

CONCLUSION

Although impaired STD appears to be a potential measurement tool for patients with chronic low back pain, further studies are required to confirm these observations as well as to assess its role in the follow-up of patients. Elevated NLR indicates that the inflammatory process persists in these patients, despite the chronic course of the disease. We believe that future studies could better define the association between biomarkers and clinical outcomes in patients with chronic low back pain. These results might change the classic biomarkers for patient follow-up. This study showed us that when planning the diagnosis and treatment of chronic low back pain, both a change in cognitive, sensory processing and the continuation of the inflammatory process should be considered. We suggest that alternative approaches and additional assessments may be required for more effective utilization of existing treatments and reducing their side effects.

Conflicts of Interest

The author declares no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Author Contributions

Plan, design: O.F. T, NT; **Material, methods and data collection:** O.F. T, NT; **Data analysis and comments:** O.F. T, NT; **Writing and corrections:** O.F. T, NT.

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Prevalence of *Ehrlichia Canis*, *Borrelia Burgdorferi* and *Dirofilaria Immitis* in Sivas Stray and Shelter Dogs

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ABSTRACT

Objective: In this study, it was aimed to investigate the prevalence of vector-transportable *Ehrlichia canis*, *Borrelia burgdorferi* and *Dirofilaria immitis* in dogs in Sivas province. **Materials and Methods:** The study was conducted on 100 dogs. Breed, age and sexes of dogs were determined and physical examinations were performed and serum samples were collected for the diagnosis of the diseases. The commercial rapid test kit SNAP* 4DX* Plus (IDEXX Laboratories) was used for the detection of *Ehrlichia canis*, *Borrelia burgdorferi* and *Dirofilaria immitis* in dogs. **Results:** In this study, it was determined that 1% of 100 dogs examined were *Ehrlichia canis* and 2% of them were seropositive to *Dirofilaria immitis*, while *Borrelia burgdorferi* seropositive animals were not detected. **Conclusion:** As a result, it was determined that *Ehrlichia canis*, *Borrelia burgdorferi* and *Dirofilaria immitis*, which can be transmitted to dogs by vectors, were observed at low rates in Sivas province. However, at the same time, necessary protective measures should be taken against these diseases that can be transmitted by vectors in this region.

Keywords: Borreliosis, *Dirofilaria immitis*, Dog, Ehrlichiosis, Sivas.

Sivas Sokak ve Barınak Köpeklerinde *Ehrlichia Canis*, *Borrelia Burgdorferi* ve *Dirofilaria Immitis*'in Prevalansı

ÖZ

Amaç: Bu çalışmada, Sivas ilindeki köpeklerde vektörle taşınabilen *Ehrlichia canis*, *Borrelia burgdorferi* ve *Dirofilaria immitis*'in prevalansının araştırılması amaçlanmıştır. **Gereç ve Yöntem:** Çalışmaya 100 köpek üzerinde yürütülmüştür. Köpeklerin ırk, yaş ve cinsiyetleri belirlenerek fiziksel muayeneleri yapılmıştır ve hastalıkların tanısı için serum örnekleri toplanmıştır. Köpeklerde *Ehrlichia canis*, *Borrelia burgdorferi* ve *Dirofilaria immitis* tespiti için SNAP* 4DX* Plus (IDEXX Laboratories) ticari hızlı test kiti kullanılmıştır. **Bulgular:** Bu çalışmada, muayene edilen 100 köpeğin 1 (%1)'inin *Ehrlichia canis* ve 2 (%2)'sinin de *Dirofilaria immitis* yönünden seropozitif olduğu belirlenirken, *Borrelia burgdorferi* yönünden seropozitif hayvan tespit edilmemiştir. **Sonuç:** Sonuç olarak, Sivas ilinde köpeklere vektörlerle taşınabilen *Ehrlichia canis*, *Borrelia burgdorferi* ve *Dirofilaria immitis*'in düşük oranda gözleendiği tespit edilmiştir. Ancak aynı zamanda, bu bölgede vektörlerle taşınabilen bu hastalıklara karşı gerekli koruyucu önlemlerin alınması gerekmektedir.

Anahtar Kelimeler: Borreliosis, *Dirofilaria immitis*, Köpek, Ehrlichiosis, Sivas.

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INTRODUCTION

Vectors and vector-borne diseases have started to gain importance due to the significant climatic and environmental changes occurring today. Vector-borne bacterial, viral, spirochetal and rickettsial diseases with zoonotic importance are transmitted by arthropods (Ataş et al., 1997; İnci and Düzlü, 2009).

Ehrlichiosis (tropical pancytopenia) is a rickettsial disease characterized by decreased formed blood elements and transmitted by ticks to dogs and humans (Eng and Giles, 1989; Matthewman et al., 1993; Friedman et al., 1997).

Lyme disease or Borreliosis; is a zoonotic disease caused by the spirochete *Borrelia burgdorferi*, transmitted mainly by ticks of the genus *Ixodes*, and affecting all organs and systems (Skotarczak and Wodecka, 2003). Lyme disease in dogs was first identified in the United States in the 1980s and has spread worldwide (Koneman et al., 1997).

Dirofilariosis is a parasitic disease transmitted by mosquitoes, especially causing cardiopulmonary diseases. Although the disease usually causes infection in dogs and canines, it rarely occurs in humans and cats (Araujo et al., 2003).

This study aimed to investigate the prevalence of vector-transportable *Ehrlichia canis*, *Borrelia burgdorferi* and *Dirofilaria immitis* in dogs in Sivas province.

MATERIALS AND METHODS

This study was conducted between April 2017-2018. During this period, a total of 100 dogs, 53 male and 47 female, from different breeds, aged between 10 months and 9 years, weighing between 9-28 kg and living in Sivas animal shelters were used as material.

10 ml of blood was taken from Vena cephalica antebrachium of dogs and put into tubes with and without anticoagulant in equal amounts. Blood samples were taken between 16:00 and 19:00. Blood samples containing anticoagulant (Edta) were examined for microfilaria on the same day. The blood taken into tubes without anticoagulant was centrifuged at 3000 rpm for 8-10 minutes, and their serum was removed and stored at -20 °C until serological testing. This study was carried out with the approval of Sivas Cumhuriyet University Animal Experiments Local Ethics Committee with the letter dated 23.02.2016 and numbered 65202830-050.040.04-26.

Modified knott method

In the modified knott method, 1 ml of blood sample with anticoagulant was mixed with 9ml of 2% formalin (2ml of 37% concentrated formaldehyde, 100 ml of distilled water) and centrifuged at 2000 rpm for 3.5 minutes. After centrifugation, the liquid on it was poured and 0.1% methylene blue was added as much as the amount of residue remaining at the bottom. Then, a few drops of this mixture were taken and examined in terms of microfilaria under the light

microscope at 10x and 40x magnifications (Wang, 1997).

Serological method

A commercial rapid test kit (SNAP* 4Dx* Plus, IDEXX Laboratories) working with ELISA principle was used for the diagnosis of *Ehrlichia canis*, *Borrelia burgdorferi* and *Dirofilaria immitis* from serum samples. In previous studies with the test kit used, specificity for *Dirofilaria immitis* antigens was 97% (Bowman et al., 2009), sensitivity 84% (Atkins, 2003), specificity for *B. Borrelia burgdorferi* antibodies 99.5% (Duncan et al., 2004), sensitivity 94.4% (O'Connor et al., 2004), specificity for *Ehrlichia canis* antibodies 100% (O'Connor et al. 2004; O'Connor et al., 2006), sensitivity 95.7% (O'Connor et al., 2002) has been determined.

Statistical analysis

The mean, minimum/maximum values and standard errors of the findings in the study were determined using the SPSS 15.0 computer program.

RESULTS

In this study, 1 out of 100 dogs (1%) were seropositive for *Ehrlichia canis* and 2 (2%) were seropositive for *Dirofilaria immitis*, but no dogs were found to be seropositive for *Borrelia burgdorferi*.

As a result of the clinical examination, the distribution of the diseases according to the age and sex of the dogs is shown in Table 1-4. While the *Ehrlichia canis* seropositive dog was male, one of the *Dirofilaria immitis* seropositive dogs was male and the other was female. In the clinical examination of the dog determined to be *Ehrlichia canis* seropositive, stagnation, loss of appetite, epistaxis, pallor of the mucous membranes and petechial hemorrhages were determined. It has also been found in dyspnea, exercise intolerance, and fatigue. Disease-related symptoms such as cough, respiratory distress and chest pain were detected in only one of the *Dirofilaria immitis* seropositive dogs.

Table 1. Distribution of *E. canis* in dogs by sex.

Gender	Number of Dogs	Number of Infected Dogs	Infection Rate
Male	53	1	1.88
Female	47	0	0
Total	100	1	1

Table 2. Distribution of *D. Immitis* in dogs by sex.

Gender	Number of Dogs	Number of Infected Dogs	Infection Rate
Male	53	1	1.88
Female	47	1	2.33
Total	100	2	2

Table 3. Distribution of *E.canis* in dogs by age.

Age	Number of Dogs	Number of Infected Dogs	Infection Rate
0-2	39	0	0
3-4	24	1	4
5-6	23	0	0
7-9	14	0	0

Table 4. Distribution of *D. Immitis* in dogs by age.

Age	Number of Dogs	Number of Infected Dogs	Infection Rate
0-2	39	0	0
3-4	24	0	0
5-6	23	1	4.34
7-9	14	1	7.14

DISCUSSION

Vector-borne diseases, which are frequently seen in our country, can cause diseases in both humans and animals and cause significant economic losses in animals (Gazyacı and Aydenizöz 2010; Issi et al., 2010). Canine monocytic ehrlichiosis, which is important in terms of veterinary medicine and spreads in carnivores all over the world, is a disease transmitted to dogs by ticks named *R. sanguineus* caused by *Ehrlichia canis* (Dodurka and Bakirel, 2002; Bremer et al., 2005). In the acute phase of ehrlichiosis, clinical symptoms such as severe weight loss, fever, anorexia, stagnation, ocular and nasal discharge, dyspnea, lymphadenopathy, epistaxis, central nervous system findings, edema in the extremities and scrotum have been reported (Eng and Giles, 1989; Dodurka and Bakirel, 2002). In the chronic phase of the disease, clinical findings such as weakness, depression, anorexia, fever, progressive weight loss, epistaxis, pallor in the mucous membranes and edema, especially in the hind legs and scrotum, can be seen commonly (Smith et al., 1975; Mylonakis et al., 2010; Ural et al., 2014; Waner et al., 2001). Ehrlichiosis in dogs has a wide distribution in the world, being more common in tropical and subtropical regions (Carrade et al., 2011; Unver et al., 2001; Waner et al., 2001). The presence of the disease has been reported in many countries in Asia, Africa, Europe and America (Carrade et al., 2011; Tsachev et al., 2006; Matthewman et al., 1993). Karagenç et al (2005) determined that *Ehrlichia canis* was positive in 41.5% of 371 dogs included in the study from various parts of the Aegean Region such as Manisa, Marmaris, Muğla, Selçuk, Aydın and Bodrum. In a study conducted by Batmaz et al. (2001) in the province of Izmir, they determined that the prevalence of ehrlichiosis was 40.6% with IFAT. Ural et al (2014), on the other hand, reported that the

disease was observed at a rate of 27.5% in a study they conducted on the seroprevalence of ehrlichiosis with the SNAP 4Dx rapid test kit in dogs in the Aegean region. In the present study, 1 out of 100 dogs were determined to be seropositive for *Ehrlichia canis* with the SNAP 4DX Plus test kit. The lower results obtained in this study compared to other studies may be due to the number of samples, the difference in the diagnostic test methods used, and the density of vector ticks that carry the disease. Lyme disease or borreliosis is a spirochete disease caused by *Borrelia burgdorferi*, which can also be seen in humans, dogs, horses, cattle and cats, especially transmitted by ticks of the genus *Ixodes* (Bowman and Nuttall, 2008). In a study conducted in the USA (Little et al., 2014), the prevalence of borreliosis was found to be 7.2%. In the study conducted in Samsun region, it was determined that 10 of 153 dogs were positive by ELISA method (Çakır and Pekmezci, 2020). Borreliosis antibodies were detected by SNAP 4Dx test in only 2 of 307 dogs in Kuşadası and Aydın (Ural et al., 2014). In the present study, borreliosis antibodies could not be detected in 100 dogs with the SNAP 4Dx Plus test in Sivas province. The inability to detect borreliosis antibodies in this study may be related to the fact that the SNAP 4DX test kit used in the diagnosis of the disease can detect borreliosis antibodies only during active infection, the test method used, the number of samples used in the study, the geographical region where the animals live and the ticks carrying the disease are not infected. Dirofilariosis is a zoonotic disease caused by *Dirofilaria immitis*, which is common in many countries of the world (Wang, 1997). Although studies have been carried out in various provinces since 1961, when the infection was first reported in our country (Oytun, 1961), it is difficult to reveal the general distribution of the disease throughout the country. Various test techniques are used in the diagnosis of dirofilariosis. These tests include the use of various antigen test kits (Ural et al., 2014), direct detection of microfilariae in blood, radiography, arteriography, doppler ultrasonography, autopsy or serology methods, which have been increasingly used recently (Wang, 1997). Among these diagnostic methods, serological method; It is widely used today due to its advantages such as ease of use, detection of amicrophilaria infection, high sensitivity and specificity against the disease (Wang, 1997). In this study; Among the test methods reported above, the modified knott and serological method (ELISA, commercial rapid test SNAP 4Dx Plus, IDEXX Laboratories) were used separately for each dog to identify positive dogs. It is stated that sometimes clinical symptoms may not be seen in dogs with dirofilariosis, and in cases of low parasite density, it may generally have a subclinical course (Wang, 1997, Raynaud, 1992). Grieve et al. (1983) stated that clinical symptoms occur when the

number of parasites is above 25 in dirofilariosis, and the infection progresses very severely when the number of parasites is above 100. Right heart failure, which occurs acutely or slowly, can cause clinical symptoms such as exercise intolerance, ascites, and fluid accumulation in the chest and peritoneal cavity in sick animals (Soulsby 1986; Yıldırım, 2004). In the picture of cardiopulmonary disease, clinical symptoms in various forms such as chronic cough, respiratory distress, abnormal lung and heart sounds and arrhythmia occur, and epistaxis may occur due to abnormal blood coagulation in the lung (Yıldırım, 2004). In parallel with the opinions of Yıldırım and Soulsby (Soulsby, 1986; Yıldırım, 2004), exercise intolerance, chronic cough, respiratory distress, abnormal lung and heart sounds were determined in one of the two dogs determined to be seropositive for *Dirofilaria immitis* in this study. No abnormal clinical symptoms were observed in the other dog. This is likely to be explained by the number of parasites, the duration of infection, and the individual susceptibility of the host, as noted by many investigators (Grieve et al., 1983; Raynaud, 1992).

In the present study, although seropositivity was determined in 2 dogs serologically, positive results were obtained in only 1 dog with the modified knott method. This situation, as stated by many researchers (Loehle, 1997; McTier, 1994), in the formation of occult infection despite the absence of microfilaria in the blood; mild infections of animals, amicrofilaremic infections caused by some drugs used for preventive purposes in animals, presence of immature *Dirofilaria* agents in circulation and Dirofilariosis disease caused by single-sex parasites are shown as reasons.

In many studies (Ağaoğlu and Şahin, 1992; Ağaoğlu et al., 2000; Ural et al., 2014; Voyvoda and Paşa, 2004; Yıldırım et al., 2006) on *Dirofilaria immitis* in different regions of Turkey, the presence of the infection has been revealed. In a study conducted around Kayseri, blood samples obtained from 280 dogs were examined for *Dirofilaria immitis* by membrane filtration and ELISA techniques, and a prevalence of 9.6% was determined (Yıldırım et al., 2006). Again, the prevalence of this parasite was found to be 46.2% in Van (Ağaoğlu et al., 2000) and 13.9% in Aydın (Voyvoda and Paşa 2004). Öncel and Vural (2005) investigated the *Dirofilaria immitis* antigen in the blood of 380 stray dogs obtained from Istanbul and Izmir by ELISA and found the seropositivity to be 1.52% in Istanbul, but could not determine seropositivity in Izmir. *Dirofilaria immitis* infection has been found in studies conducted in many different parts of the world. It has been reported to have a prevalence of 12.2% in Russia (Volgina et al., 2013), 0.3-39.7% in the USA (Theis et al., 2001) and 0.6% in Italy (Cringoli et al., 2001). In this study, a serological study was performed using the SNAP 4DX Plus

commercial test kit. The presence of *Dirofilaria immitis* was detected in only 2 (2%) of 100 dogs used as material. The findings obtained in this study are in line with the results of many researchers (Cringoli et al., 2001; Öncel and Vural, 2005; Voyvoda et al., 2004).

CONCLUSION

In conclusion, it was determined that *Ehrlichia canis*, *Borrelia burgdorferi* and *Dirofilaria immitis*, which can be transmitted to dogs by vectors, were observed at low rates in Sivas province. However, at the same time, necessary protective measures should be taken against these diseases that can be transmitted by vectors in this region. In addition, it was concluded that the low rate of *Ehrlichia canis*, *Borrelia burgdorferi* and *Dirofilaria immitis*, which can be transmitted to dogs by vectors in Sivas province, may be related to the potential vectorin population.

Conflict of Interest

The authors declare to have no conflicts of interest.

Author Contributions

Plan, design: ZTA; **Material, methods and data collection:** ZTA, OB, UA, AC; **Data analysis and comments:** OB, UA; **Writing and corrections:** ZTA.

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Fruit and Vegetable Consumption, Attitudes and Knowledge in Preschool Children and Adolescents

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ABSTRACT

Aim: The aim of the study was compared to the daily consumption servings, attitudes, and knowledge of fruit and vegetable consumption in preschool children and adolescents. **Materials and Methods:** The study sample consists of 906 participants within two groups including preschool children (n=453) and adolescents (n=453) living in Konya, Turkey. A survey model was used in the study and participants were selected randomized. The data were obtained by a questionnaire consisting of four parts. **Results:** According to results, 30.9% of the preschool children were found to be overweight and 11.7% obese, whereas 16.3% of the adolescents were overweight and 11% obese. The recommended daily consumption serving (RDS) for fruits was found to be high in both children (98.7%) (p>0.05) and adolescents (95.1%) (p=0.000), but in vegetables it was 26.7% (p>0.05) and 74.4%, respectively (p=0.000). The mean nutrition knowledge score of preschool children's parents and adolescents was found ($\bar{x}\pm SE$) 53.8±0.807 and 36.2±0.508 respectively (p=0.000). **Conclusion:** It was determined that preschool children and adolescents consumed enough for fruit, but in preschool children the vegetable consumption was low. The fruit and vegetable consumption can be increased by developing healthy eating habits in the preschool and adolescence period.

Keywords: Fruit and Vegetable Consumption, Preschool Children, Adolescents.

Okulöncesi Çocuklar ve Adölesanların Meyve ve Sebze Tüketimi, Tutum ve Bilgileri

ÖZ

Amaç: Araştırmanın amacı, okul öncesi dönem çocukları ve ergenlerin günlük tüketim porsiyonları, meyve ve sebze tüketimine ilişkin tutum ve bilgileri ile karşılaştırılmasıdır. **Gereç ve Yöntem:** Araştırmanın örneklemini Konya'da yaşayan okul öncesi çocuklar (n=453) ve ergenler (n=453) olmak üzere iki grupta 906 katılımcı oluşturmaktadır. Araştırmada tarama modeli kullanılmış ve katılımcılar rastgele seçilmiştir. Veriler, dört bölümden oluşan bir anket ile elde edilmiştir. **Bulgular:** Sonuçlara göre okul öncesi çocukların %30.9'unun hafif şişman ve %11.7'sinin obez, adölesanların ise %16.3'ünün hafif şişman ve %11'inin obez olduğu belirlendi. Meyveler için günlük önerilen porsiyon tüketimi (RDS) çocuklarda (%98.7) (p>0.05) ve adölesanlarda (%95.1) (p=0.000) yüksek bulunurken, sebzelerde ise sırasıyla %26.7 (p>0.05) ve %74.4 (p=0.000) olarak bulundu. Okul öncesi dönem çocuklarının anne-babalarının ve adölesanların beslenme bilgi puan ortalamaları sırasıyla ($\bar{x}\pm SE$) 53.8±0.807 ve 36.2±0.508 bulundu (p=0.000). **Sonuç:** Okulöncesi çocukların ve adölesanların yeterince meyve tükettikleri, ancak sebzelerde özellikle okul öncesi çocuklarda tüketim düzeyini düşük olduğu belirlendi. Okulöncesi dönem ve adölesan dönemde sağlıklı beslenme alışkanlıklarının geliştirilmesiyle meyve ve sebze tüketim düzeyi artırılabilir.

Anahtar Kelimeler: Meyve ve Sebze Tüketimi, Okulöncesi Çocuklar, Adölesanlar.

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INTRODUCTION

Vegetable consumption is a matter of importance in terms of adequate, well-balanced, and healthy nutrition in all periods of human life (Kähkönen et al., 2020; Kamphuis et al., 2006). Today, the benefits of vegetable consumption are well known. By consuming a sufficient intake of vegetables, protein, carbohydrate and lipid, vitamins and minerals are provided to the body, however, it is also used in the protection and growth of many health factors such as bioactive compounds and antioxidants (Olimat et al., 2017; Wallace et al., 2020). Vegetable consumption is considered as recommended in the nutrition guidelines all over the world, but also plays an important role in the fight against nutritional diseases (e.g., obesity, cardiovascular diseases, nutrient deficiencies) (Kelishadi and Azizi-Soleiman, 2014; Pem and Jeewon, 2015; Song et al., 2010). And also, WHO emphasize that low fruit and vegetable consumption is responsible for gastrointestinal cancer, ischemic heart disease and all stroke deaths and to decrease the risks of related diseases, 400 g daily consumption of fruit and vegetable recommended (WHO, 2015). Many studies (De Cosmi et al., 2017; Holley et al., 2017; Nicklaus, 2017; Scaglioni et al., 2018) show that the dimensions of nutrition attitudes, habits and behaviors started to be acquired in preschool age, but the profile exhibited in school and adolescence period and even in adult life is shaped depending on lifestyle, demographic and social factors and individual-related characteristics (Kähkönen et al., 2018). Primary prevention strategy for reducing the risk of chronic diseases encourage healthy eating among children and adolescent may therefore represent an effective (Boeing et al., 2012). This is caused by the development and expansion of fast-food and mass catering systems, shortening the time spent on eating, and especially family, parents, and friends (Slavin and Lloyd, 2012). Presence of vegetables different tastes is a major obstacle to gaining consumption habits, food neophobia develops prevents preschool children from accepting or leading to rejection. The taste, appearance, perception, peer environment and socio-demographic factors of vegetables continue to be important even when children are adolescents (Smith et al., 2017). Furthermore, numerous programs and interventions are being carried out on WHO recommendations, such as school programs on increasing fruit and vegetable consumption both preschool children and adolescence (Slavin and Lloyd, 2012). This paper aimed to determine the consumption, and attitudes and nutrition knowledge of fruit and vegetable in preschool children and adolescents in Turkey.

MATERIALS AND METHODS

Model

This study was designed as a survey model through face-to-face interviews in preschool children's parents and adolescents from September-December 2021.

Sample

The study sample were calculated two independent means (two groups) applied by power analysis with G*Power 3.1.9.2 software program. The parameters were entered in G*Power as 0.24 for effect length, 0.05 for error (α) and

0.95 for power ($1-\beta$). Result of the calculation, the sample size was found 453 for each group, and 906 in total. First group was constituted by 3-5-year-old preschool children ($n=453$), the second one was formed by 14-16-year-old adolescents ($n=453$). Each group has generated by randomized participants residing in Konya, Turkey. The data were obtained by the parents in preschool group and by themselves in adolescents.

Variables

The independent variables of this research are gender, age group, Body Mass Index (BMI), and schools. The dependent variable is fruit and vegetable consumption, attitudes, and knowledge.

Procedures

The questionnaire form was constituted by (Neslişah & Emine, 2011; Onur et al., 2017) and Turkish Dietary Guide (TDG) (TDG, 2016) from the literature and it was prepared with the help of 3 experts. The draft pre-applied to 15 parents and 15 adolescents, and the necessary corrections were made to form by researchers and then finalized form performed to participants. The questionnaire consists of four main parts. First part demographic variables and anthropometric measurements, second part fruit and vegetable serving consumption per day, third part was situated 25-question fruit and vegetable attitude statements, fourth part was comprised close-ended and multiple-choice 25-question fruit and vegetable knowledge test. First part consists of demographic variables such as gender, age, and the anthropometric measurements height (m), weight (kg) of all participants is based on BMI (kg/m^2) and categorized WHO Growth Standards of Children and Adolescents (WHO, 2006) and TDG by age and gender (TDG, 2016). According to TDG and WHO percentile values of BMI was used to identify children and adolescents as skinny ($\text{BMI}<3^{\text{th}}$), underweight ($3^{\text{th}}<\text{BMI}<15^{\text{th}}$), normal ($15^{\text{th}}<\text{BMI}<85^{\text{th}}$), overweight ($85^{\text{th}}<\text{BMI}<97^{\text{th}}$), and obese ($97^{\text{th}}<\text{BMI}$). The second part is for determining the consumption servings of the participants for fruits and vegetables. The 24-hour recalled food consumption record method was used and the fruit and vegetable consumed in the last 24 hours were questioned. Then consumption data (amounts) was converted into servings as 4 and above, 4, 3, 2, 1 and 0 (never) was made to reveal the daily consumption servings. In the third part, a Likert-type tool consisting of 25 statements was used to determine the attitudes of the participants towards fruits and vegetables. Statements were composed of (TDG, 2016), WHO Measuring Intake of Fruit and Vegetable Workshop and other literature outputs (Wallace et al., 2020; Wang et al., 2014) Attitudes were determined 5-point Likert-type scale (1, Strongly disagree; 2, Disagree; 3, Indecisive; 4, Agree; 5, Strongly agree) were used to identifying participant's perceived attitudes to fruit and vegetables. In last part, nutrition knowledge score was collected using nutrition knowledge questionnaire, which included 25 items on the fruit and vegetable related not only knowledge but also applications (e.g., storage, cooking techniques). All items were closed-ended, multiple choice. The score of nutrition knowledge was computed as the sum of the correctly answered items

4 points. The min and max scores are 0 to 100. Cronbach's α score was found 0.73.

Statistical analysis

The data analyze was performed using SPSS (Ver.22.0) software program. Descriptive statistics like frequency (n), ratio (%), mean (\bar{x}), standard error of mean (SE) computed for all two groups' items. Parametric (e.g., One sample independent test) and non-parametric tests (e.g., Chi-square) were used to examine the differences between predictor variables. Significance level was considered for $p < 0.05$ in all statistical analyzes.

Ethical approval

An ethical approval numbered 2021/850 received from the Selcuk University Health Sciences Faculty Ethics Committee of Non-Invasive Research.

RESULTS

The study sample consists of totally 906 participants in two groups. Preschool children group, 48.3% girl and 51.7% were boy and adolescents were 51.4% girl and 48.6% boy. Another demographic data is 84.4% of preschool children have educated in public schools and 12.6% in private schools. All adolescents were educated in public schools.

In Table 1 it was seen preschool children and percentiles for ages and for standards TDG (TDG, 2016) for girls and boys and WHO (WHO, 2006). BMI are often used to assess anthropometric measures to help evaluate children's growth and nutritional status. In preschool group, subdivided into 3, 4, and 5 ages, overweight were found 39.0%, 28.0%, and 28.1% and obese 11.0%, 8%, and 13.7% respectively. On the other hand, it has seen in 14, 15, and 16 years old in adolescent group, overweight was calculated 15.5%, 16.1%, and 18.3% and obese 10.8%, 7.2%, and 18.3% respectively (Table 1). Fruit consumption frequency of preschool children and adolescents was examined, it was determined that the 4-serving/day fruit consumption was higher in apple, banana, orange, and strawberry in both groups. Apple, banana, and pomegranate consumption is higher in adolescents than preschool children in 4 servings per day. While the fruits consumed in 4 or more servings were apricot, cherry, grape, orange, peach, pear and pomegranate in the preschool group, apple, banana, and strawberries in adolescents. All fruit consumption was differed between two group ($p=0.000$) but grape not ($p>0.05$).

Table 1. BMI groups of preschool children and adolescents*.

Preschool Children				Adolescent			
Age	BMI	n	%	Age	BMI	n	%
3 years (n=118)	Skinny	8	6.8	14 years (n=110)	Skinny	-	-
	Underweight	10	8.5		Underweight	28	25.5
	Normal	41	34.7		Normal	53	48.2
	Overweight	46	39.0		Overweight	17	15.5
	Obese	13	11.0		Obese	12	10.8
4 years (n=100)	Skinny	5	5.0	15 years (n=261)	Skinny	-	-
	Underweight	22	22.0		Underweight	49	18.8
	Normal	37	37.0		Normal	151	57.9
	Overweight	28	28.0		Overweight	42	16.1
	Obese	8	8.0		Obese	19	7.2
5 years (n=235)	Skinny	13	5.5	16 years (n=82)	Skinny	-	-
	Underweight	29	12.3		Underweight	6	7.3
	Normal	95	40.4		Normal	42	51.2
	Overweight	66	28.1		Overweight	19	23.2
	Obese	32	13.7		Obese	19	18.3

* Percentiles categorized TDG reference data for BMI.

Table 2. Fruit and vegetable recommended daily servings (RDS) of TDG for BMI.

	Group	BMI	RDS				p*
			Yes		No		
			n	%		%	
Fruit	Preschool Children ^a	Skinny	26	5.7	-	-	0.821
		Underweight	61	13.5	-	-	
		Normal	170	37.5	3	0.7	
		Overweight	138	30.5	2	0.4	
		Obese	52	11.5	1	0.2	
		Total	447	98.7	6	1.3	
	Adolescent ^b	Skinny	-	-	-	-	0.000
		Underweight	83	18.3	-	-	
		Normal	243	53.6	-	-	
		Overweight	74	16.3	3	0.7	
		Obese	31	6.8	19	4.2	
Total		431	95.1	22	4.9		
Vegetable	Preschool Children ^c	Skinny	15	3.3	46	10.2	0.162
		Underweight	7	1.5	19	4.1	
		Normal	45	9.9	128	28.3	
		Overweight	46	10.2	94	20.8	
		Obese	8	1.8	45	9.9	
		Total	121	26.7	332	73.3	
	Adolescent ^d	Skinny	-	-	-	-	0.000
		Underweight	45	9.9	38	8.4	
		Normal	200	44.2	46	10.1	
		Overweight	42	9.3	32	7.1	
		Obese	50	11.0	-	-	
		Total	337	74.4	116	25.6	

Fruit Recommended Daily Serving: ^a 2-6 age Children: Yes 1.5-2, No <-1.5; ^b 14-18 age Adolescent: Yes 2.5-3, No <-2.5

Vegetable Recommended Daily Serving: ^c 2-6 age Children: Yes 1-2, No <-1; ^d 14-18 age Adolescent: Yes 3.5-4, No <3.5

* Chi-Square Test

Table 3. Fruit and vegetable knowledge scores of groups.

Variable			$\bar{x} \pm SE$	p*
Preschool children parents (n=453)	Education level	Primary	47.6±1.856 ^a	0.000
		Secondary	47.7±1.737 ^a	
		High School	52.7±1.532 ^b	
		Bachelor	61.4±1.186 ^c	
		Total	53.8±0.807	
Adolescent (n=453)	BMI	Underweight	34.7±1.143 ^a	0.016
		Normal	38.3±0.724 ^b	
		Overweight	35.7±1.164 ^a	
		Obese	34.5±1.443 ^a	
		Total	36.2±0.508	

* Oneway ANOVA; ^{a, b, c} Differences between groups according to Duncan test.

Vegetables consumed in 4 or more portions were spinach (28.3%, 22.1%) and carrots (25.6%, 20.1%) in preschool and adolescents, respectively. At least 2 servings of green leafy vegetables (e.g., spinach, broccoli) or other vegetables such as tomatoes, citrus fruits such as oranges, lemons, or other fruits rich in antioxidants are recommended. In Table 2, the level of RDS those who supply fruit consumption in the preschool and adolescent groups was found to be 98.7% and 95.1%, respectively. All groups provided RDS of fruit and did not differ significantly between

the BMI in preschool children ($p > 0.05$). However, RDS of fruit differed in terms of BMI in the adolescents ($p = 0.000$). Otherwise, RDS of vegetable was determined 26.7% in preschool children, 74.4% in adolescents. The fruit RDS is high in adolescents, but it is the opposite for vegetables for both groups. In preschool children RDS of vegetable is not differed by BMI ($p > 0.05$), on the contrary in adolescents ($p = 0.000$). Attitude statements towards fruits and vegetables in the preschool group were taken by the parents, and findings suggest that parents have a higher

strongly agree than adolescents in terms of well and balanced nutrition, consumption, disease relationship, and production to vegetable and fruits. In addition, it's clear that result is far from the attitudes of adolescents, especially about consumption, nutrients and disease relationship to the fruits and vegetables. Moreover, both groups had a moderate or undeceive participation in the attitude statements such as the interaction of the family in terms of fruit and vegetable consumption, negative purchase and consumption behaviors, type of consumption and service, preservation applications, and their consumption in the meal patterns. It was observed that some fruit and vegetable attitudes were similar in both groups. When all 25 attitude statements were evaluated in terms of both groups, difference was determined between the Likert type participation levels ($p < 0.05$).

Another aspect of the study was to determine the fruit and vegetable knowledge levels of preschool children's parents and adolescents about fruits and vegetables. In Table 3, the mean ($\bar{x} \pm SE$) of fruit and vegetable knowledge score of preschool children's parents and adolescents was found 53.8 ± 0.807 and 36.2 ± 0.508 respectively. Parents' fruit and vegetable knowledge scores were differed by education level ($p = 0.000$). The test scores were also found to be higher according to the higher education level. It was determined that the normal group had a higher score in BMI than the others. Significant test scores were obtained among adolescents according to BMI ($p < 0.05$) (Table 3).

DISCUSSION

The study is aimed to compare the fruit and vegetable consumption, attitudes, and knowledge in preschool children and adolescents. According to the report of Monitoring the Growth of School-Age Children in Turkey 2013 (Ministry of Health, 2014), it has been reported that the overweight and obese levels of 6-year-olds are 12.4% and 5.5%. In addition, according to the Turkey Nutrition and Health Survey (TNHS) report (Ministry of Health, 2017), the prevalence of obesity is 8.5% (boys 10.1%, females 6.8%), overweight is 17.9%, overweight and obese were found to be 26.4% in 0-5-year-old children. The results of the study coincide with the data in Turkey. The BMI results of the preschool group are similar to the several study (Lasarte-Velillas et al., 2015; Mei et al., 2008; Müller et al., 2014; Nazarova & Kuzmichev, 2016) findings. On the other hand, adolescents BMI finding overlapped recent Turkish studies (Akman et al., 2010; Bahar et al., 2020; Yosmaoğlu et al., 2010) and in USA according to the National Health and Nutrition Examination Survey (NCHS) (Ogden et al., 2012) the frequency of obesity was found adolescents aged 12-19 years was 19.6% for boys, 17.1% for girls, and 14.4% on average. The prevalence of overweight and obesity in children aged under five years was found as 10.9%–17.9% in Turkey (Ministry of Health, 2017).

When discussing with the literature of fruit and vegetable consumption; in TDG (TDG, 2016) represented that; 400 g fruit and vegetable consumption

is equal to 5 serving/day and 1 serving of raw fruit and cooked vegetables recommended daily servings (RDS) is 150 g. In addition to this, at least 5 servings (at least 400 g/day) of vegetables and fruits should be consumed per day, and 2.5-3 servings should be vegetables and 2-3 portions should be fruit in adults. But another recommendation in 2-6 aged children and 14-18 aged adolescents fruit recommendation is 1.5-2 and 2.5-3 serving, vegetable is 1-2 and 3.5-4 serving per day respectively in TDG (TDG, 2016).

In Turkey Nutrition and Health Survey (Ministry of Health, 2017), daily consumption rates of vegetables, potatoes, citrus fruits, and fruits were reported to be 47.6%, 10.1%, 16.2%, and 51.5%, respectively. (Di Noia and Cullen, 2015) reported that adolescents were believe more fruit and vegetable consumption than their owns. Many studies (Cooke et al., 2004; Orlet Fischer et al., 2002; Vereecken et al., 2004) suggested that the positive association between children's and parents' fruit and vegetable intake and other studies (McClain et al., 2009; Tibbs et al., 2001; Wind et al., 2006) involving preschool-aged children and older children and adolescents and supports previous recommendations for modification of parental diet. Beech et al. (1999) implied that adolescents' fruit and vegetable consumption changed between 2.17 to 2.69 serving per day and girls reported being more confident in their ability to eat five servings of fruits and vegetables per day than did boys. On the other hand, Salwa et al. (2021) reported that 14-18 aged adolescents' the average daily consumption of fruit and vegetables was 1.22 and 1.99 servings, respectively. Only one-fifth of the respondents (21%) reported eating five servings of fruit and vegetables a day. Rasmussen et al. (2006) revealed that age, gender, parental education and occupation, parental consumption, preferences, and home availability- all contribute to adolescents' eating less fruit and vegetables. Seidu et al. (2021) found that the prevalence of adequate fruits, adequate vegetables, and adequate fruit and vegetable consumption were 35.7%, 26.8%, and 27.8%, respectively. Wyse et al. (2011) suggest that a range of factors within the home food environment appear to be associated with young children's fruit and vegetable intake. Another study (Lally et al., 2011) conducted on low-income adolescents showed that youth misperceived their own and their peers' fruit and vegetable intake (i.e., overestimated intake of fruit and underestimated intake of vegetables) and believed that peers held less favorable attitudes toward eating consumptions than was the case. Hill et al. (2020) draws attention to the fact that adolescents have attitudes towards consuming more vegetables than fruits when they are together in different environments (e.g., peer meetings). Beech et al. (1999) reported that 39% correct fruit and vegetable knowledge scores in adolescents.

CONCLUSION

Several studies and reports comparing relationship between the factors in preschool children and

adolescents' fruit and vegetable consumption. The represented study is precious because fruit and vegetable consumption is very important for well and balanced nutrition and growth as possible as important to stay healthy in the future and to control weight and prevent obesity with healthy eating habit and behaviors. BMI results were correlated to the other countries and national surveys. Many studies focused on the daily consumption but in fact, it is the serving consumption that matters. All national authorities were recommended fruit and vegetables emphasized servings for the reason it is more understandable and applicable by the society. It can be said that the consumption of fruits is very close to the recommended servings by the national and international authorities in both groups. On the other hand, vegetable consumption serving was lower than fruit consumption in both groups. While fruit consumption was found to be higher than the reference values given in both groups, it would be wrong to say the same about vegetable consumption, especially in the preschool group. It is also a fact that vegetables, whether cooked or raw, are less preferred than fruits. The fruit and vegetable knowledge score results found to be insufficient in both groups and education level also effected fruit and vegetable knowledge. This situation reveals once again the necessity of increasing the effectiveness of other informative tools, especially for fruits and vegetables, within the scope of nutrition information in the society. In future direction, considerable further works is needed in for all population groups to increase awareness, healthy eating behavior, purchasing foods and sustainable nutrition knowledge on parents and adolescences.

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Conflict of Interest

No potential conflict was declared by the authors.

Author Contributions

Plan, design: MAC; **Material, methods and data collection:** MAC; **Data analysis and comments:** EB, EB; **Writing and corrections:** MAC, EB.

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“I Forget Everything!” Diagnostic Classification of Applicants with Forgetfulness to a Secondary Care Neurology Clinic, Izmir, Turkey

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ABSTRACT

Objective: To determine the frequency of subjective cognitive decline (SCD), other neurological diseases (OND), and psychiatric disorder (PD), diagnosis among patients admitted with forgetfulness to a secondary care neurology clinic and compare their characteristic features. **Materials and Methods:** This cross-sectional study collected data from 464 patients who were admitted with forgetfulness complaints to a single neurology outpatient clinic in a secondary care hospital in Turkey between April 2017 and September 2018. Diagnostic groups were classified as SCD, OND or PD diagnosis. Age and gender-adjusted multinomial regression model was fit for the major diagnostic group. **Results:** Among admissions less than the half of the applicants were diagnosed with OND (44.4%) and it was lower in the younger age group (< 65, 7.8%). One-year increase in age decreased the risk of SCD by 14.5% (Odds Ratio (OR): 0.9, 95% CI: 0.8-0.9) and risk of PD by 14.8% (OR: 0.9, 95% CI: 0.8 - 0.9) relative to a OND diagnosis. Among women, SCD was 2.6 times higher (95% CI: 1.4 - 5.0) than men relative to OND. **Conclusion:** This study shows that more than a half of the admissions are not associated with an OND and, the population admitted to neurology clinics with forgetfulness complaints without objective deficits for cognitive decline were younger on average and the majority were women. The high number of SCD/PD admissions to a neurology clinic and gender imbalance imply lack of the referral system which is crucial for the quality of care at all levels. **Keywords:** Secondary Care, Referral, Subjective Cognitive Decline, Dementia.

“Her Şeyi Unutuyorum!” İkinci Basamak Bir Nöroloji Kliniğine Unutkanlık ile Başvuranların Tanısal Sınıflaması, İzmir, Türkiye

ÖZ

Amaç: Bir ikinci basamak nöroloji kliniğine unutkanlık yakınmasıyla başvuran olgularda öznel bellek yakınmaları (ÖBY), diğer nörolojik hastalık (DNH) ya da psikiyatrik bozukluk (PB) tanılarının sıklığını belirlemek ve olguların karakteristik özelliklerini karşılaştırmaktır. **Gereç ve Yöntem:** Bu kesitsel çalışmada Nisan 2017 ve Eylül 2018 arasında ikinci basamak tek bir nöroloji polikliniğine unutkanlık yakınması ile başvuran 464 hastanın verisi toplanmıştır. Tanı grupları ÖBY, DNH ya da PB tanılarını olarak sınıflandırılmıştır. Ana tanı grupları için yaş ve cinsiyete göre düzeltilmiş Multinomial Lojistik Regresyon modeli uygulanmıştır. **Bulgular:** Başvuranların yarısından azı bir DNH tanı grubunda yer almıştır (%44.4) ve bu tanı grubunun oranı genç yaş grubunda (<65) daha düşüktür (%7.8). DNH varlığı ile karşılaştırıldığında yaşta bir yıllık artış ÖBY’de %14.5 (OR: 0.9, %95 GA: 0.8-0.9), PB’de %14.8 (OR: 0.9, %95 GA: 0.8-0.9) azalma ile ilişkilidir. DNH varlığına göre kadınlarda ÖBY varlığı erkeklerin 2.6 katıdır (%95 GA: 1.4-5.0). **Sonuç:** Bu araştırma ikinci basamak bir nöroloji kliniğine unutkanlık yakınması ile başvuranların yarısından azında DNH hastalık tanısı konduğunu, bilişsel yetilerde nesnel azalmanın gösterilmediği grupların çoğunlukla genç ve kadınlardan oluştuğunu göstermiştir. ÖBY/PB başvurularının yüksekliği ve cinsiyet dağılımındaki farklılık tüm basamaklarda nitelikli hizmet için vazgeçilmez olan sevk zincirinin yokluğunu düşündürür.

Anahtar Kelimeler: İkinci Basamak Sağlık Hizmetleri, Sevk Sistemi, Öznel Bilişsel Yakınma, Demans.

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INTRODUCTION

Forgetfulness is one of the most common complaints in the population, particularly in old ages (Ponds et al., 1997). It is often thought to be related to dementia, however, psychiatric disorders (PD) might cause forgetfulness as well. On the other hand, there is a group of cases where conventional clinical applications don't indicate a neurodegenerative disorder. Whenever the clinical picture does not imply a psychiatric cause, this group of patients are classified as subjective cognitive decline (SCD) (Jessen et al., 2014).

SCD is a widely discussed topic in the current dementia research (Jessen et al., 2014). Advanced techniques in research clinics may show some clinic and molecular differences in this group (such as Alzheimer's disease biomarkers, low CSF amyloid-beta1-42, increased amyloid deposition on PET scans) (Slot et al., 2018). However, as these clinical imaging and molecular tests are not used yet in clinical practice, SCD is a clinical diagnosis that needs to be followed by clinicians to determine a potential neurodegenerative disease progression in clinic practice (Slot et al., 2018). There are some prevalence studies about SCD. These studies reveal that the range of the frequency is around 11% to 50% (Hao et al., 2017; Luck et al., 2018; Taylor et al., 2018). In the literature, three types of sampling method have been utilized in SCD studies such as population sampling, volunteer sampling and medical help-seeking individuals (Hao et al., 2017; Jessen et al., 2014; Luck et al., 2018). In the settings of these studies, medical help-seeking population cannot apply directly to a specialist. All participants of the studies had been referred by their general practitioner in case of a second opinion for evaluation of cognitive complaints (Hao et al., 2017; Luck et al., 2018; Slot et al., 2018).

In Turkey referral is not compulsory to apply to any level of healthcare (OECD Reviews of Health Care Quality: Turkey 2014 Raising Standards, 2014). Different financial incentives drive primary, secondary and tertiary care physicians in different ways. There is a fee-for-service performance system for secondary and tertiary care physicians (OECD Reviews of Health Care Quality: Turkey 2014 Raising Standards, 2014). These two drivers increase the total number of visits (OECD Reviews of Health Care Quality: Turkey 2014 Raising Standards, 2014). Also, the patients can apply to any specialist or even more than one specialist in secondary or tertiary care. Sum of all, several patients are consecutively assessed by different physicians even with the same diagnostic methods. This affects the daily outpatient routine, and the number of the medical help-seeking population may be increased.

Despite being one of the most common admission reasons to neurology clinics; there is no data about the diagnostic classification of the forgetfulness complaint in daily routine in Turkey. We aimed to determine the frequency of SCD, other neurological disease (OND) and PD diagnosis among patients who were admitted with forgetfulness to a secondary care neurology clinic

and compare their demographic, family history characteristics and referral status.

MATERIALS AND METHODS

Study type

This cross-sectional comparison included all cases admitted to a secondary care neurology outpatient clinic between April 2017 and September 2018 with 'forgetfulness' in Izmir, Turkey. Among all applications, 464 patients suffered from forgetfulness as the main symptom. Previously diagnosed patients were not included in the study. Only primary applications were included. All applications were assessed based on clinical diagnostic guidelines, physical and neurological examination, neuropsychological investigation and serum biochemical, thyroid, folic acid and vitamin levels (Gorno-Tempini et al., 2011; McKeith et al., 2017; McKhann et al., 2011; Rascovsky et al., 2011) and all data were collected from the case notes by one neurologist (HU).

All patients' visual atrophy rating scales on structural magnetic resonance imaging or computer tomography were measured by using visual rating scores (Harper et al., 2015; Scheltens et al., 1992). Additionally, secondary dementia aetiology such as tumour, stroke, vascular malformation, metabolic disease etc. was investigated (Koedam et al., 2011; Scheltens et al., 1995).

The patients in need were further examined. Alzheimer's Disease (AD), mild cognitive impairment (MCI) (Albert et al., 2011), frontotemporal dementia (Gorno-Tempini et al., 2011, Rascovsky et al. 2011), Lewy Body dementia (McKeith et al., 2017), vascular dementia, mix dementia (vascular and AD) (Roman et al., 1993; McKhann et al., 2011), Parkinson's disease dementia (Emre, 2003), traumatic, metabolic, toxic, alcohol and substance abuse associated cognitive decline were defined as "other neurological disease" (OND) group. Major depressive disorder, anxiety disorder, somatoform disorder, bipolar disorder, schizophrenia, autism spectrum disorder was included in the "psychiatric disorder" (PD) group. Some of the patients of this group have already been diagnosed as PD.

All remaining patients who have been pre-diagnosed as PD were referred to a psychiatrist for confirmation. The last group was diagnosed as SCD in which participants have "forgetfulness" symptoms however have no structural or functional cognitive decline. None of the participants in the SCD group fulfilled either OND or PD diagnostic criteria.

Statistical analysis

Age was categorized as younger than 65 (16-64) and older than 64 years old (65 and 65+). Gender, family history of dementia and the referral status of the patients were the remaining independent variables. The diagnostic category, demographic features and referral status of the participants were recorded for the study. Mini-Mental State Examination test results are put to define groups. A score lower than 24 was categorized as low. Categorical variables were shown as percentages. Each dichotomous independent variable was compared

by disease groups. In none of the tables the expected numbers were less than 20% of total expected numbers thus the significance of the distributions was tested by chi-square. Multinomial Logistic Regression analysis was fitted for major diagnostic group and the reference group was OND. Age was included as a continuous variable in the model. Non-parametric variance analysis and post hoc tests were used to analyze the difference in age (continuous) of each major diagnostic group. Variables were added to the model if they were significant in univariate analysis and categorical variables if there were more than ten cases in each cell. Alpha error level lower than 0.05 was accepted as significant. Statistical Package for Social Sciences 15.0 was used in the analysis.

Ethical considerations

Dokuz Eylul University Observational Studies Ethical Board approved the study protocol (2018/21-20).

RESULTS

There were 464 cases admitted with forgetfulness complaints. About two-thirds of the admitted patients were female (n=302, 65.1%). More than one-third of all admissions (n=156, 33.6%) were diagnosed with PD. More than one fifth (n=102, 22.0%) had no neurological, psychiatric or metabolic history and their cognitive, neurological, and physical examinations were normal. These patients were included in the SCD group. Only less than half of all patients were in the OND group (n=44.4%). Among these 85 had AD (18.3%), 28 had MCI (6.0%), 10 had frontotemporal dementia (2.2%) (five behavioral variant, 1.1%, 4 progressive non-fluent aphasia, 0.9%, 1 semantic dementia, 0.2%), six had Lewy-body dementia (1.3%), 19 had vascular dementia (4.1%), 36 had mix dementia (7.8%). Seven patients had Parkinson’s disease (1.5%) and 12 patients had metabolic disease (2.6%). One of the patients was diagnosed with normal pressure hydrocephalus (0.2%) and two had forgetfulness due to head trauma (0.4%).

Table 1. Associations between demographic variables, family history, referral status and major diagnostic groups.

Diagnostic group	Independent variable			p
	Age-group			
	≤64 (%)*	>64 (%)*	Total (%)*	
OND	15 (7.3)	191 (92.7)	206 (100.0)	<0.01
PD	105 (67.3)	51 (32.7)	156 (100.0)	
SCD	73 (71.6)	29 (28.4)	102 (100.0)	
Total	193 (41.6)	271 (58.4)	464 (100.0)	
	Gender			
	Men (%)*	Women (%)*	Total (%)*	
OND	93 (45.1)	113 (54.9)	206 (100.0)	<0.01
PD	48 (30.8)	108 (69.2)	156 (100.0)	
SCD	21 (20.6)	81 (79.4)	102 (100.0)	
Total**	162 (34.9)	302 (65.1)	464 (100.0)	
	Family history			
	Present (%)*	Absent (%)*	Total (%)*	
OND	40 (19.4)	166 (80.6)	206 (100.0)	<0.01
PD	6 (3.8)	150 (96.2)	156 (100.0)	
SCD	19 (18.6)	83 (81.4)	102 (100.0)	
Total	65 (14.0)	399 (86.0)	464 (100.0)	
	Referral from a physician			
	Present (%)*	Absent (%)*	Total (%)*	
OND	22 (10.7)	184 (89.3)	206 (100.0)	<0.01
PD	3 (1.9)	153 (98.1)	156 (100.0)	
SCD	1 (1.0)	101 (99.0)	102 (100.0)	
Total	26 (5.6)	438 (94.4)	464 (100.0)	
	Mini-mental state examinations			
	<24 (%)*	≥24 (%)*	Total	
OND	157 (76.2)	49 (23.8)	206 (100.0)	<0.01
PD	9 (5.8)	147 (94.2)	156 (100.0)	
SCD	1 (1.0)	101 (99.0)	101 (100.0)	
Total	167 (36.0)	297 (64.0)	464 (100.0)	

OND: Neurological disease, PD: Psychiatric disorder, SCD: Subjective cognitive decline, *: Row percentage.

When we divide the groups as younger than 65 (16-64), and older than 65 (65 and 65+) years old, 193 (41.6%) were younger than 65 years old, and 147 (76.2%) of

them were female. In this group, only 15 (7.8%) of patients were diagnosed as OND. Seventy-three (37.8%) patients were diagnosed with SCD, and 105 (54.4%) of

patients were diagnosed with PD. In contrast, in the patients that are older than 64 years old, oppositely, 29 (10.7%) patients were diagnosed with SCD, 51 (18.8%) of patients were diagnosed with PD and 190 (70.5%) of patients were diagnosed with OND. One hundred five (67.3%) of the PD group and 73 (71.6%) of the SCD group were younger than 65, however, only 15 (7.3%) of OND were in this age group (Chi-square, $p < 0.001$). One hundred thirteen (54.9%) of the OND group were women whereas 108 (69.2%) and 81 (79.4%) of PD and SCD groups were female, respectively (Chi-square, $p < 0.001$). Positive family history was present in 40 patients (19.4%) in the OND group, however, this was present in 6 (3.8%) and 19 (18.6%) of PD and SCD groups, respectively (Chi-square, $p < 0.001$). Twenty-

two patients (10.7%) in the OND group were referred by a physician whereas there were three (1.9%) and one (1.0%) patients in the PD and SCD groups, respectively (Chi-square, $p < 0.001$). Cases with an OND had significantly lower Mini-Mental State Examination scores (Chi-square, $p < 0.001$) (Table 1). In multinomial logistic regression one-year increase in age decreased the chance of having SCD compared to having an OND diagnosis by 14.5% (OR:0.855, 95% CI:0.828-0.882). Women had 2.618 (95% CI:1.359-5.044) times higher SCD diagnosis compared to men relative to having an OND diagnosis. A one-year increase in age decreased having a PD compared to having an OND by 14.8% (OR:0.852, 95%CI: 0.826-0.879). Gender was not significantly different among PD and OND cases.

Table 2: Multinomial logistic regression of major diagnostic groups.

Diagnostic group	Variable	B	p	OR (95% CI)
PD	Age	-0.160	<0.01	0.85 (0.83-0.88)
	Gender (ref: men)	0.410	0.16	1.50 (0.85-2.66)
SCD	Age	-0.160	<0.01	0.85 (0.83-0.88)
	Gender (ref: men)	0.960	<0.01	2.62 (1.36-5.04)

OR: Odds ratio, PD: Psychiatric disorder, Reference group: Other neurological disease, SCD: Subjective cognitive decline.

DISCUSSION

This cross-sectional comparison found that, in one-and-a-half-year period more than a half of the applicants were diagnosed with a non-possible/probable neurodegenerative disorder in a secondary care neurology clinic in İzmir. SCD and PD groups were both younger compared to OND. Additionally, there were more females in the SCD group than in the OND group. Applications of OND were less common than the total amount of SCD and PD. There might be some reasons for these high numbers of applications. Firstly, the referral system in healthcare is not pronounced (OECD Reviews of Health Care Quality: Turkey 2014 Raising Standards, 2014). All insured individuals can apply at any stage of hospital, even in tertiary care clinics. The most important driver of this is the lack of a gatekeeper primary care system and compulsory referral in healthcare. Performance payment for secondary care physicians may also affect supply side (OECD Reviews of Health Care Quality: Turkey 2014 Raising Standards, 2014). Secondly, according to World Health Organization (WHO) data, AD prevalence and healthcare costs are expected to increase (WHO Fact Sheet on Dementia, n.d.). As a result of increased life expectancy as well as several other factors like better access to healthcare, the incidence of Alzheimer’s disease is increasing, and the impact of burden is higher in low- and middle-income countries (WHO Fact Sheet on Dementia, n.d.). In most cases of dementia, its course is irreversible; despite significant progress and a novel therapeutic candidate (Fillit & Green, 2021), no curative treatment is available in clinical practice yet. Caregiver burden is also another stressor factor for people (Etters et al., 2008). Inevitably, concerns about dementia are also rising among populations worldwide (Slot et al., 2018). As a consequence, medical help-seeking is on the rise.

Recent studies show that SCD can be an early stage of AD (Jessen et al., 2014). Therefore, follow up is required for SCD patients. However, effective follow up can be possible by determining the patients who have risk factors and with a functional referral system which will provide enough dedicated time for patients in need. Regardless of a psychiatric diagnosis, the population-based studies have shown a relationship between concerns, worries and mood with SCD (Hao et al., 2017; Jessen et al., 2014). Concerns or worries can be a decisive factor as they may reflect a patient’s intuition that his or her subjective cognitive problems represent the beginning of a severe cognitive disorder rather than “normal ageing” (Jessen et al., 2014). We didn’t measure the mood or concerns of the patients but they are seeking medical care and all of them have worries about developing dementia. Insomuch as some of them had already admitted by more than three doctors. The PD group also constitutes a large part of applications, especially in the young age group. The high rate of PD may be a result of stigmatization against PD and the applicants may avoid applying to psychiatry clinics (Arslantaş et al., 2010). Studies on SCD mostly focus on individuals older than 60 and neurology clinic applicants are expected to be older. However, in our case, it seems that the applicants are younger and less than half of them were diagnosed with an OND. In a large series from the Amsterdam Dementia Cohort Study, the sum of the SCD and PD diagnosis prevalence was 31% in total among the applicants where an effective referral system is in place (van der Flier & Scheltens, 2018). Additionally, population-based studies show a lower rate of SCD. In the United States, the prevalence of SCD is 11.2% (6). A cross-sectional study of the Chinese population has shown the prevalence of SCD as 14.4% (Hao et al., 2017).

There were more women among admissions diagnosed with SCD compared to admissions diagnosed with OND. Studies indicate that women are more inclined to have SCD more than men (Jonker et al., 2000). Educational attainment and labor force participation are lower in women compared to men (Turkish Statistical Institute Women in Statistics 2018, n.d.). Women who are not in the labour force may have more care responsibilities compared to men which in turn brings along social isolation with an increased risk of morbidity. The relationship between these risk factors and dementia is well known and a significant number of those are also the modifiable risk factors of SCD (Aarts et al., 2011; Chen et al., 2014). Considering all these together, the higher SCD appearance in the female population in Turkey is no surprise. Only about one-tenth of patients with OND were referred by a physician however physician referral rates were too low among the other groups. Despite low referral rates, the OND group had a higher referral rate compared to other groups. The lack of an obligatory referral system results in the burden of hospital admissions in Turkey. This clarifies low referral rates especially cases with SCD. Patients are referred to a neurologist when the physician suspects a neurological disease. Positive family history for dementia was significantly higher among OND and SCD groups than PD groups. Family history and especially being a caregiver are important reasons for the applications (Liang et al., 2016). This implies a concern about having an OND such as AD among applicants. This study fitted a model to predict major diagnostic classification. A small subset of cases had a family history and was referred by a physician. This precluded fitting a model with these significant variables. A study with a higher sample size may fit such a model. Even done on small sample size and does not represent all secondary care admissions to neurology outpatient clinics in Turkey, this study reflects the daily workload of a neurologist associated with non-neurologic admissions in Turkey.

CONCLUSION

This study reveals that less than half of the forgetfulness admissions to a secondary care outpatient neurology clinic were diagnosed with OND. Individuals diagnosed with OND were older, referred by a physician more and had a more positive family history of dementia. Admissions diagnosed with SCD were mostly young and female and had a family history of dementia. The effective use of specialist care in medicine will be possible in a healthcare environment with an established referral system.

Conflict of Interest

The author declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Author Contributions

Plan, design: HU, EE; **Material, methods and data collection:** HU; **Data analysis and comments:** EE, HU; **Writing and corrections:** HU, EE.

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The Correlation Between Mother-Infant Bonding and Postpartum Depression in Women with A History of Infertility

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ABSTRACT

Objective: This study investigated the correlation between mother-infant bonding and postpartum depression in women with a history of infertility. **Methods:** The sample consisted of 169 women divided into two: infertile group (n=56) and fertile group (n=112). Data were collected using a descriptive information questionnaire, the Edinburgh Postnatal Depression Scale (EPDS), and the Mother-to-Infant Bonding Scale (MIBS). **Results:** The fertile and infertile groups had a mean age of 28.95±3.38 and 36.55±3.55, respectively (p=0.001). The infertile group had a higher mean MIBS score (3.73±2.91) than the fertile group (1.50±1.29) (p=0.001). However, there was no significant difference in EPDS scores between the two groups (p>0.05). Moreover, there was a positive correlation between MIBS and EPDS score in the fertile group (r= 0.354, p=0.001), suggesting that the higher the risk for postpartum depression, the lower the mother-infant bonding. There was no correlation between MIBS and EPDS score in the infertile group (p>0.05). **Conclusion:** Future studies should recruit larger samples of infertile women with cultural and ethnic diversity and take confounding factors into account to investigate the relationship between postpartum depression and mother-infant bonding.

Keywords: Infertility History, Postpartum Depression, Mother-Infant Bonding.

İnfertilite Hikayesi Olan Kadınlarda Anne Bebek Bağlanması ve Postpartum Depresyon Arasındaki İlişki

ÖZ

Amaç: Bu çalışmada infertilite hikayesi olan kadınlarda anne bebek bağlanması ve postpartum depresyon arasındaki ilişki incelenmiştir. **Gereç ve Yöntem:** Çalışmanın örneklemini infertil grup (n=56) ve fertil grup (n=112) olmak üzere toplam 169 kadın oluşturmuştur. Veriler Tanımlayıcı Bilgi Formu, Edinburgh Postpartum Depresyon Skalası (EPDS) ve Anne-Bebek Bağlanma Ölçeği (ABBÖ) kullanılarak toplanmıştır. **Bulgular:** Fertil ve infertil grupların yaş ortalaması sırasıyla 28.95±3.38 ve 36.55±3.55 idi (p=0.001). İnfertil grupta ABBÖ puan ortalamasının (3.73±2.91), fertil gruba göre daha yüksek (1.50±1.29) olduğu belirlenmiştir (p=0.001). Ancak iki grup arasında EPDS ortalama puanları yönünden anlamlı bir fark bulunmamıştır (p>0.05). Bunun yanı sıra, fertil grupta ABBÖ ve EPDS ortalama puanları arasında pozitif bir ilişki olduğu belirlenmiştir (r=0.354, p=0.001). Bu bulgu doğum sonrası depresyon riski ne kadar yüksek ise anne ve bebek bağlanma düzeyinin o kadar düşük olduğunu göstermiştir. İnfertil grupta ise ABBÖ ve EPDS ortalama puanları arasında ise bir ilişki belirlenmemiştir (p>0.05). **Sonuç:** Kültürel ve etnik çeşitliliğin bulunduğu daha büyük gruplarda infertil hikayesi olan kadınlarda olası karıştırıcı faktörlerin hesaba katılarak, postpartum depresyon ve anne bebek bağlanma ilişkisinin değerlendirildiği çalışmalara ihtiyaç bulunmaktadır.

Anahtar Kelimeler: İnfertilite Öyküsü, Postpartum Depresyon, Anne-Bebek Bağlanması.

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INTRODUCTION

Infertility is a complicated crisis that adversely affects social and sex life, marital relations, emotional well-being, and plans (Akyuz et al., 2010; Sahin & Gursoy, 2020). Women, especially in traditional societies, are valued and respected only when they bear children. Giving birth is also considered a sign of sexual potency (Sahin & Gursoy, 2020). Couples diagnosed with infertility experience psychosocial problems (Akyuz et al., 2010; Hess et al., 2018). Besides, infertility diagnosis and treatment are a long, painful, and financially burdensome process (Akyuz et al., 2010). Research shows that women with a history of infertility are more probably to experience depression, anxiety, isolation, and loss of control than fertile women (Hess et al., 2018; Safaei Nezhad et al., 2018). Women who conceive after infertility treatment experience more anxiety and depression than those who conceive spontaneously (Akyuz et al., 2010; Olshansky & Sereika, 2005).

Postpartum is a period of physiological changes and adaptation to new roles. The transition to motherhood can be stressful. It is more challenging for women with a history of infertility than for those who conceive spontaneously (Akyuz et al., 2010). Research shows that women with a history of infertility who experience pregnancy-specific anxiety and depression are more vulnerable to postpartum depression (PPD) (Akyuz et al., 2010; Olshansky & Sereika, 2005). PPD is defined as “a major depressive episode that can manifest itself during the first four weeks after giving birth and is characterized by dramatic mood swings that can occur up to one year later” (American Psychiatric Association, 2021). The worldwide prevalence of PPD is 5.5% to 25% (Soderquist et al., 2009). The prevalence of PPD in women with a history of infertility is 12.9% to 25% (Lee et al., 2011; Lynch & Prasad, 2014).

PPD adversely affects mother-infant bonding (MIB) (Sliwerski et al., 2020; Forman et al., 2007). Mothers with depression will probably suffer more from stress, view their children in a more negative light, and have a less intense relationship with and less secure attachment with them than those without depression (Forman et al., 2007). However, there is no published research investigating the correlation between MIB and PPD in women with a history of infertility. MIB disorders deteriorate mother-child relationships in the long term (Lehning et al., 2019). Therefore, it is essential to identify the risks affecting MIB quality early and support mothers with a history of infertility.

The research questions were as follows:

Q.1. Is there a significant difference in Mother-to-Infant Bonding Scale (MIBS) scores between women who conceive spontaneously and those who conceive after infertility treatment?

Q.2. Is there a significant difference in Edinburgh Postnatal Depression Scale (EPDS) scores between women who conceive spontaneously and those who conceive after infertility treatment? Q.3. Is there a correlation between MIBS and EPDS scores in women

who conceive spontaneously and those who conceive after infertility treatment?

MATERIALS AND METHODS

Population and sampling

This comparative descriptive study was carried out between September 2019 and June 2020 at a Gynecology Training and Research Hospital in Ankara. Prior and post-hoc power analysis were both performed based on two independent groups. Expected Cohens' *f* value for effect size was 0.2 which is considered as small effect size. For a type I error of 0.05 and an effect size of 0.2 with a minimum required power of 80%, the estimated total sample size was 152, including the infertile group (IG, *n*=51) and the fertile group (FG, *n*=102). Considering 10% sample loss, a total of 168 people, 56 in the infertile group and 112 in the fertile group, were included in the study. Power analysis was performed again at the end of the study based on mean MIBS scores and standard deviations. Post-hoc effect size of the study was calculated as 0.92 and post-hoc power 95%. For Power analyses G-power package version 3.1.9.2 was used.

Criteria for inclusion

The inclusion criteria were as follows: (1) literate, (2) Turkish speakers, (3) carrying one fetus, (4) giving birth by C-section, (5) being primiparous, (6) no having pregnancy-related complication (preeclampsia, gestational diabetes, bleeding, epilepsy, etc.); (7) no congenital malformation in the infant, (8) no mental health problems (depression, anxiety, severe psychosocial problems, etc.), (9) filling out the forms completely, and (10) voluntary.

We pre-interviewed 250 women until we reached the target sample size. A total of 168 women met the inclusion criteria [FG (*n*=112) and IG (*n*=56)]. The remaining 82 women were excluded because they could not speak Turkish (*n*=13), were illiterate (*n*=19), multiparous (*n*=25), or had a vaginal delivery (*n*=22). Twelve participants did not turn up, and therefore, the final sample consisted of 156 women in the second stage [FG (*n*=104) and IG (*n*=52)].

Data collection tools

The Descriptive Information Questionnaire-1 (DIQ-1) consisted of 15 items on the sociodemographic and obstetric characteristics of women who conceived spontaneously (Akyuz et al. 2010; Lee et al. 2011).

The Descriptive Information Questionnaire-2 (DIQ-2) consisted of 20 items on the sociodemographic and obstetric characteristics of women who conceived after ART (Akyuz et al., 2010; Lee et al., 2011; Lynch & Parasad, 2014).

The Edinburgh Postnatal Depression Scale (EPDS) was developed by Cox et al. (1987) to evaluate depressive symptoms. The EPDS was adapted to Turkish by Engindeniz et al. (1996). It consists of 10 items scored on a four-point Likert-type scale (min=0, max=30). Higher scores indicate more severe symptoms of depression. Engindeniz et al. (1996) reported the Cronbach's alpha as 0.79, 0.83 in this study.

The Mother-to-Infant Bonding Scale (MIBS) was developed by Taylor et al. (2005) to identify the level of maternal bonding. The MIBS was adapted to Turkish by Karakulak et al. (2007). It consists of eight items scored on a scale of 0 to 3. The total score ranges from 0 to 24, with higher scores indicating lower MIB. The scale has a Cronbach’s alpha of 0.71 in the 12th week postpartum (Taylor et al., 2005) and 0.68.3 in the 8th-12th week postpartum (Karakulak et al., 2007). The Cronbach’s alpha was 0.70 (in the 8th-12th week postpartum) in this study.

Procedure

The research consisted of two stages. First, we checked the files of all women admitted to the obstetric outpatient clinic for routine follow-up in the fourth-sixth weeks postpartum and determined those who got pregnant

spontaneously and those who got pregnant after ART (in vitro fertilization, artificial insemination, etc.). The fertile group (FG) consisted of women who conceived spontaneously, while the infertile group (IG) consisted of those who got pregnant after ART. The FG participants completed the DIQ-1 and EPDS, while the IF participants completed the DIQ-2 and EPDS. We also received permission from participants to log their contact details.

Second, we contacted all participants by phone to set dates for their visits to the Newborn and Child Outpatient Clinic for routine newborn follow-up in the eighth-twelfth week postpartum. Both groups completed the MIBS (Figure 1).

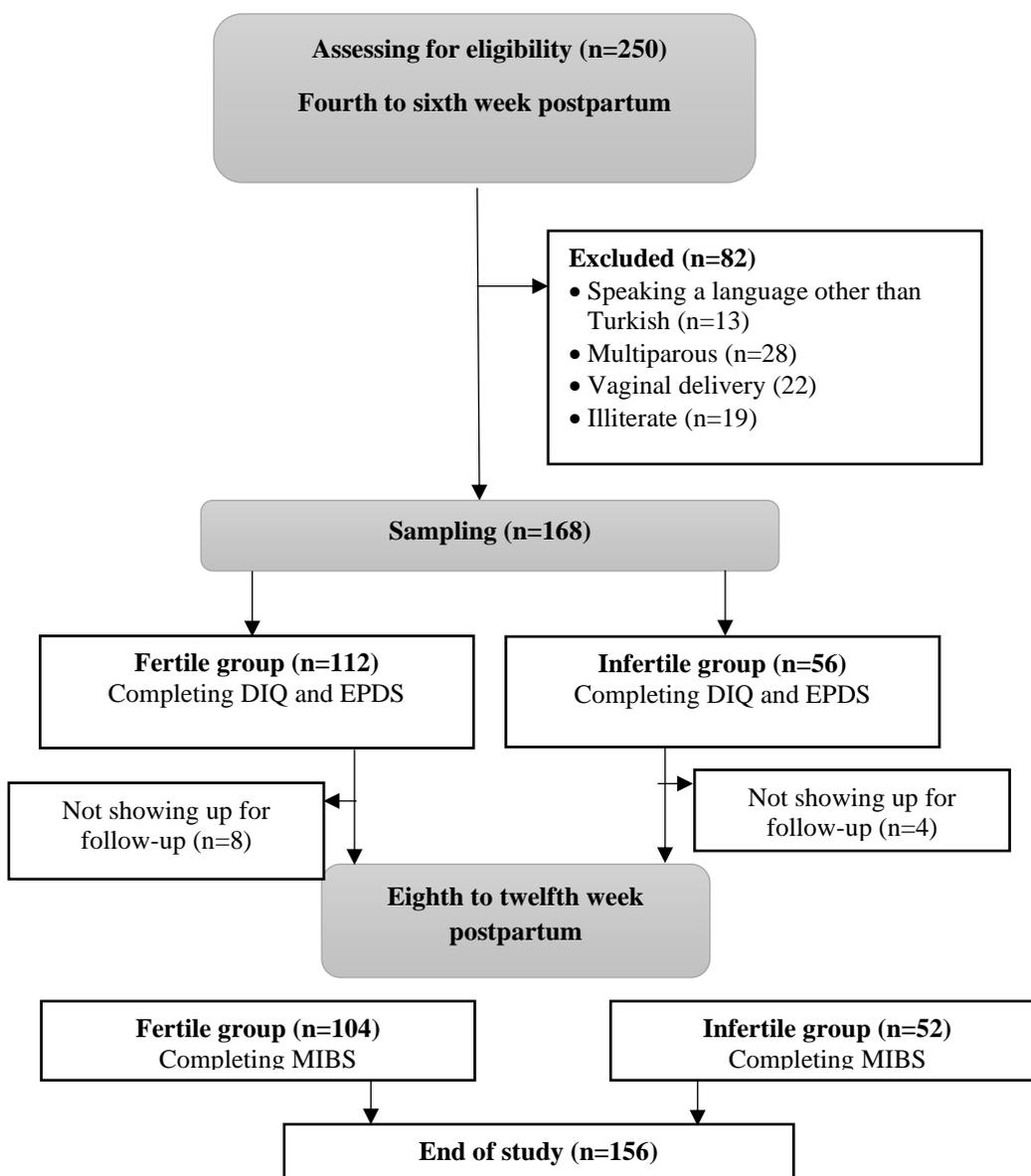


Figure 1. Flow diagram.

Data analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS, v. 23.0) at a significance level of 0.05. Percentage, median (minimum, maximum), mean (standard deviation), chi-square, and Mann-Whitney U test were used for analysis. Normality test of the study was checked using the Shapiro-Wilk test. The Spearman correlation was used to determine the correlation between scale scores.

Ethical considerations

The study was approved by an Ethics Committee (approval date/number: February 23, 2018/51). Permission was obtained from the hospital management. Prior to participation, all women were informed of the research purpose, confidentiality, and their right to withdraw. Informed consent (including permission to be interviewed) was obtained from those who agreed to participate according to the guidelines presented in the Declaration of Helsinki.

RESULTS

Demographic characteristics

Table 1 shows the socio-demographic and obstetrics characteristics of the participants. The fertile group was younger (28.95 ± 3.38) than the infertile group (36.55 ± 3.55) ($p=0.001$). The mean age of marriage was significantly lower in the fertile group (24.49 ± 2.48) than in the infertile group (28.00 ± 2.66 years) ($p=0.001$). Thirty-eight FG (36.5%) and 24 IG (46.2%) participants breastfed their babies within the first hour postpartum. The difference was statistically significant ($p<0.05$). The majority of FG participants (60.0%) received support from their relatives and friends, while slightly more than half the IG participants (53.9%) received support from their spouses/partners in the first four weeks postpartum ($p<0.05$). The two groups were demographically similar in terms of education, family type, employment, and satisfaction with the newborn's sex ($p>0.05$). Though not shown in Table 1, the IG participants had a mean infertility treatment of 4.0 ± 1.83 years (min=1-max=10). Thirty-four percent of the cases were female infertility due to female-related problems.

Distribution of scale scores

The infertile group (1.50 ± 1.29) had a lower mean MIBS score than the fertile group (3.73 ± 2.91) in the eighth to twelfth week postpartum ($p=0.001$). This result showed that the IG participants had higher levels of MIB than their FG counterparts. There was no significant difference in EPDS scores between the infertile (12.01 ± 3.39) and fertile groups (13.45 ± 6.31) in the fourth to sixth week postpartum ($p>0.05$) (Table 2).

There was a positive correlation between EPDS and MIBS scores in the fertile group ($r=0.354$, $p=0.001$), suggesting that the higher the risk for PDD, the lower the MIB. There was no correlation between EPDS and MIBS scores in the infertile group ($p>0.05$) (Table 3).

DISCUSSION

This study examined the correlation between MIB and PPD in women who conceived spontaneously and those who conceived after infertility treatment. Postpartum depression (PPD) was assessed using the EPDS in the fourth to sixth week postpartum, while mother-infant bonding (MIB) was assessed using the MIBS in the eighth to twelfth week postpartum. This study showed that the IG participants had higher levels of MIB than their FG counterparts ($p<0.05$). There was no significant difference in EPPD scores between the infertile and fertile groups in our study ($p>0.05$). We also found a positive correlation between EPDS and MIBS scores in the fertile group. It is noteworthy that there was no correlation between EPDS and MIBS scores in the infertile group.

The average age of marriage and childbearing is rising worldwide. Fertility naturally declines as women get older (Haliloğlu et al., 2014), and infertile women are more likely to enter into marriage later than fertile women (Akyüz et al., 2010). In the last two decades, Turkey has witnessed an increase in the age of marriage and fertility because women today are participating in social and economic life and pursuing their career goals more actively than before. For women, the age of marriage increased from 22 in 2000 to 25 in 2019. Similarly, the age of childbearing increased from 26 in 2000 to 29 in 2019 (TÜİK, 2019). Our results showed that the age of childbearing and marriage among fertile women was close to the national average but lower than that in women with a history of infertility. It is thought that there is relationship between fertility and maternal age.

Infertility diagnosis has adverse psychosocial impacts on couples (Akyuz et al., 2010; Hess et al., 2018). Besides, infertility diagnosis and treatment is a long, painful, and financially burdensome process (Akyuz et al., 2010). Women receiving infertility treatment are extremely worried about the process and treatment outcomes (Hasanpoor-Azghdy et al., 2014). Infertile women have difficulty adapting to the postpartum period and assuming parenting roles due to past experiences and fear of losing their babies (Ranjbar et al., 2015). There are contradicting results regarding the prevalence of PPD among women who conceive spontaneously or after infertility treatment. Ross et al. shows that women who conceive after infertility treatment have a higher prevalence of PPD than those who conceive spontaneously. On the other hand, some other studies report similar rates of PPD in the two groups (Akyuz et al., 2010; Gressier et al., 2015). There was no significant difference in EPPD scores between the infertile and fertile groups in our study ($p>0.05$). Small et al. (2003) argue that women deprived of social support in the postpartum period are more likely to experience psychosocial problems and PPD. In our study, the infertile and fertile groups had similar levels of depression, which may be because most women in both groups received support from their spouses/partners or their relatives/friends in the postpartum period.

It was determined that women in the infertile group received more support from their spousal/partners, and women in the fertile group received support from their relatives and friends in this study. According to Hasanpoor-Azghdy et al. (2014), infertile women keep their diagnosis and treatment to themselves. Akizuki and Kai (2008) maintain that spousal/partner support is essential for infertile women to become less dependent on others. In Turkish society, bearing children results in strong social approval and respect and is considered a sign of sexual potency (Sahin & Gursoy, 2020). Koçyiğit

(2012) found that infertile Turkish women described themselves as “dying trees” and “barren land.” Women stated that they were the subject of peer pressure and were bombarded with questions about having kids. Therefore, most couples with a history of infertility do not share their story with family or friends (Koçyiğit, 2012). In our study, women in the infertile group received support primarily from their spouses/partners. This result also suggests that infertile couples choose to go through the diagnosis and treatment process by themselves without sharing it with their family and friends.

Table 1. Socio-demographic and obstetrics characteristics (n=156).

Characteristics	Fertile group (n=104)		Infertile group (n=52)		Test value	p
	n	%	n	%		
Age, y, $\bar{x}\pm SD$	28.95 \pm 3.38		36.55 \pm 3.55		5.078 ^a	0.001 ^{**}
Age of marriage, y, $\bar{x}\pm SD$	24.49 \pm 2.48		28.00 \pm 2.66		6.877 ^a	0.001 ^{**}
Educational status					0.186 ^b	0.667
<High school	21	20.2	9	17.3		
\geq High school	83	79.8	43	82.7		
Employment status					0.321 ^b	0.571
Employed	55	52.9	25	48.1		
Unemployed	49	47.1	27	51.9		
Family type					2.550 ^b	0.110
Nuclear	92	88.5	41	78.8		
Extended	12	11.5	11	21.2		
First breastfeeding					9.625 ^b	0.008 [*]
Within the first hour	38	36.5	24	46.2		
First 1-2 hours	49	47.1	28	53.8		
First 2-4 hours	17	16.3	-	-		
Satisfaction with the newborn's sex					2.097 ^b	0.148
Yes	74	71.2	31	59.6		
No	30	28.8	21	40.4		
Source of support for newborn care^c					4.210 ^b	0.040 [*]
Spouse/Partner	52	40.0	35	53.9		
Relative/Friend	78	60.0	30	46.1		

^aMann-Whitney U test, ^bChi-square test, ^cPercentages are based on n. *p<0.05, **p<0.001.

Table 2. EPDS and MIBS scores (n=156).

Scales	Fertile group (n=104)		Infertile group (n=52)		Test value	p
	\bar{x}	SD	\bar{x}	SD		
MIBS	3.73	2.91	1.50	1.29	-5.367 ^a	0.001*
EPDS	13.45	6.31	12.01	3.39	-1.754 ^a	0.080

*MIBS, Mother-to-Infant Bonding Scale; EPDS, Edinburgh Postnatal Depression Scale. ^aMann-Whitney U test. *p<0.001.

Table 3. Correlation between MIBS and EPDS scores.

Scales	Fertile group (n=104)				Infertile group (n=52)			
	MIBS		EPDS		MIBS		EPDS	
	r ^a	p	r ^a	p	r ^a	p	r ^a	p
MIBS	1		0.354	0.001*	1		0.082	0.563
EPDS	0.354	0.001*	1		0.082	0.563	1	

MIBS: Mother-to-Infant Bonding Scale; EPDS: Edinburgh Postnatal Depression Scale. ^aSpearman correlation. *p<0.001.

Quality mother-infant bonding is a prerequisite for healthy relationships in the future (Barker et al., 2017). According to Bowlby’s attachment theory (1982), attachment during infancy shapes an individual’s life and affects their relationships in the future. Women with a history of infertility have difficulty transitioning to the maternal role during pregnancy. Women who conceive after infertility treatment may have special needs because they generally have concerns about motherhood and MIB (Bernstein et al., 1994). Women who become mothers after infertility treatment expresses ambivalent emotions of love and hate towards their babies. They also think that they have no right to complain about their babies (Sliwerski et al., 2020). Donarelli et al. (2012) shows that women with a history of infertility have lower MIB than those who conceive spontaneously. Our IG participants also had higher MIB than their FG counterparts (p<0.05). The baby is most sensitive in the first hours after birth. During this period, mother-infant interaction is critical for secure MIB (Donarelli et al., 2012). In our study, the IG participants may have established a more secure MIB because they started to breastfeed earlier and thus interacted more with their babies than their FG counterparts. We also think that the IG participants may have a more secure MIB because their pregnancies were planned, and they had wanted to have babies for a long time.

Research shows that PPD is negatively correlated with MIB, suggesting that the higher the levels of depression, the less the MIB (Forman et al., 2007; 2020; Nieto et al., 2017). We also found a positive correlation between EPDS and MIBS scores in the fertile group (r=0.354, p=0.001), indicating the higher the MIB in the FG participants. It is noteworthy that there was no correlation between EPDS and MIBS scores in the infertile group. There is no published research investigating the correlation between PPD and MIB in women with a history of infertility. Therefore, more evidence-based studies with larger multicultural sample sizes are

warranted to determine the relationship between PPD and MIB in women receiving infertility treatment.

Limitations of study

This study had two limitations. First, it was conducted only in one center, and therefore, the results cannot be generalized to the general population. Second, the sample consisted only of women who underwent a C-section because 97% of pregnant women receiving ART at the center undergo a C-section. We thought that the delivery type could affect the results, and therefore, we composed the fertile group from women who underwent a C-section.

CONCLUSION

The IG participants had higher levels of MIB than their FG counterparts. The risk for PPD was similar in both groups. There was no correlation between PPD and MIB in the infertile group. Future studies should recruit larger samples of infertile women with cultural and ethnic diversity and take confounding factors into account to investigate the relationship between PPD and MIB.

Conflict of Interest

The authors declare no potential conflicts of concerning the research, authorship and/or publication of this article.

Author Contributions

Plan, Design: SK, SK, EÖ; **Materials and methods:** SK, EÖ; **Data analysis and interpretation:** EÖ; **Writing and corrections:** SK, SK, EÖ.

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Is “Danger Zone” Really Dangerous? - An Endodontic Perspective

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ABSTRACT

Objective: To evaluate the accuracy of panoramic radiography in determining dentin thickness of danger zone (DZ) using cone-beam computed tomography (CBCT) imaging to examine the dentin thicknesses of the mandibular molars at different levels. **Materials and Methods:** For the study, 250 mandibular molars were selected. Dentine thicknesses were measured on panoramic radiography. On the CBCT view, dentine thicknesses of mesiobuccal (MB), mesiolingual (ML), and distal roots were measured. The reference point was determined as 3 mm, 4 mm, and 5 mm below the furcation of mandibular molars. Statistical analysis was performed. **Results:** The discrepancy between CBCT and panoramic was 0.5 mm for MB, 0.48 mm for ML, and 0.43 mm for distal. The difference in the dentin thickness between 3 mm and 5 mm is not statistically significant ($p>0.05$), whereas the 4 mm furcation level was significantly lower in either 3 mm or 5 mm for each root canal ($p<0.05$). This study showed that 0.5 mm distortion occurred in the danger zone on panoramic radiography. **Conclusion:** Our study showed that the dentin thickness of less than 1 mm in panoramic radiography is more prone to perforation because of distortion. The dentin thickness was lowest at 4 mm below the furcation. Our results can be a guide the clinicians in clinics or institutions where periapical radiography is not available.

Keywords: Cone-Beam Computed Tomography, Panoramic Radiography, Mandibular Molar, Endodontics, Root Canal Preparation.

“Tehlikeli Bölge” Gerçekten Tehlikeli mi?– Endodonik Bakış Açısı

ÖZ

Amaç: Çalışmanın amacı konik ışınli bilgisayarlı tomografi (KIBT) kullanarak tehlikeli bölgenin (TB) dentin kalınlığını belirlemede panoramik radyografinin doğruluğunu değerlendirmek ve mandibular azı dişlerinin dentin kalınlıklarını farklı seviyelerde incelemektir. **Gereç ve Yöntem:** Çalışma için 250 mandibular azı dişi seçildi. Panoramik radyografide dentin kalınlıkları ölçüldü. KIBT görüntüsünde mesiobukkal (MB), meziolingual (ML) ve distal köklerin dentin kalınlıkları ölçüldü. Referans noktası mandibular molarların furkasyonunun 3 mm, 4 mm ve 5 mm altı olarak belirlendi. İstatistiksel analiz yapıldı. **Bulgular:** CBCT ve panoramik arasındaki fark MB için 0.5 mm, ML için 0.48 mm ve distal için 0.43 mm idi. 3 mm ve 5 mm arasındaki dentin kalınlığındaki fark istatistiksel olarak anlamlı değildir ($p>0.05$), oysa 4 mm furkasyon seviyesi her bir kök kanalı için 3 mm ve 5 mm'de önemli ölçüde daha düşüktü ($p<0.05$). Bu çalışma, panoramik radyografide tehlike bölgesinde 0.5 mm distorsiyon oluştuğunu göstermiştir. **Sonuç:** Çalışmamız panoramik radyografide 1 mm'den az olan dentin kalınlığının distorsiyon nedeniyle perforasyona daha yatkın olduğunu göstermiştir. Dentin kalınlığı furkasyonun 4 mm altında en düşük seviyedeydi. Sonuçlarımız, periapikal radyografinin bulunmadığı kliniklerde veya kurumlarda klinisyenlere yol gösterici olacaktır.

Anahtar Kelimeler: Konik Işınli Bilgisayarlı Tomografi, Panoramik Radyografi, Mandibular Molar, Endodonti, Kök Kanal Şekillendirmesi.

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INTRODUCTION

Endodontic treatment of mandibular molars can be challenging for several reasons including irregular canal anatomy, severely curved roots, and inadequate dentin thickness. Curved roots and thin dentin may cause iatrogenic complications during canal preparation (De-Deus et al., 2019). The distal region of mesial roots in mandibular molars that have been termed the danger zone (DZ) can lead to strip perforations because of dentin thickness and over curved roots (Azimi et al., 2020). Excessive removal of dentin in the DZ during endodontic treatment can potentially result in strip perforation. Strip perforation (stripping) is a vertical, oblong perforation type that generally occurs in DZ of curved root canals and can be hard to diagnose. This type of perforation consequently causes complications difficult to treat such as vertical root fracture, lateral lesion, alveolar bone destruction, and extrusion of filling materials (Estrela et al., 2017).

The DZ is a risky area that needs to be examined correctly and adequately before root canal treatment to avoid impaired tooth integrity. As typically seen in the cross-sectional views, the canals are not located in the anatomical center of the root (De-Deus et al., 2019). They can be found close to furcal concavities. Overpreparation with nickel-titanium (Ni-Ti) instruments of canals increases the risk of strip perforation. Large Ni-Ti instruments are less flexible and have a worse centering ability. These files remove relatively more dentin on the thin side of the root and increase the incidence of strip perforation (Miguéns-Vila et al., 2021). On the other hand, regardless of instrumentation techniques and instruments, root dentin thickness in the DZ will be intrinsically thinner than the dentin thickness in the safe zone (mesial side of the mesial root) after instrumentation (De-Deus et al., 2021).

Panoramic radiography is the most commonly used radiographic technique for the examination of teeth in general dentistry. It is preferred due to its low exposure dose, low cost, and accessibility. Although panoramic radiography is an excellent diagnostic tool that can give the clinician an overall view of the dentoalveolar structures, significant linear image distortion that occurs in panoramic radiography, makes panoramic radiography inadequate because of the distortion that occurs in the measurement (Rahmel & Schulze, 2019). Furthermore, three-dimensional (3D) structures of dentoalveolar tissues make the two-dimensional (2D) imaging techniques inadequate. Cone-beam computed tomography (CBCT) allows 3D examination and thus has a superior ability to evaluate root anatomy than panoramic radiography in the diagnosis of endodontic pathologies. However, CBCT imaging is considered a second-level examination and does not replace panoramic radiography for every diagnostic procedure (Rahmel & Schulze, 2019).

Routinely, the most used radiographic modality to accompany endodontic therapy is the periapical

radiograph (Nardi et al., 2008). Even though most clinics and dental hospitals use periapical radiography to investigate dental structures before endodontic treatment, some institutions can only use panoramic radiography before endodontic treatment, for instance, in some military clinics or areas where the periapical modality is not available temporarily or permanently. Therefore, radiological data obtained from panoramic radiography has diagnostic importance for a successful endodontic treatment. No previous studies have demonstrated the accuracy of panoramic radiographs to evaluate DZ in mandibular molars. The present study aims to evaluate the clinical reliability of panoramic radiography with CBCT imaging as the reference standard for assessing the dentin thickness at the DZ as well as at different levels of each root of the mandibular molars. Our null hypothesis is that panoramic radiography has no diagnostic reliability for dentin thickness of the danger zone.

MATERIALS AND METHODS

Study type

The present study was designed as a retrospective study.

Study group

The study group consisted of 96 patients (49 females and 47 males) aged 30-35 years, who attended a dental clinic between June 2018 and July 2019 and were referred for panoramic radiography and CBCT examinations for various indications. Images of the patients were retrospectively evaluated. For the study, 250 mandibular molars were selected (120 mandibular first molars and 130 mandibular second molars). The inclusion criterion was the presence of at least one mandibular molar with completion of root formation. Extreme crown damage, furcation lesion, severe periodontal lesion, distal roots with two canals, mesial roots with one canal, C-canals, endodontic treated teeth, teeth with a post-core reconstructed, an undeveloped tooth with a wide-open apex, and internal and external root resorption were excluded from the study. Poor quality CBCT images with artifacts were also excluded from the study.

Procedures

All measurements were performed by the same oral maxillofacial radiologist (six-years' experience) and the endodontist (6 years' experience) blinded to patients' data. All images were evaluated twice by one specialist at two-week intervals.

Panoramic images were acquired with Orthophos (Sirona Dental Systems, Bensheim, Germany). The images were assessed in sagittal planes. Dentin thicknesses of the distal aspect of the mesial root and mesial aspect of the distal root were measured at 3 mm, 4 mm, and 5 mm below the furcation for mesial and distal canals respectively (Figure 1). Curvature in mesiobuccal (MB) and mesiolingual (ML) canals cannot be evaluated radiographically because of superimpositions and the thinnest area was recorded.

Dentin thickness was measured with Sidexis (Sirona Dental Systems, Inc, USA) at x12 magnification.



Figure 1. Measurement of mesial (left) and distal (right) dentin thicknesses on panoramic radiography 4 mm below furcation.

CBCT images were obtained using Orthophos (Sirona Dental Systems, Bensheim, Germany). Imaging parameters were set as 85 kV, 6 mA, 14.1 s exposure time, 0.2 mm voxel size, and 80x40 mm field of view. Images were examined at x12 magnification. Mandibular molars were evaluated in sagittal and axial planes. For each canal, the distance between the external surface of the root and the outer border of the canal was measured at 3 mm, 4 mm, and 5 mm below the furcation. The measurements were performed at the location, where dentin thickness was the smallest (Figure 2).



Figure 2. Measurement of dentin thicknesses on CBCT 4 mm below furcation in the axial plane (left) and the sagittal plane (right)

Statistical analysis

SPSS version 26.0 for Windows (IBM Corp., Armonk, NY, USA) was used for statistical analysis. The distribution of the data was analyzed by Bland-Altman analysis. The comparison of measurement methods, regression, and correlation analysis as a statistical method is not appropriate for the present study to not assess the relationship between CBCT and panoramic images Bland-Altman analysis was used to analyze the data as it was more suitable to compare the two methods (Bland & Altman, 1986).

One-Way-ANOVA and post hoc Bonferroni tests were utilized to compare measurements between 3 mm, 4 mm, and 5 mm below the furcation level. One-Way ANOVA was applied to compare dentin thicknesses between MB,

ML, and distal root canals. Differences were considered significant for $p < 0.05$.

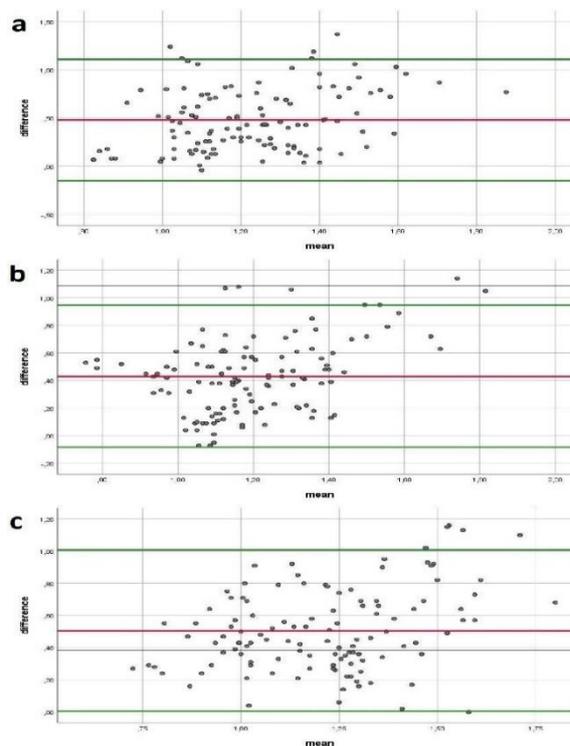
Interobserver and intraobserver agreements were evaluated using the intraclass correlation coefficient (ICC). Based on a 95% confidence interval (CI) the results were classified into 4 groups as poor (ICC values less than 0.5), moderate (ICC values between 0.5 and 0.75), good (ICC values between 0.75 and 0.9), and excellent (ICC values greater than 0.9) (Mattos et al., 2014).

Ethical considerations

The present study was accomplished in accordance with the guidelines outlined in the Declaration of Helsinki and was approved by the Akdeniz University Ethics committee (2019, #70904504/617).

RESULTS

Ninety-six (96) patients with mean ages of 33.64 ± 0.27 were included in the study. Table 1 and graphic 2 show the dentin thickness values at various levels of distal, MB, and ML root canals on CBCT images. At least half of root canals have dentin thicknesses less than 1mm at the DZ on CBCT images. The distribution of levels of thinnest dentin thickness is shown in graphic 3.



Graphic 1. Bland-Altman plots. (a) The mean difference is 0.43 mm between measurements in panoramic and CBCT for the distal canal, (b) the mean difference is 0.5 mm between measurements in panoramic and CBCT for the mesiolingual canal, (c) the mean difference is 0.48 mm between measurements in panoramic and CBCT for the mesiolingual canal.

The Bland-Altman plots show that the means of differences between panoramic and CBCT measurements for distal, MB, and ML were 0.43 mm, 0.5 mm, and 0.48 mm, respectively (Graphic 1).

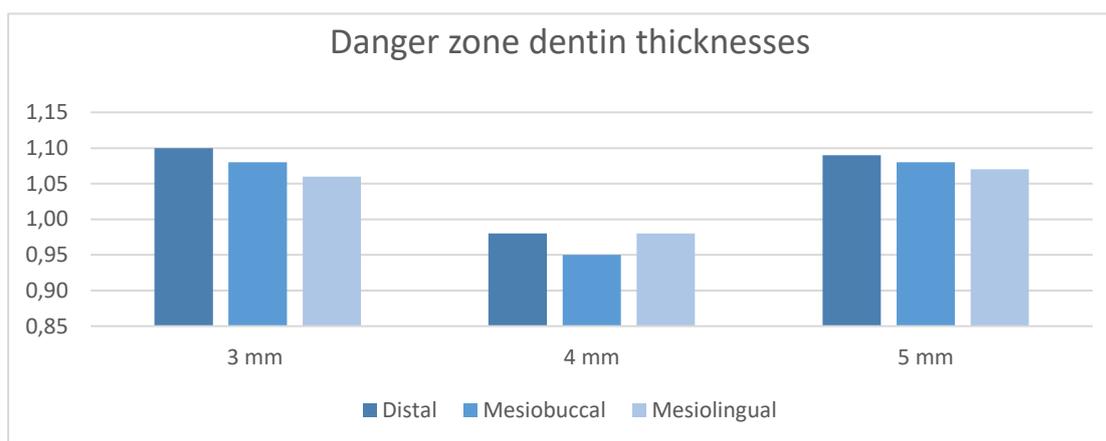
According to the Bland-Altman plots, 95% limits of agreement between the two methods ranged from 0.94

to -0.08; 1 to -0.03; and 1.1 to -0.14 for distal, MB, and ML canals, respectively. Interobserver and intraobserver agreement were excellent for both methods (ICC>0.9).

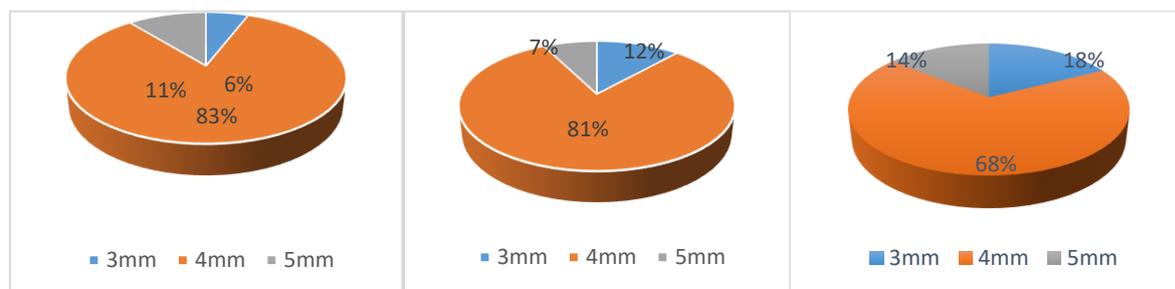
Table 1. Dentin thickness values (in millimeters) at various levels.

Root Canals	Furcation level	Mean±SD	Range	Number of canals with <1 mm of dentin
Distal canal	3mm	1.1±0.18	1.47-0.59	35 (29.1%)
	4mm	0.98±0.18	1.38-0.49	53 (44.1%)
	5mm	1.9±0.17	1.52-0.56	25 (20.8%)
Mesibuccal canal	3mm	1.08±0.2	1.6-0.67	45 (37.5%)
	4mm	0.95±0.21	1.58-0.53	64 (53.3%)
	5mm	1.08±0.21	1.57-0.59	43 (35.8%)
Mesiolingual canal	3mm	1.06±0.2	1.56-0.61	41 (34.1%)
	4mm	0.98±0.21	1.49-0.4	61 (50.8%)
	5mm	1.07±0.21	1.72-0.52	39 (32.5%)

SD: Standard deviation.



Graphic 2. Dentin thicknesses of the danger zone in the distal, mesibuccal, and mesiolingual canals at 3 mm, 4 mm, and 5 mm.



Graphic 3. Pie chart of thinnest dentine levels around the distal canal (left), the mesibuccal canal (middle), and the mesiolingual canal (right).

Based on One-Way ANOVA, there is no statistically significant difference in dentin thickness between MB, ML, and distal root canals. The difference in the dentin thickness between 3 mm and 5mm below the furcation is not statistically significant ($p>0.05$ Bonferroni test),

whereas, at 4mm below furcation level, the dentin thickness is significantly lower than that at both 3 mm and 5mm below the furcation for each of the root canals ($p<0.05$ Bonferroni test) (Table 2).

Table 2. Comparison of dentine thickness values for each level below the furcation area.

Root Canals	I(level)	J (level)	Mean Difference (I-J)
Distal canal	3mm	4mm	0.12308*
		5mm	0.00483
	4mm	3mm	-0.12308*
		5mm	-0.11825*
	5mm	3mm	-0.00483
		4mm	0.11825*
Mesiobuccal canal	3mm	4mm	0.12342*
		5mm	-0.00600
	4mm	3mm	-0.12342*
		5mm	-0.12942*
	5mm	3mm	0.00600
		4mm	0.12942*
Mesiolingual canal	3mm	4mm	0.08575*
		5mm	-0.00525
	4mm	3mm	-0.08575*
		5mm	-0.09100*
	5mm	3mm	0.00525
		4mm	0.09100*

*statistically significant differences between groups, according to ANOVA, $p<0.05$.

DISCUSSION

Our study showed a 0.5 mm distortion in the dentin thickness of the danger zone on panoramic radiography. Thus, our null hypothesis was rejected. In the literature, various methods are used to measure the DZ thickness such as periapical radiographs, CBCT, micro-computed tomography (micro-CT), and serial sections (Espir et al., 2018). Sectioning tooth is an invasive and destructive method used to determine dentin thickness. Lately, micro-CT has become a recommended technique for endodontic research (Cerqueira et al., 2021). Micro-CT has a high resolution due to the high radiation dose and allows non-destructive three-dimensional assessments. However, this technique is limited to laboratory use and is time-consuming (Junior et al., 2014). On the other hand, age is an important factor affecting dentin thickness. Results may change when the age factor is not considered in cross-sectional and micro-CT studies using stored teeth (De-Deus et al., 2019). The present study was performed in a specified age range (30-35 years) to prevent all variables and bias. Because the present study aimed to examine the accuracy of panoramic radiography of sound teeth,

micro-CT and cross-sectional methods used in extracted teeth are not suitable. CBCT provides detailed images of 3D anatomic structures without loss of information (Espir et al., 2018). In a previous study comparing micro-CT and CBCT measurements, it was shown that CBCT was sufficient to examine dentin thickness (Xu et al., 2017). For all these reasons, CBCT was preferred as the reference standard to compare the accuracy of the panoramic images.

The statistical analysis of the comparison between the two methods includes Bland-Altman, linear regression, and correlation analysis. Correlation and linear regression analysis evaluate the relationship between two quantitative measurement methods and are improper for our study. In the present study, the Bland-Altman analysis was applied to compare the two methods. The Bland-Altman analysis uses the mean difference and limits of agreement to compare the two methods. The goal of Bland-Altman analysis is to evaluate bias between mean differences in methods (Gerke, 2020).

One of the critical factors of treatment planning is diagnostic information gained from the clinical and

radiological examination. Three-dimensional imaging techniques such as CBCT present more information than 2D images because of reducing or eliminating superimpositions. Therefore, CBCT is also recommended before endodontic treatment for the high accuracy of measurements (Asgary et al., 2016). Although CBCT has a very high diagnostic accuracy, there are several disadvantages such as high radiation dose. According to “as low as reasonably achievable (ALARA)” principles some researchers do not recommend routine usage of CBCT before endodontic treatment (Hiebert et al., 2017).

Periapical radiographs are used frequently as a preoperative diagnostic tool in routine clinical examinations and an essential part of endodontic management (Asgary et al., 2016). Panoramic radiography gives insight into the morphology and anatomy of a tooth to the clinician. The results of our study provided diagnostic information for dental clinics or hospitals that only use panoramic radiography before endodontic treatment. We aimed to guide the results of our study to clinicians in institutions or military clinics where periapical radiography is not available.

This is the first study to examine the accuracy of dentin thickness in the DZ on panoramic radiography. The differences between the two methods were found to be 0.5 mm for MB, 0.48 mm for ML, and 0.43 mm for the distal canal. These results might be attributed to the image distortion and horizontal magnification properties of panoramic radiography.

The enlargement of the canal during endodontic instrumentation can cause a decrease in the dentin thickness. A previous study showed that the amount of substance loss in DZ was greater than in the safety zone (Sousa et al., 2015). This reduction is a critical factor for prognosis in areas where dentin thickness is already thin. The results of the present study were considered clinically important. Because it has been reported that 0.15-0.24 mm dentin is removed in endodontic treatment during instrumentation (Oliver et al., 2016). Besides, the minimum dentin thickness required to prevent root fracture against the compaction forces applied during filling was 0.2-0.3 mm (Lim & Stock, 1987). According to previous literature information and the results of our study, an approximate formula has been created. ‘The danger limit Formula’ was created as follows;

The danger limit (X) (mm, on panoramic radiography) = -0.5 mm (panoramic distortion effect) -[0.15~0.24](removed during endodontic instrumentation) +[0.2~0.3](required for compaction forces).

So, the danger limit (X) can be considered as 0.9~1 mm. The dentin thickness of less than 0.9-1 mm measured on the panoramic image may be considered hazardous for strip perforation. This approximate formula provides an idea of the minimum amount of dentin thickness required to maintain the integrity of the tooth after endodontic treatment without

perforation. However, this formula does not give a clear idea about the susceptibility to fracture against the masticatory forces and the survival rate of the tooth. A previous study concluded that dentin thickness of less than 1.3 mm was a risk factor for fracture (Silva et al., 2020). Another study reported that the minimum dentin thickness required for post-core treatment is 1 mm to prevent root fracture (Raiden et al., 2001). For the teeth that require post-treatment, the danger limit (Xp) can be considered as 1.9~2 mm. So the clinician should be careful when measuring 1.8-2 mm dentin thickness on panoramic radiography.

There are numerous studies to measure the dentin thickness of DZ which is between 0.78 and 1.25 (De-Deus et al., 2019; Junior et al, 2014; Kessler et al., 1983). In the present study, the DZ dentin thickness on CBCT was found to be 0.95, 0.98, and 0.98 for MB, ML, and D canals, respectively. In the current study, there is no statistically significant difference was observed in dentin thicknesses between MB, ML, and distal root canals. The results of our study were similar to those of previous studies found no differences in dentin thicknesses between MB and ML root canals (Harris et al., 2013; Garala et al., 2003).

On the other hand, there were differences in the average dentin thicknesses measured in the studies due to factors such as the method used, the method of measurement (if the measurement was repeated, how many examiners measured, etc.), the examiner's expertise, and differences in cross-sectional levels. In these studies, measurements were performed 2-5 mm below the furcation region (De-Deus et al., 2019; Junior et al, 2014; Kessler et al., 1983). Differences in cross-section levels may also result in different results. In the present study, measurements were performed at 3mm, 4 mm, and 5 mm below furcation, respectively. Some previous studies have concluded that the most sensitive levels were 3 to 4 mm below the furcation (Abou-Rass et al., 1980; Lazzaretti et al., 2006). Kessler et al. (1983) who measured 2-3 mm below furcation, found the danger zone thickness to be higher than the present study. The various results in the literature can be explained by the differences in anatomical parameters of methodologies and the root curvature changes at different levels of the root. The present study demonstrated that the measurement at 4 mm below the furcation presented significantly thinner dentin thickness compared to the other two levels (3 mm and 5 mm). In a previous study, using micro-CT, the DZ thickness was found to be 1.25 mm for the mesial root and 1.09 mm for the distal root (Junior et al., 2014). These results obtained from the middle third of the root are not similar to the results of the present study. However in that study; the measurement region, distance to furcation, how many examiners, and how many times were measured were not clearly defined. In the current study, these criteria were made clear.

The dentin thickness at 4 mm below the furcation has clinical importance because the curvature and concavity were quite prominent at this level. The average depth of concavity in DZ of the mesial roots ranges from 0.86 to 1.04 mm (Sauaia et al., 2010). Thus, this area is subject not only to strip perforation but also to the origin of the root fractures. Regions where the depth of the concavity is increased and the dentin thickness is reduced can be considered the focus of clinical failures including furcation lesions, perforation, and fractures.

Abou-Rass et al. (1980) recommended an anti-curvature technique to prevent strip perforation. The brushing motion technique to remove dentin equally from all canal walls has been recommended (Peters et al., 2008). 'Brushing' too much on the thin and concave walls can be likely to increase the risk of perforation. Numerous studies evaluated canal transportation and the centering ability of various systems and different kinematics (Liu et al. 2016; Silva et al., 2017). One of the main goals of these systems is to maintain the original form of the canal. Results of the present study have shown that without further imaging, the canal anatomy and dentin thickness cannot be accurately monitored on panoramic images. Therefore, there should not be any deviations in the shape of the canal during preparation. Our study showed that the thinnest dentin was observed 4 mm below the furcation. According to the data obtained from CBCT measurements, the MB root is the region with the thinnest dentin in DZ. Due to the superposition and distortion that occurred in the panoramic radiography, it is not possible to accurately examine the MB canal, which is the riskiest location with the thinnest dentin. Misidentification of the root canal is a predisposing factor for root perforations. Akhlaghi et al. (2010) reported the greatest ratio of dentin removal during instrumentation in the DZ on the distolingual aspect of the MB canal in the mid-root of mandibular molars compared to other walls. In the present study, CBCT measurements were performed from this distolingual wall (the thinnest part observed in the axial cross-section) and it was concluded that the panoramic film was misleading because the concave distolingual area was superposed. Consequently, clinicians should carefully examine to avoid instrumentation-related strip perforation in this concave area.

In this study, a specific age range was included for the evaluation of DZ to eliminate the effect of the deposition of the secondary dentin. DZ thickness is increased with age and pulp volume is decreased (Star et al., 2011). Due to the thin dentin thickness in young teeth, clinicians should be careful in root canal preparations in terms of strip perforation, especially in root curvature.

The limitations of this study are the small number of samples and to use of CBCT for investigating the dentin thickness of DZ. However, the strength of our study is that it is the first study to investigate the

accuracy of panoramic radiography for dentin thickness in DZ. Our results are concerning clinicians in institutions where periapical radiography does not exist and provide information about the danger zone before endodontic treatment. Further studies with a larger sample size would evaluate dentine thicknesses with different levels.

CONCLUSION

DZ on panoramic radiography. Within the limitations of this study, on panoramic radiography, 0.5 mm distortion occurred in the DZ. CBCT reveals root concavity that must be recognized before treatment to avoid strip perforation. The dentin thickness of less than 1 mm is the most prone to perforation. It is recommended to refer to CBCT data if the dentin thickness of the DZ measured less than 1 mm on panoramic radiography. The dentin thickness was lowest at 4 mm below the furcation. To reduce strip perforation during endodontic treatment, root canal morphology should be evaluated correctly by the clinician.

Conflict of Interest

The authors declare no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

Author Contributions

Plan, design: DY, ŞÖ; **Material, methods, and data collection:** DY, ŞÖ; **Data analysis and comments:** DY, ŞÖ; **Writing and corrections:** DY, ŞÖ.

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Determination of Biochemical Changes in Amniotic Fluid According to the Number of Offspring in Awassi Sheep

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ABSTRACT

Objective: The present study was carried out to investigate the difference in metabolic needs between twin and singleton pregnancies by looking at some biochemical and hormonal markers in the amniotic fluid taken at the time of birth in Awassi sheep. **Materials and Methods:** A total of 30 Awassi sheep were used in the study. The first group of the study (Group 1, n=15); ewes carrying one offspring included the second group of the study (Group 2, n=15); created sheep carrying two offsprings. From the sheep in both study groups; 10 ml of amniotic fluid was taken with a sterile syringe without disturbing the integrity of the amniotic sac hanging from the lips of the vulva at the time of delivery. Electrolyte (sodium, potassium, chlorine, calcium, phosphorus), total protein, glucose, liver (ALT and AST), kidney biomarkers (urea and keratinin) and cortisol levels were measured from the amniotic fluid. Obtained data were analyzed with independent samples t-test. **Results:** Glucose and cortisol levels were significantly higher in the twin pregnant group than in the singleton pregnant group (p<0.05). Calcium level was found to be significantly higher in the single pregnant group than in the twin pregnant group (p<0.05). As a result, a difference was observed in the parameters evaluated depending on the number of offspring in the amniotic fluid. **Conclusion:** It was concluded that considering the metabolic needs differences due to the number of offspring may be a parameter that should be evaluated in the follow-up of the pregnancy and birth process.

Keywords: Amniotic Fluid, Twin Pregnancy, Biochemical Markers, Cortisol

İvesi Irkı Koyunlarda Yavru Sayısına Göre Amniyon Sıvısındaki Biyokimyasal Değişikliklerin Belirlenmesi

ÖZ

Amaç: Sunulan çalışma İvesi ırkı koyunlarda doğum zamanı alınan amniyon sıvısı içerisindeki bazı biyokimyasal ve hormonal belirteçlere bakarak ikiz ve tekiz gebelikler arasındaki metabolik ihtiyaç farkını araştırmak için yapıldı. **Gereç ve Yöntem:** Çalışmada toplam 30 adet İvesi ırkı koyun kullanıldı. Çalışmanın birinci grubunu (Grup 1, n=15); tek yavru taşıyan koyunlar, çalışmanın ikinci grubunu (Grup 2, n=15) ise; iki yavru taşıyan koyunlar oluşturdu. Her iki çalışma gruplarındaki koyunlardan; doğum zamanı vulva dudakları arasından sarkan amniyon kesesinin bütünlüğünü bozmadan steril enjektör ile 10 ml amniyon sıvısı alındı. Alınan amniyon sıvısından elektrolit (sodyum, potasyum, klor, kalsiyum, fosfor), total protein, glikoz, karaciğer (ALT ve AST), böbrek biyomarkerları (üre ve keratinin) ve kortizol düzeyleri ölçüldü. Elde edilen veriler bağımsız gruplar t test ile analiz edildi. **Bulgular:** Glikoz ve kortizol düzeyleri ikiz gebe grubunda tekiz gebe grubuna göre anlamlı derecede yüksekti (p<0.05). Kalsiyum düzeyi tekiz gebe grubunda ikiz gebe grubuna göre anlamlı derecede yüksek olduğu görüldü (p<0.05). **Sonuç:** Yavru sayısına bağlı meydana gelen metabolik ihtiyaç farklılıklarının göz önüne alınması, gebelik ve doğum sürecinin takibinde değerlendirilmesi gereken bir parametre olabileceği kanısına varıldı.

Anahtar kelimeler: Amniyon Sıvısı, İkiz Gebeliği, Biyokimyasal Belirteçler, Kortizol

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INTRODUCTION

With the onset of implantation, structures originating from the trophoblast and turning into functional membranes over time form the fetal sacs. These membranes are from the outside to the inside; chorion, allantois and amnion (Alaçam, 1994; Assis Neto et al., 2010; Assis Neto, Santos, Pereira & Miglino 2009; Küplülü, 1990; Pfarrer et al., 2001; Rici et al., 2011; Schlafer, Fisher & Davies, 2000). Amniotic membrane; Starting from the umbilical cord, it is the innermost sac that is spherical and taut at the beginning of the pregnancy and extends according to the shape of the uterus and the offspring as the pregnancy progresses. Amniotic fluid; It is mucoid, in varying amounts depending on the type of domestic animals, and is formed by the filling of this fluid into the sac by diffusion of fluids from the mother's blood as well as fetal nasopharyngeal secretions. There is also a very small amount of fetal urine in the amniotic fluid. This fluid prevents the fetus from sticking to the mother, protects the offspring from physical impacts, and provides an energy source for the fetus in the early stages of pregnancy. In birth, it plays an important role in the lubrication of the birth canal (Alaçam, 1994; Küplülü, 1990).

Many adaptation mechanisms develop during pregnancy and these mechanisms change according to the course of pregnancy (Lain & Catalano, 2007). Pregnancy-related changes are affected by the number of developing fetuses (Mackie et al. 2019). 13-16 weeks of pregnancy in cattle and sheep. The amniotic sac is formed on the first day and then the amniotic fluid fills the amniotic sac (Robert 1986). The metabolic profile, metabolite synthesis/degradation of amniotic fluid originating from maternal, fetal and placental tissues are clear indicators of fetal maturation (especially kidneys and lungs) and biochemical changes. The metabolic composition of blood and amniotic fluid reflects these biochemical changes. (Briese et al., 1999; Underwood, Gilbert & Sherman. 2005). The biochemical profile of fetal fluids is affected by fetal metabolic and secretory activities as well as fetomaternal exchange via the placenta (Banan Khojasteh, Khadjeh, Ranjbar & Salehi, 2001; Essawi, Mostafa & El Shorbagy, 2020). Amniotic fluid is extremely important in understanding fetal metabolism and determining pathological conditions during pregnancy (Prestes, Chalhoub, Lopes & Takahira, 2001). Examination of the biochemical profile of the amniotic fluid is useful for the follow-up of the developing fetus (Moghaddam & Olfati, 2012). The biological systems of twin pregnant goats need homeostatic adaptation to meet the expected additional demands of twin pregnancies for the survival of mother and fetus. Metabolic changes during pregnancy, which may be associated with organic structural changes, are evaluated by measuring some biochemical markers and comparing them with reference values (Cuckle 2014). There is little information about the reproductive characteristics of sheep, especially with regard to the composition of the amniotic fluid. In our study, it was aimed to determine the metabolic need difference between twin and singleton pregnancies by measuring some biochemical and hormonal markers in the amniotic fluid of Awassi sheep

and to have a problem-free pregnancy period by arranging the care and feeding conditions accordingly.

MATERIALS AND METHODS

Animal selection and experimental protocol

The presented study was carried out in the month of August-February, at the Harran University Faculty of Veterinary Medicine Practice Farm in Eyyubiye district of Şanlıurfa. The animal material of the study consisted of 30 Awassi sheep, 2-4 years old, who gave birth at least once, and who did not have any genital tract problems. Sheep were housed together until birth, and their eding and water needs were met ad libitum. Progesterone-based estrus synchronization was applied during the breeding season to aggregate births within certain periods. The progesterone-containing vaginal sponge was inserted into the vagina to remain in the vagina for 12 days (day 0). On the 11th day of administration, 2 ml of PGF_{2a} (10 mg dinoprost tromethamine, Dinolytic, Pfizer) was administered intramuscularly. On the 12th day of the application, the vaginal sponge was removed and 500 IU PMSG (Ovagen PMSG, Bionhe) was injected intramuscularly. After PMSG injection, oestrus monitoring was carried out with a search ram for 30 minutes at 8-hour intervals for 3 days, and the estrus sheep were hand-crossed with rams with predetermined fertility. Pregnancy and twin/singleness examination were performed transrectally with linear array probe at 5 MHz frequency with real time ultrasound on the 35th day after breeding sheep. Sheep were divided into 2 groups according to the number of offspring by ultrasonography. The first group of the study (Group 1, n=15); ewes carrying one offspring included the second group of the study (Group 2, n=15); created sheep carrying two offsprings. One week before the expected due date, the sheep were taken into separate sections and their births were followed up. At the end of the first stage of labor, 10 ml of amniotic fluid was taken with a sterile syringe without disturbing the integrity of the amniotic sac hanging between the lips of the vulva. The collected amniotic fluid was centrifuged at 3000 rpm for 10 minutes and analyzed.

Evaluation of biochemical and hormonal changes in amniotic fluid

Sodium (Na, mmol/L), potassium (K, mmol/L), chloride (Cl, mmol/L), phosphorus (P, mmol/L) and calcium (Ca, mg/dL) electrolytes, total protein, glucose, kidney (urea and creatinine) and liver marker (Alanine aminotransferase (ALT) and aspartate aminotransferase (AST)) measurements were made using a biochemistry analyzer (SMT-120V, Chengdu Seamaty Technology™, China). Amniotic fluid cortisol level was determined spectrophotometrically (Molecular Device SpectraMax M5 Plate Reader, Pleasanton, CA, USA) using a commercial kit (Sheep Cortisol ELISA Kit, Mybiosource, USA).

Statistical analysis

Statistical analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS for Windows; version 24.0) packaged software. The conformity of the variables to normal distribution was

examined using visual (histogram and Q-Q Plot) and analytical methods (Shapiro-Wilk tests). Descriptive analyses were reported as mean \pm standard error of the mean (SEM) values for normally distributed variables. Since the data showed conformity to normal distribution, they were compared between groups using the Independent Samples t-test. The homogeneity of variances was determined using the Levene test. A value of $p < 0.05$ was accepted as statistically significant for all analyses

Ethical considerations

This study was carried out based on the permission of Harran University Animal Experiments Local Ethics Committee (HRU-HADYEK) dated 09/02/2022 and numbered 2022/001.

The biochemical and hormonal mean values in the amniotic fluid of the study groups are presented in Table 1. Accordingly, the mean Na (103.54 ± 0.19 - 103.66 ± 0.23), glucose (3.50 ± 0.13 - 7.07 ± 0.18), K (8.28 ± 0.20 - 8.49 ± 0.10), Cl (66.31 ± 0.34 - 66.63) of single and twin ewes (± 0.33), Ca (3.47 ± 0.11 - 1.35 ± 0.57), P (3.94 ± 0.97 - 3.62 ± 0.12), total protein (1.27 ± 0.86 - 1.35 ± 0.76), ALT (10.30 ± 0.18 - 10.67 ± 0.17), AST (14.17 ± 0.21 - 14.56 ± 0.21), urea (79.82 ± 0.47 - 79.20 ± 0.54), creatinine (11.58 ± 0.78 - 11.31 ± 0.13) and cortisol (4.15 ± 0.77 - 8.16 ± 0.16) values were recorded. Glucose and cortisol levels were significantly higher in twin pregnancies compared to singleton pregnancies ($p < 0.05$). Calcium level was significantly lower in sheep carrying twin pregnancies than in sheep carrying singleton pregnancies ($p < 0.05$). There was no difference between the groups in the levels of Na, K, Cl, P, total protein, ALT, AST, urea, and creatinine in the amniotic fluid ($p > 0.05$).

RESULTS

Table 1. The biochemical and hormonal mean values in the amniotic fluid of the study groups.

Amniotic fluid parameters	Single pregnancy		Twin pregnancy		*p
	Mean	SEM	Mean	SEM	
Na (mmol/L x 10)	103.54	0.19	103.66	0.23	0.672
Glucose (mg/ dL)	3.50	0.13	7.07	0.18	0.001
K (mmol/L)	8.28	0.20	8.49	0.10	0.382
Cl (mmol/ L x 10)	66.31	0.34	66.63	0.33	0.502
Ca / mg/ dL)	3.47	0.11	1.35	0.57	0.001
P (mmol/L)	3.94	0.97	3.62	0.12	0.63
Total protein (g/dLx0,1)	1.27	0.86	1.35	0.76	0.508
ALT(IU/L)	10.30	0.18	10.67	0.17	0.164
AST (IU/L)	14.17	0.21	14.56	0.21	0.214
Urea (mg/ dL)	79.82	0.47	79.20	0.54	0.396
Creatinine(mg/dL)	11.58	0.78	11.31	0.13	0.98
Cortisol(μ g/dL)	4.15	0.77	8.16	0.16	0.001

*Significance levels according to independent t-test results. Sodium (Na), potassium (K), chlorine (Cl), calcium (Ca), phosphorus (P), Alanine aminotransferase (ALT), Aspartate Aminotransferase (AST).

DISCUSSION

It is known that some changes occur in the developing fetus and mother during pregnancy (Narelle, 2017). More amniotic fluid is secreted in twin pregnancy for a suitable environment in developing fetuses (Hill et al. 2000). The large area created by twin fetuses also increases the amount of secreted fluid (Ippolito, et al., 2014). The fetus needs protein, carbohydrates, vitamins and minerals to maintain its healthy development throughout pregnancy (Alaçam, 1994; Arthur, Noakes & Peorsan. 1992; Küplülü, 1990). Glucose is the primary energy source for the fetus. In healthy pregnancies, fetal gluconeogenesis is very limited

and the fetus is dependent on maternal circulating glucose (Kalhan & Parimi, 2000). It has been reported that glucose concentration in amniotic fluid of twin pregnant goats is higher than that of single pregnant goats (Hay, 2006). In the present study, in accordance with the literature, it was

observed that the amniotic fluid glucose concentration of twin pregnant Awassi sheep was higher than that of singleton pregnant sheep. It was thought that the reason for this might be the need for glucose due to the increased number of offspring. However, the glucose requirement is parallel to the number of fetuses in the uterus, and glucose is secreted from the fetus into the amniotic fluid (Hay, 2006). With maternal stress, corticotropin-releasing hormone (CRH) is produced from the hypothalamus. This hormone causes the activation of the fetal Hypothalamus-Pituitary-Adrenal (HPA) axis and the release of fetal cortisol in the amniotic fluid (Challis, Matthews, Gibb & Lye. 2000; Mbegbu, et al., 2021), in their study on Red Sokoto goats, reported that amniotic fluid cortisol concentration was higher in twin pregnant goats compared to the singleton pregnant group. In the present study, it was higher in twin pregnancies in accordance with the literature. The reason for this may

represent the cumulative response of the two developing fetuses in twin pregnancies to the increased stress due to the decreased intrauterine volume (Herman, et al., 2016). In addition, the increase in twin pregnancies; It may also be caused by the fact that cortisol is necessary for the normal development of fetal respiratory organs, liver and kidneys (Wood & Keller-Wood, 2016). Most biological processes that require energy expenditure, such as muscle contraction, blood coagulation, and bone formation, directly require calcium, thereby reducing the amount of calcium in the amniotic fluid (Mbegbu, et al., 2021; Suttle, 2010), in their study on Red Sokoto goats, reported that amniotic fluid calcium level was lower in twin pregnancies compared to singleton pregnancies. In the present study, in accordance with the literature, amniotic fluid calcium level was found to be lower in twin pregnant ewes. The decrease in amniotic fluid calcium concentrations observed in twin pregnant ewes compared to singleton pregnant ewes may be related to increased calcium use/uptake by twin fetuses during the bone formation process (Jo, et al., 2015). In our study, no difference was found in amniotic fluid sodium, potassium, phosphorus and chlorine concentrations. It is consistent with previous studies that there were no changes in potassium, sodium, phosphorus and chlorine (Banan Khojasteh, Khadjeh, Ranjbar & Salehi 2011; Mbegbu et al., 2021). The reason why there is no difference in these minerals; It was thought that it may not have been affected by the number of offspring because the ration was sufficient for the offspring during pregnancy or because these minerals were required in very small amounts for the offspring. In the presented study, no difference was observed between twin and singleton pregnancies in urea, creatinine, AST, ALT and total protein concentrations in amniotic fluid. In previous studies, it was stated that AST, ALT and total protein levels were not affected by pregnancy and the number of developing fetuses (Carter, 1990; Pasciu et al., 2019). Since kidney and liver markers are generally related to aging and inflammation, it was thought that the relevant parameters in healthy pregnancies might not be affected by the number of fetuses. The lack of difference between the total protein values in the study is similar to the study in goats (Mbegbu et al., 2021). It has been reported that the similarity in total protein levels may be due to the fact that proteins are not filtered into fetal urine during excretion from fetal nephrons, and thus are insignificant from the number of developing fetuses (Hay, 2006).

CONCLUSION

As a result, the care-feeding conditions that will be arranged by taking into account the differences in amniotic fluid calcium, glucose and cortisol levels in twin and singleton pregnancy will contribute to the continuation and termination of the pregnancy without any problems for the mother and the offspring.

Conflict of Interest

The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Author Contributions

Plan, design: TA; **Material, methods and data collection:** TA, ÖY; **Data analysis and comments:** TA, ÖY; **Writing and corrections:** TA, ÖY.

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Prospective Comparison of the 3 Most Commonly Used Scoring Systems in Patients with Gastrointestinal Bleeding

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ABSTRACT

Objective: This study aimed to determine the most appropriate scoring system for the emergency department to facilitate the management of patients with gastrointestinal bleeding. **Materials and Methods:** Data were collected prospectively September'21-March'22 in the emergency department (ED). 117 adult patients were included. Endoscopic intervention, rebleeding, admission to the intensive care unit, and in-hospital death were recorded. **Results:** The patients median age was 75 years and 58.1% (n=68) of them were male. Of the patients, 21 were hospitalized in the intensive care unit and 85 in the ward (17.94-72.64%). All three scoring systems can determine the hospitalization place or discharge of patients with GI bleeding. A positive and significant correlation was found between Glasgow-Blatchford, AIMS-65 and length of hospitalization (p<0.05). In-hospital mortality developed in 15 (12.82%) of the patients. Rockall, Glasgow-Blatchford, and AIMS-65 scoring systems were found to be significant for mortality prediction (AUC= 0.745 - 0.777 - 0.851). Seventy-one (60.68%) of patients received ES transfusion, and the Rockall, Glasgow-Blatchford, and AIMS-65 scoring systems were found to be significant for the prediction of ES transfusion (AUC= 0.624 - 0.826 - 0.653). Rebleeding developed in 16 (13.67%) patients. Glasgow-Blatchford and AIMS-65 scoring systems were found to be significant for rebleeding (p=0.03-0.04). The Rockall classification was found to be insignificant (p=0.57). **Conclusion:** All scoring systems were successful in terms of management of patients with GI bleeding and survey estimation. The AIMS-65 scoring system stands out only in terms of rebleeding prediction. For this reason and it is more practical, we recommend the AIMS-65 scoring system for the emergency department.

Keywords: Gastrointestinal Bleeding, Scoring Systems, Emergency Department, Complications.

Gastrointestinal Sistem Kanamalı Hastalarda En Sık Kullanılan 3 Skorlama Sisteminin Prospektif Karşılaştırılması

ÖZ

Amaç: Bu çalışmanın amacı gastrointestinal kanamalı hasta yönetimini kolaylaştırmak için acil servise en uygun skorlama sistemini saptamaktır. **Gereç ve Yöntem:** Veriler, acil serviste 6 aylık (22.09.2021-31.03.2022) bir süre boyunca ileriye dönük olarak toplandı. GI kanaması olan yetişkin 117 hasta dahil edildi. Bileşik klinik sonuçlar, cerrahi veya endoskopik müdahale ihtiyacı, tekrar kanama, yoğun bakım ünitesine yatış veya hastane içi ölümden oluşuyordu. **Bulgular:** Hastaların ortanca yaşı 75 olup, %58.1'i (n=68) erkekti. Hastaların 21'i yoğun bakım, 85'i servise yatırıldı (% 17.94-72.64). Her üç skorlama sistemide hastalarının yatış yeri veya taburculuğunu belirleyebilmektedir. Glasgow-Blatchford ve AIMS-65 ile yatış süresi arasında pozitif anlamlı bir ilişki bulunmuştur (p<0.05). Hastaların 15'inde (%12.82) hastane içi mortalite gelişmiştir, Rockall, Glasgow-Blatchford, AIMS-65 skorlama sistemleri mortalite tahmini yönünden anlamlı bulunmuştur (AUC= 0.745 – 0.777 – 0,851). Hastaların 71'ine (%60.68) ES transfüzyonu yapılmıştır ve Rockall, Glasgow-Blatchford, AIMS-65 skorlama sistemleri ES transfüzyonu tahmini açısından anlamlı saptanmıştır (AUC=0.624 – 0.826 – 0.653). Hastalardan 16'sında (% 13.67) yeniden kanama gelişmiştir. Glasgow-Blatchford ve AIMS-65 skorlamaları yeniden kanama için anlamlı (p=0.03-0.04), Rockall sınıflaması ise anlamsız saptanmıştır (p=0,57). **Sonuç:** Tüm skorlama sistemleri GIS kanama hastalarının yönetimi ve survey tahmini açısından başarılıdır. Sadece yeniden kanama tahmini açısından AIMS-65 ön plana çıkmaktadır. Bu sebepten ve daha pratik olduğundan AS için biz AIMS-65 skorlama sistemini önermekteyiz.

Anahtar Kelimeler: Gastrointestinal Kanama, Skorlama Sistemi, Acil Servis, Komplikasyonlar.

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INTRODUCTION

Patients with Gastrointestinal System Bleeding (GSB) are one of the groups with high morbidity and mortality in emergency service (ED) admissions if they are not intervened rapidly. Depending on the severity of the bleeding, it can end with a wide variety of clinics and outcomes. The annual incidence of the disorder is 50-170/100000 people (Park et al., 2016; Atkinson & Hurlstone, 2008). Although its mortality varies between 3-and 15%, this rate is even higher in patients with hemodynamic instability (Tang et al., 2018). While bleeding caused by erosive gastritis and peptic ulcer can sometimes be controlled with acid-suppressing drugs, severe bleeding, hemorrhagic shock, and even mortality may occur in conditions, such as esophageal or gastric variceal bleeding, a peptic ulcer caused by large arterial erosion, Dieulafoy's disease, and gastrointestinal tumors (Zhong et al., 2016). Therefore, determining the severity of GSB is important for optimizing patient care and efficient use of resources. Although endoscopy-based triage has been recommended to reduce the length of hospital stay and costs, the possibility of continuous endoscopy in ED is limited. Various risk scoring systems have been developed to predict the need for intervention or the probability of death and to develop an optimal management strategy. Scoring systems are important in terms of choosing the appropriate treatment method, reducing medical costs, and improving prognosis (Çay & Çetinkaya, 2022). AGA (American Gastroenterological Association) recommends early classification for the management of patients with GSB (Abougergi et al., 2016). Various scoring systems have been developed to predict risks, such as mortality, rebleeding, timing of endoscopy, time of discharge, and level of care, and to help decision-making (Zhong et al., 2016). Although there are many scoring systems, the most commonly used ones are AIMS65, Glasgow-Blatchford Bleeding Score (GBS), and pre-endoscopic Rockall Score. Risk scores will be useful for emergency physicians in situations where emergency endoscopy cannot be performed. In our study, we aimed to investigate which of these scores was more useful in ED and superior in predicting clinical outcomes.

MATERIALS AND METHODS

Study type

This descriptive study was conducted with Balikesir University Medical Faculty Emergency Department patients from September 22, 2021- March 31, 2022.

Study group

The research universe consisted of were collected prospectively over 6 months (September 22, 2021-March 31, 2022) in the ED of a university hospital. Balikesir University is located in Balikesir province, at the northern west coast of Turkey. The sample size was not calculated as the researchers attempted to

reach the maximum study size. Participation in this research was voluntary, patients who did not agree to participate in the study were excluded and the study was conducted with 117 patients.

Dependent and independent variables

The independent variables of this research are age, gender, comorbidities, clinic, endoscopic diagnosis, hospitalization (ICU, ward etc.), length-of-stay, in-hospital mortality, ES transfusion, rebleeding. The dependent variables are Glasgow-Blatchford, AIMS-65 and pre-endoscopic Rockall score.

Procedures

Blood counts were tested by Beckman Coulter Hematology Analyzer LH780. All serum biochemical parameters were tested by Beckman Coulter Chemistry Analyzer AU680. Blood transfusion was applied to patients with shock, hypotension, clinical deterioration or hemoglobin below 7 g/dl. Scoring systems were filled out by the patient's physician with the data obtained at the time of admission to the ED. Data about patients who developed mortality, their re-bleeding status, endoscopic intervention report, and length of hospital stay were obtained from epicrisis report and hospital management software.

Statistical analysis

Skewness and kurtosis coefficients were examined for the assumption of normality. Since these values were in the range of ± 1 , the assumption of normality was met. Continuous variables with normal distribution were expressed as mean \pm standard deviation values, and comparisons between independent groups were performed with the independent t-test and one-way ANOVA test. ROC curve analysis was performed to evaluate and compare the performances of diagnostic markers. Youden J index was used to obtain the optimal cut-off value, and related sensitivity, specificity, positive predictive, and negative predictive values were presented. The significance level was taken as $\alpha = 0.05$. Statistical analyses were performed on IBM SPSS Statistics version 22.0 (IBM Corp., USA).

Ethical considerations

The study was approved by Clinical Research Ethics Committee with the decision number 2021/192. An 'informed consent form' was obtained from each patient participating in the study.

RESULTS

117 patients were included in the study. The median age of the patients was 75 (min-max: 18-97) years, and 58.1% (n=68) of them were male. The most common diagnosis of the patients in ED was melena (64.10%, n=75). When the patients were evaluated in terms of comorbidities, the most common ones were hypertension, diabetes mellitus type 2, and coronary artery diseases. The frequencies of comorbidities and bleeding patterns are shown in Table 1.

Table 1. The frequencies of comorbidities and bleeding patterns.

Comorbidity	n	%
Hypertension	37	31.62
Diabetes Mellitus type 2	27	23.07
Coronary Artery Disease	22	18.80
CVD	10	8.54
Congestive Heart Failure	9	7.69
Atrial Fibrillation	8	6.83
Chronic renal failure	8	6.83
COPD	6	5.12
Cirrhosis	6	5.12
Covid-19	2	1.70
Other	34	29.05
No Comorbidity	30	25.64
Total	117	100
Clinic	n	%
Hematemesis	29	24.79
Hematochezia	21	17.95
Melena	75	64.10
Hemoptysis	1	0.85
No clinic	3	2.56
Total	117	100

CVD: Cardiovascular Disease, COPD: Chronic Obstructive Pulmonary Disease.

Of the patients diagnosed with GI bleeding in ED, 21 were hospitalized in the intensive care unit (ICU) and

85 were hospitalized in the ward. While 9 patients were discharged, 2 patients refused treatment (17.94%-72.64%-7.69%-1.70%). The scores of the scoring systems in terms of predicting ICU admission, ward admission, and discharge were found significant as follows, respectively: pre-endoscopic Rockall, 4.19-3.15-2.89 (2.112=3.66, $p<0.05$); Glasgow-Blatchford 13.86-8.96-5.11 (2.112=18.21, $p=0.00$); AIMS-65, 2.90-1.32-0.67 (2.112=25.47, $p=0.00$). All three scoring systems can determine the hospitalization place or discharge of patients with GI bleeding during ED admission. This situation is shown in Table 2.

Pre-endoscopic Rockall, Glasgow-Blatchford, and AIMS-65 scoring systems were found to be significant in determining whether patients with a diagnosis of GI bleeding needed hospitalization and, if they did, whether they would be admitted to the ICU or ward ($p=0.039-0.00-0.00$). An intra-group analysis of hospitalized patients was carried out in terms of the relationship between the scoring systems and length of stay. Accordingly, a positive and significant correlation was found between Glasgow-Blatchford and AIMS-65 and length of hospital stay ($r=0.22$, $p<0.05$, $r=0.22$, $p<0.05$), but there was no significant relationship between pre-endoscopic Rockall Score and length of hospital stay ($r=-0.01$, $p=0.93$). This is shown in Table 3.

Table 2. Ability of scoring systems to determine hospitalization.

Scoring System	Conclusion	n	Mean	Std. Deviation	95% Confidence Interval for Mean		F	p
					Lower Bound	Upper Bound		
Rockall	ICU Hosp.	21	4.19	1.692	3.42	4.96	3.66	0.029*
	Ward Hosp.	85	3.15	1.562	2.82	3.49		
	Discharged	9	2.89	2.315	1.11	4.67		
	Total	115	3.32	1.689	3.01	3.63		
Glasgow - Blatchford	ICU Hosp.	21	13.86	3.678	12.18	15.53	18.21	0.000*
	Ward Hosp.	85	8.96	3.983	8.11	9.82		
	Discharged	9	5.11	5.349	1.00	9.22		
	Total	115	9.56	4.615	8.70	10.41		
AIMS-65	ICU Hosp.	21	2.90	1.375	2.28	3.53	25.47	0.000*
	Ward Hosp.	85	1.32	0.916	1.12	1.52		
	Discharged	9	0.67	0.500	0.28	1.05		
	Total	115	1.56	1.186	1.34	1.78		

ICU: Intensive Care Unit, Hosp.: Hospitalization.

One-way-ANOVA, * $p<0.05$.

Table 3. Relationship between scoring systems and length of stay.

Scoring System	Length of Stay	
	r	p
Rockall	-0.01	0.93
Glasgow-Blatchford	0.22	0.01*
AIMS-65	0.22	0.01*

r: Pearson correlation test, * $p<0.05$

66 patients received an endoscopic intervention after the diagnosis of GI bleeding (56.41%). Bleeding patterns were classified according to the Forrest classification. Accordingly, Forrest 2C type bleeding was the most frequently observed one. The classification of endoscopy results is shown in Table 4.

Table 4. Diagnoses after endoscopic intervention.

Endoscopy Diagnosis	n	%
No Active Bleeding	1	1.52
Mallory Weiss Syndrome	1	1.52
Forrest 1 A	5	7.58
Forrest 1 B	9	13.64
Forrest 2 A	6	9.09
Forrest 2 B	5	7.58
Forrest 2 C	21	31.82
Forrest 3	13	19.70
Lower GIS Bleeding	2	3.03
Crohn's Disease	2	3.03
Ulcerative Colitis	1	1.52
Total	66	100

Discriptive statistics

17 (14.52%) patients diagnosed with GI bleeding in ED were referred to another center. In-hospital mortality developed in 15 (12.82%) of all patients. Pre-endoscopic Rockall, Glasgow-Blatchford and AIMS-65 scoring systems were compared with ROC analysis for in-hospital mortality prediction, and all scoring systems were found to be significant in terms of mortality prediction (AUC= 0.745 - 0.777 - 0.851, p=0.002 - 0.001 - 0.000) (Table 5 and Figure 1).

Table 5. ROC curve analysis results on mortality estimation of scoring systems.

Accuracy index	Rockall	Glasgow-Blatchford	AIMS-65
AUC	0.745	0.777	0.851
p-value	0.002	0.001	0.000
Cut-off value	2.5	13.5	1.5
Youden J index	0.22	0.52	0.57
Sensitivity (95% CI)	28.4	85.3	63.7
Specificity (95% CI)	93.3	66.7	93.3

AUC: Area Under the Curve
ROC Curve Analysis, p< 0.05

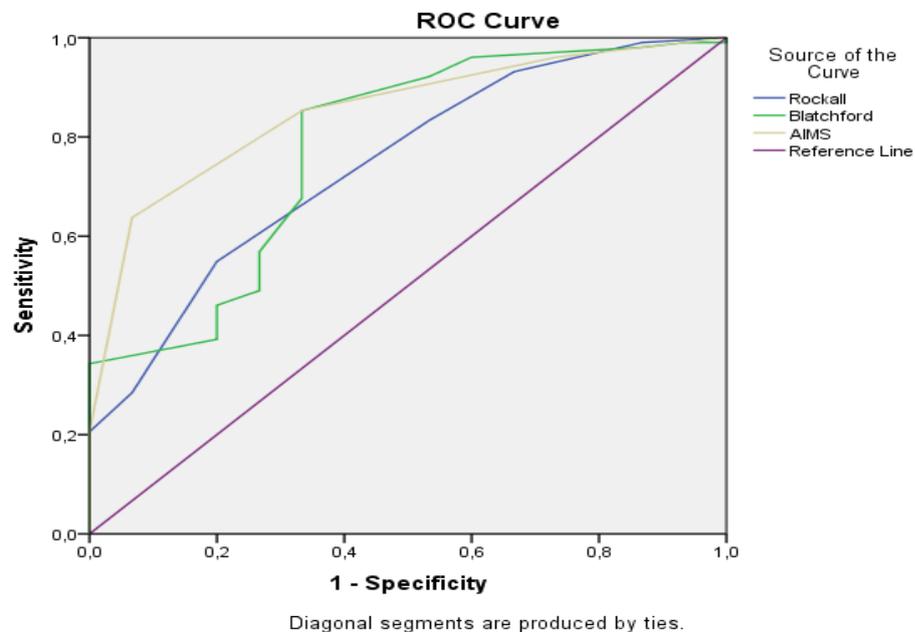


Figure 1. ROC curve analysis chart of scoring systems in terms of in-hospital mortality.

71 (60.68%) patients diagnosed with GI bleeding in ED received erythrocyte suspension (ES) transfusion. When pre-endoscopic Rockall, Glasgow-Blatchford,

and AIMS-65 scoring systems were compared in terms of ES transfusion prediction, all three scoring systems were found to be significant. AUC= 0.624 - 0.826 - 0.653, p=0.023-0.000-0.005 (Table 6 and Figure 2).

Table 6. ROC curve analysis results on estimation of ES transfusion by scoring systems.

Accuracy index	Rockall	Glasgow- Blatchford	AIMS-65
AUC	0.624	0.826	0.653
p-value	0.023	0.000	0.005
Cut-off value	4.50	15.50	2.5
Youden J index	0.19	0.1	0.17
Sensitivity (95% CI)	28.2	12.7	28.2
Specificity (95% CI)	91.3	97.8	89.1

AUC: Area Under the Curve
ROC Curve Analysis, *p < 0.05

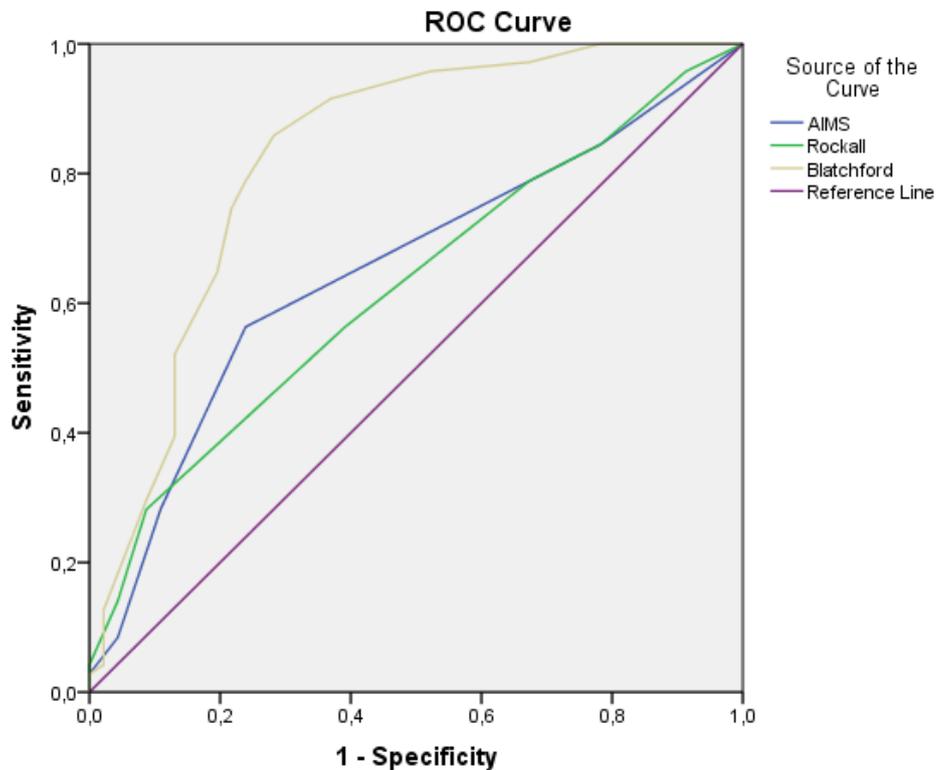


Figure 2. ROC curve analysis plot on estimation of ES transfusion by scoring systems.

Rebleeding developed in 16 (13.67%) patients diagnosed with GI bleeding in ED. When pre-endoscopic Rockall, Glasgow-Blatchford, and AIMS-65 scoring systems were compared in terms of

rebleeding prediction, they were found to be significant. AUC= 0.544 - 0.669 - 0.661, p=0.571-0.030-0.040 (Table 7 and Figure 3).

Table 7. ROC curve analysis results on rebleeding estimation of scoring systems.

Accuracy index	Rockall	Glasgow- Blatchford	AIMS-65
AUC	0.544	0.669	0.661
p-value	0.571	0.030	0.40*
Cut-off value	3.50	9.50	4.5
Youden J index	0.08	0.39	0.05
Sensitivity (95% CI)	56.3	87.5	6.3
Specificity (95% CI)	51.5	51.5	99

AUC: Area Under the Curve, ROC Curve Analysis, *p < 0.05.

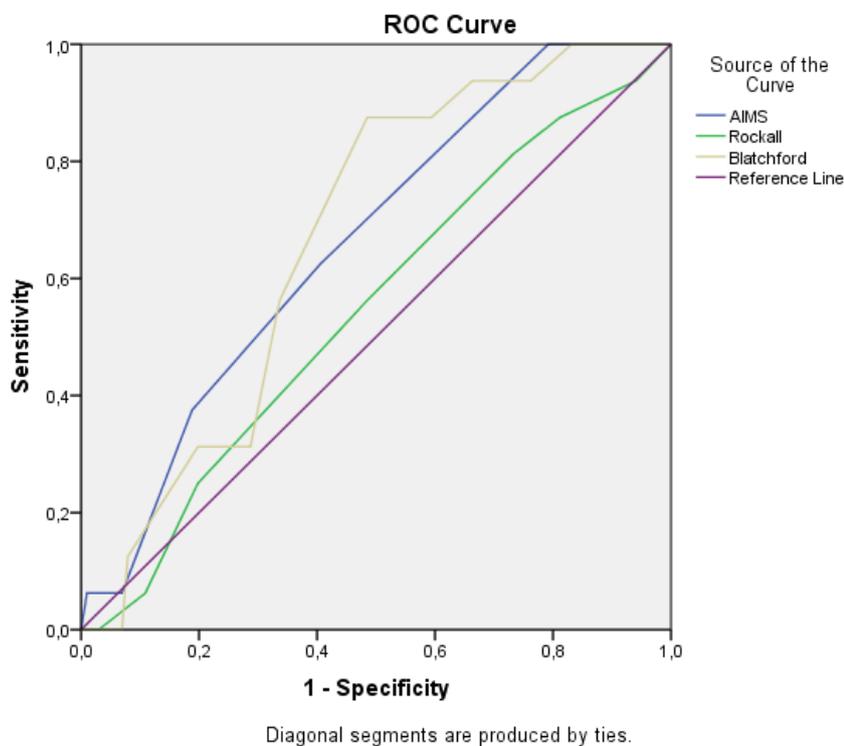


Figure 3. ROC curve analysis chart on rebleeding estimation of scoring systems.

DISCUSSION

The majority of patients diagnosed with GI bleeding in the ED are male (71.9%, 70.6%, 76.6%, 68.4%) and their mean age has been reported as approximately 60 years (64, 57.4, 61.3 years) (Kim et al., 2019; Lewis et al., 2002; Gu et al., 2018; Jung et al., 2019). In our study results, similar to the literature, the majority of them were male and the mean age was 75 years. The most common clinical presentation of patients has been reported as melena (27.5%-74.2%) (Kim et al., 2019; Lewis et al., 2002). According to our study results, the most common clinical presentation was melena, too. The most common comorbidities in patients have been reported as DM type2 (38%) and HT (22.1%) (Jung et al., 2019), and HT and DM type2 were found to be the most common ones in our study, as well.

During the admission of 16.8% of patients to the ICU, the scoring systems have been reported to be successful in identifying patients who will be admitted to the ICU (The AUC values for predicted admission were: AIMS65=0.73 (95% CI, 0.69–0.77), PRS = 0.70 (95% CI, 0.66-0.74), and GBS=0.71 (95% CI, 0.67–0.75) (Kim et al., 2019). Consistent with the literature, we found that all three scoring systems were significant in determining ICU admissions. Regarding the relationship between the length of stay and scoring systems, it has been stated that the AIMS-65 score is superior (Abougergi et al., 2016). Consistent with the literature, in addition to the AIMS-65 score, we found the Glasgow-Blatchford score correlated in our study. According to the literature, most patients (86%) diagnosed with GI bleeding do not need endoscopic

intervention (Stanley et al., 2011), but the majority of the patients in our study underwent endoscopic intervention. We think that the approach of the gastroenterology clinic was effective in this result. In addition, our hospital had the only active gastroenterology clinic in the province during this period, which may also be effective. In line with the classifications of patients undergoing endoscopy, the most common etiology has been reported as gastric ulcer (Kim et al., 2019; Lewis et al., 2002; Jung et al., 2019). In our study, as we made the endoscopic classification based on Forrest classification, the most common etiology was 'pigmented lesion on ulcer', which is class 2C. This finding was consistent with the literature.

Although there is no study in the literature regarding the referral rate of patients diagnosed with GI bleeding, the high referral rate in our study is striking. The first reason for this is that the clinical responses of gastroenterology were periodically shared with different hospitals in the province. In addition, the lack of beds due to patient density may have played a role. It has been reported in the literature that mortality rates vary between 3 and 7% (Stanley et al., 2017; Shafaghi et al., 2019). AIMS-65 Score was found to be more successful in predicting in-hospital mortality than GBS and pre-endoscopic Rockall (AIMS65=0.84, PRS=0.74, and GBS=0.72) (Abougergi et al., 2016; Kim et al., 2019). In a study, it was reported that all three scoring systems were able to predict mortality with similar rates (AIMS65, GBS, and RS; AUC:0.76 - 0.78 - 0.78) (Martínez-Cara et al., 2016). In our study results, similar to the literature, we determined that all three scoring systems were significant in terms of mortality prediction, and similarly, we found that the AIMS-65 score was superior to GBS and pre-endoscopic Rockall in predicting mortality.

The need for urgent ES transfusion for patients with GI bleeding has been reported at rates ranging from 40% to 77% in the literature. GBS has been found to be superior to other scoring systems in determining the need for ES transfusion in many studies (Kim et al., 2019; Lewis et al., 2002; Stanley et al., 2011; Stanley et al., 2017). In our study, all three scoring systems were found to be successful in terms of ES transfusion prediction, and similar to the literature, GBS came to the fore as the best predictive scoring system. According to the results of the current studies in the literature, rebleeding has been detected in 2.5% of patients (Park et al., 2016; Lewis et al., 2002). In many studies, scoring systems have not been found to be superior to each other, but they are still successful in estimating rebleeding (Martínez-Cara et al., 2016; Wang et al., 2013). In addition, there are studies indicating that pre-endoscopic Rockall and GBS are better predictors than AIMS-65 (8,13). In our study, similar to the literature, AIMS-65 and GBS scoring systems were successful in predicting rebleeding, but the pre-endoscopic Rockall score was found to be

insignificant in terms of rebleeding. AIMS-65 and Glasgow-Blatchford scoring systems were not found superior to each other, either.

CONCLUSION

Gastrointestinal bleeding is one of the leading diseases with high morbidity and mortality rates in patients that present to the ED. It is recommended that scoring systems be used for patient management and survey estimation. The most commonly used scoring systems, namely pre-endoscopic Rockall, Glasgow-Blatchford and AIMS-65 yielded similar results in terms of serious outcomes, such as mortality, ES transfusion, and ICU hospitalization. However, AIMS-65 and Glasgow-Blatchford scoring systems were found to be more successful in terms of rebleeding. For this reason, we recommend the AIMS-65 scoring system as more practical for emergency services due to easier applicability and calculation for all possible serious outcomes.

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Conflict of Interest

The author declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Author Contributions

Plan, design: TA; **Material, methods and data collection:** TA, SK; **Data analysis and comments:** TA, SK; **Writing and corrections:** TA, SK.

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Overview of Probiotics Available in Pharmacies in Turkey

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ABSTRACT

Objective: In this study, we aimed to provide a general assessment of the probiotic preparations actively sold in pharmacies in Turkey in December 2021 to health professionals and interested parties. **Materials and Methods:** We examined the probiotic preparations that were actively sold in pharmacies in Turkey in December 2021 by using the Rx Mediapharma® 2021 Interactive Drug Information Resource Program. **Results:** There were 249 preparations in the program. Most of them were capsules (n=103). 39% of preparations contained one type of microorganism (n:97) and the rest more than one type. Genres of Lactobacillus and Bifidobacterium are the main content (84%) in the probiotic preparations. 97% of preparations were approved as Food Supplements by the Ministry of Agriculture, and 99% of the probiotics are sold without a prescription. 59% of them were imported. **Conclusion:** Probiotics are evaluated into categories as food/supplement that can claim or not claim to be beneficial to health, and probiotic drugs. The probiotics we examined are evaluated under the title of 'Nutritional Products' and are not separated according to the grouping in the consensus. Investigation and inspection by the Ministry of Health will make the use of probiotics safer.

Keywords: Probiotics, Microbiota, Nutraceutical, Pharmacy.

Türkiye'deki Eczanelerde Bulunan Probiyotiklere Genel Bakış

ÖZ

Amaç: Bu çalışma, Türkiye'de eczanelerde aktif olarak satılan probiyotik preparatları hakkında sağlık profesyonellerine ve ilgililere genel bir değerlendirme sağlamak amacıyla yapıldı. **Gereç ve Yöntem:** Çalışmada Rx Mediapharma® 2021 İnteraktif İlaç Bilgi Kaynak Programını kullanılarak, Aralık 2021'de Türkiye'de eczanelerde aktif olarak satılan probiyotik preparatlar incelendi. **Bulgular:** Değerlendirmeye alınan 249 preparatın, çoğunun kapsül formunda olduğu tespit edildi (n:103). Preparatların %39'u tek tip mikroorganizma (n=97) içerirken geri kalanı birden fazla tip mikroorganizma içermekteydi. Probiyotik preparatların içeriğini başlıca Lactobacillus ve Bifidobacterium türlerinin (%84) oluşturduğu tespit edildi. Preparatların %97'si Tarım Bakanlığı tarafından Gıda Takviyesi olarak onaylanmış olduğu ve neredeyse tamamının (%99) reçetesiz satıldığı görüldü. Tüm preparatların %59'unu ithal ürünler oluşturmaktaydı. **Sonuç:** Probiyotikler sağlık otoritelerince; sağlığa yarar iddiası olan ve olmayan gıda/takviye maddeleri ile probiyotik droglar olarak sınıflandırılmaktadır. İncelediğimiz programda probiyotiklerin "Nutrisyonel Ürünler" başlığı altında listelenmekte olduğu herhangi bir sınıflandırma yapılmadığı görüldü. Sağlık Bakanlığı tarafından yapılacak değerlendirme ve denetlemeler probiyotik kullanımını daha güvenli hale getirecektir.

Anahtar Kelimeler: Probiyotikler, Mikrobiyota, Nutrasötik, Eczacılık.

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INTRODUCTION

Probiotics are live microorganisms that are beneficial to health; When it comes to probiotics, fermented products come to mind first. Findings on the use and benefit of fermented products date back to prehistoric times. In the excavations made in different continents of the world, it has been determined that the preparation and use of fermented foods are depicted in many figures, which are generally determined to belong to the neolithic age. The use of fermented foods obtained from milk dates back to 10000 BC and was first found to be used in the Middle East and Ancient Egypt (Gasbarrini et al., 2016). Yogurt, one of the most important fermented milk products, according to legends, was discovered by a Turkish shepherd who carried his milk in a goatskin pouch while traveling in the desert. In addition, the word yogurt is derived from the Turkish word “yoğuşmak”, which means to solidify and condense (Ozen & Dinleyici, 2015). The findings belong to 7000-5000 BC show, that fermented beverages were made from rice in Asia and fruits, cereals, and honey in whereas Mesopotamia and Ancient Egypt (Gasbarrini et al., 2016). The production and use of fermented foods have continued until today; yogurt, kefir, pickles, cheese, wine and beer are among the most popular fermented foods (Bell et al., 2017).

It was discovered by the scientist Pasteur in 1864 that the fermentation of foods is carried out by microorganisms (Barnett, 2000). In 1905, Bulgarian Doctor Grigoroff found that the microorganism that enables the formation of yogurt by fermentation was a bacillus, and this microorganism was named *Lactobacillus bulgaricus* (Grigoroff, 1905). Nobel Prize-winning scientist Elie Metchnikoff described the hypothesis that the longevity of Bulgarians is due to *Lactobacillus* found in yogurt in his book “The Prolongation of Life” in 1907 (Metchnikoff, 1908). Then, many scientists began to be interested in this subject and search for beneficial microorganisms.

The definition of these beneficial bacteria as probiotics was first made by R. Fuller in 1989 and was used for animal feeds containing beneficial microorganisms (Fuller, 1989). The definition of probiotics was finalized by scientists working on behalf of the United Nations Food and Agriculture Organization (FAO) and the World Health

Organization (WHO) as “live microorganisms that provide health aid to the host organism when taken in sufficient quantities”, in 2001 (Cordoba, 2001). As of this date, almost more than 1000 studies have been published annually (McFarland, 2015). Thereby, the number of probiotic products on the market and the interest of society in these products have increased rapidly.

In this study, we aimed to provide a general assessment of the probiotic preparations actively sold in pharmacies in Turkey in December 2021 to health professionals and interested parties.

MATERIAL AND METHODS

The present study examined the probiotic preparations that were actively sold in pharmacies in Turkey in December 2021 by using the Rx Mediapharma® 2021 Interactive Drug Information Resource Program. By searching the data bank on “Pharmacological Group Structure”; “Probiotics” under the sub-title of “Vitamins, Minerals and Other Nutritional Products” were evaluated in the study. Preparations whose production or import was stopped were excluded from the study. In addition, only one of the preparations with the same drug names and pharmaceutical forms but with a different package amount was included in the evaluation. The numbers, pharmaceutical forms, contents (single/combined microorganism), microorganism types in its content, approval status by official institutions, sales (prescription/nonprescription), and production status (domestic/imported) of selected probiotic preparations were investigated in the present study.

Statistical analysis

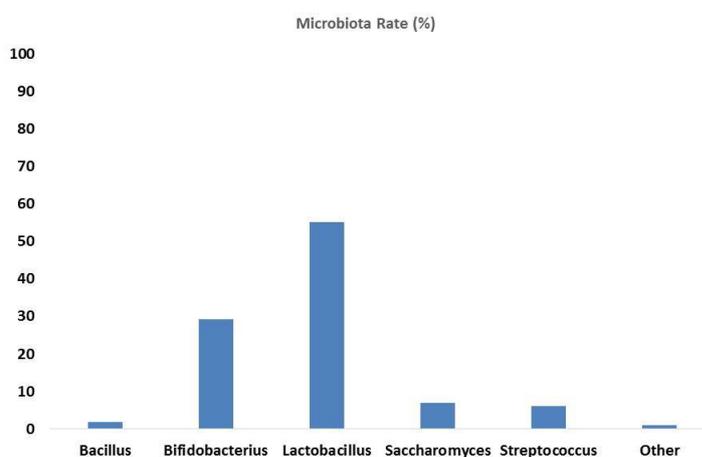
Statistical calculations were not used in our article.

Ethics committee approval

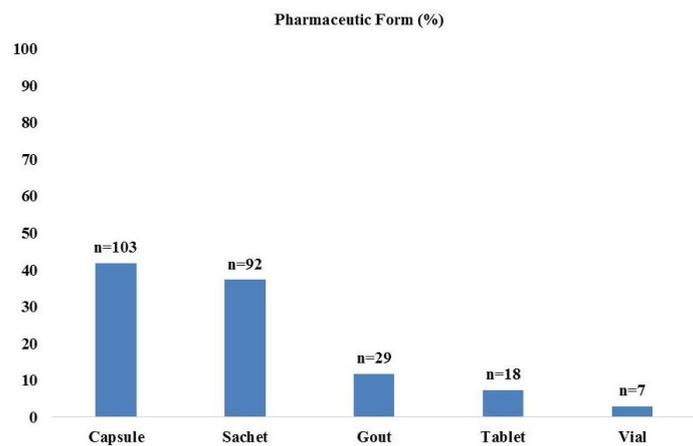
The author declares that this study does not include any experiments with human or animal subjects.

RESULTS

There were 323 probiotic preparations registered in the program. 35 of them have not been imported or produced, and one of them has been approved by the Ministry of Health but has not been put into use yet.



Graph 1. Microbiota type rate of probiotics (n=764).



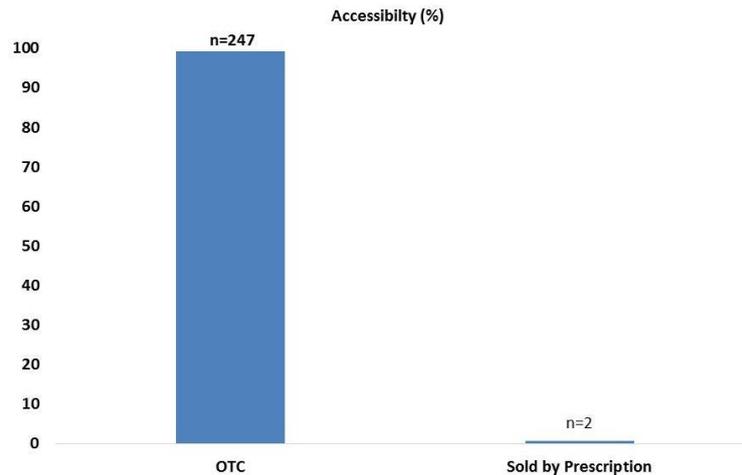
Graph 2. Pharmaceutical form of probiotics (n=249).

The remaining (n:287) preparations are actively sold in pharmacies. Only one of the same preparation and pharmaceutical forms with different package quantities were included in the study, it was determined that 249 preparations met our criteria. The most common

bacteria of microorganisms in preparations used as probiotics are *Lactobacillus* and *Bifidobacterium* species, but there are other bacteria like some strains of *Streptococcus* and yeast such as *Saccharomyces boulardii* (Table 1, Graph 1).

Table 1. Type of microorganism of probiotics (n:764).

Type of microorganism	n
<i>Lactobacillus</i>	n:420
<i>Lactobacillus rhamnosus</i>	118
<i>Lactobacillus acidophilus</i>	117
<i>Lactobacillus plantarum</i>	34
<i>Lactobacillus casei</i>	29
<i>Lactobacillus paracasei</i>	28
<i>Lactobacillus bulgaricus</i>	24
<i>Lactobacillus reuteri</i>	24
<i>Lactobacillus salivarius</i>	10
<i>Lactobacillus helveticus</i>	7
<i>Lactobacillus gasseri</i>	6
Other	23
<i>Bifidabacterium</i>	n:223
<i>Bifidabacterium lactis</i>	61
<i>Bifidabacterium longum</i>	59
<i>Bifidabacterium bifidum</i>	48
<i>Bifidabacterium infantis</i>	26
<i>Bifidabacterium breve</i>	17
Other	12
<i>Streptococcus</i>	n:47
<i>Streptococcus salivarius</i>	1
<i>Streptococcus thermophilus</i>	46
<i>Saccharomyces</i>	n:53
<i>Saccharomyces cerevisia</i>	1
<i>Saccharomyces boulardii</i>	52
<i>Bacillus</i>	n:13
<i>Bacillus clausii</i>	7
<i>Bacillus coagulans</i>	5
<i>Bacillus subtilis</i>	1
Other	n:8



Graph 3. Accessibility of probiotics (OTC; Over the Counter/ Sold by Prescriptions) (n=249).

Most of the 249 probiotic preparations were pharmaceutical in capsule form (n:103) and followed by sachet (n:92), drop forms (n: 29) (Graph 2). When evaluated in terms of content, it was seen that 39% contained only one type of microorganism (n:97), and 61% contained more than one type of microorganism (n:152). It was determined that almost all of the probiotics (97%) in the active sale were approved as Food Supplements by the Ministry of Agriculture, and the number of products approved by the Ministry of Health was just seven (3%). In addition, 99% of the probiotics were sold without a prescription (n:247), and only two of them were sold under prescription (Graph 3). We also established that 59% (n:146) of the 249 preparations sold actively were imported.

DISCUSSION

Demonstration of many benefits in the studies on probiotics, the interest of the society in fermented foods such as yogurt, kefir, boza, and pickles that can contain probiotics has increased (Bell et al., 2017). In order for the beneficial effects of probiotics to occur, they must contain a certain number of live microorganisms; The reasons such as the inability to determine the amount and type of microorganisms in fermented foods, the fact that they may contain toxins due to poor storage conditions or contamination, and that they may lose their nutritional value with pasteurization and heat treatments applied for food safety make the effectiveness of these foods controversial (Motarjemi, 2002). In order to rationalize the benefit to be obtained from probiotics, the basic features that these microorganisms must have are; a) reaching the target organ alive and in the desired amount; b) providing health benefits to its host; c) having an anti-pathogenic effect; d) be safety; e) can be produced and stored (McFarland, 2015). As a result, the fact that a product contains beneficial microorganisms does not mean that it will have a

beneficial effect on health unless it has all the properties specified.

Lactobacillus and Bifidobacterium genuses are the microorganisms that meet these specifications and are most frequently used as probiotics. these microorganisms are also most frequently (84%) used in our country. However, only certain species and even certain strains of the Lactobacillus and Bifidobacterium genuses show probiotic properties. In the study of Domig et al. (Domig et al., 2014), just 3% of the lactobacilli obtained by vaginal isolation showed probiotic properties; Gu et al. (Gu et al., 2008) examined 567 lactobacilli in their study and found that only 36 of them could reach the intestine without being affected by stomach and bile acids. In order for the beneficial effects of probiotics can be occurred; scientific studies have shown that it should contain a minimum of 10^6 - 10^8 colony-forming units (CFU)/gram or 10^8 - 10^{10} CFU/day live microorganisms (may differ according to the type of and strain the microorganism it contains), at the time of consumption (Champagne et al., 2011). Probiotics have positive effects on many diseases such as inflammatory bowel diseases, ulcerative colitis, Crohn's disease, antibiotic-associated diarrhea, Clostridium difficile-associated diarrhea, infective diarrhea, constipation, obesity, hypercholesterolemia, allergy, eczema, atopic dermatitis, depression, sleep disorder and anxiety (Hill et al., 2014; Bermúdez-Humarán et al., 2019). However, probiotics' some theoretical and clinical adverse effects have been reported. Probiotics may theoretically cause autoimmune disease by excessive immunostimulation, pathogenicity and antibiotic resistance by gene transfer, colon cancer by toxin production however, no evidence of these effects was found in clinical studies (Snydman, 2008; Doron et al., 2015). The most common clinically reported adverse effects are; bacteremia, fungemia, endocarditis, and intestinal ischemia (Didari et al.,

2014). It has been emphasized that these effects are frequently observed in immunosuppressed individuals, premature infants, those with short bowel syndrome, elderly patients, and patients with heart valve disease or with central venous catheters, and therefore caution should be exercised when using probiotics in these individuals (Snydman, 2008).

Probiotics are evaluated into three basic categories as food or supplement that do not claim to be beneficial to health, food or supplement that claim to benefit health, and probiotic drugs in the Consensus Statement held in 2013 (Hill et al., 2014).

The probiotics we examined in our study are evaluated under the title of “Vitamins, Minerals and Other Nutritional Products” and are not separated according to the grouping in the consensus. Of the 249 probiotic preparations we examined, 7 were approved by the Ministry of Health, and only 2 are sold by prescription. The remaining 242 preparations are subject to the approval of the Ministry of Agriculture.

According to the regulation numbered 26221 in the Turkish Food Codex (TGK) dated 07/07/2006, for a product to be probiotic, it must contain a sufficient number of live probiotics (at least 1.0×10^6 CFU/g) microorganisms until the end of its shelf life (Turkish Food Codex, 2006). The contents of only 99 of the 249 preparations in our study were specified CFU, and we observed that this information was not included in the prospectus of the remaining preparations.

In order to provide the desired benefit and minimize possible side effects, content determination and inspection by the Ministry of Health will make the use of probiotics safer. In addition, studies have shown that probiotics have the potential to change the bioavailability of drugs taken together (Koziolek et al., 2019). Therefore, some arrangements should be made in the sale and use of probiotics, and at least they should meet the quality, safety, and efficacy conditions sought in modern drugs.

The increasing interest in probiotics in recent years has also affected its place in the global market; The market volume, which was 49.4 million dollars in 2018, is expected to increase to 69.3 million dollars in 2023 (Trush et al., 2020). In our study, we found that 59% of the preparations we examined were imported. It is important for the economy of the country that this situation is evaluated by the relevant authorities and that domestic products play a competent role in the domestic market and even in the global market by making the necessary investments.

CONCLUSION

In this study, in which we examined probiotics that are actively sold in pharmacies in Turkey as of December 2021, we identified 249 different preparations meeting our criteria. This number may vary depending on the changes that may occur in the production, import, and license processes. The

number of studies on the subject in our country is limited, and we expect our current study to provide a general assessment of the probiotics sold in pharmacies in Turkey to healthcare professionals and interested parties.

Conflict of Interests

The author declares that for this article they have no actual, potential, or perceived conflict of interests.

Author Contributions

Plan, design: OG, FS; Material, methods and data collection: FS; Data analysis and comments: OG; Writing and corrections: OG, FS.

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Predictive Value of CCR3 Expression on Diagnosis and Prognostic Classification of the Prostate Adenocarcinoma

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ABSTRACT

Objective: Chemokines are factors secreted from damaged or infected tissues to induce an inflammatory and immunological reaction. Both chemokines and their receptors are considered as critical actors of tumor formation. Our study aims to investigate CCR-3 expression, which is a chemokine receptor that has recently been found to be secreted by most cancer cells, in prostatic adenocarcinoma (PCa), and reveal any possible relation between the Gleason prognostic grade and CCR-3 levels. **Material Method:** The study included 25 patients with PCa who underwent prostatectomy, and 25 patients with benign prostate hyperplasia (BPH). CCR-3 was detected at mRNA level by the Real-Time PCR, and at protein level by immunohistochemical (IHC) staining in both PCa and BPH patients. Results from PCa and BPH groups were compared. In PCa group, any correlation between the CCR-3 expression and Gleason prognostic grade was also searched. **Results:** Although CCR-3 mRNA levels in PCa group were found to be significantly higher than the BPH group ($p=0.001$), the difference was not significant at the protein level ($p=0.205$). The difference between the CCR3 expression levels of patients with different Gleason prognostic grades was not significant at both mRNA and protein levels. A statistically significant positive correlation was found between the CCR-3 IHC staining and total PSA levels ($p=0.001$) in PCa patients. **Conclusion:** We concluded that the CCR-3 mRNA levels may be useful in the diagnosis of PCa.

Keywords: CCR-3, Prostate Cancer, Gleason Prognostic Grade, Chemokine.

Prostat Adenokarsinomunun Tanısında ve Prognostik Sınıflandırmasında CCR3 Ekspresyonunun Prediktif Değeri

ÖZ

Amaç: Kemokinler, enflamatuar ve immünolojik bir reaksiyonu indüklemek için hasarlı veya enfekte dokulardan salgılanan faktörlerdir. Hem kemokinler hem de reseptörleri, tümör oluşumunun kritik aktörleri olarak kabul edilir. Çalışmamız, prostat adenokarsinomunda (PCa) son zamanlarda çoğu kanser hücresi tarafından salgılandığı tespit edilen bir kemokin reseptörü olan CCR-3 ekspresyonunu araştırmayı ve Gleason prognostik derecesi ile CCR-3 seviyeleri arasındaki olası ilişkiyi ortaya koymayı amaçlamaktadır. **Gereç ve Yöntem:** Çalışmaya, prostatektomi yapılan PCa'lı 25 hasta ve iyi huylu prostat hiperplazisi (BPH) olan 25 hasta dahil edildi. Her iki hasta grubunda da CCR-3 ekspresyonu hem Real-Time PCR ile mRNA düzeyinde, hem de immünohistokimyasal (IHC) boyama ile protein düzeyinde saptandı. PCa ve BPH gruplarının sonuçları karşılaştırıldı. PCa grubunda CCR-3 ekspresyonu ile Gleason prognostik derecesi arasında herhangi bir ilişki de araştırıldı. **Bulgular:** Her ne kadar PCa hastalarında CCR-3 ekspresyonu mRNA düzeyinde anlamlı olarak yüksek bulduysa da ($p=0.001$), fark protein düzeyinde anlamlı değildi ($p=0.205$). Yine, farklı Gleason prognostik derecelerine sahip hastaların CCR3 ekspresyon seviyeleri arasındaki fark hem mRNA hem de protein seviyelerinde anlamlı değildi. PCa hastalarında CCR-3 IHC boyaması ile toplam PSA düzeyleri ($p=0.001$) arasında istatistiksel olarak anlamlı pozitif korelasyon bulundu. **Sonuç:** CCR-3 mRNA düzeylerinin PCa tanısında faydalı olabileceği sonucuna varıldı.

Anahtar Kelimeler: CCR-3, Prostat Kanseri, Gleason Prognostik Derecesi, Kemokin.

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INTRODUCTION

Prostate cancer (PCa) is the second most common type of cancer in men (Castillejos-Molina and Gabilondo-Navarro, 2016). The only way to confirm the diagnosis of the disease is by a prostate biopsy in patients with abnormal findings on digital rectal examination or high prostate-specific antigen (PSA) levels (Munjal and Leslie, 2021). Age and family history stand as the main risk factors for the disease. Androgen and estrogen hormones, metabolic syndrome, smoking and diet are the other risk factors (Bostwick et al., 2004). Although no causal relationship between chronic inflammatory process and PCa has been shown so far, it is still suggested as a risk for the development of the disease (Munjal and Leslie, 2021).

Chemokines are the factors secreted from the damaged or infected tissues to induce the inflammatory and immunological reactions. Approximately 50 different types of chemokines are reported in the literature. Recently, it has been shown that most cancer cells secrete chemokines (Balkwill, 2004). The effect of chemokines on cancer is highly complicated. While many chemokines show anti-tumor activity through the activation of the immune system cells or the suppression of the angiogenesis, others found to increase the cancer growth and metastasis by direct activation of the cancer cell proliferation, migration and angiogenesis (Araújo et al., 2020).

Chemokines create their effects by binding their specific receptors. Chemokine receptors have seven transmembrane domains and, of which 23 different types are currently known, are classified as typical or atypical chemokine receptors according to their G protein binding. Nineteen of them are G-protein bound and are classified as typical chemokine receptors. The remaining four are classified as atypical chemokine receptors because, although they have the same membrane structure as typical ones, they are not G protein bound. Consequently, they cannot induce classical intracellular signaling and act as scavengers targeting chemokines for lysosomal degradation (Legler and Thelen, 2018).

In tumor formation process, chemokines and their receptors are considered as critical actors. Both tumor proliferation and metastasis are facilitated by chemokines and their receptors. However, we do not know much about the role of neither chemokines nor their receptors in the formation and growth of malignant PCa. Recently, C-C chemokine receptor type 3 (CCR-3), which is a receptor for C-C type chemokines and binds to several of them; namely CCL4, CCL5, CCL7, CCL11, CCL13, CCL15, CCL24, CCL26, CCL28, is found to be highly expressed in eosinophil and basophils, and has been shown to be expressed in melanoma, glioblastoma and kidney cancers, and has also shown to be expressed in PCa (Lee et al., 2010; Tian et al., 2016; Vela et al., 2015; Zhu et al., 2014).

The aim of this study is to determine the CCR-3 expression level in PCa and to compare it with its

expression level in BPH molecularly and immunohistochemically in order to answer whether this molecule may be used in diagnosis and/or differential diagnosis of the PCa. Evaluation of the presence of any correlation between the CCR3 levels and prognostic Gleason classification in PCa is also aimed to investigate the predictive value of its expression level in prognosis of PCa patients.

MATERIALS AND METHODS

In this study, 25 PCa patients and 25 BPH patients were evaluated in terms of age, total PSA level and CCR-3 expression. CCR-3 expression levels of PCa patients in different Gleason prognostic grades were also compared.

Specimen selection and retrospective data collection

This study included 10% neutral buffered formaldehyde (10% NBF) fixed and paraffin embedded prostate specimens obtained from 50 patients, between January 2016 and December 2017.

Half of the patients (n=25) were diagnosed with PCa and the other half (n=25) with BPH by the examination of hematoxyline and eosin (H&E) stained slides of their surgical specimens. The data about the age, total PSA level, and Gleason prognostic grade of patients were collected from the patient files retrospectively. Gleason prognostic grades of the PCa patients were determined as described by Epstein et al. (2016a).

Immunohistochemistry

In order to determine CCR3 levels, IHC staining was applied to the 5 µm thick paraffin sections of the PCa and BPH patients. Briefly, after being hydrated and incubated in 3% hydrogen peroxidase (H₂O₂) for 15 minutes, sections were washed by phosphate buffered saline (PBS). In order to prevent nonspecific staining, blocking solution was applied to them. Next, sections were incubated by anti-CCR-3 antibody (1:100, ab36827, Abcam, Boston, USA) at 37°C for 1 hour. After washing away of the unbound primary antibody, secondary antibody (iVIEW DAB Detection Kit, 05266157001, Roche Diagnostics GmbH, Germany) was applied to the sections for 30 min. Fresh 3, 3'-diaminobenzidine (DAB) chromogen (GBI Labs, Mukilteo, WA, USA) was applied to the washed sections for 1-2 minutes. After the removal of the chromogen, nuclear counter stain, hematoxyline, was applied, and slides were dehydrated and cleared. Mounted slides were evaluated under light microscopy (Nikon Eclipse CI) by a blind pathologist who verified and scored the CCR3 IHC staining intensity of each sample by a scale from 0 to 3 (0; negative, 1; low, 2; moderate and 3; high staining intensity).

Real time PCR

After the completion of IHC staining, RNA was isolated from the paraffin embedded prostate tissues of the 25 PCa and 25 BPH patients. Additionally, paraffin blocks from 10 patients, that have shown totally normal prostate histology on H&E-stained sections, were also used for RNA isolation to constitute the control group of the real-time PCR experiments.

Total RNA of all samples was purified with the use of the RNeasy FFPE Kit (QIAGEN, Germany) as described by the manufacturer. After RNA isolation, cDNA synthesis was performed by Hyperscript First strand Synthesis kit (Cat no: 601-005, Lot no: FS015B04002, USA). The Real-Time PCR (GeneAllSybr Green Master Mix, Cat No: 801-520, Lot No: QP116G25001) was conducted by StepOnePlus™ Real-Time PCR System (Applied Biosystem).

Real-Time PCR was repeated at least three times for each sample. To trigger reactions, their own genes and actin beta (ACTB) were used. 7500 Fast Real-Time Sequence detection system Software (Applied Biosystems, Foster City, CA) was used to quantify the levels of gene expressions. The threshold cycle (Ct) was utilized to define gene expressions. In addition, ACTB was considered as a reference gene functioning as an internal reference to make the RNA expressions normal, measured as $2^{-\Delta\Delta CT}$. We present the primer sequences below:

ACTB (103 bp)

5'CCTGACTGACTACCTCATGAAGATCCTC3' (forward), 5'CGTAGCACAGCTTCTCCTTAATGTCA C3' (reverse).

CCR-3 (180 bp)

5'GGTTTTATCACACAGGCTTG3'(forward), 5'AGCTCTTCCTGAATTTATCT3'(reverse)

Statistical Analyses

SPSS v.24.0 (SPSS Inc., Chicago, Illinois, USA) package program was used for statistical analysis of the data. Student t test and ANOVA were used to compare the continuous variables of PCa and BPH patients. Mann Whitney U and Kruskal Wallis tests were used when the data was not normally distributed. Bonferroni post hoc test was performed when necessary. The correlation analysis was performed by Spearman's rho test. p values less than 0.05 were considered as statistically significant.

RESULTS

The mean age of PCa patients was found as 64.88 ± 4.52 , while it was 66.12 ± 6.19 in BPH patients. No significant difference in terms of age was found between them ($p=0.423$) (Table 1). While the mean total PSA level was 24.68 ± 21.90 ng/mL in PCa patients, it was 7.23 ± 8.86 ng/mL in BPH group. Total PSA level was significantly higher in PCa ($p=0.001$) (Table 1).

Table 1. Age and total PSA level of BPH and PCa patients.

Data	BPH		PCa		p
	M	SD	M	SD	
Age (year)	66.12	6.19	64.88	4.52	0.423
Total PSA level (ng/mL)	7.23	8.86	24.68	21.91	0.001

Student t test was used. M: Mean, SD: Standard deviation

IHC stained tissue sections from BPH patients (Figure 1a) showed weaker staining when compared with the sections from PCa patients (Figure 1b). The mean staining intensity scores of BPH and PCa were 1.48 ± 0.65 and 1.80 ± 0.87 respectively. Although staining intensity was found higher in PCa, the difference between them was not significant ($p=0.205$) (Figure 1c). Similarly, when the IHC staining intensities of the PCa patients in different Gleason prognostic grades were compared, the

difference was again not significant ($p=0.20$, Figure 1d). The correlation between the IHC staining for CCR-3 and total PSA levels was also evaluated and a significant positive correlation was found demonstrating that CCR-3 expression goes up with increasing total PSA level ($p=0.001$) (Table 2). The other correlation analyzed was the one between the IHC staining for CCR-3 and Gleason prognostic grade of the patient, which was insignificant ($p=0.375$) (Table 2).

Table 2. Correlation between the IHC CCR-3 staining intensities and total PSA level and Gleason prognostic grade in PCa patients.

Correlation (CCR-3 expression)	Rho*	p
Total PSA	0.448	0.001
Gleason prognostic grade	0.185	0.375

*Spearman's Rho test was used.

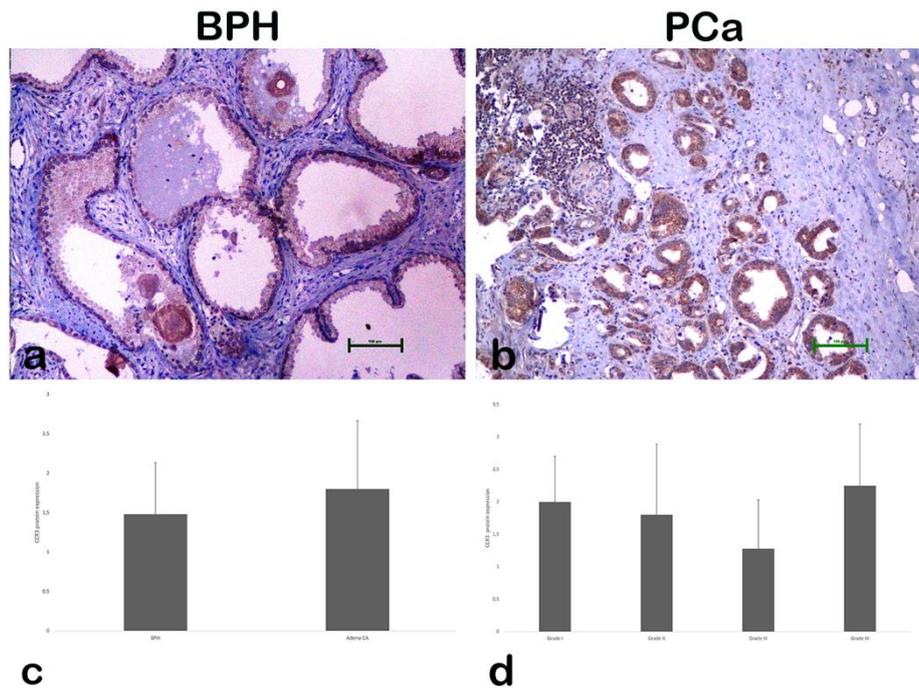


Figure 1. CCR-3 IHC staining of a) BPH tissue sample b) PCa tissue sample c) Comparison of IHC staining intensities of BPH and PCa groups d) Comparison of IHC staining intensities of PCa patients having different Gleason prognostic grades. Black and green bars in figures 1a and 1b represents 100 micrometre.

When mRNA levels of PCa patients were compared with BPA patients and control group, CCR-3 was found to be significantly higher in PCa patients ($p=0.001$) (Figure 2a). Comparison of the CCR-3 mRNA levels obtained from the PCa tissues of patients in different Gleason prognostic grade groups

showed that the difference between Gleason scoring and CCR-3 mRNA levels of patients in different groups was insignificant (Fig 2b). This situation made us think that CCR-3 expression level could not contribute to determining the prognosis of the patient.

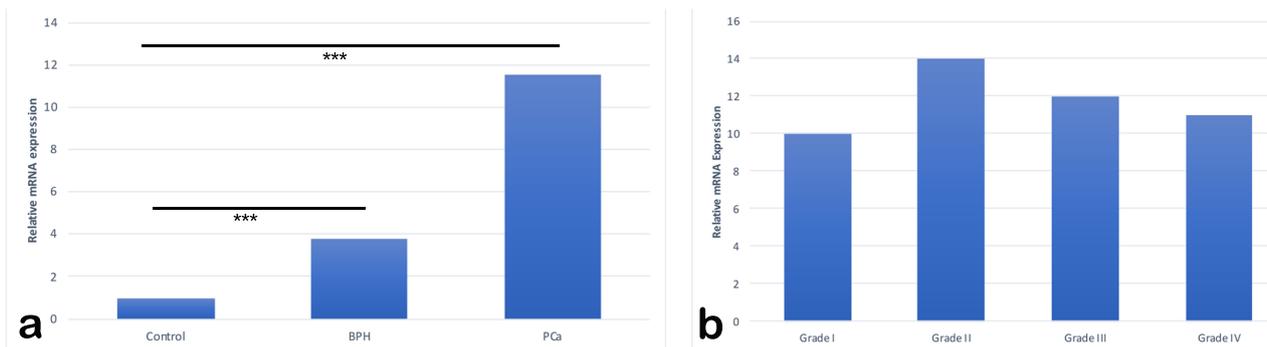


Figure 2. a) Comparison of CCR-3 mRNA levels measured by qRT-PCR and normalized to ACTB levels in BPH and PCa patients, b) Comparison of CCR-3 mRNA levels measured by qRT-PCR and normalized to ACTB levels in different Gleason prognostic grades.

DISCUSSION

In this study, the diagnostic and prognostic importance of CCR-3 expression in PCa patients was

investigated. CCR-3 expression levels were determined and compared with prognostic parameters such as Gleason prognostic grade and total PSA levels in PCa patients. CCR-3 expression at mRNA

level was significantly higher in PCa patients. There was no relationship between Gleason prognostic grade and CCR-3 expression. The high total PSA level was found to be correlated with the increased CCR3 expression. These findings suggest that CCR-3 expression may contribute to the diagnosis of PCa. Chemokines, which are peptide signaling cytokines, are known to guide cell migration. Inflammation, infection, tissue repair, cancer progression, and organogenesis of lymph nodes are the other examples of important physiological and pathological events in which chemokines have been shown to play a role (Guan, 2015).

Laurent et al., in their study published in 2016; demonstrated that CCR3 was expressed in PCa and was not expressed in normal prostate epithelium, with the IHC. They also showed that there is a correlation between the CCR3 level and the Gleason score. In our study, we examined CCR3 expression not only with IHC methods but also with qPCR. Again, in addition to normal and PCa tissues, we included samples of BPH in our study. We showed that there is CCR3 expression in the prostate gland in BPH. This expression was significantly less at the mRNA level than in PCa. Contrary to Laurent et al. (2016), we could not reveal a relationship between CCR3 levels and Gleason score in our study. We think that this may be due to the small number of our subjects. Laurent et al. (2016) showed that in prostate cancer, expression of the CCR3 receptor is associated with the occurrence of aggressive disease with local dissemination and a higher risk of biochemical recurrence (Laurent et al., 2016).

For cancer to spread, cancer cells must transform normal stromal cells, thereby shifting the microenvironment to a metastasis-supporting state (Alizadeh et al., 2014). Zhu et al. (2014) showed that eotaxin-1 promotes prostate cancer cell invasion and migration, as a result of increasing MMP-3 expression via the CCR-3-ERK pathway. In the same study, they also showed that mesenchymal stromal cells increase the invasive potential of PCa cells via increased eotaxin-3/CCR-3 expression (Zhu et al., 2014). Other studies have also proven that, there are some other cells in which CCR3 activates the ERK pathway such as smooth muscle cells and large cell lymphoma cells (Markwick et al, 2012, Miyagaki et al., 2011). The importance of the ERK pathway in proliferation, invasion and metastasis, which are important stages of tumor progression, is well known (Reddy et al., 2003).

In 2014, a new terminology was adopted by the World Health Organization (WHO) for the urinary tract tumors and male genital organs through using Gleason prognostic grade, a new grading system, between grades 1-5 (Mottet et al., 2017). The current modified Gleason classification, which forms the basis of new classification of groups, has little similarity to the original Gleason system. With this new classification, it is aimed to correct the accurate

classification of PCa patients and to reduce the excessive treatment of cancer (Epstein et al., 2016b). Gleason prognostic grade was associated with the risk of death and metastasis of PCa in terms of tumor management strategies including definitive treatment, conservative treatment and androgen blockade (Leapman et al., 2017). In our study, there was no significant relationship between CCR-3 expression and Gleason prognostic grade which is important for prognostic and treatment management. It is known that leukocyte infiltration occurs in many tumors. Although the degree of inflammation may vary, it exists in almost every tumor (Coussens & Werb, 2002). Even if the inflammatory reaction is considered to be protective, the inflammatory cells may also show tumor-promoting activity by producing growth factors or by causing additional DNA damage (Zhao et. al., 2021). For example, cyclooxygenase-2 (COX-2) enzyme, which is responsible for the conversion of arachidonic acid to prostaglandins, is produced by inflammatory stimuli and this enzyme is high in colon cancer and other cancers (Kumar et. al., 2014). Acetylsalicylic acid (ASA), a nonsteroidal anti-inflammatory drug, has been shown to inhibit COX-2, thereby reducing the release of prostaglandins responsible for different forms of tissue damage. With this effect, ASA prevents the formation of tissue and/or cell damage in many diseases with similar antioxidant properties. Clinical and preclinical studies show that ASA can reduce oxidative damage and have a role in cancer prevention (Xu et al., 2012; Demirel and Kılıksız, 2011). Perhaps a possible link between CCR3 and COX2 pathways may be a new topic for future studies.

Life-style changes such as smoking cessation, weight loss and regular exercise are recommended for males with prostate cancer. However, recent studies have suggested that regular use of aspirin may prevent the development of prostate cancer (Cuzick et al., 2014). CCR-3 expression in PCa tumors has been reported to have potential benefits in terms of preventing the local spread, high biochemical recurrence risk and the emergence of aggressive disease (Laurent et al., 2016). In their study, Lee et al. (2010) demonstrated that CCR-3 was more expressed in malignant melanoma. It is also claimed that the presence of CCR-3 protein may increase the aggressive potential of malignant cutaneous tumors and may facilitate proliferative effect in tumor cells. In another study, it has been shown that CCR-3 and its ligand eotaxin/CCL11 expression play a role in the spreading and binding of CD30+ malignant T cells (Kleinhans et al., 2003). On the other hand, in a study investigating CD4+ (TH1 and TH2) cell groups in the blood of gastric cancer patients in terms of chemokine receptor expression, it was stated that CCR5 in TH1 and CCR3 in TH2 were extremely limited for clinical evaluation due to the low CCR3 expression (Andalib et al., 2013). There was a significant difference in

terms of CCR-3 expression between BPH and PCA patients in our study. CCR-3 expression was significantly increased in PCA patients.

The study has some limitations. First of all, the number of subjects is small, and we think that this is the main reason why significant differences when CCR3 expression was examined at the mRNA level were not observed at the protein level. In addition, we think that another limitation is not questioning the chronic pathologies of the subjects such as obesity, hypertension and diabetes. In summary, we think that it is necessary to conduct new studies that include more subjects and examine the comorbidities of the subjects. Again, we believe that it will be meaningful to evaluate serum CCR3 levels in new studies to be planned.

CONCLUSION

In conclusion, CCR-3, a member of the chemokine family, was found to have increased expression in PCA patients. No significant CCR-3 effect was detected in terms of Gleason prognostic grade which has a role in predicting tumor morphology and prognosis. On the other hand, increased CCR-3 expression which is correlation to increased total PSA is thought to be used to identify potential therapeutic strategies and to predict conditions favorable to tumor and secondary to inflammation.

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Conflict of Interest

The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Author Contributions

Plan, design: ASA, EA; **Material, methods and data collection:** ASA, EA, **Data analysis and comments:** ASA, EA, FB; **Writing and corrections:** ASA, EA, FBS.

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Aerosol Management in Dentistry on YouTube

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ABSTRACT

Aim: The novel coronavirus continues to spread around the world despite all efforts. Dental professionals and staff are exposed to high infection risks and have the potential to spread these infections. Web-based portals are widely used in dentistry for obtaining information. YouTube is one of the most popular web platforms provides a wide variety of information, however, concerns are raised regarding the quality of the videos. The purpose of this study is to analyze the content of YouTube videos on aerosol management in dentistry during the COVID-19 pandemic. **Materials and Methods:** YouTube search was performed using the terms “dental aerosol management and COVID-19”, “dental aerosol control and COVID-19” and “dental aerosol reduction and COVID-19” with the default filter set to “sort by relevance”. The first 100 videos for each term were viewed and analyzed by 2 independent research. The Global Quality Scale (GQS) and the video information and quality index (VIQI) INDEX were performed to evaluate video quality. Viewers’ interactions and Viewing Rate index were chosen to evaluate the video popularity and also other parameters (number of views, duration, time since upload, and likes and dislikes, country of origin, source of upload) were evaluated. **Results:** After the exclusion criteria 57 videos were evaluated. 11 of the 57 videos had excellent quality and flow. It was observed that as the duration increased, the quality of the videos in both evaluation indexes (VIQI-GQS) were increased. There was no significant difference in terms of loading sources for VIQI and GQS index. **Conclusion:** Besides high-quality videos on aerosol management, there were some low-quality videos that may cause spread of misleading information. Healthcare professionals should play a more active role in the educational videos about infection prevention and aerosol management during pandemic on YouTube.

Keywords: COVID-19, Dental Aerosol Management, Dental Aerosol Reduction, YouTube.

YouTube’da Diş Hekimliğinde Aerosol Yönetimi

ÖZ

Amaç: Yeni tip koronavirüs tüm çabalara rağmen dünyaya yayılmaya devam etmektedir. Diş hekimleri ve çalışanları yüksek enfeksiyon risklerine maruz kalmakta ve bu enfeksiyonları yayılmasına sebep olmaktadır. Web tabanlı portallar diş hekimliğinde bilgi edinmek için yaygın olarak kullanılmaktadır. YouTube, çok çeşitli bilgiler sağlayan en popüler web platformlarından biridir. Bu çalışmanın amacı, COVID-19 salgını sırasında diş hekimliğinde aerosol yönetimine ilişkin YouTube videolarının içeriğini analiz etmektir. **Gereç ve Yöntem:** YouTube araması, “dental aerosol management and COVID-19”, “dental aerosol control and COVID-19” ve “dental aerosol reduction and COVID-19” terimleri kullanılarak, varsayılan filtre “sıralama ölçütü” olarak ayarlanarak yapıldı. Her terim için ilk 100 video 2 bağımsız araştırma tarafından görüntüledi ve analiz edildi. Video kalitesini değerlendirmek için Global Quality Scale (GQS) ve video bilgi ve kalite indeksi (VIQI) INDEX uygulandı. Video popülerliğini değerlendirmek için izleyici etkileşimleri ve Görüntüleme Oranı endeksi seçildi ve ayrıca diğer parametreler (görüntüleme sayısı, süre, yüklemeye sonrakı süre ve beğeniler ve beğenmemeler, ülke, yükleme kaynağı) değerlendirildi. **Bulgular:** Yetersiz olan videolar çıkarıldıktan sonra sonra 57 video değerlendirildi. 57 videonun 11’i mükemmel kalite ve akışa sahipti. Süre arttıkça her iki değerlendirme indeksindeki (VIQI-GQS) videoların kalitesinin arttığı gözlemlendi. VIQI ve GQS endeksi için yükleme kaynakları açısından anlamlı bir fark yoktu (P>0.05). **Sonuç:** Aerosol yönetimiyle ilgili yüksek kaliteli videoların yanı sıra, yanıltıcı bilgilerin yayılmasına neden olabilecek bazı düşük kaliteli videolar da vardı. YouTube’da pandemi sırasında enfeksiyon önleme ve aerosol yönetimi ile ilgili eğitici videolarda sağlık çalışanları daha aktif rol oynamalı.

Anahtar Kelimeler: COVID-19, Diş Hekimliğinde Aerosol Yönetimi, Diş Hekimliğinde Aerosol Azaltma, YouTube.

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INTRODUCTION

Dentistry has been significantly impacted by the novel Coronavirus Disease (COVID-19) as the other healthcare professions. It has been reported that the virus causing COVID-19 disease binds to ACE 2 receptors that found in saliva and mouth structures like tongue, floor of the mouth (Khurshid et al. 2020; Xu et al. 2020). Dentists are directly in contact with the source of the disease since they perform the treatment procedures in and about the entire mouth and face area (Peng et al. 2020). Most dental procedures pose high infection risk for both patients and dental staff due to the aerosol spread of saliva, blood, and secretions (Teichert-Filho et al. 2020). Moreover, this aerosol production also contaminates the surfaces and instruments used in the dental environment which could also facilitate transfer of coronavirus (Izzetti et al. 2020). For these reasons, dental health professionals feel somatic and cognitive anxiety considering that their patients may be infected or contacted with the virus during the pandemic (Ather et al. 2020; Meng et al. 2020). This anxiety may prevent dentists to diagnose correctly (8). In a recent study, it was reported that 87% of dentists were afraid of being infected with the coronavirus in the dental clinic (Ahmed et al. 2020a). Dentists should increase the knowledge of aerosol management to prevent COVID-19 transmission during treatment procedures and reduce the anxiety of infection risk (Olivieri et al. 2020).

One of the most important objectives identified by the World Health Organization (WHO) to prevent COVID-19 transmission is to share accurate information to all communities and avoid the disseminate of misinformation (Dutta et al. 2020). The internet is growingly being used as the most accessible source of information for people involving healthcare information. It has been reported that YouTube (<http://www.youtube.com>) as the second most visited web portal after Google.com with over 2 billion users generating billions of daily views. Compared to other social media platforms, YouTube stands out with its advantages such as using audio and visual communication logically and being able to access easily by many people from all demographic backgrounds (Li et al. 2020). Although YouTube is very useful to guide community during the pandemic, some misleading information can provoke panic and even increase the spread of the disease, as it does not have a regulatory system to evaluate the content quality of uploaded videos (Kocyigit et al. 2020). The “coronavirus” and “COVID-19” terms became the most searched on YouTube with the beginning of the COVID-19 pandemic. Despite the increased interest in studies on YouTube and social media coverage on the COVID-19 outbreak (Ahmed et al. 2020b; D'Souza et al. 2020; Li et al. 2020; Szmuda et al. 2020), the number of studies related to dentistry is still limited (Ozdede and Peker 2020; Yuce et al. 2020).

To the best of our knowledge, there is no study investigating the reliability of YouTube content of aerosol management in dentistry during the COVID-19 outbreak. The purpose of this study was to analyze the content of YouTube videos on aerosol management in dentistry during the COVID-19 pandemic.

MATERIALS AND METHODS

Video selection

This cross-sectional study was approved by the Ministry of Health, Republic of Turkey (No: 2020-11- 18T21_32_23). The research aimed to evaluate the YouTube videos comprising information about aerosol management in dentistry during COVID-19 pandemic after December 31, 2019. YouTube search was performed using the terms “dental aerosol management and COVID-19”, “dental aerosol control and COVID-19” and “dental aerosol reduction and COVID-19” with the default filter set to “sort by relevance”. The search term ‘dental aerosol management and COVID-19’ yielded 607 results, and the ‘dental aerosol control and COVID-19’ term yielded 671 results, while the term ‘dental aerosol reduction and COVID-19’ showed 571 results. It has been reported that the most users searched on YouTube consider and evaluate the first 60-200 videos found (Desai et al. 2013). The videos are not in English, not related to the subject, no audio, a duplicate of another were excluded.

Data analysis

Since this study included the evaluation of publicly accessible videos, ethical approval was not required. The first 100 videos for each term were viewed and analyzed by 2 independent research (NBK and EK). The researchers were not aware of the number of likes, dislikes, or comments before completing their reviews to assess objectively. The interaction index ($[\text{number of likes} - \text{number of dislikes}] / \text{total number of views} \times 100$ percent) and the viewing rate ($\text{amount of views} / \text{number of days since upload} \times 100$ percent) were used to calculate viewer interactions. The American Dental Association (ADA), dental health professionals/centers, information websites, and commercial videos were all classified according to the source of submission. To assess the video's quality and substance, the video information and quality index (VIQI) (20) was chosen. The VIQI scale assesses the following video features: flow of information, information accuracy, quality (1 point each for the use of still images, animation, community interviews, video captions, and a report summary), and precision using a 5-point Likert scale ranging from 1 (poor quality) to 5 (high quality). The YouTube videos meeting our inclusion criteria were then assessed using the Global Quality Scale (GQS) (Singh et al. 2012) for the following content:

- Is it recommended using any mouthwashes before oral examination?

- Is it recommended Personal protective equipment (PPE) (Gloves, masks, gowns, and face shields) and antiseptic application for dentists?
- Is it mentioned the importance of using Rubber-Dam?
- Is it recommended high vacuum suction?
- Is minimally invasive procedure recommended to reduce the treatment time? (Azim et al. 2020)

The overall quality of the videos was subjectively assessed using a 5-point Likert-type GQS, which assigned a score of: 1. Poor video quality: the video has a poor flow, the majority of the content is missing, and it is not at all informative. 2. Poor overall quality and flow: some material is given, but many crucial topics are missing; of limited utility. 3. Moderate quality: weak flow; some vital information is adequately discussed, while other material is inadequately discussed; information is somewhat useful. 4. Good quality and overall flow: the majority of significant material is given, but certain topics are not; useful. 5. Outstanding quality and flow; quite beneficial.

Statistical analysis

Statistical software (SPSS 22.0 software IBM, Armonk, NY) was used to analyze the data. Descriptive statistics for each variable were calculated and presented as 'mean \pm standard deviation' or 'median (min-max)' for continuous variables and n, n% for categorical variables. Fisher-Freeman-Halton test was used to examine the distribution of frequencies for categorical variables since the expected values were less than 5.

Kruskal Wallis test was used to examine the scores of video characteristics and quality index between source of upload and GQS groups. Dunn test was used as post-hoc procedure after any significant differences. Mann Whitney U test was used to test video information and quality indexes between country of origin. Correlation of the variables was investigated using Spearman's rank correlation analysis. Quadratically weighted kappa coefficients were calculated to assess the agreement among two quality scoring variables (VIQI vs GQS). The inter-observer agreement was calculated as a kappa score. A probability value of less than 0.05 was considered significant.

RESULTS

The first 100 videos containing each relevant search items were screened 247 videos were excluded because of not meeting the inclusion criteria. Descriptive analysis of the characteristics and VIQI scores was presented in Table 1 and descriptive statistics according to country of origin, source of upload and GQS score categories were shown in Table 2. The mean number of views of the videos was 12.780. The mean duration of videos was 29.07 (1.5- 157) min. The mean distributions of viewer's interaction were found as 182.94 (range 0 - 2638.3), 113.44 likes (0-2100), 6.14 (range 0 - 90) dislike. Viewing Rate were found as 6018.19 (range 34.65-73856.6) The mean VIQI index score was 11.63 (between 4 and 19). The mean score of GQS index was 2.94 (between 1-5). Most videos (%66.7) were uploaded by users in the USA.

Table 1. Descriptive analysis of the characteristics and quality.

Characteristics of the videos	n	Mean \pm Std. Dev	Median (Min. - Max.)
Number of Views	57	12780.49 \pm 26410.93	2735 (70 - 166916)
Number of Likes	57	113.44 \pm 288.35	38 (0 - 2100)
Number of Dislikes	57	6.14 \pm 14.01	1 (0 - 90)
Duration (min.)	57	29.07 \pm 30.6	20 (1.5 - 157)
Number of Days since upload	57	207.4 \pm 46.65	212 (30-324)
ViewingRate	57	6018.19 \pm 11835	1474.42 (34.65-73856.6)
Viewers' Interaction	57	182.94 \pm 356.96	98.85 (0 - 2638.3)
Video information and quality indexes			
Flow of information	57	2.89 \pm 1.19	3 (1 - 5)
Information accuracy	57	3 \pm 1.3	3 (1 - 5)
Quality	57	2.81 \pm 1.25	3 (1 - 5)
Precision	57	2.93 \pm 1.37	3 (1 - 5)
Total	57	11.63 \pm 4.78	11 (4 - 19)

Table 2. Descriptive statistics according to country of origin, source of upload and GQS score categories.

		n (%)
Country of origin	Other	19 (33.3)
	USA	38 (66.7)
Source of upload	ADA	7 (12.3)
	Dental Health Centers	11 (19.3)
	Information Website	16 (28.1)
	Commercial	23 (40.4)
GQS SCORE	Poor quality	8 (14)
	Generally poor quality and poor flow	12 (21.1)
	Moderate quality	11 (19.3)
	Good quality and generally good flow	15 (26.3)
	Excellent quality and flow	11 (19.3)

Table 3. The evaluation of the videos' characteristics according to the GQS score.

Characteristics of videos	Poor quality (n=8)	Generally poor quality and poor flow (n=12)	Moderate quality (n=11)	Good quality and generally good flow (n=15)	Excellent quality and flow (n=11)	p*
	Median (Min. - Max.)	Median (Min. - Max.)	Median (Min. - Max.)	Median (Min. - Max.)	Median (Min. - Max.)	
Number of Views	2120 (125 - 44200)	8153.5 (93 - 166916)	2323 (88 - 8357)	4287 (70 - 78866)	2536 (364 - 28243)	0.495
Number of Likes	39 (3 - 330)	48.5 (2 - 2100)	18 (3 - 114)	47 (1 - 535)	49 (0 - 190)	0.755
Number of Dislikes	2 (0 - 18)	2.5 (0 - 90)	0 (0 - 9)	1 (0 - 52)	2 (0 - 13)	0.493
Duration (min.)	2.5 (2 - 20) c	5.38 (1.5 - 30) c	25.7 (7 - 61.5) b	33 (2 - 81) b	58 (22 - 157) a	<0.001
Viewers' interaction index	138.06 (22.52 - 2638.3)	95.67 (2.71 - 131.49)	142.52 (30.13 - 427.35)	98.72 (46.31 - 476.19)	107.94 (0 - 815.22)	0.544
Number of Days since upload	230.5 (179 - 259)	217 (30 - 266)	216 (136 - 264)	215 (79 - 324)	200 (160 - 242)	0.474
Viewing rate	903.34 (60.98 - 17198.44)	3998.93 (46.97 - 73856.64)	1025.38 (41.9 - 3482.08)	1895.33 (34.65 - 36011.87)	1297 (158.26 - 13777.07)	0.37

Table 4. The evaluation of VIQI index and viewers' interaction and viewing rate in terms of uploading sources.

Video information and quality indexes	Source of Upload				p
	ADA (n=7)	Dental Health Centers (n=11)	Information Website (n=16)	Commercial (n=23)	
	Median (Min. - Max.)	Median (Min. - Max.)	Median (Min. - Max.)	Median (Min. - Max.)	
Flow of information	3 (2 - 4)	2 (1 - 5)	2.5 (1 - 5)	3 (1 - 5)	0.68
Information accuracy	3 (1 - 5)	3 (1 - 5)	3 (1 - 5)	3 (1 - 5)	0.981
Quality	2 (1 - 4)	2 (1 - 5)	2.5 (1 - 5)	3 (1 - 5)	0.407
Precision	3 (2 - 5)	2 (1 - 5)	3 (1 - 5)	3 (1 - 5)	0.801
Total	12 (8 - 18)	9 (5 - 19)	10.5 (4 - 19)	14 (4 - 19)	0.828

Table 4. (continue). The evaluation of VIQI index and viewers' interaction and viewing rate in terms of uploading sources.

Video information and quality indexes	Source of Upload				p
	ADA (n=7)	Dental Health Centers (n=11)	Information Website (n=16)	Commercial (n=23)	
	Median (Min. - Max.)	Median (Min. - Max.)	Median (Min. - Max.)	Median (Min. - Max.)	
Viewers' Interaction	54.53 (22.52 - 95.3) ^d	120.42 (0 - 815.22) ^{ab}	147.79 (30.13 - 2638.3) ^a	92.81 (2.71 - 340.91) ^{bc}	0.003
Viewing Rate	8798.76 (1554.79 - 23028.08) ^a	2386.59 (90.96 - 73856.64) ^{ab}	787.25 (122.4 - 17198.44) ^b	1662.2 (34.65 - 36011.87) ^{ab}	0.044

^{a,b,c,d}: Values in the same row with different superscripts show statistical significance (p<0.05).

* Kruskal Wallis test.

Table 5. Correlation coefficients of values for VIQI, GQS and other factors.

		Number of Views	Duration (min.)	Number of days since uploading	Flow of information	Information accuracy	Quality	Precision
Number of Views	r	1						
	p	.						
Duration (min.)	r	0.004	1					
	p	0.978	.					
Number of days since uploading	r	0.06	-0.073	1				
	p	0.66	0.592	.				
Flow of information	r	0.029	0.59	-0.036	1			
	p	0.828	0	0.792	.			
Information accuracy	r	0.103	0.604	-0.175	0.785	1		
	p	0.446	0	0.196	0	.		
Quality	r	0.065	0.655	-0.098	0.892	0.795	1	
	p	0.632	0	0.474	0	0	.	
Precision	r	0.076	0.725	-0.083	0.887	0.885	0.827	1
	p	0.576	0	0.544	0	0	0	.
Total	r	0.07	0.698	-0.1	0.942	0.918	0.934	0.957
	p	0.607	0	0.463	0	0	0	0
viewers' interaction index	r	-0.557	0.081	-0.198	-0.096	0.051	0.022	-0.017
	p	0	0.55	0.143	0.476	0.707	0.872	0.899
viewing rate	r	0.978	0.02	-0.064	0.037	0.111	0.063	0.084
	p	0	0.885	0.639	0.785	0.417	0.642	0.538
GQS SCORE	r	-0.033	0.724	-0.18	0.869	0.817	0.889	0.849
	p	0.808	0	0.185	0	0	0	0

The evaluation of the videos' characteristics according to the GQS score was showed in Table 3. It was observed that the GQS score increases significantly as the duration of the video increases. (p

<0.001). The evaluation of VIQI index and viewers' interaction and viewing rate in terms of uploading sources is shown in Table 4. Correlation coefficients of values for VIQI, GQS and other factors were given

in Table 5. While VIQI and GQS showed a strong positive correlation with duration of videos, there was a weak correlation with parameters such as likes, dislikes, views, viewing Rate, viewer's interaction. The agreement of VIQI and GQS indexes was showed between 0.81- 1 = excellent agreement. In terms of total Scores Spearman correlation coefficient between two observers was found as $r=0.727$; $p<0.001$.

DISCUSSION

Aerosols and droplets contaminated with bacteria, viruses and blood are produced during dental treatments. It has been reported that dental professionals and staff are exposed to high infection risks and have the potential to spread these infections (Harrel and Molinari 2004). Transmission occurs through droplets from aerosols produced during clinical dental procedures.

Therefore, understanding aerosol management and implementing some special precautions in addition to standard precautions is very crucial for dental practice during this pandemic period (Ge et al. 2020). Hand hygiene, personal protective equipment (PPE), disinfection of clinical settings and the management of medical waste are one of the most important issues to prevent COVID-19 transmission during oral examination and dental treatment (Peng et al. 2020). However, it should be known that even the protective equipment may not protect the patient and the physician from the spread of the virus; therefore, precautions should be taken carefully by considering each patient as a COVID-19 case. On the other hand, this challenging situation after COVID-19 has provided the opportunity to increase our knowledge and understanding of dentistry-related infection control procedures (Rosales-Mendoza et al. 2020) and has made it necessary to use additional methods for aerosol management in addition to PPE (Gloves, masks, gowns, and face shields). It is important for dental professionals to access updated information and to use this information in their clinical environments in terms of infection control. YouTube is a valuable tool for reaching this information and has become popular in dentistry and medicine. It has been reported that in past outbreaks such as the Ebola outbreak in 2014, the Zika virus outbreak in 2016, and the H1N1 Flu outbreak in 2009 YouTube had a positive impact on the education of professionals and the public, and the videos have been viewed millions of times (Tang et al. 2018). Millions of videos, such as educational, professional or non-professional videos for dentistry are shared on YouTube. Since the videos uploaded on YouTube are not evaluated and reviewed before sharing, it is inevitable to reach both true and false information. Therefore, this present study aimed to investigate the reliability of the content of YouTube videos, which reduces the concerns of dentists about the aerosol management during dental examination and treatment after

COVID-19 outbreak and formed a guide for this issue. It is recommended to apply antimicrobial mouthwash before treatment to reduce the number of microorganisms in the oral cavity (Marui et al. 2019). Using PPE (gloves, masks, gowns and face shields) are crucial to protect against aerosols that come out during the use of high or low speed devices (Samaranayake et al. 1989). The application of rubber dam alone decreases aerosol spread up to 90% (Cochran et al. 1989). Using high vacuum suction removes air up to 2.83 m³/min and reduces aerosols and contamination by 90% (Narayana et al. 2016). Another protective method is to shorten the treatment procedures as much as possible and to use minimally invasive procedures (Azim et al. 2020). The evaluation of these criteria which has been verified by CDC, WHO, OSHA was provided by GQS index in this present study. The average value of the GQS index was 2.94 (between 1-5) and 19.3% (n=11) of the videos were Excellent quality and flow, 26.3% (n=15) Good quality and generally good flow, 19.3% (n=11) Moderate quality, 21.1% (n=12) Generally poor quality and poor flow and 14% (n=8) had Poor quality in our study, No significant difference was found between the viewers' Interaction, Viewing rate, likes, dislikes, number of views, source of upload, origin of country according to GQS index (Table 3). However, it was observed that as the duration of the video increased, the GQS score significantly increased ($p < 0.001$). The VIQI index evaluating the quality of a universal video in terms of visual, auditory and information was chosen since it has been used in previous studies analyzing the quality of YouTube videos (Hatipoğlu and Gaş 2020; Ozdede and Peker 2020). There was a positive correlation with duration and a statistically significant correlation ($r=0.698$, $p < 0.001$), while there was no correlation with parameters such as the number of views, Viewing Rate and Viewers' Interaction (Table 5). In our study, we did not limit the video duration as the previous YouTube studies (Cesur Aydin and Gunec 2020) in order to evaluate the YouTube video content transparently and objectively. It was observed that as the duration increased, the quality of the videos in both evaluation indexes (VIQI-GQS) increased. No significant difference was found in terms of uploading sources for VIQI and GQS index. Viewer's interaction for the Information website source was significantly higher than the other sources except dental health professionals/centers. Although viewer's interaction of ADA was significantly lower than other sources ($p=0.003$), viewing rate of ADA was significantly higher than other sources ($p=0.044$). Yuce et al. (Yuce et al. 2020) analyzed the quality of YouTube videos on additional preventive procedures in dentistry at the beginning of the COVID-19 outbreak as educational sources for dental professionals. They reported that 43.6% YouTube videos were in a poor quality and no significant difference was found between groups in the

comparison on GQS means. Similarly, when evaluating the quality of the videos, no significant difference was found in terms of sources of upload in our study. Ozdede et al. (Ozdede and Peker 2020) reported that at the beginning of COVID-19 pandemic, YouTube videos had high views in dentistry, but the videos were moderate in terms of quality and usefulness. Conversely, the quality of the videos was higher in our study. This discrepancy may be due to the fact that these two studies were conducted at the beginning of the COVID-19 pandemic. Since the number of studies on COVID-19 pandemic increased during this period, more literature-based videos may have been uploaded by healthcare professionals. It is not possible to make comparisons completely since there is no study on aerosol management in dentistry during COVID-19 pandemic. YouTube studies have some limitations like not evaluating by a single observer, performing subjective ratings. Videos displayed until the time of the research are evaluated. YouTube is a dynamic platform and new videos are added and displayed over time. Therefore, more research is needed to evaluate video changes relative to the upload time of existing YouTube videos during and after the COVID-19 outbreak. While YouTube is a valuable resource for medical information about the COVID-19 outbreak, the CDC, WHO, OSHA, academic institutions, and other government health authorities should explore using it to distribute correct medical information to the general public.

Also, dental professionals should be encouraged to provide more reliable and useful resources, especially to upload educational videos on trusted sites with a high audience, such as YouTube.

CONCLUSION

Increasing the knowledge of aerosol management in dentistry can help dental professionals implement special precautions that prevent disease transmission during pandemic. For this purpose, it may be necessary to enhance educational content in social media areas such as YouTube to reach more people. Healthcare professionals should play a more active role in the educational videos on infection prevention and aerosol management during pandemic on YouTube. Evaluating the content of YouTube videos before uploading may contribute to reaching more accurate information for the healthcare professionals and general public.

Conflict of Interest

The authors deny any conflict of interest related to this study.

Author Contributions

Plan, design: NBK, EK; **Material, methods and data collection:** NBK, EK; **Data analysis and comments:** NBK, EK; **Writing and corrections:** NBK, EK.

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Effects of Cold Storage in Krebs-Henseleit Solution at +4 °C on Vasoreactivity of the Rat Thoracic Aorta

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ABSTRACT

Objective: This study aimed to investigate whether endothelium-dependent and -independent relaxation responses can be preserved intact in the rat thoracic aorta after storage for 3, 6, and 18 hours in Krebs-Henseleit solution at +4 °C. **Materials and Methods:** Isolated organ bath model and 10-12 weeks old male Wistar rats were used to perform the experiments. To investigate the effect of endothelium-dependent relaxation factors, the cyclooxygenase inhibitor indomethacin was added to the Krebs-Henseleit solution to inhibit endogenous prostanoid synthesis. Submaximal contraction response was obtained with a single dose of phenylephrine and then acetylcholine was administered cumulatively (10^{-9} - 10^{-4} M) to induce endothelium-dependent relaxation responses. Besides, smooth muscle-dependent relaxation responses were obtained by applying sodium nitroprusside cumulatively (10^{-9} - 10^{-5} M) following precontraction induced by phenylephrine. The statistical significance level was considered as $p < 0.05$. **Results:** Thoracic aorta segments (0 hours, control) exhibited a mean contraction response of 3123 mg against 10^{-5} M phenylephrine. Although the responses slightly reduced in vascular rings stored for 3, 6, and 18 hours, no significant difference was observed ($p > 0.05$). Besides, cumulatively administered acetylcholine did not cause a significant change in endothelium-dependent relaxation responses ($p > 0.05$). Similarly, sodium nitroprusside did not modulate the endothelium-independent relaxation responses in aortic segments after storage for 3, 6, or 18 hours ($p > 0.05$). **Conclusion:** In the present study, the first physiological findings have been obtained that the endothelium-dependent and -independent contraction-relaxation responses of rat thoracic aortas can be preserved intact after storage periods of 3, 6, or 18 hours in Krebs-Henseleit solution at +4°C.

Keywords: Cold Storage, Rat Thoracic Aorta, Vascular Smooth Muscle, Vasoreactivity.

+4 °C’de Krebs-Henseleit Solüsyonunda Soğuk Depolamanın Sıçan Torasik Aortunun Vazoreaktivitesi Üzerindeki Etkileri

ÖZ

Amaç: Bu çalışma, Krebs-Henseleit solüsyonunda +4 °C’de 3, 6 ve 18 saat süreyle saklanan sıçan torasik aortlarında endotel-bağımlı ve -bağımsız gevşeme yanıtının bozulmadan korunup korunamayacağını araştırmayı amaçlamıştır. **Gereç ve Yöntem:** Deneyler izole organ banyosu modeli ve 10-12 haftalık erkek Wistar sıçanlar kullanılarak gerçekleştirilmiştir. Endotel-bağımlı gevşeme faktörlerinin etkisini araştırmak için, endojen prostanoid sentezini inhibe etmek üzere Krebs-Henseleit çözeltisine siklooksijenaz inhibitörü indometazin eklenmiştir. Tek doz fenilefrin ile submaksimal kasılma yanıtı elde edilmiş ve ardından endotel-bağımlı gevşeme yanıtını indüklemek üzere kümülatif olarak (10^{-9} - 10^{-4} M) asetilkolin uygulanmıştır. Ayrıca, fenilefrin ile indüklenen prekontraksiyonu takiben sodyum nitroprussid kümülatif (10^{-9} - 10^{-5} M) uygulanarak düz kasa bağıl gevşeme yanıtları elde edilmiştir. İstatistiksel anlamlılık düzeyi $p < 0.05$ olarak belirlenmiştir. **Bulgular:** Torasik aort segmentleri (0 saat/kontrol), 10^{-5} M fenilefrine karşı 3123 mg’lık ortalama kasılma yanıtı göstermiştir. 3, 6 ve 18 saat saklanan vasküler halkalarda yanıtlar biraz azalmasına rağmen, anlamlı bir fark gözlenmemiştir ($p > 0.05$). Ayrıca, kümülatif uygulanan asetilkolin, endotel-bağımlı gevşeme yanıtlarında anlamlı bir değişikliğe neden olmamıştır ($p > 0.05$). Benzer şekilde, sodyum nitroprussid 3, 6 veya 18 saatlik saklamadan sonra aort segmentlerinde endotel-bağımsız gevşeme yanıtlarını modüle etmemiştir ($p > 0.05$). **Sonuç:** Bu çalışmada, Krebs-Henseleit solüsyonunda +4 °C’de 3, 6 veya 18 saatlik saklama sürelerinden sonra sıçan torasik aortlarının endotel-bağımlı ve -bağımsız kasılma-gevşeme yanıtının bozulmadan korunabildiğine dair ilk fizyolojik bulgular elde edilmiştir.

Anahtar Kelimeler: Soğuk Depolama, Sıçan Torasik Aortu, Vasküler Düz Kas, Vazoreaktivite.

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INTRODUCTION

The limitation of isolated tissue bath experiments, in which physiological and pharmacological responses of vascular tissues can be examined, is the necessity of using cellular functions without disruption. Many studies are reporting that the vascular endothelium may be damaged during isolation and storage (Kristek et al., 1993; Török et al., 1993). Therefore, the vascular preparations should be hung in tissue baths rapidly after isolation. This requirement limits the number of studies to be carried out with vascular segments isolated from the same animal and allows an animal to be used for only one vessel type in experimental animal models where cardiovascular studies are performed. This issue causes a greater constraint for physiological and pharmacological research where human vessels are used, as these vessels are more difficult to obtain (Cupitra et al., 2021; Wise et al., 2015).

Preservation of vascular tissues has an important role not only in facilitating experimental research but also in terms of organ transplantation success. The vascular endothelium is a dynamic structure responsible for the synthesis and release of many vasoactive substances necessary for the physiological functions of the vessel (Yetik-Anacak & Catravas, 2006). The preservation of endothelial integrity is of great importance in terms of both the integrity of the organ and the transplantation of the vascular graft. In cases where vascular tissues are not well protected and endothelial integrity is impaired, decreased nitric oxide synthesis, vascular spasm, and dysfunction of the transplanted tissue due to increased thrombogenicity may be observed (Chong et al., 2001; Dragun et al., 2001). In addition, it is important to preserve the viability of vascular tissues to evaluate the long-term effects of drugs.

In previous studies, researchers have used refrigerators (Kristek et al., 1993; Shibata, 1969; Török et al., 1993) or freezing at lower temperatures to preserve vessels intact. However, conditions in which endothelium- and vascular smooth muscle-related responses could remain intact could not be provided in these studies (Ku et al., 1994). The preservation of the functions of vascular tissues mainly depends on the preferred storage solution and duration (Jahania et al., 1999). It has been reported that contraction and endothelium-dependent relaxation responses are impaired in isolated vascular rings stored in the cold for a few days (Kristek et al., 1993). Tissues that were stored cold overnight had better results, although there were differences depending on the storage solutions used. As for the preservation solution, the most used in physiology is Krebs-Henseleit at 4 °C, because the arteries placed in it show just a tendency to decrease vascular reactivity after 24 h (Cupitra et al., 2021). This study aimed to investigate whether endothelium-dependent and -independent relaxation responses can be preserved intact in the rat thoracic aorta after storage for 3, 6, and 18 hours in Krebs-Henseleit solution at +4 °C.

MATERIALS AND METHODS

Test animals

The study was carried out using 10-12 weeks old male Wistar Albino rats obtained from Bursa Uludag University Experimental Animal Breeding Application and Research Center.

Chemicals and reagents

Phenylephrine (PE), acetylcholine (ACh), sodium nitroprusside (SNP), indomethacin (INDO), and chemicals in Krebs-Henseleit solution were purchased from Sigma Aldrich (St Louis, MO, USA). PE, ACh, SNP, and INDO were dissolved in distilled water.

Isolated organ bath experiments

Animals were sacrificed by decapitation without anesthesia. The thoracic cavity of the animals was rapidly opened. Then, the thoracic aorta was removed delicately and placed in a 0-4 °C Krebs-Henseleit solution in a Petri dish. The Krebs-Henseleit solution consisted of 119.0 mM NaCl, 4.7 mM KCl, 1.5 mM MgSO₄, 1.2 mM KH₂PO₄, 2.5 mM CaCl₂, 25.0 mM NaHCO₃, and 11.0 mM glucose. The isolated aortas were cleared of adherent tissues and separated into 2-3 mm segments. Thus, 3-4 aortic segments were obtained from each animal. Aortic segments were hung immediately (0 hours, control) or after stored for 3 hours, 6 hours, or 18 hours in Krebs-Henseleit solution at +4 °C to the isometric force transducer (BIOPAC Systems, 42 Aero Camino-Goleta, USA) of the four-chamber tissue bath (Commat, Ankara, Turkey) via stainless steel wires to measure the isometric contraction-relaxation responses. The temperature of the baths was adjusted to 37 °C (±0.5 °C) by employing a thermocirculator. The tissue baths were continuously aerated with a gas mixture of 95% O₂-5% CO₂. The Krebs-Henseleit solution, containing the tissues, was refreshed every 10 min during the resting period. After the equilibration period, the active agents were precisely administered into the baths using an adjustable automatic pipette (Jespersen et al., 2015). In preliminary experiments, the submaximal contraction dose for PE was determined as 10⁻⁵ M.

To investigate the effect of endothelium-dependent relaxation factors, the cyclooxygenase inhibitor INDO (1 mM) was added to the Krebs-Henseleit solution to inhibit endogenous prostanoid synthesis. Submaximal contraction response was obtained with a single dose of PE and then ACh was administered cumulatively (10⁻⁹-10⁻⁴ M) to induce endothelium-dependent relaxation responses. Besides, smooth muscle-dependent relaxation responses were obtained by applying SNP cumulatively (10⁻⁹-10⁻⁵ M) following precontraction induced by PE. The tissues were rested for 45 min between the concentration-response curves and the Krebs-Henseleit solution was refreshed every 10 min during this time.

Eight aortic rings were used for each group (n=8).

Statistical analysis

SPSS 23.0 program (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. All data are expressed as mean±SD (standard deviation). Statistical significance between two independent groups was analyzed by independent sample t-test; a one-way analysis of variance

(one-way ANOVA) test was used for multiple comparisons. Dunnett's T3 post hoc test was used to determine the differences between the groups. The statistical significance level was considered as $p < 0.05$.

Ethical Consideration

This study was approved by the decision of Bursa Uludag University Animal Experiments Local Ethics Committee (BUÜ HADYEK) dated 05/04/2022 and numbered 2021-05/05. All experiments were conducted following the legislation on the Welfare and Protection of Animals Used for Experimental and Other Scientific Purposes.

RESULTS

In preliminary experiments, the submaximal contraction dose for PE was determined as 10^{-5} M.

Rat thoracic aorta segments hanged rapidly after isolation (0 hours, control) exhibited a mean contraction response of 3123 mg against 10^{-5} M PE. Although the responses

slightly reduced in vascular rings stored for 3 hours, 6 hours, and 18 hours in Krebs-Henseleit solution at $+4^{\circ}\text{C}$, no statistically significant difference was observed ($p=0.245$, $p=0.179$, and $p=0.134$, respectively) (Figure 1).

Cumulatively administered (10^{-9} - 10^{-5} M) ACh did not cause a statistically significant change in endothelium-dependent relaxation responses in rat thoracic aortas precontracted by submaximal (10^{-5} M) PE dose after storage in Krebs-Henseleit solution at $+4^{\circ}\text{C}$ for 3 hours ($p=0.884$ for 10^{-9} M; $p=0.835$ for 10^{-8} M; $p=0.571$ for 10^{-7} M; $p=0.610$ for 10^{-6} M; $p=0.619$ for 10^{-5} M), 6 hours ($p=0.591$ for 10^{-9} M; $p=0.532$ for 10^{-8} M; $p=0.346$ for 10^{-7} M; $p=0.377$ for 10^{-6} M; $p=0.424$ for 10^{-5} M), or 18 hours ($p=0.589$ for 10^{-9} M; $p=0.541$ for 10^{-8} M; $p=0.264$ for 10^{-7} M; $p=0.195$ for 10^{-6} M; $p=0.288$ for 10^{-5} M) (Figure 2).

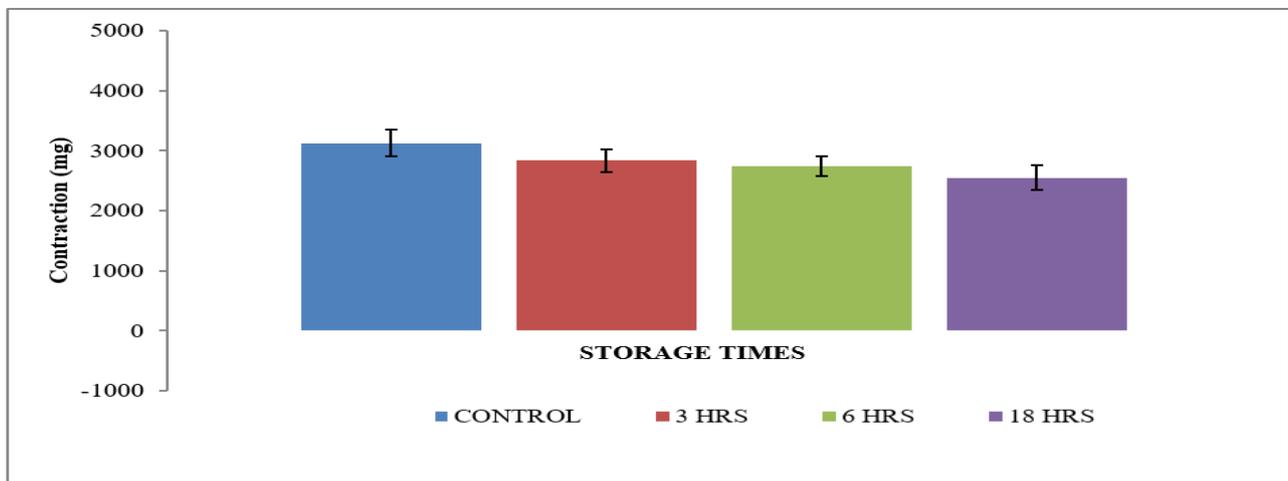


Figure 1. Comparison of maximal absolute (milligram) contractions in response to 10^{-5} M PE in rat thoracic aortas stored for 0 hours (control), 3 hours, 6 hours, and 18 hours in Krebs-Henseleit solution at $+4^{\circ}\text{C}$. The data were expressed as the mean \pm SD. $n=8$ in each group.

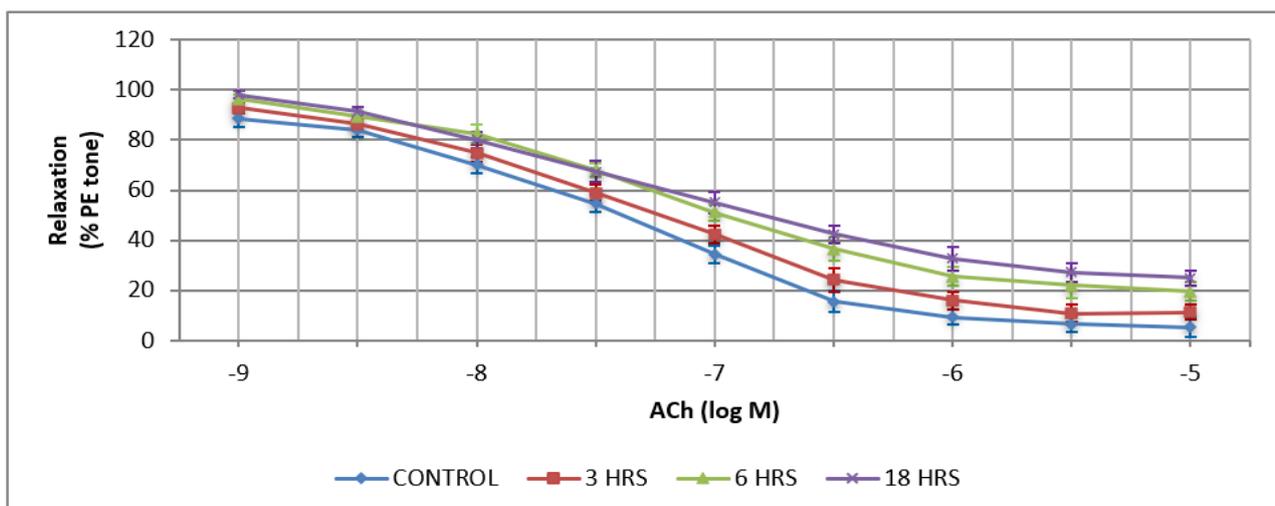


Figure 2. Endothelium-dependent relaxation responses induced by cumulatively administered (10^{-9} - 10^{-5} M) ACh in rat thoracic aortas. Aortic segments stored for 0 hours (control), 3 hours, 6 hours, and 18 hours in Krebs-Henseleit solution at $+4^{\circ}\text{C}$ were precontracted with submaximal (10^{-5} M) PE dose. The relaxant effects were assessed as a percentage of the contraction in response to 10^{-5} M PE. The data were expressed as the mean \pm SD. $n=8$ in each group. PE: phenylephrine. ACh: acetylcholine.

Cumulatively administered (10^{-9} - 10^{-5} M) SNP did not cause a statistically significant change in endothelium-independent relaxation responses in rat thoracic aortas precontracted by submaximal (10^{-5} M) PE dose after storage in Krebs-Henseleit solution at +4 °C for 3 hours ($p=1.000$ for 10^{-9} M; $p=1.000$ for 10^{-8} M; $p=1.000$ for

10^{-7} M; $p=1.000$ for 10^{-6} M; $p=1.000$ for 10^{-5} M), 6 hours ($p=0.579$ for 10^{-9} M; $p=0.591$ for 10^{-8} M; $p=0.604$ for 10^{-7} M; $p=0.345$ for 10^{-6} M; $p=0.339$ for 10^{-5} M), or 18 hours ($p=0.574$ for 10^{-9} M; $p=0.531$ for 10^{-8} M; $p=0.523$ for 10^{-7} M; $p=0.310$ for 10^{-6} M; $p=0.408$ for 10^{-5} M) (Figure 3).

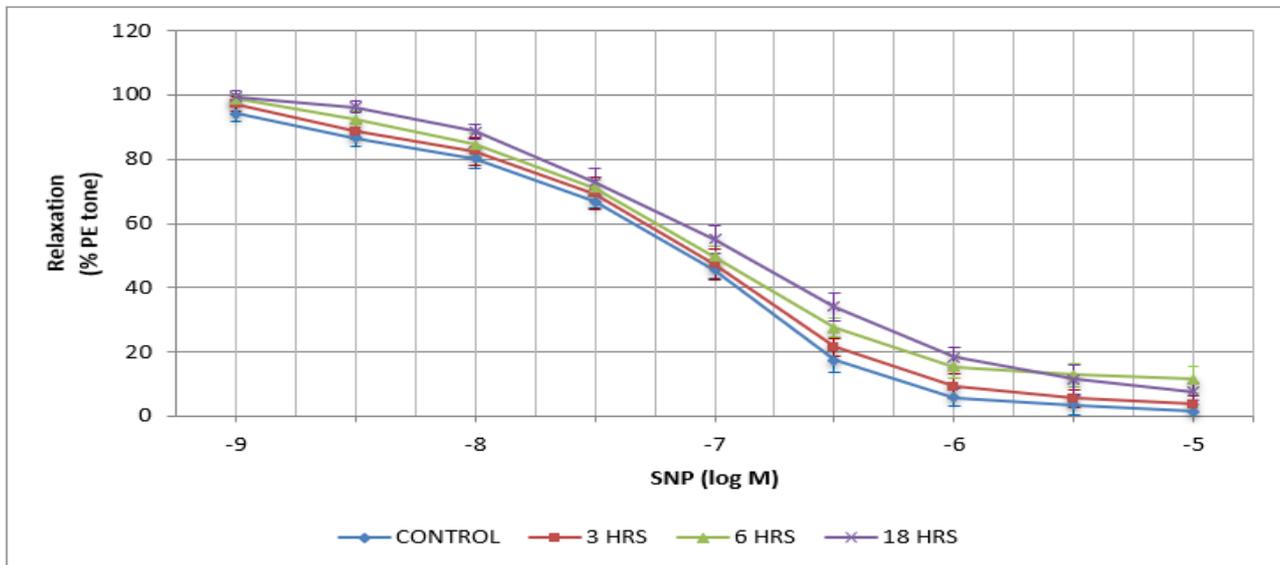


Figure 3. Endothelium-dependent relaxation responses induced by cumulatively administered (10^{-9} - 10^{-5} M) SNP in rat thoracic aortas. Aortic segments stored for 0 hours (control), 3 hours, 6 hours, and 18 hours in Krebs-Henseleit solution at +4 °C were precontracted with submaximal (10^{-5} M) PE dose. The relaxant effects were assessed as a percentage of the contraction in response to 10^{-5} M PE. The data were expressed as the mean \pm SD. n=8 in each group. PE: phenylephrine. SNP: sodium nitroprusside.

DISCUSSION

This study aimed to investigate the effects of cold storage on the vascular activity of rat thoracic aorta. In this regard, it was shown for the first time that both endothelium-dependent and -independent relaxation responses can be preserved intact in rat thoracic aorta after storage for 3, 6, and 18 hours at +4 °C in Krebs-Henseleit solution.

It is important for the efficiency of experimental studies that vascular tissues can be stored for longer periods without disturbing their viability. The ability to preserve the functional effects of tissues without deterioration allows the use of different vessels/tissues of the same animal in isolated tissue bath studies. Thus, it can also be possible to investigate the long-term effects of drugs. Moreover, since the transplanted organs are usually transplanted together with the surrounding vascular beds, it is important for the success of the transplantation that the functions of the vessels can be preserved intact. In the literature, for this purpose, methods of culturing vascular tissues, freezing, or storing them in cold (+4 °C) have been tried. Many studies are showing that contraction responses differ in cultured vessels (Binko et al., 1999; Thyberg, 1996). Another method is to freeze the vessels in liquid nitrogen. However, it has been reported that relaxation responses and smooth muscle contractions related to endothelium and smooth muscle are impaired in this method (Langerak et al., 2001;

Rendal et al., 2004; Rendal Vázquez et al., 2004a; Rendal Vázquez et al., 2004b).

Recently, studies have been conducted to minimize functional loss by adding preservative agents such as dimethyl sulfoxide (DMSO) and sucrose to the storage solutions of tissues during the freezing process or by gradually freezing and thawing them. However, none of these studies found that endothelium- or smooth muscle-derived relaxation responses of vascular tissues were impaired, as well as contraction responses (Langerak et al., 2001; Rendal et al., 2004). In studies using the cold storage method, vascular functions were tested in various storage solutions, periods, and temperatures (Kristek et al., 1993; Neil et al., 2002; Payne et al., 2002; Piepot et al., 2002; Rinaldi, 2001; Török et al., 1993). It has been reported that contraction responses, as well as endothelium-dependent relaxation responses, are impaired in isolated vascular preparations that are kept cold (for a long time) for days (Kristek et al., 1993; Török et al., 1993). In tissues that were stored for a shorter period, overnight, better results were obtained, depending on the storage solution used. For instance, with the University of Wisconsin (UW) solution, endothelium- or smooth muscle-dependent relaxation responses in canine coronary microvascular vessels were protected (Murphy et al., 1997).

It has been reported that the sensitivity to ACh did not change in rat aortas stored in Hepes-Krebs-Henseleit

solution for 24-48 hours, but the maximal response to ACh and contraction agents decreased (Stanke-Labesque et al., 1999). Similarly, in the present study, ACh sensitivity was shown to be unchanged in rat thoracic aortic rings stored in Krebs-Henseleit solution at +4°C for 3, 6, or 18 hours. This study also demonstrated that ACh-induced maximum relaxation response was not affected by cold storage for these times.

It is known that in cases where endothelial functions are impaired and therefore nitric oxide (NO) production decreases, contraction responses increase due to the decrease in basal relaxation provided by nitric oxide (Amerini et al., 1995; Mendizabal et al., 1999; Vo et al., 1992). Therefore, in the current study, the absence of any increase in PE-induced contractile responses indicates that endothelial function is preserved. Besides, the absence of a significant decrease despite a slight attenuation in PE-mediated contraction responses indicates that the dynamics in smooth muscle contraction physiology are still effective and vascular functions can continue without deterioration.

In this study, 3, 6, or 18 hours of cold storage did not cause a significant change in relaxation responses to SNP. These findings are consistent with studies showing that endothelium-independent relaxation responses do not change after cold storage in rat aortas (Stanke-Labesque et al., 1999). The fact that 3, 6, or 18 hours of cold storage did not affect the contraction-relaxation responses in rat thoracic aortas suggests that the characteristic properties of smooth muscle do not change.

The main difference in storage solutions is that the ion concentrations in the solutions mimic the extracellular ion content, such as Krebs-Henseleit solutions, or the intracellular ion content, such as Euro Collins or UW solutions (Mühlbacher et al., 1999). Preservation of vasoactive properties after storage at +4 °C indicates that Krebs-Henseleit solution is a suitable storage solution for cold storage of vessels.

In conclusion, this study shows that ideal conditions can be provided in the rat thoracic aorta by using the appropriate solution and time, in which vasoactive functions can be kept intact. In this regard, the first physiological findings have been obtained that the endothelium-dependent and -independent contraction-relaxation responses of rat thoracic aortas can be preserved intact after storage periods of 3, 6, or 18 hours in Krebs-Henseleit solution at +4°C. These results suggest that different types of vessels may also be isolated from an animal for later use in isolated organ bath experiments. Besides, the results of this experimental study may also provide a prediction regarding the viability of vascular tissues used in transplant surgeries. However, further studies using different animal models, vessel types, solutions, and temperatures are needed to obtain more precise results regarding storage times.

Conflict of Interest

The author declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author Contributions

Plan, design: SD; Materials and Methods: SD; Data analysis and interpretation: SD; Writing and corrections: SD.

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Comparison of PCR Methods for Determination of Different Types of Milk Added to Goat Milk

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ABSTRACT

Objective: This study, it was aimed to determine which of the multiplex conventional PCR and Real-Time PCR methods are more suitable for the detection of cow and sheep milk mixed with goat milk. **Materials and Methods:** For this purpose, one liter of each goat, cow, and sheep milk was obtained from farms in Van province. PCR experiments were carried out by adding cow's milk and sheep's milk in the same proportions into goat milk (1%, 2%, 5%, 0.1%, and 0.5%). Multiplex conventional and Real-Time PCR were used in these trials. **Results:** In the cow and sheep milk trials, it was determined that the presence of 1%, 2%, and 5% cow and sheep milk added to goat milk could be determined by the multiplex conventional PCR method. However, it was observed that the positivity of the gel image of the milk mixtures added at the rate of 0.5% was unclear, and the mixtures at the rate of 0.1% could not be detected. In the Real-Time PCR method, the presence of cow and sheep milk was detected in all the mixtures and positive graphics were determined. **Conclusion:** This showed that the Real-Time PCR method gives more reliable results even when 0.1% cow or sheep milk is mixed with commercially available goat milk.

Keywords: Goat milk, Multiplex conventional PCR, Real Time PCR.

Keçi Sütüne Eklenen Farklı Süt Türlerinin Belirlenmesi İçin PCR Yöntemlerinin Karşılaştırılması

ÖZ

Amaç: Bu çalışma, keçi sütüne karıştırılmış inek ve koyun sütünün tespiti için multiplex konvansiyonel PCR ve Real Time PCR yöntemlerinden hangisinin daha uygun olduğunun belirlenmesi amacıyla yapılmıştır. **Gereç ve Yöntem:** Bu amaçla Van ilindeki çiftliklerden keçi, inek ve koyun sütünden birer litre süt temin edilmiştir. Keçi sütüne aynı oranlarda inek sütü ve koyun sütü (0.5%, 0.1%, 1%, 2%, 5%) ilave edilerek PCR deneyleri yapılmıştır. Bu denemelerde multiplex konvansiyonel ve Real Time PCR kullanıldı. **Bulgular:** İnek ve koyun sütü denemelerinde keçi sütüne ilave edilen %1, %2 ve %5 inek ve koyun sütünün varlığının multiplex konvansiyonel PCR yöntemi ile belirlenebileceği ortaya konmuştur. Ancak %0.5 oranında eklenen süt karışımlarının jel görüntüsünün pozitifliğinin belirsiz olduğu ve %0.1 oranındaki karışımların tespit edilemediği görülmüştür. Real Time PCR yönteminde ise tüm karışımlarda inek ve koyun sütü varlığı tespit edildi ve pozitif grafikler belirlendi. **Sonuç:** Bu sonuçlar, Real Time PCR yönteminin, ticari olarak satılan keçi sütü ile %0.1 inek veya koyun sütü karıştırıldığında bile daha güvenilir sonuçlar verdiğini gösterdi.

Anahtar Kelimeler: Keçi sütü, Multiplex konvansiyonel PCR, Real Time PCR.

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INTRODUCTION

Goat milk, it contains less trans fatty acids than cow's milk, it is easier to digest and has less allergic effects. Since a large amount of milk can be obtained from goat breeds with high milk yield, goat breeding and goat milk production are increasing in our country and in the world. Accordingly, consumers tend to buy more goat milk and its products (Paszczyk and Łuczyńska, 2020).

Depending on the seasons, the production of goat milk mostly by families engaged in farming and small-scale enterprises and the increasing demand of consumers for goat milk cause this milk to be offered for sale at higher prices compared to cow and sheep milk. For this reason, it has come to the fore that the milk of different kinds of animals, especially cow's milk, can be mixed with goat's milk, since it is cheaper and more plentiful. If this situation is not reported on the label of the product, it is considered as adulteration and is prohibited according to the legislation of many countries (Golinelli et al., 2014; Alikord et al., 2018).

It is reported that the information on the labels of some foods in the world does not reflect the truth, and it is stated that such practices negatively affect food safety (Di Pinto et al., 2017). Food labels should enable consumers to make informed choices about the products they buy and should always contain accurate information (TFC, 2017). In addition, illegal trade should be avoided and the origin of the milk types should be verified so that unfair profits can be prevented (Golinelli et al., 2014; TFC, 2017). Species detection in dairy products has recently attracted great interest, as the identification of species substitutes or mixtures is important for consumer protection and public health (Bottero et al., 2009). In Türkiye, the Turkish Food Codex Communiqué on Drinking Milk (TFC, 2019) states that the labels of other milks, excluding cow's milk, which are offered to the market, should include the product name and the information from which animal the milk is obtained from.

Many analytical methods, including immunological approaches have been developed to determine which animal species the milk belongs to (Abbas et al., 2018). These methods are chemical, Enzyme-Linked Immunosorbent Assay, (ELISA) (López-Calleja et al., 2007; Stănciuc (Sava) and Răpeanu, 2010; González-Martínez et al., 2018), Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC) (Ten-Domenech et al., 2015), Urea Polyacrylamide Gel Electrophoresis (Urea PAGE) (Duarte-Vázquez, 2018), Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) (Ma et al., 2019) and Isoelectric Focusing Methods (Chen et al., 2004).

Electrophoretic methods based on differences in chromatic and protein profiles have disadvantages such as time consuming and not being economical (Alikord et al., 2018). The polymerase chain reaction

(PCR) method on the other hand, is used as an alternative method to determine whether different milk or dairy products have been added to the examined milk (Bottero et al., 2003; López-Calleja et al., 2004; Cheng et al., 2006; Mašková and Paulíčková, 2006; Rodrigues et al., 2012; Di Pinto et al., 2017). The PCR method is a fast and sensitive method used for determination and verification of milk type, and it is also the method with the lowest margin of error (Cosenza et al., 2019). In addition, DNA-based methods such as PCR have also been applied to ripened cheeses and heated dairy products, compared to protein-based methods that are not always applicable and must be carefully selected (López-Calleja et al., 2007; Stănciuc (Sava) and Răpeanu, 2010; Agrimonti et al., 2015; Kara et al., 2016).

Although the European Union proposes protein-based methods for species identification (CR, 2001), nucleic acid-based techniques have been used instead of protein for species identification, especially in foods of animal origin (Kumari et al., 2015). Among these techniques, PCR, multiplex PCR, Restriction Fragment Length Polymorphism (RFLP), PCR-RFLP and Real Time PCR are the most widely used molecular techniques (Bottero et al., 2003; Natonek-Wiśniewska and Krzyścin, 2019).

With the PCR method, which is among the DNA-based technologies, small amounts of DNA can be amplified quickly and specifically (Rodríguez-Ramírez et al., 2011). In addition, since the gene regions targeted by PCR are relatively small, DNA molecules degraded by the thermal, chemical and/or physical processes in which the food is prepared can also be detected (Drummond et al., 2013).

Complicated mixes can be determined in a single step when using the multiplex conventional PCR method, provided that the specific amplicons are of different lengths and are readily determined by agarose gel electrophoresis. Multiplex conventional PCR method can be applied for the separation of cow, goat, sheep and buffalo milk (Bottero et al., 2003). However, conventional techniques allow the qualitative detection of different species in the presence of a defined detection limit. On the other hand, in the Real Time PCR method, the identification of species is quantitative and this method has more sensitivity (Natonek-Wiśniewska and Krzyścin, 2019).

This research study was carried out to determine which of the multiplex conventional and Real Time PCR methods is more suitable for the detecting of cow and sheep milk mixed with goat milk.

MATERIALS AND METHODS

Material

In this study, cow, sheep and goat milk (one liter each) was obtained from farms in Van. Cow and sheep milks were added to goat milk at the rates of 0.5%, 0.1%, 1%, 2%, and 5%, respectively.

Method

DNA extraction

Commercial kit (GeneAll, Exgene™ Cell SV, South Korea) was used for DNA extraction from milk. Pure DNA samples obtained in accordance with the manufacturer's recommendations were stored at -20 ± 1 °C until the analysis.

Multiplex conventional PCR method

In this research, specific primers for goat, sheep and cow species were used, which target 12 and 16 mitochondrial rRNA and designed by Bottero et al. (2003) (Table 1).

Ready commercial master mix (abm 2X PCR Taq Plus Master Mix, Canada) was used for the multiplex conventional PCR analysis used to determine the DNAs of the species. In the preparation of PCR mixes, 1 µl (10 µM) of each primer and 5 µl of genomic DNA were added to the 10 µl master mix and the total volume was completed to 25 µl with PCR water. For

DNA amplification of the PCR mixture created for the determination of cow and sheep milk in goat milk, the DNA amplification was carried out by a thermocycler (Qiagen Rotor-Gene Corbett Research, USA) with an initial denaturation of DNA at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 30 sec., annealing at 58 °C for 60 sec., extension at 72 °C for 60 sec., followed by a final extension at 72 °C for 5 min. Multiplex conventional PCR analysis was performed in five replications.

Agarose gel electrophoresis

Gel-red (abm, Safe View Classic™ G108, Canada) stained 1.5% agarose gel was prepared for gel electrophoresis of amplicons obtained as a result of multiplex conventional PCR process. Specific DNA and positive control bands obtained with the help of DNA marker were observed in the gel imaging device (Genesis®; England).

Table 1. Oligonucleotides used as multiplex conventional PCR primers.

Species and genes	Oligonucleotide primers	bp
Goat (<i>Capra hircus</i>) M55541 ^a	Sens 144 F: 5'CGCCCTCCAAATCAATAAG 3' Antisens 469 R: 5'AGTGTATCAGCTGCAGTAGGGTT3'	326 bp
Sheep (<i>Ovis aries</i>) NC001941 ^a	Sens959 F: 5'ATATCAACCACACGAGAGGAGAC 3' Antisens 1130 R: 5'TAAACTGGAGAGTGGGAGAT3'	172bp
Cow (<i>Bos taurus</i>) NC001567 ^a	Sens 916 F: 5'GTACTACTAGCAACAGCTTA 3' Antisens 1171 R: 5'GCTTGATTCTCTTGGTGTAGAG3'	256 bp

^a GenBank accession number

Real Time PCR method

In order to determine the DNA of the species, a commercial kit (DIAGEN 2103, 2104, 2110, Türkiye) was used for Real Time PCR analysis. For this purpose, PCR mix consisting of 10 µl mix A, 5 µl mix B and 5 µl DNA of each species was prepared in line with the manufacturer's recommendations. The DNA amplification was carried out by a thermocycler (Qiagen Rotor-Gene Corbett Research, USA) with an initial denaturation of DNA at 95 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 10 sec., annealing at 59 °C for 30 sec., extension at 72 °C for 5 sec., followed by a final extension at 25 °C for 1 min.

Real-time PCR analysis was performed in five replications.

Ethical consideration

Ethics committee approval is not required as no living material was used in the study.

RESULTS

The results of multiplex conventional PCR analysis of the samples added to goat milk at the rate of 0.5%, 0.1%, 1%, 2%, and 5%, cow's milk and also sheep's milk in the same proportions are shown in Figures 1 and 3, and also Real Time PCR analysis results are shown in Figures 2 and 4.

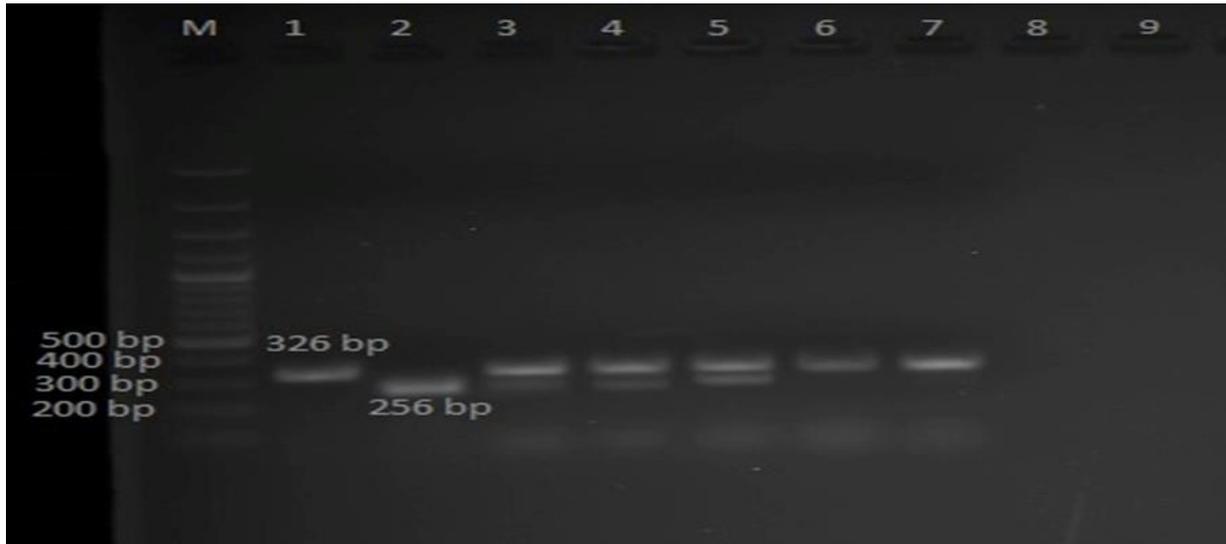


Fig 1. The image of the presence of cow's milk obtained by multiplex conventional PCR method in experimentally prepared goat milk. (M: 100 bp marker; 1: Goat milk (%100) (326 bp); 2: Cow milk (%100) (256 bp); 3: Goat and cow milk (1%) mix; 4: Goat and cow milk (2%) mix; 5: Goat and cow milk (5%) mix; 6: Goat and cow milk (0.1%) mixture; 7: Goat and cow milk (0.5%) mix; 8: Goat primer + cow milk DNA; 9: Cow primer + goat milk DNA)

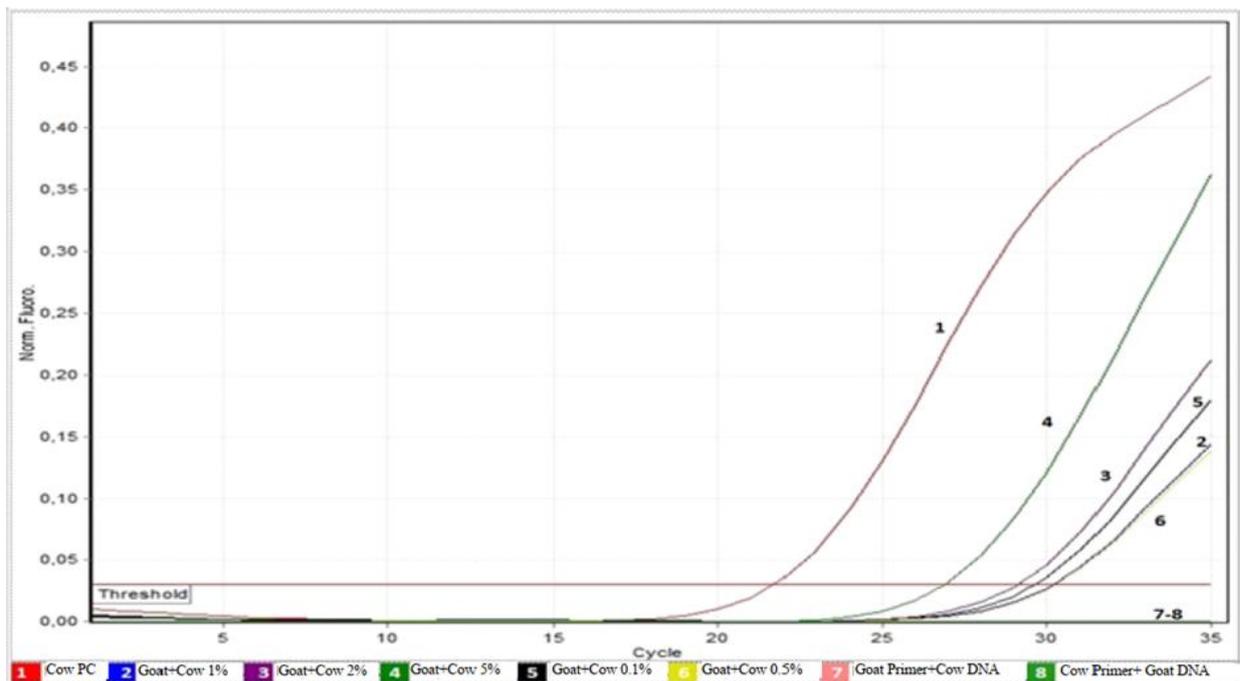


Fig 2. The image of the presence of cow's milk obtained by Real Time PCR method in experimentally prepared goat milk. (1: Cow's milk PC; 2: Goat and cow milk (1%) mix; 3: Goat and cow milk (2%) mix; 4: Goat and cow milk (5%) mix; 5: Goat and cow milk (0.1%) mixture; 6: Goat and cow milk (0.5%) mix; 7: Goat primer + cow milk DNA; 8: Cow primer + goat milk DNA).

Ct values of Real Time PCR results obtained from goat and cow milk mixtures are given in Table 2, and

Ct values of Real Time PCR results obtained from goat and sheep milk mixtures are given in Table 3.

Table 2. Ct values of Real Time PCR results obtained from goat and cow milk mixtures.

No.	Colour	Name	Type	Ct
1	Red	Cow milk	Positive Control	21.73
2	Blue	Goat's milk + 1% cow's milk	-	30.20
3	Purple	Goat's milk + 2% cow's milk	-	29.15
4	Green	Goat's milk + 5% cow's milk	-	26.90
5	Black	Goat's milk + 0.1% cow's milk	-	29.68
6	Yellow	Goat's milk + 0.5% cow's milk	-	30.29
7	Red	Goat primer + cow milk DNA	Negative Control	
8	Green	Cow primer + goat milk DNA	Negative Control	



Figure 3. The image of the presence of sheep milk obtained by multiplex conventional PCR method in experimentally prepared goat milk. (M: 100 bp marker; 1: Goat milk (%100) (326 bp); 2: Sheep milk (%100) (172bp); 3: Goat and sheep milk (1%) mix; 4: Goat and sheep milk (2%) mix; 5: Goat and sheep milk (5%) mix; 6: Goat and sheep milk (0.1%) mixture; 7: Goat and sheep milk (0.5%) mixture; 8: Goat primer + sheep milk DNA; 9: Sheep primer + goat milk DNA).

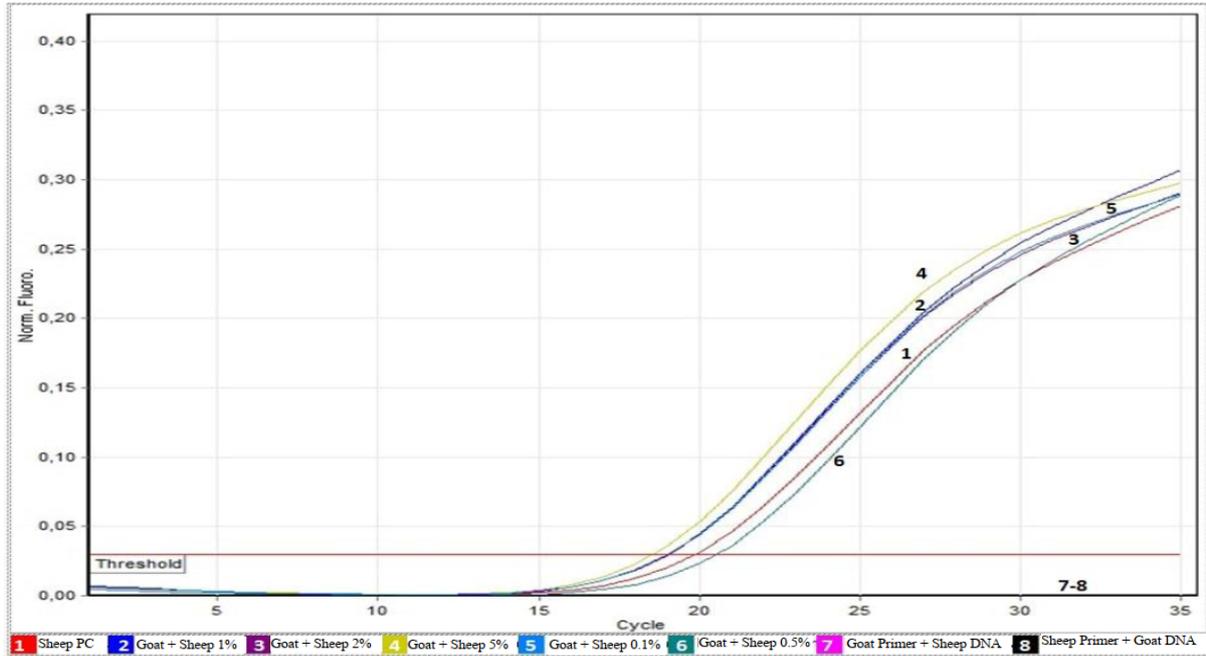


Fig 4. The image of the presence of sheep milk obtained by Real Time PCR method in experimentally prepared goat milk. (1: Sheep milk PC; 2: Goat and sheep milk (1%) mix; 3: Goat and sheep milk (2%) mix; 4: Goat and sheep milk (5%) mix; 5: Goat and sheep milk (0.1%) mixture; 6: Goat and sheep milk (0.5%) mixture; 7: Goat primer + sheep milk DNA; 8: Sheep primer + goat milk DNA).

Table 3. Ct values of Real Time PCR results obtained from goat and sheep milk mixtures.

No.	Colour	Name	Type	Ct
1	Red	Sheep milk	Positive Control	19.87
2	Blue	Goat's milk + 1% sheep's milk	-	19.05
3	Purple	Goat's milk + 2% sheep's milk	-	19.05
4	Yellow	Goat milk + 5% sheep milk	-	18.57
5	Cyan	Goat milk + 0.1% sheep milk	-	19.02
6	Teal	Goat milk + 0.5% sheep milk	-	20.54
7	Magenta	Goat primer + sheep milk DNA	Negative Control	
8	Black	Sheep primer + goat milk DNA	Negative Control	

DISCUSSION

Milk, which contains most of the nutrients in sufficient amounts and is considered the closest food to perfection, has an important place in the nutrition of individuals of all ages (FDA, 1995). Many proteins in milk and dairy products are potential allergens and have a significant effect on the formation of food allergies. In addition, cow's milk has been reported to be a dairy product responsible for the adverse reaction (Rance et al., 2005). Species definitions in milk and dairy products are important in terms of preventing health risks that may occur in people who are sensitive to some dairy products and ensuring food

safety. However, in commercial milk production, the use of cheaper milk instead of high quality and costly milk is a common practice that deceives the consumer (Khatun et al., 2021). For these reasons, the use of analytical methods that give fast and accurate results in the analyses and adequate inspections will contribute to the protection of public health (Derinöz et al., 2021). In this study, the detection limit was determined as 1% in the multiplex conventional PCR method. Cheng et al. (2006), in their study with the PCR method, stated that they found the detection limit in goat milk powder to be 0.5-1% after adding 0.5%, 0.1%, 1% and 2% cow's milk into the goat milk

powder. Mašková and Paulíčková (2006), in their study on the determination of the presence of cow's milk in goat and sheep cheeses using the multiplex PCR method, reported that cow's milk was found in 3 of 17 goat cheeses and 1 of 7 sheep cheeses, and the detection limit in the study was determined as 1%. It is seen that the detection limits determined in different studies are similar to the detection limits determined in this study. Bottero et al. (2003) reported that the minimum detection limit was determined as 0.5% in the multiplex PCR method they applied for species identification in the curd cheese they produced by adding 1%, 0.5% and 0.1% cow's milk to goat milk. In a study by López-Calleja et al. (2004), in which cow's milk (0.5%, 0.1%, 1.5%, 10% and 100%) was added to sheep and goat milk to identify species, the multiplex PCR method was used to determine the percentage of different milks in raw and heat-treated milk and dairy products, it has been stated that it can be detected at a rate of 0.1%. Rodrigues et al. (2012) reported that the detection limit was 0.5% in their study by adding cow's milk at 0%, 0.1%, 0.5%, 1%, 5%, 10%, 50% and 100% concentrations to fresh goat milk to determine the analytical sensitivity of the duplex PCR method. It is thought that the differences between the studies may have arisen from the devices, consumables, materials and methods used in the analysis. In this study, the detection limit was determined as 0.1% in the Real Time PCR method. Agrimonti et al. (2015), in their study using the quadruplex PCR (qPCR) test to detect cheats in dairy products, added buffalo, sheep and goat milk to cow's milk at a rate of 0.1%, 0.5%, 1%, 1%, 1% and 25%. They reported that they found the qPCR detection limit to be 0.1% in their study. In this study, the detection limit determined by the Real Time PCR method in milk is similar to the findings of different studies. Di Pinto et al. (2017) reported that 58 of the 80 goat milk products they examined using the end-point PCR method were not compatible with the information specified on the labels. In the same study, they stated that both Real Time PCR results confirmed the end-point PCR results and 64 out of 80 products examined according to the Real Time PCR method were inconsistent with the information specified on the labels. The fact that Di Pinto et al. (2017) reported that the Real Time PCR method gave more sensitive results is in line with the findings of this study. In addition, some other researchers (Drummond et al., 2013) have also stated that the Real Time PCR method has more sensitivity.

CONCLUSION

As a result of this research, it was determined that the presence of cow and sheep milk (at the rate of 1%, 2% and 5%) added to goat milk could be detected by multiplex conventional PCR method. However, it was observed that the positivity of the gel image of the milk mixtures added at the rate of 0.5% was unclear, and the mixtures at the rate of 0.1% could not

be detected. The high detection limit in the multiplex conventional PCR method based on gel electrophoresis makes it difficult to determine the different milks mixed into the milk in very small amounts. Conventional techniques allow only qualitative detection of different types of milk with a defined detection limit. In addition, the fact that this method requires more than one repetition while optimizing the temperature and time caused wastage of time and consumables. In the Real Time PCR method, the presence of cow and sheep milk was detected in all of the mixtures (1%, 2%, 5%, 0.5% and 0.1%) and positive graphics were determined. This showed that the presence of even 0.1% cow and sheep milk in commercially available goat milk can be detected more reliably by the Real Time PCR method. The lower detection limit in the Real Time PCR method can be attributed to the originality and higher sensitivity of this method. As a result, it has been seen that the detection limit is high in the multiplex conventional PCR method, and it requires more than one repetition while optimizing the temperature and time, which causes waste of time and consumables. In the Real Time PCR method, it has been determined that the detection limit is lower, even cow and sheep milk, which is added to goat milk at very low rates, can be determined and fast results can be obtained in a short time. For these reasons, it has been concluded that the Real Time PCR method is a fast and effective method that can be used routinely for the detection of cow and sheep milk mixed with goat milk.

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Conflict of Interest

The author declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Author Contributions

Plan, design: RMT, YCS; **Material, methods and data collection:** RMT; **Data analysis and comments:** RMT, YCS; **Writing and corrections:** RMT, YCS.

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COVID-19 Pandemi Döneminde Hemşirelik Öğrencilerinin Uzaktan Eğitim Sürecine İlişkin Görüş ve Tutumlarının Değerlendirilmesi

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ÖZ

Amaç: Bu araştırma, COVID-19 pandemi döneminde hemşirelik bölümü lisans öğrencilerinin uzaktan eğitim sürecine ilişkin görüş ve tutumlarının değerlendirilmesi amacıyla yapılmıştır. **Gereç ve Yöntem:** Araştırma tanımlayıcı bir çalışma olarak 16-25 Temmuz 2020 tarihleri arasında yapılmıştır. Araştırma örneklem grubu 434 hemşirelik öğrencisinden oluşmaktadır. Verilerin toplanmasında; "Bireysel Bilgi Formu" ve "Çevrimiçi Öğrenme Tutum Ölçeği (ÇÖTÖ)" kullanılmıştır. Verilerin analizinde; sayı, yüzde dağılımları, ortalama ve standart sapma değerleri hesaplanmış, karşılaştırmalarda bağımsız örneklem t testi, tek yönlü varyans analizi ve Pearson korelasyon analizi kullanılmıştır. **Bulgular:** Araştırmaya katılan öğrencilerin ÇÖTÖ puan ortalaması 54.81±16.63 olup, çoğunluğunun COVID-19 pandemi sürecinde uzaktan eğitimden memnun olduğu (%75.6), uzaktan eğitimde sınav ve ödevler hakkında zamanında geri bildirim aldığı (%81.5) ve uzaktan eğitimde bağlantı sorunu yaşamadığı (%81.5) bulunmuştur. Araştırmaya katılan öğrenciler arasında dördüncü sınıf öğrencilerinin birinci sınıf öğrencilere göre ÇÖTÖ puan ortalamasının daha yüksek olduğu belirlenmiştir. Bunun yanı sıra yüz yüze eğitimi tercih edeceğini belirtenlerin diğer eğitim yöntemlerini tercih edenlere göre ÇÖTÖ puan ortalamalarının daha düşük olduğu bulunmuştur. ÇÖTÖ puan ortalaması ile yaş arasında pozitif yönde ilişki bulunduğu belirlenmiştir ($r=0.153$, $p<0.05$). Aynı zamanda öğrencilerin akademik ortalaması ile ÇÖTÖ puan ortalaması arasında negatif yönde anlamlı ilişki bulunmuştur ($r=-0.159$, $p<0.05$). **Sonuç:** Hemşirelik öğrencileri çevrimiçi öğrenmeye yönelik kararsız tutum gösterse de hibrit eğitim sistemini istedikleri görülmektedir. İleri çalışmalarda her iki eğitim sistemine dair öğrenci görüşlerinin eğitim çıktılarıyla birlikte değerlendirilmesi önerilmektedir.

Anahtar Kelimeler: COVID-19, Hemşirelik, Uzaktan Eğitim, Hemşirelik Öğrencisi.

Evaluation of Nursing Students' Views and Attitudes on Distance Education Process During the COVID-19 Pandemic Period

ABSTRACT

Objective: This study was conducted to evaluate the views and attitudes of undergraduate nursing students regarding the distance education process during the COVID-19 pandemic period. **Materials and Methods:** The research was conducted between 16-25 July 2020 as a descriptive study. The research sample group consists of 434 nursing students. In the collection of data; "Personal Information Form" and "Online Learning Attitude Scale (OLAS)" were used. In the analysis of data; numbers, percentage distributions, mean and standard deviation values were calculated, and independent sample t test, one-way analysis of variance and Pearson correlation analysis were used for comparisons. **Results:** The mean score of the students participating in the research was 54.81±16.63, and it was found that the majority of the students were satisfied with distance education (75.6%) during the COVID-19 pandemic, received timely feedback about exams and homework in distance education (81.5%), and did not experience connection problems in distance education (81.5%). Among the students participating in the research, it was determined that the fourth grade students had a higher mean score of OLAS compared to the first grade, while those who preferred face-to-face education had a lower mean score of OLAS than those who preferred other education methods. It was determined that there was a positive correlation between the mean score of OLAS and age ($r=0.153$, $p<0.05$). At the same time, a significant negative correlation was found between the academic average of the students and the mean score of the OLAS ($r=-0.159$, $p<0.05$). **Conclusion:** Even though nursing students show an indecisive attitude towards online learning, it is seen that they want the hybrid education system. In further studies, it is recommended to evaluate students' views on both education systems together with their education outcomes.

Keywords: COVID-19, Distance Education, Nursing, Nursing Student.

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GİRİŞ

SARS-CoV-2 virüsünün neden olduğu COVID-19 pandemisi, dünya genelinde günlük yaşamdaki kısıtlamalarla, sağlık, lojistik, turizm sektörlerinin yanı sıra eğitim alanında da önemli değişikliklere neden olmuştur. Bu dönemde virüs yayılım hızını kontrol altına almaya yönelik alınan önlemler kapsamında yüz yüze eğitime ara verilerek uzaktan eğitime geçiş yapılmıştır (Chen ve ark., 2020; Fawaz ve Samaha, 2020). Ülkemizde de COVID-19 pandemi dönemiyle birlikte, Yükseköğretimde zorunlu uzaktan eğitim uygulamaları çevrimiçi (senkron) ve/veya asenkron olarak yürütülmüştür (YÖK, 2020a). Üniversitelerde uzaktan eğitim alt yapı sistemleri oluşturularak, çevrimiçi ders, ders kaynaklarının sisteme yüklenmesi, devam kontrolü, ödev atama, not verme ve mesajlaşma gibi özellikleri olan uzaktan eğitim sistemleri aracılığıyla eğitim faaliyetleri yürütülmüştür (YÖK, 2020a).

Uzaktan eğitim kapsamında verilen çevrimiçi eğitim, eğitimci ve öğrencinin aynı anda senkron ve interaktif olarak etkileşimde bulunduğu eğitim yaklaşımı olarak tanımlanmaktadır. Çevrimiçi eğitim, eğitimci ve öğrencinin iş birliğine, tartışma, soru-cevap etkinlikleri düzenleme gibi faaliyetlere imkân sağlamaktadır. Online eğitim, çevrimiçi eğitim ile eş anlamlı kullanılmaktadır. Bunun yanı sıra uzaktan eğitim kapsamında verilen asenkron eğitim ise bilginin önceden üretildiği ve depolandığı, öğrencinin istediği zaman tekrar yapmasına olanak sağlayan eğitim yaklaşımıdır (Chen ve ark., 2020; Basilaia & Kvavadze, 2020).

COVID-19 pandemi döneminde uzaktan eğitime ivedilikle geçilmesi, eğitim kurumlarının gerekli fiziki alt yapıya sahip olma durumu, öğrencilerin internete erişim imkânı, teknolojik kaynakların varlığı, eğitimcilerin dijital beceri ve deneyimleri eğitimin amaçlanan hedeflerine ulaşılması bakımından ele alınması gereken önemli hususlar arasında yer almıştır (Isaac ve ark., 2019). Bununla birlikte hemşirelik gibi insan hayatına dokunması bakımından kritik öneme sahip ve klinik beceriye dayalı mesleklerde, uzaktan eğitimde beklenen öğrenim çıktularına erişimin değerlendirilmesi oldukça önemli bir husustur.

Konu ile ilgili COVID-19 pandemisi öncesinde yapılan bazı araştırmalarda, hemşirelik öğrencilerinin çoğunluğunun e-öğrenmeye karşı olumsuz ya da tarafsız tutumları olduğu bildirilirken, bazı çalışmalarda ise bu tutumun olumlu olduğu bildirilmiştir (Hvalič - Touzery ve Lobe, 2015; Elbasuony ve ark., 2018; Ali ve ark., 2016; Akimanimpaye ve Fakude, 2015). Pandemi döneminde yapılan çalışmalar incelendiğinde; Kozan ve ark. (2021) uzaktan öğrenme sürecinde video konferans yönteminin, eğitimci ile etkileşim sağlaması ve geleneksel sınıf ortamına en çok benzeyen yöntem olması nedeniyle öğrenciler tarafından öğrenmede en etkili yol olarak bildirilmiştir. Bunun yanı sıra sağlık alanında eğitim

gören öğrencileri kapsayan bazı araştırmalarda pandemi döneminde uzaktan eğitime yönelik öğrenci tutumlarının olumsuz olduğu belirtilmektedir (Abbasi ve ark., 2020; Oducado ve Soriano, 2020; Diab ve ark., 2020; Olum ve ark., 2020; Gaur ve ark., 2020). Beklenmedik şekilde gelişen ve sürece uyum sağlanmasının zorunlu olduğu pandemi döneminde 2019-2020 Yılı Bahar Dönemi için hemşirelik eğitiminin simülasyon, proje, vaka analizi vb. faaliyetlerle uzaktan eğitim yoluyla sürdürülmesi kararı alınmıştır (YÖK, 2020b). Pandemi sürecinde hemşirelik eğitiminde klinik uygulama veya yüz yüze eğitim dışında kullanılan yöntemler arasında simülasyon (%61), yüksek kaliteli mankenler (%12), telesağlık (%10) ve vaka çalışmaları, hibrit çevrimiçi uygulamalar ve diğer (% 17) eğitim faaliyetleri yer almıştır (Kalanlar 2022). Pandemi döneminde verilen uzaktan eğitime yönelik hemşirelik öğrencilerinin görüşlerinin incelendiği çalışmalarda; öğrenciler tarafından, uzaktan eğitime yönelik ekonomik olması, bireysel öğrenmeyi desteklemesi, zaman ve mekân sınırını en aza indirmesi ve ileri teknoloji kullanma imkânı sağlaması gibi olumlu görüşler ifade edilirken, eğitimci ile öğrenciler arasında etkileşimin azalması, sanal ortamı yönetmede zorluk, öğrenme güçlüklerinin hızlıca çözümlenemesi, uygulamaya dayalı konuların pekiştirilmesinde sınırlılıklar gibi olumsuz görüşler de bildirilmiştir (Kalanlar, 2022; Kozan ve ark., 2021; Uzelli Yılmaz, 2021; Keskin Kızıltepe ve Kurtgöz, 2020). Bunun yanı sıra hemşirelik gibi uygulamalı bilimler için klinik uygulamalar ve laboratuvar modüllerinin yürütülmesi konularında uzaktan eğitimin yeterli olmadığı yönünde görüşler de bildirilmiştir (Kozan ve ark., 2021). Nitekim Hemşirelik Eğitimi Derneği (HEMED) nitelikli hemşirelik eğitiminin COVID-19 pandemisi ile mücadeleye katkı sağlayacak önemli bir hizmet olduğunu vurgulamakla birlikte, yeterli hazırlık, denetim ve destek olmadan mezun edilecek hemşirelik öğrencilerinin kendilerini, hastalarını ve sağlık profesyonellerini daha fazla risk altına sokabileceklerini belirtmiştir (HEMED, 2020). Literatürde hemşirelik öğrencilerinin COVID-19 pandemi dönemi uzaktan eğitim deneyimleri üzerine sınırlı araştırmalar bulunsa da hemşirelik öğrencileri, uzaktan eğitimin hemşirelik eğitimi ve temel uygulamalı hemşirelik becerilerine uygun olmadığını, uzaktan eğitim sisteminin yeterince iyi işlemediği ve sanal hastanelerde hemşirelik klinik uygulamalarının yapılmasının uygun olmadığını düşündükleri belirtilmektedir (Ramos-Morcillo ve ark., 2020; Wallace ve ark., 2021; Kaya ve Işık, 2021; Terzi ve ark., 2021; Özkan ve ark., 2021). Ayrıca pandemi sürecinde uzaktan eğitim alan hemşirelik öğrencileri, klinik uygulamalara yönelik telafi programlarının gerekli olduğunu aksi halde kendilerini mesleki olarak yetersiz hissedeceklerini bildirmişlerdir (Özkan ve ark., 2021).

Bu bağlamda ülkemizde hemşirelik lisans öğrencilerinin uzaktan eğitime yönelik görüş ve

tutumlarının değerlendirilmesi dijital çağa özgü eğitim gereksinimlerinin belirlenmesi açısından önemlidir. Hemşirelik öğrencilerinin eğitim sistemine yönelik deneyimlerini ve beklentilerini anlamak, uzaktan eğitim sistemine özgü planlamalarda yönlendirici olabilir. Bu yönüyle araştırmanın hemşirelik eğitim ve öğretim faaliyetlerinin planlanmasında literatüre katkı sunacağı düşünülmektedir.

Araştırmanın amacı

Bu araştırma, COVID-19 pandemi döneminde hemşirelik lisans öğrencilerinin uzaktan eğitim sürecine ilişkin görüş ve tutumlarının değerlendirilmesi amacıyla yapılmıştır.

Araştırma soruları

- COVID-19 pandemi döneminde hemşirelik öğrencilerinin uzaktan eğitim sürecine yönelik görüşleri nedir?
- COVID-19 pandemi döneminde hemşirelik öğrencilerinin çevrimiçi öğrenmeye yönelik tutumları nedir?
- COVID-19 pandemi döneminde hemşirelik öğrencilerinin çevrimiçi öğrenmeye yönelik tutumları bireysel özelliklere göre farklılaşmakta mıdır?

GEREÇ VE YÖNTEM

Araştırmanın tipi, yeri ve zamanı

Bu araştırma, Ankara'da bir devlet üniversitesinin hemşirelik lisans programı öğrencileriyle, 16-25 Temmuz 2020 tarihleri arasında tanımlayıcı olarak yapılmıştır.

Araştırma evren ve örnekleme

Araştırma evreni ilgili üniversitenin hemşirelik lisans programında 2019-2020 akademik yılı bahar döneminde eğitim alan tüm öğrenciler oluşturmaktadır (N = 550). Araştırmada öğrencilerin tamamına ulaşılması hedeflenmiş olup, örneklem hesabına gidilmemiştir. Araştırma kapsamında evrenin %78.9'unu temsil eden 434 öğrenciyeye ulaşılmıştır.

Örnekleme büyüklüğünü hesaplamak için G*Power 3.1.9.6 yardımıyla, .25 etki büyüklüğü, .05 birinci tür hata ve .75 güç için 448 olarak elde edilmiştir. Çalışmaya 434 öğrenci katılmış, çalışma sonunda yapılan post-hoc güç analizi sonucunda; yaş ve akademik ortalamasının ÇÖTÖ ile ilişkisi için güç değerleri yaş için .888, not ortalaması için .915 elde edilmiştir.

Araştırma uygulamasının yapıldığı üniversitede, 30 Mart 2020 tarihinde uzaktan eğitime geçilmiş olup, 2019-2020 Yılı Bahar Döneminde yer alan Hemşirelik Lisans dersleri çevrimiçi (senkron) ve/veya asenkron yürütülmüştür. Hemşirelik birinci, ikinci ve üçüncü sınıflarda hemşirelik mesleğine özgü seçmeli ve mesleki uygulamalı zorunlu dersler asenkron eğitim şeklinde yürütülmüştür (power-point sunum, kitap, makale, ödev, vaka değerlendirmesi, video demonstrasyon gibi kaynak ve materyaller öğrencilerin otomasyon sistemine yüklenmiştir).

Dördüncü sınıflarda ise hemşirelik mesleğine özgü temel derslerde asenkron eğitim yönteminin yanı sıra, mesleki uygulama dersi çevrimiçi/senkron eğitim şeklinde yürütülmüştür. Bunun yanı sıra hemşirelik bölümünde Türk Dili ve Türk Tarihi dersleri gibi lisans bölümleriyle ortak zorunlu yürütülen dersler pandemi öncesinde çevrimiçi/senkron eğitim yöntemiyle yürütülmekte olup, pandemi döneminde aynı eğitim yöntemiyle verilmiştir.

Veri toplama araçları

Çalışma verilerinin toplanmasında; 'Bireysel Bilgi Formu' ve 'Çevrimiçi Öğrenme Tutum Ölçeği (ÇÖTÖ)' kullanılmıştır.

Bireysel Bilgi Formu: Araştırmacılar tarafından ilgili literatür doğrultusunda hazırlanan (Chen ve ark., 2020; Akimanimpaye ve Fakude, 2015; Gaur ve ark., 2020; Ramos-Morcillo ve ark., 2020) 'Bireysel Bilgi Formu' iki bölümden oluşmaktadır. Birinci bölümde; öğrencilerin sosyo-demografik özelliklerini belirlemeye yönelik dört soru maddesi (yaş, cinsiyet, sınıf düzeyi, genel not ortalaması) yer almaktadır. İkinci bölümde; öğrencilerin COVID-19 pandemi sürecinde almış oldukları uzaktan eğitime yönelik deneyimlerini belirlemeye ilişkin beş soru maddesi (uzaktan eğitim sürecine dair bilgiye erişim durumu, hazırlanan ödev sayısı, çevrimiçi eğitimde kullanılan araçlar, eğitim sürecinde kullanılan program, öğrencilerin eğitim yöntem tercihleri) yer almıştır. Aynı zamanda bu bölümde öğrencilerin uzaktan eğitime yönelik görüşlerinin belirlenmesi amacıyla 5'li Likert tipte araştırmacılar tarafından hazırlanan yedi soru yer almıştır.

Çevrimiçi Öğrenme Tutum Ölçeği (ÇÖTÖ): Ölçek Usta ve arkadaşları (2016) tarafından öğrencilerin çevrimiçi öğrenmeye karşı tutum düzeylerini belirlemek amacıyla geliştirilmiştir. Ölçek, 20 maddeden ve "Genel Kabul", "Bireysel Farkındalık", "Kullanışlılık (Zaman-Emek-Maliyet)", "Uygulama Etkililiği (Etkin Katılım)" olmak üzere dört alt boyuttan oluşmaktadır. Ölçek soru maddeleri 5'li likert tipte olup, (1) Kesinlikle Katılmıyorum, (2) Katılmıyorum, (3) Kararsızım, (4) Katılıyorum, (5) Kesinlikle Katılıyorum şeklinde puanlanmaktadır. Ölçekten alınan toplam puan 20 ile 100 arasında değişmektedir. Elde edilen toplam puanın yükselmesi, öğrencilerin çevrimiçi öğrenmeye yönelik olumlu tutuma sahip olduklarını göstermektedir. Ölçeğin kesme puanı veya tutum düzeyi gruplandırması bulunmamaktadır. Usta ve arkadaşlarının çalışmasında ölçeğin iç tutarlık katsayısı olan Cronbach alfa değeri 0.904 olarak bulunmuştur (Usta ve ark., 2016). Bu araştırmada ise ölçeğin Cronbach alfa değeri 0.943 olarak belirlenmiştir.

Veri toplama araçlarının uygulanması

Araştırma veri toplama formları "Google Formlar" ile hazırlanan online anket aracılığıyla toplanmıştır. Hemşirelik öğrencilerine online anket bağlantısı e-posta ve WhatsApp grupları aracılığıyla ulaştırılmıştır. Hemşirelik öğrencileri sosyal medya

aracılığıyla araştırmaya davet edilmiş, araştırmaya katılmayı kabul eden ve sorulara eksiksiz yanıt veren öğrenciler çalışmaya dahil edilmiştir.

Verilerin analizi

Verilerin analizi IBM SPSS Statistics 21.0 (IBM Corp.Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) programı ile yapılmıştır. Analizler sonucunda elde edilen tanımlayıcı istatistikler üzerinden post-hoc güç analizi GPower 3.1.9.6. ile elde edilmiştir. Kategorik değişkenler için frekans ve yüzde, sürekli değişkenler için ortalama ve standart sapma raporlanmıştır. Merkezi limit teoremi gereği birey sayısının 30'dan büyük olduğu kategoriler için parametrik testler (bağımsız örneklem t testi ve tek yönlü varyans analizi), 30'dan az olduğu durumda Kruskal-Wallis H testi kullanılmıştır (Alpar, 2020). Gruplar arasındaki farkı belirlemede post-hoc testi yapılmıştır. ÇÖTÖ ile öğrencilerin bazı özellikleri arasındaki ilişki için Pearson korelasyon katsayısı hesaplanmıştır. Anlamlılık düzeyi .05 olarak alınmıştır.

Araştırmanın etik boyutu

Araştırmanın yürütülebilmesi için bir devlet üniversitesi Etik Kurulundan (09.07.2020 tarih ve 42 karar no ile) yazılı izin alınmıştır. Öğrencilere veri toplama araçları doldurulmadan önce veri toplama formunun ilk sayfasında araştırma hakkında bilgilendirme yapılmış, öğrencilere çalışmanın herhangi bir aşamasında ayrılma hakkına sahip oldukları ve çalışmaya katılmanın gönüllülük esasına

dayandığı belirtilmiştir. Öğrencilerden çalışmaya katılmayı kabul etmeleri durumunda “çalışmaya katılmayı onaylıyorum” beyanını işaretlemeleri istenmiştir. Formu online ortamda tamamlayan öğrenciler araştırmaya katılmayı kabul etmiş sayılmıştır. Araştırma amacıyla öğrencilerden herhangi bir ücret talep edilmeyeceği ve/veya öğrencilere herhangi bir ücret ödenmeyeceği belirtilmiştir. Öğrencilere çalışma sonuçlarının ders notlarını etkilemeyeceği açıklanmıştır. Araştırma uygulaması ders ve sınavlar tamamlandıktan sonra gerçekleştirilmiştir. Araştırmacılarla öğrenciler arasında çıkar ilişkisi bulunmamaktadır. Çalışma kapsamında araştırmaya katılan öğrencilerin cevaplarının gizliliği sağlanmıştır.

BULGULAR

Araştırmaya katılan öğrencilerin (n=434) yaş ortalaması 20.78 ± 1.71 olup, öğrencilerin akademik başarı ortalaması 3.03 ± 0.45 'tir. Öğrencilerin %89.2'sinin cinsiyeti kadın olup, %26.3'ü dördüncü sınıfta eğitim almaktadır. Öğrencilerin %34.6'sının uzaktan eğitim sürecinde sınıf gruplarında yapılan duyurular aracılığıyla bilgi edindiği belirlenmiştir. Öğrencilerin uzaktan eğitim derslerine katılmak için çoğunlukla (%83.4) akıllı telefon kullandığı, çevrimiçi eğitim yapılan derslerde ise en çok (%79.1) Zoom programını kullandıkları belirlenmiştir. Öğrencilerin %53.9'unun pandemi döneminde “yüz yüze eğitim” almak istedikleri saptanmıştır (Tablo 1).

Tablo 1. Öğrencilerin bazı bireysel özelliklerinin dağılımı (n=434).

Bireysel özellikler	Sayı	%
Cinsiyet		
Kadın	387	89.2
Erkek	47	10.8
Sınıf		
Birinci	100	23.0
İkinci	111	25.6
Üçüncü	109	25.1
Dördüncü	114	26.3
Uzaktan eğitim sistemi bilgisine erişim kaynağı		
Sınıf gruplarında yapılan duyurular	150	34.6
Üniversite UZEM Youtube/Twitter/Instagram	139	32.0
Arkadaş	66	15.2
Üniversite-okul web sitesi	56	12.9
Danışman bilgilendirmesi	23	5.3
Hazırlanan toplam ödev (X±SS)		8.33±3.53
Kullanılan araç*		
Akıllı telefon	362	83.4
Bilgisayar	317	73.0
Tablet	28	6.5
Smart TV/diğer	4	0.9
Kullanmayan/katılmayan	3	0.7

Tablo 1. (Devam) Öğrencilerin bazı bireysel özelliklerinin dağılımı (n=434).

Uzaktan eğitim sürecinde kullanılan program		
Zoom	284	79.1
Üniversite Uzaktan Eğitim Merkezi	25	7.0
Google Meet	23	6.4
Microsoft Teams	20	5.6
Wisco web	4	1.1
Skype	3	0.8
Tercih edilen eğitim yöntemi		
Yüz yüze eğitim	234	53.9
Hibrit: Uzaktan eğitim ve yüz yüze eğitim eğitim	98	22.6
Çevrimiçi ve asenkron eğitim birlikte	55	12.7
Uzaktan asenkron	36	8.3
Uzaktan çevrimiçi eğitim	11	2.5

*Birden fazla seçenek işaretlenmiştir.

Öğrencilerin uzaktan eğitime ilişkin görüşleri incelendiğinde; çoğunluğunun (%75.6) COVID-19 pandemi sürecinde uzaktan eğitimden memnun olduğu, %81.5'inin uzaktan eğitimde sınav ve ödevler hakkında zamanında geri bildirim aldığı, %91.4'ünün uzaktan eğitim sürecinde derslerde verilen ödev, rapor, proje gibi faaliyetleri dersin konusu ile alakalı bulduğu ve %81.5'inin uzaktan

eğitimde internet bağlantı sorunu yaşamadığı belirlenmiştir. Bunun yanı sıra öğrencilerin %31.4'ünün uzaktan eğitim sürecinde dersleri takip etmede kendini kolayca disipline edemediği, %51'inin uzaktan eğitim sürecinin derslere olan ilgisini artırmadığı ve %42.2'sinin uzaktan eğitimde ders konularını kolaylıkla öğrenemediği belirlenmiştir (Tablo 2).

Tablo 2. COVID-19 pandemi döneminde verilen uzaktan eğitime ilişkin hemşirelik öğrencilerinin görüşleri (n=434).

Görüşler	Tamamen katılıyorum		Katılıyorum		Kısmen katılıyorum		Katılmıyorum		Hiç katılmıyorum	
	Sayı	%	Sayı	%	Sayı	%	Sayı	%	Sayı	%
Uzaktan eğitimden memnunum.	98	22.6	116	26.7	114	26.3	65	15.0	41	9.4
Uzaktan eğitimde dersleri takip etmede kendimi kolayca disipline edebiliyorum.	76	17.5	117	27.0	105	24.2	78	18.0	58	13.4
Uzaktan eğitim derslere olan ilgimi artırdı.	48	11.1	63	14.5	102	23.5	124	28.6	97	22.4
Uzaktan eğitimde kolaylıkla öğrenebiliyorum.	51	11.8	93	21.4	107	24.7	104	24.0	79	18.2
Uzaktan eğitimde sınav ve ödevler hakkında zamanında geri bildirim alabiliyorum.	88	20.3	166	38.2	100	23.0	43	9.9	37	8.5
Uzaktan eğitim kapsamında verilen ödev, rapor, proje vb. dersin konusu ile alakalıdır.	146	33.6	187	43.1	64	14.7	23	5.3	14	3.2
Uzaktan eğitimde bağlantı sorunu yaşamıyorum.	110	25.3	145	33.4	99	22.8	44	10.1	36	8.3

Çalışmaya katılan öğrencilerin çevrimiçi öğrenme tutumları ÇÖTÖ ile değerlendirilmiştir. Öğrencilerin ÇÖTÖ toplam puan ortalaması 54.81±16.63'tür. ÇÖTÖ alt ölçek puan ortalamaları incelendiğinde; “Genel

Kabul” puan ortalaması 20.50±5.72; “Bireysel Farkındalık” puan ortalaması 15.91±6.39; “Kullanışlılık” puan ortalaması 9.20±3.32; “Uygulama Etkililiği” puan ortalaması ise 12.51±3.91'dir (Tablo 3).

Tablo 3. Öğrencilerin Çevrimiçi Öğrenme Tutum Ölçeği puan ortalamalarının dağılımı.

Alt Ölçek Grupları	Min	Maks	$\bar{x} \pm SS$
Genel Kabul	7	35	20.50±5.72
Bireysel Farkındalık	6	30	15.91±6.39
Kullanışlılık (Zaman-Emek-Maliyet)	3	15	9.20±3.32
Uygulama Etkililiği (Etkin Katılım)	4	20	12.51±3.91
Toplam	20	100	54.81±16.63

Tablo 4. Öğrencilerin Çevrimiçi Tutum Ölçeği puan ortalaması ile bazı özelliklerinin karşılaştırılması (n=434).

Özellikler	ÇÖTÖ	Test değeri* /p
	$\bar{x} \pm SS$	
Cinsiyet		
Kadın	58.42±16.84	t=1.032; p=0.303
Erkek	55.68±19.86	
Sınıf		
Birinci ^a	53.41±16.86	F=4.167; p=0.006
İkinci	57.58±16.99	
Üçüncü	59.86±18.83	
Dördüncü ^b	61.13±15.21	
Tercih edilen eğitim yöntemi		
Yüz yüze eğitim ^a	51.98±14.79	H=76.590; p<.001
Uzaktan asenkron eğitim ^b	62.56±20.97	
Uzaktan çevrimiçi eğitim ^b	68.18±12.68	
Çevrimiçi ve asenkron eğitim birlikte ^b	69.45±18.50	
Hibrit: Uzaktan eğitim ve yüz yüze eğitim ^b	63.67±14.63	

ÇÖTÖ:Çevrimiçi Öğrenme Tutum Ölçeği, **t:** Bağımsız Örneklem t testi, **F:**Tek Yönlü Varyans Analizi, **H:**Kruskal-Wallis H Testi, ^{a,b} Farklı harfli gruplar arasında anlamlı farklılık elde edilmiştir.

Öğrencilerin ÇÖTÖ puan ortalaması ile bireysel özellikleri karşılaştırıldığında; birinci sınıf öğrencilerde ÇÖTÖ puan ortalaması en düşük (53.41±16.86) iken, bu ortalama dördüncü sınıf öğrencilerinde en yüksek elde edilmiştir (61.13±15.21) (p=0.006). Ayrıca ÇÖTÖ puan ortalaması yüz yüze eğitimi tercih edeceğini belirten öğrencilerde 51.98±14.79 iken, çevrimiçi ve asenkron eğitim birlikte olmasını istediğini belirten öğrencilerde en yüksek (69.45±18.50) elde edilmiştir (p<0.001). Araştırmada kadın öğrencilerin daha yüksek ÇÖTÖ puan ortalamasına sahip olduğu belirlenirken, cinsiyetler arasındaki farkın istatistiksel olarak anlamlı olmadığı bulunmuştur (Tablo 4).

ÇÖTÖ puanı ile sınıf düzeyi arasında yapılan çoklu karşılaştırma sonucunda, birinci sınıf öğrencilerine göre dördüncü sınıf öğrencilerde ÇÖTÖ puan ortalamasının

anlamlı olarak daha yüksek olduğu belirlenmiştir (p=0.006). ÇÖTÖ puanı ile tercih edilen eğitim yöntemi arasında yapılan çoklu karşılaştırma sonucunda; ÇÖTÖ puan ortalamasının yüz yüze eğitim tercih edenlerle diğer yöntemleri (uzaktan asenkron, uzaktan çevrimiçi, hibrit, çevrimiçi ve asenkron eğitim birlikte) tercih edenler arasında anlamlı farklılık elde edilmiş olup yüz yüze eğitimi tercih edenlerin ÇÖTÖ puan ortalaması diğer yöntemlere göre düşük elde edilmiştir. Bu araştırmada ÇÖTÖ puanı ile yaş ortalaması arasında pozitif yönde anlamlı ilişki olduğu belirlenmiştir (r=0.153, p=0.001). Aynı zamanda öğrencilerin akademik ortalaması ile ÇÖTÖ puan ortalaması arasında negatif yönde anlamlı ilişki bulunmuştur (r=-0.159, p=0.001). Bunun yanı sıra ders ve ödev sayısı ile ÇÖTÖ puan ortalaması arasında bir ilişki bulunmamıştır (Tablo 5).

Tablo 5. Çevrimiçi Öğrenme Tutum Ölçeği ile öğrencilerin bazı özellikleri arasındaki ilişkinin incelenmesi.

Özellikler	ÇÖTÖ	
	r*	p
Yaş	0.153	0.001
Akademik ortalama	-0.159	0.001
Ders sayısı	-0.016	0.740
Ödev sayısı	0.003	0.952

ÇÖTÖ: Çevrimiçi Öğrenme Tutum Ölçeği, *Pearson Korelasyon Katsayısı.

TARTIŞMA

Bu çalışmada hemşirelik öğrencilerinin COVID-19 pandemi döneminde uzaktan eğitime yönelik görüş ve tutumları değerlendirilmiştir. Yapılan çalışmalar uzaktan eğitimin, bireysel öğrenmeyi desteklediği, zaman ve mekân sınırlılığını ortadan kaldırdığı, eğitime devam ederken aile sorumluluklarını yerine getirebilme imkânı tanıdığı, düzenli ders çalışma alışkanlığı kazandırdığı ve zaman yönetimini geliştirdiğini göstermektedir (Ramos-Morcillo ve ark., 2020; Subedi ve ark., 2020; Şenyuva, 2013; Michel ve ark., 2021). Uzaktan eğitimin bu fırsatlarının yanı sıra bağlantı sorunları yaşama, yeterli çalışma alanı eksikliği, eğitime uygun olmayan ev ortamı, bireysel çalışma sürecinde bilgiyi öğrenmede zorlanma, motivasyon eksikliği, derslere ayrılan sürenin az veya yetersiz olması, dersleri anlamada güçlük, eğitici ile sınırlı iletişim kurma, uygulamalı dersler için yeterli beceri kazanamama, ekran süresinin artmasıyla fiziksel sorunların ortaya çıkması gibi olumsuzluklarının olduğu da bildirilmektedir (Ramos-Morcillo ve ark., 2020; Subedi ve ark., 2020; Şenyuva, 2013; Michel ve ark., 2021). Bu çalışmada ise öğrencilerin COVID-19 pandemi döneminde uzaktan eğitim sürecine yönelik olumlu görüşleri bulunurken bireysel motivasyona dair olumsuz görüşler belirtilmiştir. Her ne kadar COVID-19 pandemi sürecinde başarılı bir uzaktan eğitim sistemi kurulsa da öğrencilerin uzaktan eğitimde daha fazla güdülenmesi ve motive edilmesi gerektiği düşünülmektedir.

Öğrencilerin ÇÖTÖ puanı çevrimiçi öğrenmeye karşı “kararsız” tutuma sahip olduklarını düşündürmüştür (54.81±16.63). Selçuk ark. (2021) tarafından üniversite öğrencileri ile yapılan çalışmada üniversite öğrencilerinin Çevrimiçi Öğrenme Tutum Ölçeği (ÇÖTÖ) puan ortalaması 58.35±15.95 olarak bulunmuştur. Bu doğrultuda ‘çevrimiçi öğrenmeye ilişkin olarak, öğrencilerin genel tutumlarının zayıf ve ortalamanın üzerinde olduğu belirlenmiştir’ ve ‘Bu durum öğrencilerin çevrimiçi öğrenmeye karşı “kararsız” oldukları şeklinde yorumlanabilir, yani tutumları olumsuz yöne olduğu kadar olumlu yöne de eğilimlidir’ şeklinde yorum yapılmıştır. Bu çalışmada öğrencilerin ÇÖTÖ puan ortalamasının daha düşük olması nedeniyle öğrencilerin ‘kararsız’ tutuma sahip olduğu şeklinde yorum yapılmıştır. COVID-19 pandemi döneminde çevrimiçi öğrenmeye yönelik öğrenci tutumlarının değerlendirildiği çalışmalar incelendiğinde; Selçuk ve ark. (2021) çalışmasında ilahiyat bölümü öğrencilerinde ÇÖTÖ ortalama

puanının 58.35±15.95 olduğu, Hergüner ve ark. (2020) çalışmasında ÇÖTÖ ortalama puanının hukuk fakültesi öğrencilerinde 64.81±13.31, İngilizce öğretmenliği öğrencilerinde 62.37±16.09 ve beden eğitimi ve spor bilimleri öğrencilerinde 64.01±15.01 olduğu bildirilmiştir. Bu çalışmada ÇÖTÖ puan ortalamasının daha düşük olması, hemşirelik eğitiminin klinik ve uygulamalı bir alan olmasından kaynaklanmış olabilir. Nitekim katılımcıların sadece %2.5’inin çevrimiçi eğitim almak istediğini belirtmesinin yanı sıra yaklaşık yarısı yüz yüze eğitim almak istediğini belirtmiştir. Literatürde hemşirelik öğrencilerinin yüz yüze eğitimi çevrimiçi eğitime tercih ettikleri (Ramos-Morcillo ve ark. 2020); tıp ve hemşirelik öğrencilerinin yalnızca %36.5’inin çevrimiçi öğrenme konusunda olumlu tutuma sahip olduğu (Li ve ark. 2021) yönünde bulguların yanı sıra tıp ve hemşirelik öğrencilerinin çevrimiçi eğitimin derslerin amaç ve hedeflerini karşıladığı, ders içeriğinin doğru bir şekilde iletildiği, zamanın uygun şekilde kullanıldığı, içeriğin kalitesinin geleneksel öğretimle kıyaslanabilir olduğu (Dutta ve ark. 2020); öğrencilerinin %69.9’unun asenkron eğitimi tercih ettiği (Armstrong-Mensah vd. 2021); COVID-19 sürecinde çevrimiçi öğrenmeye devam edilmesi gerektiğini düşündükleri bildirilmiştir (Koirala ve ark., 2020). Bu bilgilerden yola çıkarak çevrimiçi öğrenmeye dair literatürde yer alan araştırma bulgularının farklılaştığı söylenebilir.

Bu çalışmada hemşirelik öğrencilerinin akademik başarıları ile çevrimiçi öğrenmeye yönelik tutumları arasındaki negatif ilişki bulunduğu belirlenmiştir. Bu bulgu öğrencilerin akademik başarıları arttıkça çevrimiçi öğrenmeye yönelik daha olumsuz tutuma sahip olduklarını göstermektedir. Benzer olarak; Ergün ve Kurnaz (2019) çalışmalarında akademik başarı ile aktif öğrenme stili arasında pozitif yönde ilişki, e-öğrenme stili açısından negatif yönde ilişki bulunduğunu belirlemişlerdir. Hemşirelik eğitimi bilişsel, duyuşsal ve davranışsal öğrenme alanlarını bütün olarak ele almaktadır (Warner, 2018). Özellikle mesleki uygulama derslerinin laboratuvar ve klinik ortamlarda eğitimci mentörlüğünde yürütülmesi öğrenme çıktılarının başarıları yönünden önemlidir (Ramos-Morcillo ve ark., 2020; Michel ve ark., 2021). Bu bakımdan akademik başarıları yüksek öğrencilerin uzaktan eğitime ve çevrimiçi öğrenmeye yönelik olumsuz tutumları, davranışsal alanda öğrenme gereksinimlerinin ve farkındalıklarının daha yüksek olmasıyla ilişkili olabilir.

Bu araştırmada mesleki uygulamalı dersler kapsamında çevrimiçi ders alan dördüncü sınıf öğrencilerinin ÇÖTÖ puan ortalamasının birinci sınıf öğrencilerine göre daha yüksek olduğu belirlenmiştir. Bu durum çevrimiçi dersi deneyimleme fırsatı elde eden öğrencilerin çevrimiçi öğrenme açısından tutumunun daha olumlu olabileceği bilgisini desteklemektedir (Hvalič-Touzery & Lobe). Benzer olarak Selçuk ve ark. (2021) tarafından üniversite öğrencileri ile yapılan çalışmada, daha önce çevrimiçi öğrenme deneyimine sahip öğrencilerin çevrimiçi öğrenmeye yönelik tutumlarının, bu konuda deneyime sahip olmayan öğrencilere göre daha olumlu olduğu belirlenmiştir. Güven Ozdemir ve Sönmez (2020) çalışmasında hemşirelik öğrencilerinin %43.8'inin e-öğrenme deneyiminin bulunduğu, e-öğrenmeye karşı hafif derece olumlu tutuma sahip oldukları ve %65'nin e-öğrenmeden kaçındıkları belirlenmiştir. Aynı zamanda bu araştırmada yaş ile çevrimiçi öğrenmeye yönelik tutum puanları arasında anlamlı pozitif ilişki olduğu bulunmuştur. Bu bulgu öğrencilerin yaşları arttıkça çevrimiçi öğrenmeye yönelik daha olumlu tutuma sahip olduklarını göstermiştir. Çevrimiçi öğrenme puan ortalamasının dördüncü sınıf öğrencilerinde en yüksek olması bu bulguyu desteklemektedir. Wallace (2021) çalışmasında, pandemi döneminde hemşirelik öğrencilerinin uzaktan eğitim yoluyla öğrenim hayatını devam ettirebilmesinin yanı sıra değişen koşulların aile ve ev sorumluluklarını arttığını belirlemiştir. Bu bağlamda hemşirelik öğrencilerinin uzaktan eğitim sisteminde öğretim faaliyetlerinin yanında ev ve aile sorumluluklarını da yürütebileceği düşünülmektedir. Literatürde hemşirelik dördüncü sınıf öğrencilerinin birinci sınıf öğrencilere göre çevrimiçi öğrenmeden daha fazla memnun olduğu, birinci sınıf öğrencilerinin mesleki eğitim sürecini tamamlamadaki belirsizlikler, dersleri anlamaya yönelik endişe ve strese bağlı olarak daha fazla olumsuz görüşe sahip oldukları belirtilmektedir (Diab ve ark., 2020; Dutta ve ark., 2021). Hemşirelik öğrencilerinin sınıf düzeylerine göre farklılaşan araştırma bulguları, çevrimiçi öğrenmeyi deneyimlemenin yanı sıra mesleki bilgi düzeyinin ilerleyen yıllarda artmasının yeni öğrenme stillerine yönelik tutumu etkileyebileceği şeklinde değerlendirilebilir. Bu araştırmada, COVID-19 pandemi döneminde uzaktan eğitim yöntemi olarak; çevrimiçi ve asenkron veya hibrit eğitim (uzaktan eğitim ve yüz yüze eğitim) verilmesi yönünde görüş bildiren öğrencilerin yüz yüze eğitimi tercih eden öğrencilere göre çevrimiçi öğrenme yönelik tutumlarının daha olumlu olduğu belirlenmiştir. Uygulamalı bir alan olan hemşirelik eğitiminde, klinik ve saha uygulaması yerine elektronik ortamda teorik ve uygulamalı derslerin verilmesi hemşirelik öğrencilerinin memnuniyet düzeyleri üzerinde etkili olurken (Wallece ve ark., 2021; Kaya ve Işık, 2021) mesleki beceriye odaklı hemşirelik eğitiminde çevrimiçi eğitimin tek başına bir yöntem olarak tercih

edilmesinden ziyade yüz yüze eğitimi destekleyen ve yaşam boyu öğrenmeyi pekiştiren, hibrit bir öğrenme ortamı geliştirilmelidir. COVID-19 pandemi sürecinde hemşirelikte klinik ve laboratuvar uygulamalarının etkili bir şekilde sürdürülebilmesi nedeniyle, çevrimiçi ve yüz yüze eğitim yöntemlerinin birlikte kullanıldığı hibrit eğitim modeli önerilmektedir (Kozan ve ark., 2021). Nitekim Yükseköğretim Kurulu (YÖK) tarafından teorik derslerin çevrimiçi olarak, uygulamalı eğitimlerin ise yüz yüze yürütülmesi yönünde yapılan açıklamada (YÖK, 2021), COVID-19 pandemi sürecinde etkili ve verimli eğitim-öğretim faaliyetleri için uygulamalı meslek dersleri olan bölümlerde hibrit eğitim modeli kullanımının önemi vurgulanmaktadır. Bu araştırmada da öğrencilerin %22.6'sı hemşirelik eğitiminin çağın teknolojisine uygun olarak hibrit eğitim modeli ile verilebileceği yönünde görüş bildirmişlerdir. Bu bulgu zaman tasarrufu, etkin insan gücü yönetimi ve maliyet dengesi bakımından avantajlı olduğu düşünülen hibrit eğitim modeline yönelik hemşirelik öğrencilerinin tercihlerini ortaya koyması nedeniyle önemlidir. Hibrit eğitim modelinin, geleceğin eğitim yaklaşımı olarak ön plana çıkacağı düşünülmektedir. Teknolojik çağın gereksinimleri, öğrenci istek ve tercihlerinin gözetildiği bir eğitim sisteminde hemşirelik eğitim müfredatının hibrit eğitim sistemine uygun revize edilebileceği söylenebilir.

Araştırmanın sınırlılıkları

Araştırma, bir devlet üniversitesinin hemşirelik bölümü öğrencileri ile sınırlandırılmış olup, çalışma sonuçları genellenememektedir. Araştırma verileri öğrencilerin beyanı ile sınırlıdır. Çalışmada pandemi döneminde uzaktan eğitimin ileri sonuçlarının değerlendirildiği izlem basamağının olmaması diğer sınırlılıktır.

SONUÇ

Bu araştırmada hemşirelik öğrencilerinin çevrimiçi öğrenmeye yönelik tutumlarının kararsız olduğu belirlenmiştir. Ayrıca hemşirelik öğrencilerinin uzaktan eğitim sürecine yönelik olumlu ve olumsuz görüşleri ortaya konulmuştur. Hemşirelik öğrencileri, çevrimiçi öğrenmeye yönelik kararsız tutum gösterebilir. Hibrit eğitim sistemini istedikleri görülmektedir. İleri çalışmalarda her iki eğitim sistemine dair öğrenci görüşlerinin eğitim çıktılarıyla birlikte değerlendirilmesi önerilmektedir.

Teşekkür

Araştırmacılar çalışmaya katılan öğrencilere ve okul idaresine teşekkür etmektedir.

Çıkar Çatışması

Yazarlar, gerçek veya potansiyel çıkar çatışması bildirmemektedir.

Yazar Katkıları

Plan ve tasarım: EK; **Gereç yöntem ve Veri toplama:** EK, AA, SK; **Veri analizi ve yorum:** AA EK, AA, SK; **Yazım ve eleştirel inceleme:** EK, AA, SK.

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Ebelik Öğrencilerinin Anatomi Eğitimi Hakkındaki Görüşlerinin Değerlendirilmesi

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ÖZ

Amaç: Bu çalışmada, Sağlık Bilimleri Fakültesi'nde öğrenim gören ebelik bölümü öğrencilerinin anatomi eğitimine ilişkin görüşlerinin değerlendirilmesi amaçlanmaktadır. **Gereç ve Yöntem:** Araştırmaya, Balıkesir Üniversitesi, Sağlık Bilimleri Fakültesi, 2019-2020 yılları arasında öğrenim gören Ebelik bölümü öğrencilerinden yaş ortalaması 20.71±1.88 olan 294 gönüllü katılımcı dahil edilmiştir. Öğrencilere anatomi eğitimini değerlendirmeleri amacıyla 3'ü açık uçlu, 23'ü ise kapalı uçlu olmak üzere 26 sorudan oluşan anket formu yöneltilmiştir. Edinilen veriler, SPSS (Statistical Package for Social Sciences) 22.0 paket programına aktarılarak analiz edilmiştir. **Bulgular:** Çalışmaya katılan öğrencilerin %76.9'u ebelik bölümünü isteyerek seçtiğini belirtmiştir. Çalışmaya katılan öğrencilerden %64.3'ü anatomi eğitiminin 10:20–12:00 saatleri arasında verilmesini ve %75.5'i anatomi sınavının “çoktan seçmeli”, %12.2'si “kısa cevaplı”, %10.2'si “klasik yazılı”, %2'si ise “sözlü” olması gerektiğini ifade etmiştir. Çalışmada, 11'i sağlık meslek lisesi, 56'sı ise Anadolu lisesi mezunu olan 67 öğrenci, anatomi öğrenimi konusunda güçlük çektiğini bildirmiştir. Sağlık meslek lisesinden mezun olan öğrencilerin anatomi eğitimi vize-final başarı ortalamalarının diğer liselere göre fazla olduğu tespit edilmiştir. **Sonuç:** Anatomi eğitiminin niteliğinin artırılmasında öğrenci geri bildirimleri oldukça değerlidir. Öğrencilerden alınan bu geri bildirimler doğrultusunda anatomi eğitimi ders planı ve içeriklerinin yeniden düzenlenmesinin, anne ve bebek sağlığı açısından önemli görevler üstlenen ebelik öğrencilerinin insan anatomisini daha iyi öğrenmelerine katkı sağlayacağı düşünülmektedir.

Anahtar Kelimeler: Anatomi Eğitimi, Geri Bildirim, Ebelik Öğrencileri.

The Evaluation of the Students' Opinions on Anatomy Education

ABSTRACT

Objective: In this study, it is aimed to evaluate the views of midwifery students studying at the Faculty of Health Sciences on anatomy education. **Materials and Methods:** 294 volunteer participants with an average age of 20.71±1.88 from the midwifery department students studying at Balıkesir University, Faculty of Health Sciences between 2019-2020 were included in the study. In order to evaluate the anatomy education, the students were asked a questionnaire consisting of 26 questions, 3 of which were open-ended and 23 of which were closed-ended. The obtained data were analyzed by transferring them to the SPSS (Statistical Package for Social Sciences) 22.0 package program. **Results:** 76.9% of the students who participated in the study stated that they chose the midwifery department willingly. 64.3% of the students who participated in the study stated that they wanted anatomy education to be given between 10:20-12:00. 75.5% of the students preferred the anatomy exam to be "multiple choice", 12.2% to be "short answer", 10.2% to be "classical written" and 2% to be "oral". In the study, a total of 67 students, 11 of whom graduated from health vocational high school and 56 of whom graduated from Anatolian high school, reported that they had difficulty in learning anatomy. In addition, it was determined that the anatomy education midterm-final success averages of the students who graduated from the health vocational high school were higher than the other high schools. **Conclusion:** Student feedback is very valuable in increasing the quality of anatomy education. In line with the feedback received from the students, it is possible to rearrange the anatomy education lesson plan and its contents. It is thought that this arrangement will contribute to the better learning of human anatomy by midwifery students, who take on important tasks in terms of maternal and infant health.

Keywords: Anatomy Education, Feedback, Midwifery Students.

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GİRİŞ

Anatomi bilimi, insan vücudunun normal yapısını ve şeklini, organlarını ve bu organların birbirleri ile olan ilişkilerini inceleyen bilim dalıdır (Erdem, 2020). Anatomi eğitimi, yükseköğretim kurumlarının; öznesi insan ve insan sağlığı olan bölümlerinde lisans ve ön lisans düzeyinde öğrenim gören öğrenciler için, en temel ve anlaşılması en zor derslerden biridir. Bununla birlikte anatomi eğitimi, sağlık alanında eğitim gören tüm öğrencilerin insan vücudu ve organlarıyla ilk kez karşılaştıkları ders olması nedeniyle sağlık bilimleri alanında eğitim gördüklerini hissettiren derslerin en başında yer almaktadır. Öğrencilerin meslek hayatlarında daha öz güvenli ve bilinçli olabilmeleri açısından anatomi bilgilerinin yeterli düzeyde olması gerekmektedir. Gerekli tanı, tedavi ve girişimlerde doğru yöntemi kullanmak; engin bir anatomi bilgisi ile mümkündür (Acar, 2018; Acuner ve ark., 1999; Arı ve ark., 2003; Bolatlı, 2021; Erdem, 2020).

Öğrenci geri bildirimleri, modern eğitim sistemlerinde uygulama alanı gittikçe artan ve eğitim etkinliğini değerlendirmede sıkça kullanılan bir kontrol sistemi olmaya devam etmektedir (Cankur ve Turan, 2000; Gönüllü ve ark., 2021). Eğitim sürecinde, program üzerinde yapılması planlanan düzenleme ve iyileştirmeler için ilgili tarafların görüşlerinin alınması gerekmektedir (Sarıkaya ve ark., 2002). Öğrencilerden alınan geri bildirimler; eğitmenlerin eğitici özelliklerinin iyileştirilmesinde, geliştirilmesinde ve yöneticiler tarafından eğitici personel ve programlar ile ilgili verilecek kararlarda veri kaynağı olarak kullanılmaktadır (Kulik, 2001). Bu bağlamda sağlık alanında öğrenim gören öğrencilerden alınan geri bildirimler sayesinde ders içerikleri ve ders planları üzerinde gerekli düzenlemelerin yapılabilmesi oldukça gerekli ve değerli görülmektedir. Bu amaç doğrultusunda gerçekleştirilen yenilik ve düzenlemeler ile anatomi eğitiminde ulaşılması hedeflenen kazanımların niteliği ve eğitimdeki kalite doğru orantılı olarak iyileştirilebilecektir (Öğertürk ve ark., 2003).

Çalışma kapsamında araştırmaya dahil edilen öğrenciler Balıkesir Üniversitesi, Sağlık Bilimleri Fakültesi, Ebelik bölümünde öğrenim görmektedir. Fakültenin eğitim müfredatının ebelik bölümü ders programında, anatomi eğitimi teorik dersi birinci sınıfın güz döneminde ve haftalık 3 ders saati olarak yer alırken, anatomi eğitimi pratik uygulama dersi yer almamaktadır. Bu çalışmada Balıkesir Üniversitesi, Sağlık Bilimleri Fakültesi, Ebelik bölümü öğrencilerinin anatomi eğitimi hakkındaki görüşlerinin değerlendirilmesi amaçlanmaktadır. Elde edilecek sonuçların eğitim-öğretim faaliyetleri ile ilgili düzenlemelerde yol gösterici olarak kullanılması hedeflenmektedir.

GEREÇ VE YÖNTEM

Araştırma grubu

Araştırmamızın evrenini, Balıkesir Üniversitesi, Sağlık Bilimleri Fakültesinde 2019-2020 yılları arasında öğrenim gören ve anatomi dersini almış olan bütün

ebelik bölümü öğrencileri oluşturmaktadır. Çalışmamızda örnek büyüklüğü hesaplaması yapılmamış, evrenin tamamına ulaşılması hedeflenmiştir. Araştırmamıza katılım tamamen gönüllülük esas alınarak gerçekleştirilmiştir. Araştırmamıza katılmayı kabul eden öğrencilerimizden sözlü olarak onamları alınmıştır. Etik kurul başvuru sürecinde onamların sözlü olarak alınacağı belirtilmiştir. Bu kapsamda araştırmamıza, derslere devam eden toplam 328 ebelik öğrencisinden 294'ü (%89.6) katılım göstermiştir. Ebelik öğrencilerinin tamamı kadın olup, yaş ortalamaları 20.7±1.88 (en az: 18, en fazla: 30) olarak tespit edilmiştir.

Anket formu

Katılımcılara, araştırmacılar tarafından literatür taraması yapılarak geliştirilen anket formu uygulanmıştır (Arı ve ark., 2003; Gözil ve ark., 2006; Özcan ve Vatansever, 2018; Turan ve ark., 2001). Öğrencilere uygulanan anket formu, 3'ü açık uçlu ve 23'ü kapalı uçlu olmak üzere toplam 26 sorudan oluşmaktadır. Araştırmamıza katılan öğrencilerin kendilerini baskı altında hissetmemeleri ve düşüncelerini özgür bir şekilde ifade edebilmeleri amacıyla anket formu üzerine hiçbir şekilde ad, soyad ve öğrenci numarası yazmaları istenmemiştir.

İstatistiksel analiz

Katılımcıların yanıt verdiği anket soruları SPSS (Statistical Package for Social Sciences) 22.0 paket programına aktarılarak gerekli istatistiksel analizler yapılmıştır. Elde edilen verilerin tanımlayıcı istatistik değerleri hesaplanmıştır. Sürekli değişkenler Shapiro-Wilk normallik testi ile analiz edilmiştir. Sürekli değişkenlerin normallik varsayımının sağlanmaması nedeniyle, tek değişkenli analizlerde Mann-Whitney U testi kullanılmıştır. Normal dağılım göstermeyen değişkenlerin birbirleriyle olan ilişkisini değerlendirmek amacıyla Spearman's Rho korelasyon analizi uygulanmıştır. Verilerin özeti; ortalama ± standart sapma olarak ifade edilmiştir. Uygulanan testte, p değerinin 0.05'ten küçük olduğu sonuçlar, istatistiksel olarak anlamlı kabul edilmiştir.

Etik onay

Bu çalışma, Balıkesir Üniversitesi Girişimsel Olmayan Klinik Araştırmalar Etik Kurulu tarafından değerlendirilerek tıbbi açıdan etik olduğu uygun görülmüştür (2020/58).

BULGULAR

Araştırmaya katılan öğrencilere ait demografik değişkenlerinin frekans dağılımları Tablo 1'de verilmiştir.

Çalışmaya katılan öğrencilerden 277'si (%94.2) anatomi eğitiminin işlenişi ile ilgili olarak geri bildirim alınmasını gerekli görürken, sadece 17 öğrenci (%5.8) gerekli olmadığını belirtmiştir. Öğrencilere yöneltilen "Anatomi dersinin ders programında hangi saatlerde olması daha iyi öğrenilmesine neden olur?" sorusuna verdikleri yanıtlar Şekil 1'de gösterilmiştir.

Öğrencilerin %33.7'si lise düzeyinde anatomi eğitimi almış olarak ebelik bölümüne başlarken, %66.3'ü ebelik

bölümüne başladıktan sonra anatomi eğitimini almıştır. Anatomi eğitimi sınavının nasıl yapılmasının daha etkili olabileceği sorusuna öğrencilerin, %75.5'i çoktan seçmeli, %12.2'si klasik yazılı, %10.2'si kısa cevaplı ve %2'si ise sözlü olması gerektiğini vurgulamıştır. Anatomi dersi sınav şeklinin sınıflara göre dağılımı Tablo 2'de gösterilmiştir.

Tablo 1. Örneklemin frekans dağılımı.

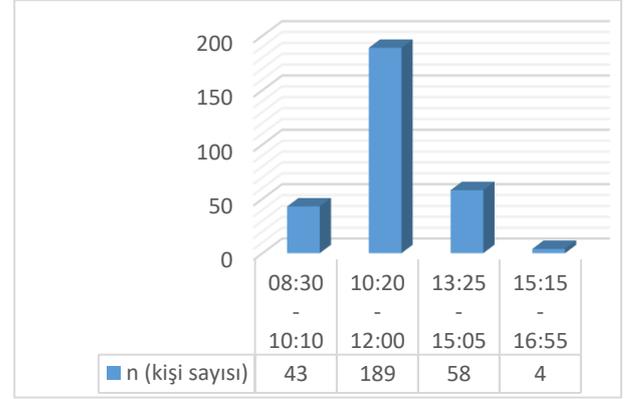
	n	%
Sınıf		
1.sınıf	65	22.1
2.sınıf	67	22.8
3.sınıf	74	25.2
4.sınıf	88	29.9
Mezun olunan lise türü		
Anadolu lisesi	150	51
Sağlık meslek lisesi	99	33.7
Genel lise	25	8.5
Diğer liseler	17	5.8
Teknik meslek lisesi	3	1
Sınıf tekrarı yapma durumu		
Tekrar yapanlar	10	3.4
Tekrar yapmayanlar	284	96.6

Tablo 2. Anatomi dersi sınav şeklinin sınıflara göre dağılımı.

	Klasik yazılı	Sözlü	Çoktan seçmeli	Kısa cevaplı	Toplam
1.sınıf	1	0	63	1	65
2.sınıf	8	0	48	11	67
3.sınıf	15	6	46	7	74
4.sınıf	12	0	65	11	88
	36	6	222	30	294

Katılımcılardan, anatomi derslerinin süresini değerlendirmeleri istenildiğinde, 238 öğrenci (%81) yeterli olduğunu, 35 öğrenci (%11.9) az olduğunu ve 21 öğrenci (%7.1) ise fazla olduğunu ifade etmiştir (Tablo

3). “Öğretim üyelerinin Anatomi derslerini anlatma süresi kaç dakika olmalıdır?” sorusuna öğrenciler ortalama 51.21±16.77 dakika (en az: 30, en fazla: 120) olmasının uygun olacağını belirtmişlerdir. Sınıflar arasında karşılaştırma yapıldığında istatistik açıdan anlamlı bir fark tespit edilmemiştir ($p>0.05$) (Tablo 3).

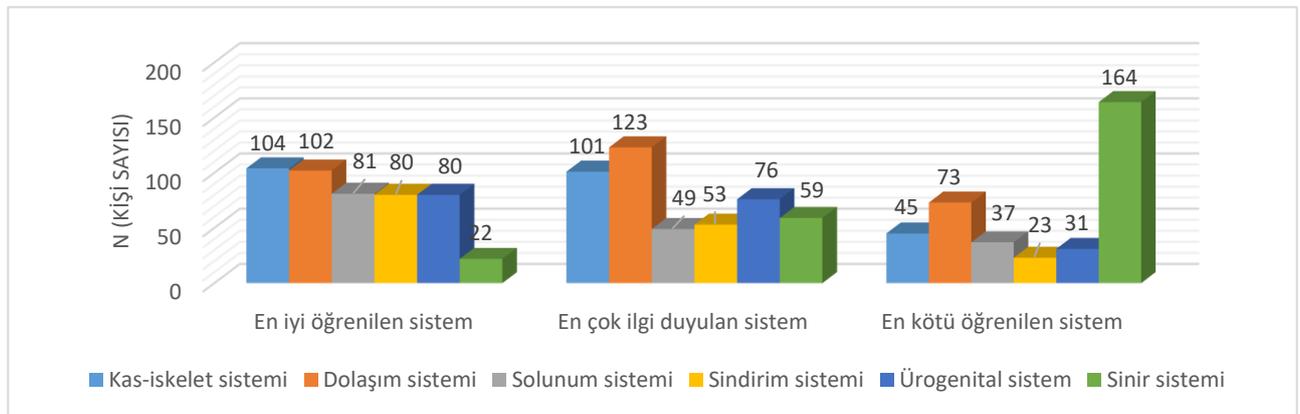


Şekil 1. Ders programında anatomi dersleri için uygun saatler.

Ebelik bölümünde öğrenim gören öğrencilerin, mezun olunan lise türüne göre vize ve final sınavı başarı ortalamaları karşılaştırılmıştır. Buna göre, sağlık meslek lisesinden mezun olan öğrencilerin başarı ortalamaları ile genel lise, Anadolu lisesi, meslek lisesi ve diğer liselerden mezun olan öğrencilerin başarı ortalamaları arasında anlamlı bir fark tespit edilmiştir ($p<0.05$). Sağlık meslek lisesinden mezun olan öğrencilerin anatomi eğitimi vize-final başarı ortalamalarının diğer liselere göre fazla olduğu görülmüştür (Tablo 3).

Çalışmaya katılan öğrencilerin tümü anatomi eğitimini tamamlamıştır. Bu kapsamda öğrencilerin en iyi öğrendikleri konunun kas – iskelet sistemi, en çok ilgi duydukları konunun dolaşım sistemi ve en yetersiz oldukları konunun ise sinir sistemi olduğu tespit edilmiştir (Şekil 2).

Katılımcıların anatomi eğitimini değerlendirmeleri amacıyla yöneltilen anket sorularına verdikleri yanıtlar Tablo 4'te gösterilmiştir.



Şekil 2. Öğrencilerin anatomik sistemler hakkındaki görüşleri.

Tablo 3. Mezun olunan lise türüne göre vize-final başarı ortalamalarının Mann Whitney-U testi ile karşılaştırılması.

Mezun Olunan Lise	Vize				Final				Vize	Final
	n	Ort.	Min-Max	Std. Sapma	n	Ort.	Min-Max	Std. Sapma	p	p
Sağlık Meslek Lisesi Genel Lise	99	82	48 – 100	13	99	75	40 – 98	12	0.006	0.007
	25	71	28 – 95	18	25	67	32 – 96	15		
Sağlık Meslek Lisesi Anadolu Lisesi	99	82	48 – 100	13	99	75	40 – 98	12	0.000	0.000
	150	75	15 – 100	14	150	67	30 – 96	13		
Sağlık Meslek Lisesi Meslek Lisesi	99	82	48 – 100	13	99	75	40 – 98	12	0.031	0.022
	3	66	60 – 70	6	3	60	59 – 60	1		
Sağlık Meslek Lisesi Diğer Liseler	99	82	48 – 100	13	99	75	40 – 98	12	0.034	0.000
	17	72	30 – 92	18	17	59	30 – 80	11		

Tablo 4. Anatomi eğitimi ile ilgili anket soruları.

Anket Soruları		Evete	Kararsızım	Hayır
1-Anatomi ders sayısı ve süresi yeterli mi?	n	199	68	27
	%	67.7	23.1	9.2
2-Anatomi derslerine devamsızlık yapmamak ders başarısı için gerekli midir?	n	223	46	25
	%	75.9	15.6	8.5
3-Öğretim üyeleri ile derslerde rahatça iletişim kurabiliyorum.	n	183	86	25
	%	62.2	29.3	8.5
4-Anatomi dersinin sonunda dersin özetinin yapılması faydalı olur mu?	n	273	19	2
	%	92.9	6.5	0.7
5-Anatomi derslerini zaman kaybı olarak görmüyorum.	n	222	22	50
	%	75.5	7.5	17
6-Anatomiye hatırlatma dersi olarak tekrar almak ister misiniz?	n	102	119	73
	%	34.7	40.5	24.8
7-Anatomi dersinin sadece 1.sınıf ilk döneminde olmasını uygun buluyor musunuz?	n	126	85	83
	%	42.9	28.9	28.2
8-Anatomi dersinin pratiğinin olması ders başarısı için gerekli midir?	n	261	29	4
	%	88.8	9.9	1.4
9-Anatomi çalışırken sıkılıyor musunuz?	n	39	88	167
	%	13.3	29.9	56.8
10-Anatomi öğrenimi konusunda günlük çekiyor musunuz?	n	85	86	123
	%	28.9	29.3	41.8
11-Anatomi derslerinin stajlarınızda eğitim sürecinde faydalı olacağını düşünüyor musunuz?	n	229	48	17
	%	77.9	16.3	5.8
12-Seçmeli bir ders olarak anatomi dersini alır mıydınız?	n	145	88	61
	%	49.3	29.9	20.7
13-Anatomi derslerinde radyolojik görüntüler yardımcı ders aracı olarak kullanılmalı mıdır?	n	232	56	6
	%	78.9	19	2

TARTIŞMA

Dünya Sağlık Örgütü (DSÖ) ebeliği; doğum öncesi, doğum ve doğum sonrası dönemlerde annelere gerekli olan tüm bakım ve danışmanlığı sağlamak, normal doğumları yaptırmak ve yeni doğan bebeklerin bakımını yapmak amacıyla eğitim almış olan birey olarak tanımlanmaktadır (World Health Organization, 1983). DSÖ verileri, ebelerin aktif bir şekilde çalıştıkları ülkelerde anne ve perinatal bebek ölümleri ile sezaryen doğum oranlarının azaldığını ortaya koymaktadır. Bu

nedenle ebelik öğrencilerinin meslek hayatına katılmadan önce mutlaka iyi bir eğitim alması gerekmektedir (Kaya ve Yurdakul, 2007).

Fransa'da III. Henry 1560 yılında ebelerin pratik eğitimlerinin yanında mutlaka kadın pelvis anatomisi ve kadın genital sistem fizyolojisi konusunda da eğitim almalarına zorunluluk getirmesi (Eren ve Uyer, 1991), anatomi eğitiminin önemini ortaya koymaktadır (Arı ve ark., 2003; Carmichael and Pawlina, 2000).

Araştırmamıza katılan 294 öğrenciden %75.5'i anatomi derslerinin zaman kaybı olmadığını, mesleki yeterlilikleri konusunda oldukça gerekli olduğunu bildirmiştir. Yapılan diğer çalışmalarda da (Arı ve Şendimir, 2003; Ögetürk ve ark., 2003; Özcan ve Vatansver, 2018; Turan ve ark., 2001) bu sorulara verilen yanıtların oransal olarak yüzdesinin yüksek olması (Arı ve Şendimir, 2003; %89.6 olarak bildirmiştir.) anatomi eğitimin önemini vurgulamaktadır. Mutluay ve Açıkgöz'ün 2020 yılında yaptıkları çalışmada, ebelik öğrencilerine verilen anatomi derslerinde uygulama derslerinin olmasını katılımcıların neredeyse tamamının (%93.8) istediği belirtilmiştir. Bu çalışmamızda da öğrencilerin %88.8'i uygulama derslerinin anatomi eğitiminde başarıyı arttıracığını belirtmiştir. Bununla birlikte hem tıp fakültesi hem de sağlık bilimleri fakültesi bölümlerinde yapılan araştırmalarda; katılımcıların büyük bir çoğunluğu (%76.4), bizim çalışmamızda da olduğu gibi anatomi derslerinin "10:20 ile 12:00" arasında yapılmasının, her bir ders saatinin ise ortalama 45-60 dakika arasında olmasının uygun olacağını belirtmiştir (Arı ve Şendimir, 2003; Ögetürk ve ark., 2003; Özcan ve Vatansver, 2018; Turan ve ark., 2001). Sonuçlarımızı destekler nitelikte verilerin bulunması nedeniyle, ebelik öğrencilerinin uygulama derslerinin ebelik bölümü eğitim müfredatına yeri ve saatine dikkat edilerek eklenmesi; gelecekte mezun olacak ebelerin daha bilgili ve tecrübeli bir şekilde meslek hayatına geçiş yapacaklarını göstermektedir. Mutluay ve Açıkgöz (2020) ve Acar'ın (2018) yaptıkları çalışmada, ebelik öğrencileri eğitim öğretim müfredatında yer alan anatomi derslerinin sayısı ve süresini yeterli bulmazken, çalışmamızdaki katılımcıların %67.7'si yeterli bulmuştur. Bu durumun, katılan öğrencilerin %33.7'sinin lise düzeyinde anatomi eğitimi almasından kaynaklandığı düşünülmektedir. Acar'ın 2018 yılında yaptığı çalışmada, katılımcılara yöneltilen "Öğretim görevlileri-üyeleri ile rahat iletişime geçebiliyorum" önermesine ebelik öğrencilerinin %29'u "Katılıyorum", %4'u ise "Kesinlikle Katılıyorum" yanıtını vermiştir. Çalışmamızda ise öğrencilerin %62.2'si "Evet" yanıtı vermiştir. Anatomi derslerinin iyi öğrenilmesinde öğretim görevlileri-üyelerinin rolünün oldukça önemli olması nedeniyle, öğrencilerin bu soruya yüksek oranda olumlu yanıt vermesi değerlidir. Öğrencilerin aldıkları anatomi eğitiminin ne kadar kalıcı olduğunu belirlemek amacıyla ders sınavları uygulanmaktadır. Anatomi ders sınavlarının nasıl olması gerektiği ile ilgili yapılan çalışmalarda (%75.5) olduğu gibi (Gözil ve ark., 2006; Ögetürk ve ark., 2003; Özcan ve Vatansver, 2018; Sindel ve ark., 2008; Tuygar ve ark., 2015) bizim çalışmamızda da öğrencilerin büyük bir çoğunluğu anatomi ders sınavının çoktan seçmeli olarak yapılmasını istemektedir. Öğrencilerin anatominin bazı konularında bilgilerinin yetersiz olduğunu hissetmeleri durumunun; radyolojik veya üç boyutlu görüntüler gibi yardımcı araçlardan yararlanılarak derslerin iyileştirilmesi ve daha kalıcı bir anatomi eğitimi verilmesi ile çözüme ulaştırılabileceği düşünülmektedir.

SONUÇ

Bu kapsamda geri bildirim çalışmaları ile ilgili tüm ortak sonuçlar değerlendirildiğinde, anatomi eğitiminin daha kaliteli ve nitelikli bir duruma getirilmesinin, öğrencilikten meslek hayatına geçen donanımlı ebelerin yetişmesine katkı sağlayacağı kanaatindeyiz.

Teşekkür

Çalışmamıza katılan tüm ebelik öğrencilerine katkılarından dolayı teşekkürlerimizi sunarız.

Çıkar Çatışması

Yazarlar arasında çıkar çatışması bulunmamaktadır.

Yazar Katkıları

Plan, tasarım: EÖ, ÖK, RÇ; **Gereç yöntem ve veri toplama:** EÖ, ED, AÇ; **Veri analizi ve yorum:** EÖ, ED, RÇ, AÇ; **Yazım ve eleştirel değerlendirme:** EÖ, ED, İK.

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Formaldehitin Sıçan Testislerine Zararı Üzerine Üzüm Çekirdeği Ekstraktının (*Vitis vinifera*) Koruyucu Etkisi

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ÖZ

Amaç: Bu çalışmanın amacı formaldehitin testis dokusu üzerine olan toksik etkileri ve oluşan bu toksik etkilere karşı üzüm çekirdeği ekstraktının koruyucu etkisini biyokimyasal ve histopatolojik düzeylerde araştırmaktır. **Gereç ve Yöntem:** Bu araştırma için toplam 21 adet *Wistar-Albino* cinsi erkek sıçan kullanıldı. Rastgele olarak yedişerli üç gruba ayrıldı. Grup I'deki sıçanlar kontrol grubu olarak belirlendi. Grup II'deki sıçanlara gün aşırı olarak formaldehit intraperitoneal olarak enjekte edildi. Grup III'deki sıçanlara ise gün aşırı formaldehit enjeksiyonu ile birlikte her gün intragastrik olarak üzüm çekirdeği ekstraktı uygulandı. 30 günlük deney süresi sonunda tüm sıçanlar kanları alındıktan sonra dekapitasyon yöntemi ile öldürüldü ve testisleri diseke edilerek diğer dokulardan ayrıldı. Alınan testis dokularının bir kısmı süperoksitdismutaz (SOD), total antioksidant status (TAS), testosteron ve malondialdehit (MDA) seviyelerinin analizi için ayrıldı. Diğer kısımları ise histopatolojik çalışmada kullanılmak üzere ayrıldı. **Bulgular:** Formaldehit uygulanan sıçanlara ait SOD ve TAS değerlerine bakıldığında; kontrol grubu ve üzüm çekirdeği ekstraktı ile tedavi edilen gruba göre istatistiksel olarak anlamlı bir şekilde azalmadığı görüldü. Testosteron düzeyleri incelendiğinde ise üzüm çekirdeği ekstraktı uygulanan grubun kontrol grubuna göre istatistiksel olarak anlamlı bir şekilde arttığı saptandı. Ayrıca formaldehit maruziyeti sonrası intersitisyel ödemin ve seminifer tübül lümenlerindeki genişlemenin azaldığı, seminifer tübül içindeki germ hücre sayısının normale yakın olduğu tespit edildi. **Sonuç:** Formaldehitin testis dokusunda zararlı etkiler oluşturduğu ve üzüm çekirdeği ekstraktının bu etkilere koruyucu etki gösterdiği belirlendi. **Anahtar Kelimeler:** Formaldehit, Sıçan, Testis, Üzüm çekirdeği (*Vitis Vinifera*).

Protective Effect of Grape Seed Extract (*Vitis Vinifera*) on the Damage of Formaldehyde to Rat Testes

ABSTRACT

Objective: The aim of this study is to investigate the toxic effects of formaldehyde on testicular tissue and the protective effect of grape seed extract against these toxic effects at biochemical and histopathological levels. **Materials and Methods:** For this purpose, 21 male Wistar-Albino rats were divided in to three groups. First group rats were used as control. Second group rats were injected intraperitoneal every other day with formaldehyde. Third group rats were administered grape seed extract with intragastric injection of formaldehyde. After thirty days, all rats were killed by decapitation after blood samples were taken. The testes of rats were removed and dissected from the surrounding tissue. The activities of superoxide dismutase (SOD), total antioxidant serum (TAS), testosterone and malondialdehyde (MDA) were determined in the some of testicular tissue specimens. The remaining testicular tissue specimens were used for histopathological examination. **Results:** The activities of SOD and TAS were examined in second group; they were not significantly decreased. It was seen decrease of testosterone degrees in rats administered grape seed extract with exposure of formaldehyde compared to control group. Furthermore, histopathological damages caused by formaldehyde were restored in this group. **Conclusion:** Formaldehyde exposure caused to harmful effects in testes tissue and grape seed extract have protective effects to these damages.

Keywords: Formaldehyde, Rat, Testes, Grape Seed (*Vitis Vinifera*).

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GİRİŞ

Formaldehit; aldehitler ailesinden, keskin kokulu ve renksiz ayrıca suda iyi çözünebilen kimyasal bir bileşiktir. Formaldehit çok reaktif bir bileşik olduğu için kolaylıkla her ortamda gaz haline dönüşebilmektedir (Feron ve ark., 1991; Smith, 1992). Formaldehit, vücuda başlıca solunum, deri ve sindirim yoluyla alınmaktadır. Egzoz gazı, sigara dumanı ve ortamdan buharlaşan formalin solunum yoluyla formaldehitin vücuda girişini oluştururken, sindirim yoluyla girişini gıdalarda bulunan katkı maddeleri, meyve sebzeler ve içme suyu sağlar

(www.cdc.gov/nceh/drywall/docs/WhatYouShouldKnowaboutFormaldehyde.pdf., 2015). Formaldehit vücuda giriş yaptığımda karaciğer ve kan hücrelerinden biri olan eritrositlerde formaldehit dehidrojenaz enzimi (FDH) katalizörlüğü ile formik aside dönüşür. FDH enzimi bu reaksiyonda glutatyonukofaktör olarak kullanır. Formik aside dönüşerek idrar ve feçes yoluyla veya karbondioksite okside olarak solunum yoluyla vücuttan atılımı gerçekleşir (Smith, 1992; Zararsız ve ark., 2007). Formaldehit'in organizmadaki sistemlerin birçoğunda zararlı etkilerinin olduğu yapılan birçok çalışmada gösterilmiştir (Canbilen ve ark., 1999; Sarsılmaz ve ark., 2007). Formaldehit üreme sistemi üzerine de olumsuz etkilere sahiptir. Formaldehit'in üreme hücrelerine zarar verdiği, dişi ve erkek üreme hücrelerinde primer ve sekonder infertiliteye neden olduğu bildirilmiştir (Thrasher ve Kilburn, 2001). Formaldehit maruziyetinin sperm sayısı ve miktarında (Chowdhury ve ark., 1992), testis ağırlıkları ve seminer tübül çaplarında azalmaya neden olduğu ve Leydig hücrelerinde histopatolojik değişiklikler oluşturduğu rapor edilmiştir. Bununla birlikte serum testosteron, çinko, bakır ve demir değerlerinde düşüklüğe sebep olduğu belirlenmiştir (Özen ve ark., 2005; Sarsılmaz ve ark., 1999).

Günümüzde bitkilerden elde edilen özütler tedavi amacıyla sıklıkla kullanılmaktadır (Zhou, 2006). Üzüm çekirdeği ekstraktı da bu amaçla kullanılan maddelerden bir tanesidir. Üzüm ve üzüm ürünleri monomerik flavanol, dimerik, trimerik ve polimerik prosiyanidin gibi flavonoidler ile gallik ve epigallik asit benzeri fenolik asit müştaklarını kapsamaktadır. Üzümün kabuk ve çekirdek kısmında ise yüksek oranda kondanse tanenler olarak bilinen proantosiyanidler bulunur (Kaya, 2008). Cos ve ark. (2004) göre proantosiyanidinlerin metalleri yakalayıcı etkisinde B-halkasının rolü olduğu iddia edilmiştir. Proantosiyanidinler prooksidatif enzimlerin inhibisyonu vasıtasıyla da antioksidan aktivite gösterebilirler.

Taze meyve ve yeşil sebze tüketiminin artması insan vücudunda antioksidan durumunu geliştirir. Diğer taraftan sigara içme ve fiziksel stres gibi sebepler serbest radikal üretimini artırarak dejeneratif hastalıklara yol açabilir. Bütün bunlar bir arada ele alındığında, araştırmalar üzüm çekirdeği ekstraktının biyoyararlanımının fazla olduğunu ve çeşitli kimyasalların, çevresel faktörlerin ve ilaçların neden olduğu hedef organ toksisitesine karşı koruyucu rol

oyunabileceğini göstermektedir. (Bagchi ve ark., 2002).

Sunulan çalışmada formaldehitin testis dokusu üzerindeki zararlı etkileri incelenmiştir. Ayrıca bu zararlı etkilere karşı üzüm çekirdeği ekstraktının muhtemel koruyucu etkileri de değerlendirilmiştir.

GEREÇ VE YÖNTEM

Hayvan materyali

Araştırmada ortalama vücut ağırlığı 250-300 gr, toplam 21 adet Wistar-Albino cinsi erkek sıçan kullanıldı. Sıçanlar oda sıcaklığı $21 \pm 2^{\circ}\text{C}$ olan 12 saat aydınlık (07.00-19.00) ve 12 saat karanlık (19.00-07.00) kafeslerde barındırıldı. Araştırma boyunca hayvanlar besin ihtiyacı temin edilen hazır pelet yem ve normal çeşme suyu ile karşılandı.

Deney protokolü

Çalışmada kullanılan sıçanlar üç gruba ayrıldı. Grup I (Kontrol grubu)'de bulunan yedi adet sıçana deney süresi boyunca hiçbir şey uygulanmadı. Sıçanlar pelet yem ve içme suyu ile beslenmelerini sürdürdüler. Grup II (formaldehit grubu)'de bulunan yedi adet sıçana serum fizyolojik ile 0.1 oranında sulandırılmış 10 mg/kg formaldehit intraperitoneal olarak 30 gün boyunca gün aşırı olarak uygulandı. Grup III (formaldehit ve üzüm çekirdeği ekstraktı grubu)'de bulunan yedi adet sıçana gün aşırı uygulanan serum fizyolojik ile 0.1 oranında sulandırılmış 10 mg/kg formaldehitin yanı sıra, serum fizyolojik ile 0.1 oranında sulandırılmış 25 mg/kg dozundaki üzüm çekirdeği ekstraktı intragastrik gavaj yoluyla 30 gün boyunca her gün uygulandı. 30 gün sonunda eter anestezi altında kanları alındıktan sonra tüm hayvanlar dekapite edilerek öldürüldü. Sıçanlara ait testis dokuları histolojik analizler için kullanıldı.

Histolojik analiz

Histopatolojik değerlendirme için alınan testis dokusu, %10'luk formaldehit ile tespit edildi ve 0.3 cm boyutunda kesitler alınarak doku kasetlerine konuldu. Doku kasetleri, formol-alkol-xylo-parafin setinden geçirilerek blokama yapıldı. Parafin kesitler beş mikron kalınlığında kesilerek, lam üzerine konuldu ve etüvde $60-70^{\circ}\text{C}$ 'de parafin eriyene kadar tutuldu. Kesitler daha sonra, önceden hazırlanan xylo içerisinde yavaş bir şekilde geçirildi. Bu işlem sonrası kesitler farklı yüzdelerdeki alkol solüsyonlarından geçirildi. Kesitler parafinden tamamen arındırılana kadar distile su ile yıkandı (deparafinizasyon). Bu kesitler, Mayer Hemotoksilen çözeltisi içinde 45 saniye ile 1.5 dakika bekletilerek boyandı. Daha sonra çeşme suyunda yıkandı ve %70'lik alkolde hazırlanan; %0.5'lik HCL solüsyonuna batırıldı. Çeşme suyu ile yıkanan lamlar ardından %1'lik NH_3 'ten geçirildi. %50'lik alkol solüsyonundan geçirilen lam üzerindeki dokuların boyama işlemi eozin içinde 5-15 saniye bekletilerek yapıldı. Boyama işlemi gerçekleştikten sonra dokular tekrar çeşme suyu ile yıkandı. Daha sonra %70-99.9'luk alkol solüsyonlarından geçirildi (dehidrasyon). Hazırlanan lamlar, minimum 10 dakika xylo'de bekletildi ve mikroskop altında uygun inceleme için

üzerlerine lameller kapatıldı. Testis dokuları eksize edilerek hazırlanan histopatolojik kesitler ışık mikroskopuyla değerlendirildi. Kesitlerden rastgele seçilen semifer tubül çapları ışık mikroskopunda Lecia Application Suit (acquire image) programı kullanılarak incelendi.

Biyokimyasal parametrelerin analizi

Testis dokuları, öncelikle soğuk (+4°C) 0.15'lik M'lık potasyum klorür (KCL) ile yıkanarak kurutma kâğıdı ile kurutuldu. Homojenizatör (Ultra Turrax Type T25-B, IKA Labortechnik, Germany) vasıtasıyla 0.15'lik M'lık KCL çözeltisi içerisinde 16000 rpm'de üç dakikada homojenize edildi. Homojenizasyon bir buz kabı içerisinde yapıldı. Homojenat 5000 xg'de bir saat (+4°C) santrifüjlenerak süpernatant elde edildikten sonra analiz zamanına kadar -40°C'de bekletildi. Süpernatanda antioksidan enzimlerden süperoksitdismutaz (SOD) ve total antioksidan status (TAS) ile oksidatif hasarın bir göstergesi olan malondialdehit (MDA) ölçümleri spektrofotometrik olarak belirlendi.

SOD analizi

Sun ve ark. (1988) tarafından geliştirilen metoda göre çalışıldı. Hazır ticari kit (Cayman assay kit 70600002 USA) ile ELISA (Thermo Multiskan FC Microplate Photometer, USA)'da süperoksitdismutaz enzim değeri analizleri çalışıldı. Sunulan çalışmada SOD düzeyi ünite/gram (U/g) doku proteini olarak ifade edildi.

TAS analizi

TAS analizleri, hazır ticari kit (Total Antioxidant Status Assay kit, Rel Assay Diagnostics, Turkey) ile ELISA (Thermo Multiskan FC Microplate Photometer, USA)'da çalışıldı. TAS yeni otomatik ölçme yöntemleri kullanılarak ölçüldü. TAS düzeyi, mmol trolox Equiv./mg protein olarak belirtildi.

MDA analizi

MDA analizi Yoshioka ve ark. (1979) bildirdiği yöntemle çalışıldı. Çıkan sonuçlar nanomol/gram (nmol/g) doku proteini ile ifade edildi.

Testosteron analizi

Testosteron analizleri hazır ticari kit (Cayman Testosterone EIA Kit 582701 USA) ile ELISA (Thermo Multiskan FC Microplate Photometer, USA)'da çalışıldı. Çalışmamızda testosteron birimi, pg/mg protein ile ifade edildi.

İstatistiksel analiz

Biyokimyasal parametrelerin değerlendirilmesi "IBM SPSS Statistics 21" programı ile yapıldı. Non-parametrik testlerden one-sample Kolmogorov-Smirnov Test kullanılarak grupların dağılımları değerlendirildi. Gruplar normal dağılım gösterdiği için değerler karşılaştırılırken parametrik testlerden one-way ANOVA ve Post Hoc testlerden LSD kullanıldı. P<0.05 olan değerlerin anlamlı olduğu kabul edildi. Hesaplanan veriler aritmetik ortalama ±standart sapma (SS) şeklinde tabloda belirtildi.

Araştırmanın etik yönü

Çanakkale On sekiz Mart Üniversitesi Deneysel Araştırmalar Uygulama ve Araştırma Merkezi Etik

Kurulunun 30.05.2013 tarihinde karar numarası 2013/05-08 olan etik kurul kararı sonucu çalışmaya başlanmıştır.

BULGULAR

Deney süresi boyunca kontrol grubu olarak sıçanların motor hareketlerinde herhangi bir değişiklik gözlenmezken formaldehit uygulanan grupta, motor hareketlerde yavaşlama ve kilo kaybı şeklindeki bulgular tespit edildi. Formaldehit maruziyetinin yedinci gününden itibaren ise deneklerin kıllarında sararma olduğu ve bu sararmanın deney süreci boyunca devam ettiği görüldü. Formaldehit ile birlikte üzüm çekirdeği ekstraktı uygulanan sıçanlarda ise vücut ağırlığı artışı motor hareketlerinde iyileşme ve kıllardaki sararmaların azaldığı görüldü (Tablo 1).

Tablo 1. Gruplarda vücut ağırlığı değerleri.

	Ortalama (g)	Standart Sapma
Kontrol grubu	304.60	18.62
Formaldehit grubu	299.45	32.90
Formaldehit ve Üzüm çekirdeği ekstraktı grubu	319.86	48.66

Biyokimyasal bulgular

Serum TAS düzeyi ortalaması istatistiksel olarak kontrol grubunda 1.34, formaldehit grubunda 0.74 ve formaldehit ve üzüm çekirdeği ekstraktı grubunda 1.12 mmol trolox Equiv./mg olarak hesaplandı. Formaldehit uygulaması kan serum TAS düzeyini kontrol grubuna göre anlamlı şekilde azalttı (p<0.05). Formaldehit ile formaldehit ve üzüm çekirdeği ekstraktı grubu ve kontrol grubu ile formaldehit ve üzüm çekirdeği ekstraktı grubu karşılaştırıldığında çıkan sonuçların istatistiksel olarak formaldehit uygulanan gruba göre arttığı görüldü (p>0.05).

Serum SOD düzeyi ortalaması istatistiksel olarak kontrol grubunda 1.31, formaldehit grubunda 1.25 ve formaldehit ve üzüm çekirdeği ekstraktı grubunda 1.22 U/g olarak hesaplandı. Formaldehit uygulanmasına bağlı olarak kontrol grubu ile formaldehit grubu; formaldehit grubu ile formaldehit ve üzüm çekirdeği ekstraktı grubu ve kontrol grubu ile formaldehit ve üzüm çekirdeği ekstraktı grubu serum SOD değerleri istatistiksel olarak formaldehit grubu ile karşılaştırıldığında anlamlı ölçüde arttığı belirlendi, ancak sonuç istatistiksel olarak anlamlı değildi (p>0.05). Serum Testosteron düzeyi ortalaması istatistiksel olarak kontrol grubunda 316.63, formaldehit grubunda 269.36 ve formaldehit ve üzüm çekirdeği ekstraktı grubunda 263.75 pg/mg olarak hesaplandı. Formaldehit uygulanması sonucunda kontrol grubu ile formaldehit ve üzüm çekirdeği ekstraktı ve kontrol grubu ile formaldehit grubundaki istatistiksel sonuçlar anlamlı bulundu (p<0.05).

Tablo 2. Gruplarda biyokimyasal bulguların verileri.

	TAS düzeyi ortalaması (Equiv./mg)		SOD düzeyi ortalaması (U/g)		Testesteron düzeyi ortalaması (pg/mg)		MDA düzeyi ortalaması (nmol/g)	
	Serum verileri	Doku verileri	Serum verileri	Doku verileri	Serum verileri	Doku verileri	Serum verileri	Doku verileri
Kontrol grubu	1.34	1.21	1.31	0.2928	316.63	202.10	17.57	7.87
Formaldehit grubu	0.74	1.25	1.25	0.2965	269.36	198.74	18.30	8.28
Formaldehit ve üzüm çekirdeği ekstraktı grubu	1.12	1.41	1.22	0.2902	263.75	194.27	18.08	8.20

Serum MDA düzeyi ortalaması kontrol grubunda 17.57, formaldehit grubunda 18.30 ve formaldehit ve üzüm çekirdeği ekstraktı grubunda 18.08 nmol/g olarak tespit edildi. Formaldehit uygulanması sonucunda kontrol grubu ile formaldehit grubu; formaldehit grubu ile formaldehit ve üzüm çekirdeği ekstraktı grubu ve kontrol ile formaldehit ve üzüm çekirdeği ekstraktı grubu serum MDA değerleri istatistiksel olarak formaldehit grubu lehine arttı ($p>0.05$). Üzüm çekirdeği ekstraktı uygulanmasıyla serum MDA düzeyinde anlamlı bir azalma olmadığı görüldü.

TAS düzeyi ortalaması istatistiksel olarak kontrol grubunda 1.21, formaldehit grubunda 1.25 ve formaldehit ve üzüm çekirdeği ekstraktı grubunda 1.41 trolox Equiv./mg olarak bulundu. Formaldehit uygulanmasına bağlı olarak kontrol grubu ile formaldehit grubu; formaldehit grubu ile formaldehit ve üzüm çekirdeği ekstraktı grubu ve kontrol ile formaldehit ve üzüm çekirdeği ekstraktı grubu TAS değerleri istatistiksel olarak formaldehit grubu lehine arttı ($p>0.05$). İstatistiksel olarak, üzüm çekirdeği ekstraktının dokuların TAS değerleri üzerine anlamlı bir düzeltici etkisinin olduğu gözlemlendi.

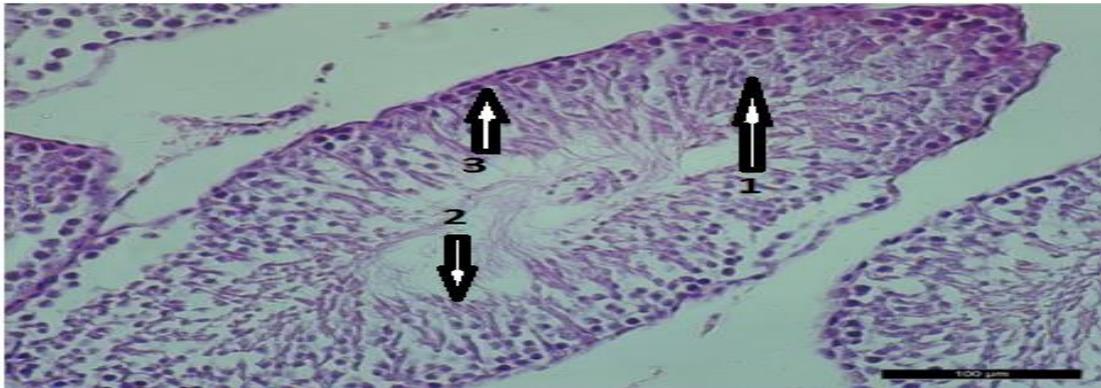
SOD düzeyi ortalaması istatistiksel olarak kontrol grubunda 0.2928, formaldehit grubunda 0.2965 ve formaldehit ve üzüm çekirdeği ekstraktı grubunda 0.2902 U/g olarak hesaplandı.

Formaldehit uygulanması sonucunda olarak kontrol grubu ile formaldehit grubu; formaldehit grubu ile formaldehit ve üzüm çekirdeği ekstraktı grubu ve kontrol ile formaldehit ve üzüm çekirdeği ekstraktı grubu SOD değerleri istatistiksel olarak formaldehit grubu lehine arttı, ancak elde edilen sonuç istatistiksel olarak anlamlı değildi ($p>0.05$).

Testosteron düzeyi ortalaması istatistiksel olarak kontrol grubunda 202.10, formaldehit grubunda 198.74 ve formaldehit ve üzüm çekirdeği ekstraktı grubunda 194.27 pg/mg olarak hesaplandı. Formaldehit uygulanması bağlı olarak istatistiksel olarak hesaplanan sonuçlara göre kontrol grubu ile formaldehit ve üzüm çekirdeği ekstraktı grubu testosteron değerleri anlamlı bulundu ($p<0.05$). Kontrol grubu ile formaldehit grubu ve formaldehit ile formaldehit ve üzüm çekirdeği ekstraktı grubu arasındaki değerler istatistiksel olarak anlamlı değildi ($p>0.05$).

MDA düzeyi ortalaması istatistiksel olarak kontrol grubunda 7.87, formaldehit grubunda 8.28 ve formaldehit ve üzüm çekirdeği ekstraktı grubunda 8.20 nmol/g olarak tespit edildi. Yapılan deney sonucuna göre kontrol grubu ile formaldehit grubu; kontrol grubu ile formaldehit ve üzüm çekirdeği ekstraktı grubu ve formaldehit ile formaldehit ve üzüm çekirdeği ekstraktı grupları arasındaki istatistiksel olarak çıkan sonuçlar anlamlı olarak bulundu ($p<0.05$) (Tablo 2).

1



Şekil 1. Kontrol grubu sıçan testis dokusunun ışık mikroskopik görüntüsü (bar: 200 µm, H&E x 4 BBA).
1: Sertoli hücresi, 2: Spermatojenik hücre serileri, 3: Leydig hücreleri (µm: Mikrometre, H&E x 4 BBA: Hemotoksilen ve Eozin ile 4'lük büyük büyütme alanı)

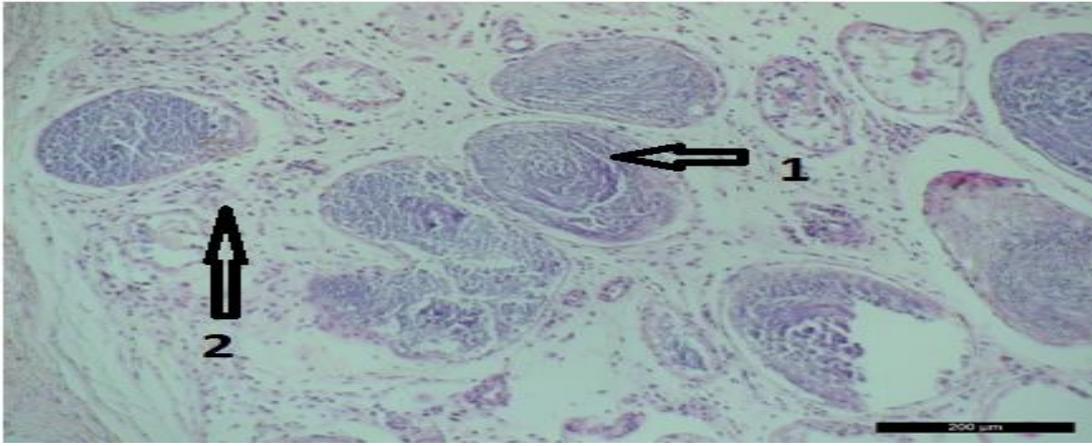
Histopatolojik bulgular

Çalışmamızda, histopatolojik değişiklikleri açığa çıkarmak amacı ile her üç gruba ait testis doku örnekleri incelendi.

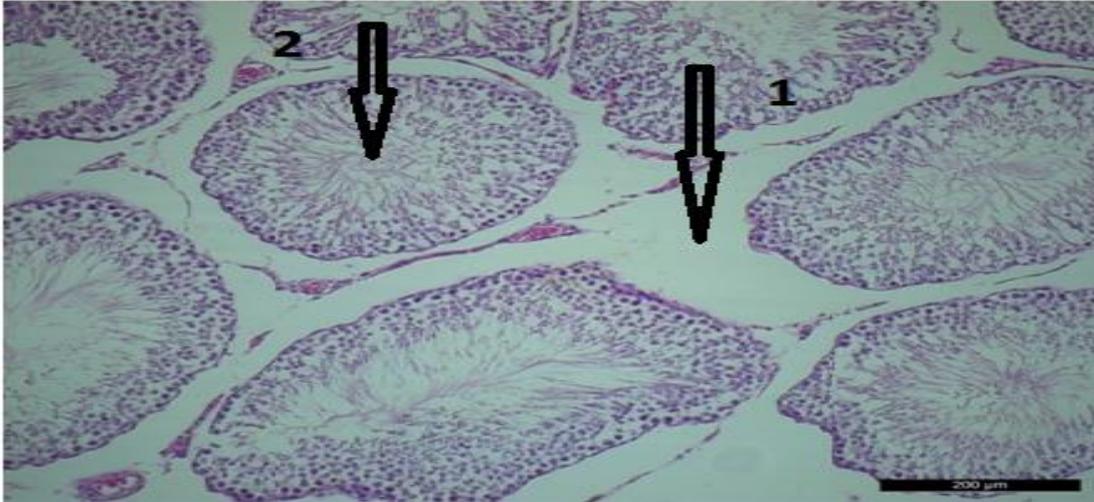
Kontrol grubunu oluşturan sıçanların testis doku örneklerinin mikroskopik incelemelerinde, tubuli seminiferi kontorti ve intersitisyel alanların normal histolojik görünümde olduğu belirlendi. Seminifer tubül sınırlarının düzgün, sertoli hücrelerinin belirgin olduğu tubüllerde; spermatogenik hücrelerinin düzenli dizildiği, spermiyogenezin tüm basamaklarının belirgin olduğu, intersitisyel alanlarda özellikle damar çevrelerinde gruplar halinde oval çekirdekli Leydig hücrelerinin bulunduğu görüldü (Şekil 1).

Formaldehit verilen sıçanların testis doku örneklerinde, tubuli seminiferi kontortiler de yaygın ve şiddetli nekroz

ile intersitisyel bölgede ödem, fibrin iplikleri ve kanama ile Leydig hücrelerinde nekroz tespit edildi. Sıçanların altısında tubuli seminiferi kontorti'de yaygın ve şiddetli nekroz sebebiyle sertoli hücre ve spermatogenik hücrelerin tamamen gözden silinerek geriye sadece bazal tabakanın kaldığı görüldü. Yoğun nekrozun şekillendiği bazı tubüllerde dökülmüş nekrotik hücreler nedeniyle dilatasyon şekillenmişti. Daha az şiddetli nekrozun şekillendiği dört vakada ise, intersitisyel alanda ödem, hiperemi ile birlikte bazı tubüllerde nekrotik hücrelerin yanında az sayıda sağlam kalmış sertoli ve spermatogenik hücrelerin bulunduğu, kanal lümenlerinde spermatozoa yoğunluğunda azalma şekillendiği dikkati çekti (Şekil 2).



Şekil 2. Formaldehit uygulanan sıçan testis dokusunda yaygın ve şiddetli nekroz, intersitisyel bölgede yoğun ödem nedeniyle genişleme (bar: 200 µm H&E x 4 BBA). 1: Nekrotik hücreler, 2: İntersitisyel ödem (µm: Mikrometre, H&E x 4 BBA: Hemotoksilen ve Eozin ile 4'lük büyükbüyükme alanı)



Şekil 3. Formaldehit ve üzüm çekirdeği ekstraktı verilen sıçan testis dokusu (bar: 200 µm, H&E x 4 BBA). 1: İntersitisyel ödem, 2: Spermatogenetik hücreler (µm: Mikrometre, H&E x 4 BBA: Hemotoksilen ve Eozin ile 4'lük büyükbüyükme alanı).

Formaldehit ile birlikte üzüm çekirdeği ekstraktı verilen sıçanların testis dokusu, sadece formaldehit verilen sıçanlarla kıyaslandığında patolojik bulguların şiddeti ve sayısında azalma saptandı. Bu grupta ayırıcı bulgu tubuli seminiferi kontortiler de, formaldehit grubunda görülen yaygın ve şiddetli nekrozun yerine segmental nekrozların şekillenmesiydi. Segmental nekroz şekillenen beş vakada tubül lümenlerinde pembe renkli hiyalinize materyal, nekroze olup dökülmüş hücreler ile birlikte sağlam kalan alanlarda dejeneratif sertoli hücrelerine rastlandı. Segmental nekroz görülen tubüllerde spermiyogenezis tamamen kaybolmuş görünürken, etkilenmenin daha az olduğu tubüllerde farklı derecelerde de olsa spermiyogenezin değişik aşamalarına rastlandı.

İntersitisyel alanda ise ödemin yanı sıra eozinofilik sitoplazmalı dejeneratif Leydig hücreleri ile tek tük nekrotik hücreler belirlendi. Segmental nekroz görülmeyen deneklerin testis dokularında, intersitisyel alanlarda belirgin ödem, tubüllerde lümenine yakın sertoli ve spermatogenik hücrelerde nekroz nedeniyle dökülme ile çok sayıda spermatozolar görüldü (Şekil 3).

TARTIŞMA

Formaldehit; aldehitler grubundan, oldukça reaktif, renksiz, keskin kokulu ve ayrıca suda iyi çözünen bir kimyasal bileşiktir. Literatürde yaygın kullanım alanı olan formaldehit ile ilgili yapılan çalışmalarda, formaldehit'in uygulama şekli, dozu ve etki süresi ile ilgili çeşitli çalışmalar ortaya çıkmıştır. Yapılan literatür taramalarında, 0.1 ppm ile 1000 ppm aralığında farklılık gösteren ve uygulama süresi on altı gün ile iki yıl arasında süren çalışmaların olduğu görülmüştür (Canbilen ve ark., 1999; Çelik ve ark., 2001; Sarsılmaz ve ark., 1999).

Balu ve ark. (2005a-2005b) yılında yapmış olduğu çalışmaya göre; genç ve yaşlı sıçanlara günlük 100 mg/kg oral üzüm çekirdeği ekstraktının 30 gün süresince verilmesi, yaşlı olan sıçanlarda omurilik, serebral korteks, striatum ve hippokampus alanlarında artan; protein karbonil, lipid peroksidasyonu ve DNA hasarını önemli ölçüde azaltmıştır. Azalan enzimatik (SOD, GPx ve CAT) ve non-enzimatik (GSH, E ve C vitamini) antioksidanlar, üzüm çekirdeği ekstraktı verilmeyen yaşlı sıçanlara göre önemli ölçüde artmıştır. Ancak sunulan deneysel çalışmada üzüm çekirdeği grubuna ait SOD değerinin kontrol ve formaldehit grubuna göre istatistiksel olarak anlamlı bir şekilde artmadığı görüldü. Iwasaki ve ark. (2004) yapmış olduğu bir çalışmada gastrik mukoza hasarı oluşturulan sıçanlara içme suyunda %0.002, %0.02, % 0.2 veya %1 oranında iki hafta boyunca adlibitum üzüm çekirdeği ekstraktı verilmiştir. Deneysel çalışma sonunda üzüm çekirdeği ekstraktı uygulanmasının gastrik mukoza hasarı ve MPO aktiviteleri önemli ölçüde baskıladığı, SOD aktivitesini ise artırdığı belirtilmiştir. Sunulan çalışmada formaldehite maruz kalan testis doku örneklerinde SOD aktivitelerinin gruplara göre istatistiksel olarak formaldehit uygulanan grupta anlamlı bir şekilde arttığı, TAS değerinin ise formaldehit ile üzüm çekirdeği

uygulanan grupta anlamlı olarak arttığını, formaldehit uygulanan grupta ise azaldığı gözlemlendi.

Testosteron, Leydig hücrelerinden salgılanan ve germinal hücrelerin bölünme ve gelişmesinde etkili olan bir hormondur. Bu hormon erkek cinsiyet organlarının gelişmesine neden olur (Hall ve Guyton, 2006). Yapılan çalışmada formaldehit uygulanan gruptaki testosteron düzeyi formaldehit uygulanan grupta istatistiksel olarak anlamlı bir şekilde azalmış ve formaldehit ile birlikte üzüm çekirdeği ekstraktı verilen grupta ise artmıştır. Çalışmamızın biyokimyasal verileri incelendiğinde formaldehit ile birlikte üzüm çekirdeği ekstraktı uygulanan sıçanlara ait testis dokusundaki testosteron değerinin formaldehit uygulanan gruptaki sıçanların testosteron değerlerine göre istatistiksel anlamlı şekilde arttığı görülmüştür.

Metabolizma, oksidan streslere karşı kendi antioksidan sistemlerini ortaya çıkararak oksidatif strese karşı çıkmaktadır. Oluşan oksidatif stres, enzimatik ve enzimatik olmayan antioksidan mekanizmaları ile tespit edilmektedir. TAS değeri bütün bu bileşimlerin hepsini temsil etmektedir. Deneysel olarak yapmış olduğumuz bu çalışmada, testis dokusundaki TAS değerlerini karşılaştırdığımızda istatistiksel açıdan anlamlı bir değişme olmadığı saptandı. Sadece serum TAS değerinin, kontrol grubu ile formaldehit uygulanan grup arasında istatistiksel olarak anlamlı olduğu belirlendi.

Lipid peroksidasyonu sonucu oluşan ve oksidatif hasarı gösteren parametrelerden en önemlisi malondialdehittir (MDA). Zhou ve ark. (2006) yılında yaptıkları çalışmaya göre formaldehitin oksidatif stresi yükselterek testisin yapı ve fonksiyon bozukluğuna yol açtığı bu durumun E vitamini ile düzeltildiği gösterilmiştir. Tang ve ark. (2003) yaptığı çalışmaya göre farelere beş gün boyunca intraperitoneal olarak formaldehit uygulanması sonucunda testis doku MDA değerlerinde anlamlı bir artış meydana gelmiştir. Ayrıca Zararsız ve ark. (2006) yaptığı bazı deneysel çalışmada formaldehit uygulanması sonucu dokularda oksidatif hasar oluştuğu gözlemiştir. Yürütülen çalışmanın doku MDA verileri; Zhou ve ark. (2006), Tang ve ark. (2003) ve Zararsız ve ark. (2006) belirttiği sonuçlar ile paraleldir. Ancak sadece serum MDA analizinde istatistiksel olarak anlamlı bir düzelme olmadığı görülmüştür. Deneysel olarak yapılmış olan birçok çalışmada, formaldehit maruziyeti sonrasında morfolojik olarak sıçanların testislerinde farklılıklar gözlenmiştir (Chowdhury ve ark., 1992; Özen ve ark., 2005; Tang ve ark., 2003; Zhou ve ark., 2006). Zhou ve ark. (2006) yaptığı deneysel bir çalışmada iki hafta boyunca formaldehit inhalasyonu sonrasında seminifer tubüllerde atrofi olduğu görülmüştür. Özen ve ark. (2005) ile Sarsılmaz ve ark. (1999) yapmış olduğu çalışmalara göre formaldehit maruziyetinin testis ağırlıkları ve seminifer tubül çaplarında azalma meydana getirdiği belirtilmiştir. Benzer şekilde, deneysel olarak yapılan başka çalışmalarda da formaldehitin sperm sayıları ve miktarında azalma neden olduğu görülmüştür (Chowdhury ve ark., 1992; Hall ve Guyton, 2006; Iwasaki ve ark., 2004; Tang ve ark., 2003; Zhou ve ark., 2006).

Sunulan çalışmada literatür bildirimleriyle uyumlu sonuçlar ortaya çıkmıştır. Formaldehit maruziyetiyle testis hücrelerindeki interstisyel ödemin arttığı, tubül lümenlerinin genişlediği, tubüllerdeki germ hücreleri ile spermatozoa sayısında ve de Leydig hücrelerinde azalma olduğu görülmüştür.

SONUÇ

Elde edilen bulgular ışığında formaldehit maruziyetiyle birlikte üzüm çekirdeği ekstraktı uygulanan sıçanlara ait testis doku örneklerindeki SOD değerleri ve TAS parametrelerinin anlamlı bir şekilde artmadığı; testosteron değerinin ise kontrol grubuna göre anlamlı bir şekilde arttığı belirlendi. MDA seviyelerinin ise istatistiksel olarak anlamlı bir şekilde azaldığı görüldü. Ayrıca histopatolojik olarak yapılan incelemelerde, formaldehit ile meydana gelen patolojik hasarın, üzüm çekirdeği ekstraktının antioksidan özelliği sayesinde kısmen giderildiği tespit edilmiştir.

Çıkar Çatışması

Yazarlar bu makalenin araştırılması, yazarlığa ve/veya yayınlanması ile ilgili olarak çıkar çatışması beyan etmemiştir.

Yazar Katkıları

Plan, tasarım: MC, GA; **Gereç, Yöntem ve veri toplama:** GA, EÖ; **Analiz ve yorum:** MC, GA; **Yazım ve eleştirel değerlendirme:** GA.

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Üniversite Öğrencilerinde İnteraktif Online Flört Şiddeti Eğitimi

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ÖZ

Amaç: Flört şiddeti gençler arasında yaygın olarak görülen ve yaşamlarını etkileyen önemli bir psikososyal sorundur. Bu araştırmanın amacı Balıkesir Üniversitesi Sağlık Bilimleri Fakültesi Hemşirelik ve Fizyoterapi ve Rehabilitasyon bölümü öğrencilerinin flört ilişkilerinde şiddetin önlenmesini hedefleyen bir eğitim programının etkinliğini değerlendirmektir. **Gereç ve Yöntem:** Araştırma Balıkesir Üniversitesi Sağlık Bilimleri Fakültesi'nde Hemşirelik ve Fizyoterapi ve Rehabilitasyon bölümlerinde öğrenim gören, Toplumsal Cinsiyet Eşitliği dersini alan 85 öğrenci üzerinde yürütüldü. Çalışma kapsamında öğrencilere iki oturumluk interaktif online flört şiddeti eğitimi verildi. Eğitim öncesinde Flört Şiddeti Tutum Ölçeği" (FŞTÖ) ile flört şiddeti tutumları ve "Şiddet Eğilim Ölçeği" (ŞEO) ile şiddet eğilimleri; eğitim sonrasında FŞTÖ tekrar değerlendirildi. **Bulgular:** Katılımcıların %14.3'ü flört ilişkisinde şiddete uğradığını, %4.7'si ise şiddete başvurduğunu bildirmiştir. Çalışmaya katılanların %96.48'i interaktif online flört şiddeti eğitimi faydalı bulduğunu belirtmiştir. Katılımcıların FŞTÖ ortalama değerinin eğitim sonrası istatistiksel olarak anlamlı düzeyde düştüğü gözlenmiştir ($p<0.05$). **Sonuç:** Çalışmanın sonuçları, eğitimin katılımcıların flört şiddeti tutumlarında değişiklik yaratmada etkili olabildiğini göstermiştir. Bu çalışma, Türkiye'de flört şiddetini önleme konusunda interaktif online bir eğitim ortaya koyan ve eğitimin etkinliğini inceleyen ve önemini vurgulayan ilk çalışmalardan biridir.

Anahtar kelimeler: Flört Şiddeti, Üniversite Öğrencileri, İnteraktif Online Eğitim.

Interactive Online Dating Violence Training for University Students

ABSTRACT

Objective: Dating violence is an important psychosocial problem that is common among young people and affects their lives. The aim of this research is to evaluate the effectiveness of a training program aimed at preventing violence in dating relationships among students from Balıkesir University Faculty of Health Sciences, Department of Nursing and Physiotherapy and Rehabilitation. **Materials and Methods:** The research was carried out on 85 students studying in the Department of Nursing, Physiotherapy and Rehabilitation at Balıkesir University, Faculty of Health Sciences and taking the Gender Equality course. Within the scope of the study, two sessions of interactive online dating violence training were given to the students. Attitudes to dating violence with the "The Dating Violence Scale-DVS" and violence tendencies with the "the Violence Tendency Scale" before the training; After the training, the DVS was re-evaluated. **Results:** 14.3% of the participants reported that they were subjected to violence in a dating relationship, and 4.7% reported that they resorted to violence. 96.48% of the participants in the study stated that they found interactive online dating violence training useful. It was observed that the mean DVS values of the participants decreased statistically significantly after the training ($p<0.05$). **Conclusion:** The results of the study showed that the training could be effective in creating a change in the participants' attitudes towards dating violence. This study is one of the first studies in Turkey that presents an interactive online training on the prevention of dating violence, examines the effectiveness of the training and emphasizes its importance.

Keywords: Dating Violence, University Students, Interactive Online Training.

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GİRİŞ

Flört şiddeti, ısrarlı takip de dahil olmak üzere evli olmayan bir flört ilişkisinde ortaya çıkan fiziksel, psikolojik, duygusal, ekonomik veya cinsel istismarı ifade etmektedir (CDC,2021). Flört şiddeti beraberlik sırasında ya da sonrasında kişilerin diğeri üzerinde kontrol kazanmaya çalıştığı, zarar verici davranışları içermektedir. Zarar verici davranışlar; partneri kontrol etmek için sözel, duygusal, sanal, fiziksel ya da cinsel şiddet, tehdit ya da sosyal kısıtlamalar gibi farklı biçimlerde ortaya çıkabilir. Çoğu zaman bir şiddet türü ortaya çıktıktan sonra diğeri türler de ortaya çıkmakta ve artarak devam etmektedir (Koçak ve Can, 2019; Set, 2020).

Flört şiddeti gençler arasında yaygın olarak görülen ve yaşamlarını etkileyen önemli bir psikososyal sorundur. Genç bireylerin yaklaşık %20-37'sinin ilişkilerinde flört şiddetine maruz kaldığı tahmin edilmekte ve bu gençler yalnızca şiddet içeren davranışların kurbanları olarak değil, aynı zamanda failer olarak da karşımıza çıkmaktadır (Cornelius ve Resseguie, 2007). Üniversite öğrencilerinin %8-37'sinin fiziksel, %2-10'unun cinsel ve %43-86'sının psikolojik flört şiddetine maruz kaldığı bildirilmektedir. Bu şiddet deneyimlerinin kısa ve uzun vadede fiziksel yaralanma, kronik baş ve boyun ağrısı, artan depresyon ve anksiyete bulguları, post travmatik stres bozukluğu, düşük akademik performans, madde ve alkol kullanımı ve cinsel yolla bulaşan hastalıklar gibi pek çok akademik, fiziksel ve psikolojik riskle ilişkili olduğu bilinmektedir (Brewer, Thomas ve Higdon, 2018; Eshelman ve Levendosky, 2012; Üstünel, 2020). Gençlerle flört şiddetinin yaygınlığı ve sonuçları, onu erken ve etkili önleme gerektiren bir halk sağlığı sorunu haline getirmektedir. Flört şiddetini önlemeye yönelik yapılan araştırmalarda birey, ilişkiler, topluluk ve toplum dahil olmak üzere birden fazla düzeyde var olan flört şiddeti risk faktörlerini incelenmektedir. Araştırmalar, gençlerin ailelerinde şiddete maruz kalma deneyimlerinin (ebeveynler arası şiddete tanık olma; çocuklara kötü muamele), flört şiddeti ve akran ve suçbağı şiddet uygulanması ile bağlantılı olduğunu göstermektedir. Bununla birlikte şiddet içeren ilişkileri olan arkadaşlara sahip olmanın, ebeveynler arası şiddete tanık olmaktan ziyade flört şiddeti uygulama ve mağduriyetini daha güçlü bir şekilde yordadığı da düşünülmektedir. Sosyal ilişkilerde çoklu şiddet deneyimleri, olumlu rol modellerinin eksikliği ve şiddeti destekleyici akran grubu normları, akran ve flört şiddeti yaşama veya uygulama riskini artıran etkileşimli faktörlerdendir (Ball ve arkadaşları 2012; Brendgen, Vitaro, Tremblay ve Wanner, 2002; Resnick, Ireland ve Borowsky, 2004).

Flört şiddetinin yaygınlığı ve ortaya çıkardığı olumsuz sonuçlar görüldükçe önlenmesi için yapılan toplum araştırmaları geçtiğimiz yıllarda hızlanmaya başlamıştır. Flört şiddeti ile ilgili bilgilendirme çalışmaları ileride ortaya çıkabilecek şiddet davranışlarının önüne geçmeye ve gençlerin bilinçlenerek kendilerini korumalarına yardımcı olacaktır. Bu nedenle eğitim sistemi içerisinde flört şiddetini önlemeyi hedefleyen

programların geliştirilmesi ve öğrencilerin içerisinde yer alacağı aktivitelerin düzenlenmesi ile şiddet algısının değişeceği ve öğrencilerin bilincinin artacağı böylece şiddetin önüne geçilebileceği düşünülmektedir (Özdere ve Kürtül, 2018; Selçuk, Dilek ve Mercan, 2018; Set, 2020; Wong, Bouchard ve Lee, 2021). Son dönemlerde ülkemizde de flört şiddetinin gençler arasında yaygın bir sorun olduğu ortaya konulmuş, bu konudaki araştırmaların sayısı artmış, bilgilendirici ve farkındalık artırıcı çalışmaların gerekliliği ortaya çıkmıştır. Ancak ulusal literatüre bakıldığında flört şiddeti eğitim programının etkinliğini inceleyen araştırma sayısının oldukça kısıtlı olduğu görülmektedir.

Bu araştırmanın amacı Balıkesir Üniversitesi Sağlık Bilimleri Fakültesi Hemşirelik ve Fizyoterapi ve Rehabilitasyon bölümü öğrencilerinin flört ilişkilerinde şiddetin önlenmesini hedefleyen bir eğitim programının etkinliğini değerlendirmektir.

Araştırmanın hipotezleri

H₁: Sağlık Bilimleri Fakültesi Hemşirelik ve Fizyoterapi ve Rehabilitasyon bölümü öğrencilerine flört şiddeti konusunda uygulanan interaktif online eğitimin flört şiddeti tutumu puan ortalamasına etkisi vardır.

H₂: Sağlık Bilimleri Fakültesi Hemşirelik ve Fizyoterapi ve Rehabilitasyon bölümü öğrencilerine flört şiddeti konusunda uygulanan interaktif online eğitimin kız öğrencilerde flört şiddeti tutumu puan ortalamasına etkisi erkek öğrencilere göre daha yüksektir.

GEREÇ VE YÖNTEM

Araştırma Balıkesir Üniversitesi Sağlık Bilimleri Fakültesi'nde Hemşirelik ve Fizyoterapi ve Rehabilitasyon bölümlerinde öğrenim gören, Toplumsal Cinsiyet Eşitliği dersini alan öğrenciler ile gerçekleştirildi. Toplumsal cinsiyet açısından geleneksel bakış açısı, eşitsizliklerin ve şiddetin temel kaynaklarından biri olarak kabul gördüğünden, özellikle Toplumsal Cinsiyet Eşitliği dersi alan öğrencilerde dersi almayan öğrencilere göre toplumsal cinsiyet açısında temel bir bilgi birikimi ders süreci içerisinde olduğundan dersi alan öğrencilerde yapılması tercih edilmiştir.

Araştırmanın tipi

Çalışma ön test ve son test biçiminde ve tek grupta yarı deneysel tipte uygulandı.

Araştırmanın yeri ve zamanı

Çalışma Balıkesir Üniversitesi Sağlık Bilimleri Fakültesi'nde 2020-2021 Güz Eğitim ve Öğretim yarıyılıının sonunda gerçekleştirilmiştir.

Araştırmanın evreni ve örnekleme

Araştırmanın evrenini Fizyoterapi ve Rehabilitasyon ve Hemşirelik bölümünde Toplumsal Cinsiyet Eşitliği Dersi alan tüm öğrenciler oluşturmakta olup (n=102), örneklem seçimi için Toplumsal Cinsiyet Eşitliği dersini almış olmak dışında herhangi bir kriter belirlenmemiştir. Çalışmaya katılmaya gönüllü olan toplam 95 öğrenciyle 2020-2021 Güz Eğitim ve Öğretim yarıyılıının sonunda iki oturumluk, her oturum

60 dakika olacak şekilde flört şiddeti eğitimi verildi. Yapılan incelemelerde 10 kişinin ölçekleri doğru bir şekilde doldurmadığı görüldü. Bu nedenle 85 kişinin verileri analiz edildi. Evrenin %83.3'üne ulaşılmıştır.

Veri toplama araçları ve yapılan işlem

İnteraktif yöntemler kullanılarak oluşturulan online eğitim öncesinde Flört Şiddeti Tutum Ölçeği" (FŞTÖ) ile flört şiddeti tutumları ve "Şiddet Eğilim Ölçeği" (ŞEÖ) ile şiddet eğilimleri; eğitim sonrasında ise Flört Şiddeti tutumları tekrar değerlendirildi.

Online eğitim içeriği: Çalışmanın amacı doğrultusunda interaktif online öğretim yöntemleri kullanıldı. Bu yöntemlerden ilkini, farklı konuları içeren teorik materyaller oluşturdu. Bu kapsamda Flört şiddetinin tanımı ve türleri, risk faktörleri, görülme oranları, etkileri, flört şiddetine yönelik tutum ve algılar, koruma programları, flört şiddetini besleyen mitler, güvenli ilişki cetveli, flört hakları, sağlıklı sosyal ve duygusal ilişki gibi teorik konuları içeren okumalar yapıldı, örnek olaylar incelendi ve bunlara ilişkin tartışmalar yapıldı (Ball ve ark., 2012; CDC, 2021, Üstünel, 2020). İkincisini ise, teorik okumaları destekler biçimde olan Flört şiddeti ile ilgili 40 Tilki Blog tarafından oluşturulmuş olan 10 kısa video içeriğinin paylaşılması ve tartışılması oluşturdu (40 Tilki, 2021). Video içeriğinin paylaşılması için gerekli izinler alındı. Ayrıca bu materyallerin dışında, öğrencilerin gözlemleri ve deneyimleri eğitim içi etkinlikte başvuru olan canlı kaynakları oluşturdu.

Eğitim videoları: Video içerikleri; Aşk, sevgi nedir, ne değildir? Flört şiddeti nerede başlar? Öfke nedir?, Şiddet nedir?, Sanal şiddet nedir?, Israrlı takip nedir?, Flört şiddeti nedir? Konularını içermektedir.

Videolar (40 Tilki, 2021):

- Tehdit ve Suçlu Hissettirme
- Öfke
- Aşırı Kontrol
- Sanal Şiddet
- Israrlı Takip 1
- Israrlı Takip 2
- Bir Flört Şiddeti Hikayesi / My Story About My Abusive Boyfriend
- Aşk Bu Değil / That's Not Love
- Seni Seviyorum O Halde Sil 1
- Seni Seviyorum O Halde Sil 2

Tanımlayıcı Bilgi Formu: Tanımlayıcı Bilgi Formu araştırmacılar tarafından literatür taranarak oluşturuldu (Foshee ve ark., 2004; Selçuk ve ark., 2018). Araştırmaya katılan öğrencilere cinsiyet, anne-baba eğitim durumu, yaşadığı yer gibi sosyo-demografik özellikleri, şiddet uygulama/maruz kalma durumu, flört ilişkisi varlığı ve süresine ilişkin sorulardan oluşan tanımlayıcı bilgi formu uygulanmıştır.

Flört Şiddeti Tutum Ölçeği (FŞTÖ): Katılımcıların eğitim öncesi ve sonrası flört şiddetine yönelik tutumlarını değerlendirmek için Terzioğlu ve arkadaşlarının (2016) geliştirdiği "Flört Şiddeti Tutum Ölçeği" kullanıldı. Beş alt boyuttan oluşan ölçekte cinsel şiddet 7, duygusal şiddet 6, genel şiddet 5, ekonomik şiddet 5 ve fiziksel şiddet 5 madde olmak

üzere toplamda 28 madde yer almaktadır. Flört Şiddeti Tutum Ölçeği'ne verilen yanıtlar her bir maddeye verilen puanlar üzerinden değerlendirilmektedir. Ölçekte maddeleri 5'li likert tipte ve 1) "kesinlikle katılmıyorum", 2) "Katılmıyorum", 3) "Kararsızım", 4) "Katılıyorum", 5) "Tamamen Katılıyorum" şeklindedir. Her madde için alınabilecek en yüksek puan 5, en düşük puan ise 1'dir. Puan ortalamasının 5'e yaklaşması bireylerin flört şiddetine yönelik sahip olduğu tutumlarının, flört şiddetini desteklemediğini göstermektedir (Gönenç ve arkadaşları, 2016).

Şiddet Eğilimi Ölçeği (ŞEÖ)

Öğrencilerin şiddet eğilimlerinin değerlendirilmesi Göka, Bayat ve Türkçapar tarafından 1995 yılında Milli Eğitim Bakanlığı tarafından yürütülen çalışmada kullanılmak üzere geliştirilen "Şiddet Eğilimi Ölçeği (ŞEÖ)" ile yapılmıştır. ŞEÖ öğrencilerin şiddet eğilimlerini ölçmek için kullanılan geçerli ve güvenilir bir ölçektir. ŞEÖ'de toplam 20 madde bulunmaktadır. "Hiç uygun değil", "Biraz uygun", "uygun" ve "çok uygun" maddeleri sırasıyla bir ile dört arasında puanlanan ölçekten en az 20 ve en fazla 80 puan alınmaktadır. Yüksek puan, bireyin şiddet eğiliminin yüksek olduğunu göstermektedir. 1-20 arasındaki puan öğrencinin Şiddet eğiliminin "çok az", 21-40 arasındaki puan "az"; 41-60 arasındaki puan "fazla", 61-80 arasındaki puan "çok fazla" olduğunu göstermektedir. (Başbakanlık Aile Araştırma Kurumu, 1998).

İstatistiksel analiz

Verilerin değerlendirilmesinde SPSS 22.0 programı kullanıldı. Verilerin analizinde standart sapma, aritmetik ortalama, frekans ve yüzde değerleri hesaplandı, veriler normal dağılıma uygun olarak dağılmadığından eğitim öncesi ve sonrası ölçek sonuçlarındaki değişimi incelemek için Wilcoxon Sıralı testi uygulandı. Ölçek değerlerinin cinsiyetler arasındaki karşılaştırmaları için Mann Whitney U testi kullanılmıştır (Hayran, 2011).

Araştırmanın etik yönü

Çalışmamız, 24.02.2021 tarihinde E-94025189-050.01.04-10806 sayı numarası ile Balıkesir Üniversitesi Tıp Fakültesi Klinik Araştırmalar Etik Kurulu tarafından onaylanmıştır. Araştırma Helsinki Deklarasyonuna uygun şekilde yürütülmüş olup, katılımcılardan online ortamda yazılı olarak onam alınmıştır. Kullanılan eğitim içerikleri için içerik üreticilerinden, ölçekler için ölçek sahiplerinden gerekli izinler alınmıştır.

BULGULAR

Katılımcıların demografik özelliklerine göre dağılımını Tablo 1'de gösterilmiştir. Toplam 85 katılımcının %71.8'i (n=61) kadın, %28.2'si (n=24) erkektir. Katılımcıların %75.3'ünün (n=64) geçmişte, %27.1'inin (n=23) ise günümüzde flört ilişkisi olduğu belirlenmiştir. Flört ilişkisi yaşamış 64 kişiden %46.9'u (n=30) flört süresinin 1 yıldan az, %40.6'sı (n=26) 1-3 yıl arası, %12.5'i (n=8) ise dört yıldan uzun sürdüğünü belirtmiştir. Katılımcıların %14.3'ü (n=11) flört ilişkisinde şiddete uğradığını, %4.7'si ise (n=4) şiddete başvurduğunu bildirmiştir (Tablo 1). Çalışmaya

katılanların 96.48'i (n=82) interaktif online flört şiddeti eğitimi faydalı bulunduğunu belirtmiştir.

Tablo 1. Katılımcıların demografik özelliklerine göre dağılımı.

Demografik Özellikler		n	%
Cinsiyet	Kadın	61	71.8
	Erkek	24	28.2
Bölüm	Fizyoterapi ve Rehabilitasyon	53	62.4
	Hemşirelik	32	37.6
Geçmiş flört ilişki durumu	Var	64	75.3
	Yok	21	24.7
Güncel flört ilişki durumu	Var	23	27.1
	Yok	62	72.9
Flört ilişki süresi	Bir yıldan az	30	46.9
	1-3 yıl	26	40.6
	Dört yıldan fazla	8	12.5
Flört ilişkisinde şiddete uğrama	Evet	11	14.3
	Hayır	66	85.7
Flört ilişkisinde şiddete başvurma	Evet	4	4.7
	Hayır	81	95.3
Şiddet eğilimi	Çok az	0	0
	Az	69	81.2
	Fazla	15	17.6
	Çok fazla	1	1.2
TOPLAM		85	100.0

Katılımcıların eğitim öncesi ve sonrası Flört Şiddeti Tutum Ölçeği alt parametrelerindeki değişim Tablo 2'de gösterilmiştir. FŞTÖ ortalama değerinin eğitim

sonrası istatistiksel olarak anlamlı düzeyde düştüğü gözlenmiştir ($p<0.05$, Tablo 2).

Tablo 2. Katılımcıların eğitim öncesi ve sonrası Flört Şiddeti Tutum Ölçeği alt parametrelerindeki ortalama puan değişim.

Alt Parametreler	Ön Test (n=85) X±SS	Son Test (n=85) X±SS	p
Genel Şiddet	4.77±0.42	4.76±0.43	0.82
Fiziksel Şiddet	4.65±0.47	4.73±0.47	0.04*
Duygusal Şiddet	4.58±0.48	4.62±0.56	0.22
Ekonomik Şiddet	4.36±0.62	4.45±0.62	0.11
Cinsel Şiddet	4.64±0.56	4.70±0.52	0.19
Toplam	4.60±0.37	4.65±0.42	0.011*

FŞTÖ: Flört Şiddeti Tutum Ölçeği, *Wilcoxon işaretli sıralar testi, $p<0.05$, X:Ortalama, SS:Standart sapma.

Katılımcıların Flört Şiddeti Tutum Ölçeği ortalamalarının cinsiyete göre karşılaştırmasına ilişkin bilgiler tablo 3'te gösterilmiştir. FŞTÖ ön test ve son test toplam değerlerinin kadınlarda istatistiksel olarak anlamlı düzeyde daha yüksek olduğu belirlenmiştir. Ön test sonuçlarında FŞTÖ'nin ekonomik ve cinsel şiddet alt parametrelerinde, son test sonuçlarında ise fiziksel, ekonomik ve cinsel şiddet alt parametrelerinde kadın

katılımcıların puanlarının istatistiksel olarak anlamlı düzeyde daha yüksek olduğu görülmüştür ($p<0.05$, Tablo 3).

Flört şiddetine maruz kalan 11 öğrencinin online interaktif flört şiddeti eğitimi sonrası FŞTÖ ortalamalarının 4.60'tan 4.65'e yükseldiği ve bu artışın istatistiksel olarak anlamlı olduğu saptanmıştır ($p=0.028$).

Tablo 3. Katılımcıların Flört Şiddeti Tutum Ölçeği puan ortalamalarının cinsiyetlere göre karşılaştırması.

Ölçekler n (85)	Kadın (n=61) X±SS	Erkek (n=24) X±SS	p
FŞTÖ Toplam - Ön Test	4.70±0.29	4.36±0.46	0.000*
Genel Şiddet	4.82±0.34	4.66±0.57	0.80
Fiziksel Şiddet	4.70±0.42	4.53±0.56	0.24
Duygusal Şiddet	4.62±0.43	4.46±0.58	0.44
Ekonomik Şiddet	4.53±0.48	3.92±0.70	0.001*
Cinsel Şiddet	4.80±0.38	4.25±0.74	0.000*
FŞTÖ Toplam – Son Test	4.75±0.31	4.42 ±0.56	0.000*
Genel Şiddet	4.82±0.31	4.61±0.62	0.09
Fiziksel Şiddet	4.70±0.42	4.52±0.70	0.019*
Duygusal Şiddet	4.65±0.51	4.54±0.69	0.905
Ekonomik Şiddet	4.59±0.53	4.07±0.70	0.001*
Cinsel Şiddet	4.83±0.37	4.38±0.68	0.002*

FŞTÖ: Flört Şiddeti Tutum Ölçeği, **ŞEÖ:** Şiddet Eğilim Ölçeği, *Mann Whitney U Testi, p<0.05, **X:**Ortalama, **SS:**Standart sapma.

TARTIŞMA

Bu çalışma Balıkesir Üniversitesi Hemşirelik ve Fizyoterapi ve Rehabilitasyon bölümü öğrencilerinde İnteraktif Online Flört şiddeti önleme eğitimin öğrencilerin Flört Şiddeti tutumları üzerindeki etkisini araştırmayı hedeflemiştir. Bu amaçla, flört şiddeti konusundaki farkındalığı artıracak ve güvenli ve sağlıklı ilişkiler kurma konusunda bilgilendirme sağlayacak bir flört şiddeti önleme programı hazırlanmıştır. Çalışmanın sonuçları, eğitimin katılımcıların flört şiddeti tutumlarında değişiklik yaratmada etkili olabildiğini göstermiştir. Bu çalışma, Türkiye’de flört şiddetini önleme konusunda interaktif online bir eğitim ortaya koyan ve eğitimin etkinliğini inceleyen ilk çalışmalardan biri olma özelliğini taşıdığı için ulusal literatüre katkı sağlamaktadır. Bu çalışma ile flört şiddetine ilişkin önleyici yaklaşımların önemi vurgulanmaktadır.

Flört şiddetinin potansiyel fiziksel, psikolojik ve sosyal sonuçlarından dolayı bu saldırganlık biçimini önlemeye ve iyileştirmeye yönelik çalışmalar yapılmaktadır. Günümüzde flört ilişkilerindeki şiddeti önleme veya mağdurlarını korumaya yönelik çeşitli programlar geliştirilmiştir. Flört şiddetini önleme programlarında kullanılan teorik temeller ve teknikler açısından çok fazla çeşitlilik olmasına rağmen, önleme programları genellikle iki alt boyutta değerlendirilmektedir (Cornelius ve Resseguie, 2007). Birincil koruma programları genellikle risk faktörlerini belirleyerek bu doğrultuda bir kurumdaki ya da sınıftaki tüm popülasyonu veya daha sonra flört ilişkisinde şiddet görme ihtimali yüksek olan bireyleri hedefleyen önleme programlarıdır. Bu programda riskli bireylere eğitimler verilerek flört ilişkisi içerisinde ilk şiddet olayının önlenmesi amaçlanır. Birincil önleme programlarında genellikle sağlıklı ilişki stratejileri ve toplumsal cinsiyet eşitliği hakkında bilgilendirmeler yer alır. Flört ilişkilerinin 18-24 yaş aralığında görülme oranı yüksek

olduğundan, genellikle birincil önleme programları bu yaş grubunu hedef alarak uygun tutum ve davranışları arttırmayı amaçlar. Araştırmalar birincil koruma yöntemlerinin farkındalığın artmasında etkili olduğunu göstermektedir. İkincil koruma programları ise, mevcut şiddeti durdurmaya odaklanmıştır. Flört şiddeti vakalarına veya mevcut zararlı tutumların sürdürülmesine müdahale etmek için geliştirilen güvenli ve uygun yollar anlatılır bununla birlikte flört şiddetinin mevcut olduğunu gösteren uyarı işaretleri öğretilir. Her iki önleme programı da genellikle grup tartışmaları, rol yapma senaryoları, yazılı bilgilendirme metinleri veya videolar gibi etkileşimli etkinlikleri içerir (Wong ve arkadaşları, 2021). Bizim çalışmamızda da literatürdeki benzer çalışmalar örnek alınarak şiddet ve flört şiddeti konusunda yazılı bilgilendirme metinlerine, grup tartışmalarına, örnek senaryolara ve çözümlerine, eğitim videolarına ve tartışmalara yer verilerek interaktif bir eğitim ortamı oluşturuldu.

Flört şiddetini önlemeye yönelik programların etkinliğini inceleyen en güncel meta analizde, yapılan çalışmalarda çoğunlukla tek seanslık kısa eğitimlerin yer aldığı ve ön test- son test yöntemi ile tutum değişikliklerinin incelendiği ve bu eğitimlerin flört şiddeti tutumlarında değişime sebep olurken davranışları değiştirme açısından yeterli olmadığı bildirilmiştir (Wong ve arkadaşları, 2021). Ball ve ark. 144 öğrencide 24 haftalık bir flört şiddeti önleme programı geliştirmiş ve programın sonunda öğrencilerin sağlıklı ilişki becerilerinde önemli artışlar görüldüğünü ancak flört şiddeti uygulama ve maruz kalma oranlarında bir değişim olmadığını bildirmişlerdir (Ball ve arkadaşları, 2012).

Davranış değişimini etkilemek, bilgi veya tutumları değiştirmekten çok daha karmaşık bir süreç olduğundan bu da öngörülebilir bir durumdur. Bizim çalışmamızda da benzer şekilde ikişer saatlik iki oturumdan oluşan bir eğitim öncesinde ve sonrasında katılımcıların flört şiddeti tutumları değerlendirilmiş ve eğitim sonucunda

flört şiddeti tutumlarında bir gelişme olduğu katılımcıların flört şiddetini artık daha az desteklediği görülmüştür. Bununla birlikte flört şiddeti mağduru olan öğrencilerin tutumları ayrıca analiz edilmiş ve flört şiddetini kabullenici tutumlarının azaldığı görülmüştür. Üniversite öğrencilerinde flört şiddetine yönelik tutumlarının değişmesinin istismarcı davranışların gelişimini dolaylı olarak önleyeceğini ve bunun da şiddetin ortadan kaldırılabilmesi için önemli bir adım olacağını düşünmekteyiz.

Çalışmamızda interaktif online flört şiddeti eğitimi sonucunda üniversite öğrencilerinde flört şiddetine ilişkin kabullenici tutumların azaldığı görülmüştür. Çalışmada kullandığımız Flört Şiddeti Ölçeği genel, fiziksel, duygusal, ekonomik ve cinsel olmak üzere flört şiddetini inceleyen beş alt parametreden oluşuyordu Eğitim öncesi ve sonrası öğrencilerin flört şiddeti tutumlarındaki değişim alt parametreler düzeyinde incelendiğinde genel olarak flört şiddetini destekleyici tutumların azaldığı ve en büyük farkın da fiziksel flört şiddetinde olduğu görüldü. Literatürdeki benzer çalışmalar incelendiğinde farklı ölçekler kullanılsa da yine de bezer sonuçlar elde edildiği görülmektedir. Özdere ve ark. 319 üniversite öğrencisine tek oturumluk bir flört şiddeti eğitimi vermiş ve eğitim sonrasında hem kadınlar tarafından hem de erkekler tarafından uygulanan fiziksel flört şiddeti kabullenici tutumlarında azalma olduğunu bildirmiştir (Özdere ve Kürtül, 2018). Üstünel ve ark. 90 öğrenci ile gerçekleştirdiği 8 oturumluk flört şiddeti önleme programı sonrası ise kadınlar tarafından uygulanan fiziksel şiddeti kabullenici tutumlarda bir azalma olurken, erkekler tarafından uygulanan fiziksel şiddete yönelik tutumlarda bir değişiklik olmadığını bildirmiştir (Üstünel, 2020). Foshee ve ark. ise 460 öğrenciye Güvenli Flört eğitimi verdiği ve bu öğrencileri 4 yıl süreyle izlediği çalışmanın sonucunda öğrencilerin uyguladığı ve maruz kaldığı fiziksel, duygusal ve cinsel flört şiddeti oranlarının; Wolfe ve ark. da 5 oturumluk eğitim sonrası fiziksel ve duygusal flört şiddeti oranlarının azalmasını sağladığını göstermiştir (Foshee ve arkadaşları, 2004; Wolfe, 2006). Hem bu çalışmayı hem de literatürdeki benzer çalışmaları incelediğimizde etkili bir flört şiddeti eğitimi ile başta fiziksel olmak üzere flört şiddetinin tüm alt parametrelerine dair öğrenci tutumlarında bir gelişme sağlandığı, öğrencilerin eğitimler sonrası flört şiddetini daha az desteklediği ve flört şiddeti uygulama /maruz kalma oranlarının azaldığı görülmektedir.

Flört şiddeti, romantik ilişkilerde, taraflardan birinin ya da her iki ikisinin de diğerine, fiziksel, cinsel, ekonomik, psikolojik ya da dijital şiddet uygulamasını ifade etmektedir. Literatür incelendiğinde flört ilişkilerinde şiddet görülme oranlarının oldukça yüksek olduğu ve şiddete uğrayan tarafın çoğunlukla kadın olduğu görülmektedir (Koçak ve Can, 2019; Set, 2020). Dünya genelinde kadınların %20-40 oranlarında fiziksel ve cinsel flört şiddetine, yaklaşık %90 oranında da psikolojik şiddete maruz kaldığı bilinmektedir (Tjaden ve Thoennes, 2000). Bizim ülkemizde yapılan araştırmalarda ise kadınların %84'ünün cinsel şiddeti

deneyimledikleri, üniversite öğrencilerinde ise hem fiziksel hem de cinsel şiddet uygulama oranlarının erkeklerde daha yüksek olduğu bildirilmiştir (Kayı, Yavuz ve Arıcan, 2000; Set, 2020). Bizim çalışmamızda da kadın öğrencilerin %16.7'si erkek öğrencilerin ise %8.7'si flört şiddetine maruz kaldığını bildirmiştir. Sadece şiddet uygulama ve maruz kalma oranları değil şiddete dair tutum ve algılarda da cinsiyetler arası farklılık olduğu görülmektedir. Ulusal yayımlar incelendiğinde erkek öğrencilerin fiziksel ve duygusal flört şiddetini kadınlara göre daha çok desteklediği görülmektedir (Özdere ve Kürtül, 2018; Selçuk ve arkadaşları, 2018; Üstünel, 2020). Bizim çalışmamızda ise kadın ve erkek öğrenciler şiddet eğilimleri ve eğitim öncesi fiziksel ve duygusal şiddeti kabullenme düzeyleri açısından benzerdi. Ancak ekonomik ve cinsel şiddeti kabullenme düzeyleri erkeklerde daha yüksekti. Eğitim sonrası bu değerler eğitim öncesi değerler göre azalsa da yine de kadın öğrencilere kıyasla erkek öğrencilerin hem fiziksel hem ekonomik hem de cinsel şiddeti desteklemeye daha çok meyilli olduğu görüldü. Cinsiyetler arası bu tutum farklılıklarının Türk toplumunun erkek egemen bir toplum olmasından ve üniversite öğrencilerinin yetiştikleri süreçte toplumsal cinsiyet eşitsizliği normlarını benimsemiş olmalarından kaynaklandığını düşünmekteyiz.

Ülkemizde flört şiddeti eğitiminin etkilerinin incelendiği çalışmaların flört şiddetinin yalnız psikolojik ve fiziksel parametrelerine odaklandığı ancak hiçbir çalışmada ekonomik ve cinsel şiddet tutumlarının incelenmediği görülmüştür. Bizim çalışmamız flört şiddeti eğitimi öncesinde ve sonrasında öğrencilerin hem ekonomik hem de cinsel flört şiddeti tutumlarını inceleyen ilk çalışma olma özelliğini göstermekte ve bu yönden ulusal literatüre önemli bir katkı sunmaktadır. Bununla birlikte uluslararası literatür incelendiğinde flört şiddetinin nedenlerini, risk faktörlerini, sonuçlarını ve ilişkili etmenleri inceleyen pek çok çalışma ile karşılaşılmakta flört şiddetinin çok yaygın ve önlenmesi gereken bir sorun olduğu belirtilmektedir. Flört şiddetinin erken yaşlarda başladığı ve önüne geçilmesi gereken önemli bir sorun olduğu belirtilmesine karşın flört şiddetini önlemeye yönelik yapılan araştırma sayısı oldukça kısıtlıdır. İnteraktif online flört eğitiminin öğrencilerin flört şiddeti tutumlarına ilişkin etkilerini inceleyen bu çalışmamızın hem ulusal hem de uluslararası literatüre önemli katkıları olduğunu düşünmekteyiz. Bu çalışma üniversite öğrencilerinin flört şiddeti ile ilgili görüşlerinin saptanması ve flört şiddetinden korunmaları için gerekli bilgilendirme ve farkındalığın oluşturulması açısından önemli bulgular sunmaktadır.

Araştırmanın Sınırlılıkları

Çalışmamızın bazı sınırlılıkları bulunmaktadır. Bunlardan birincisi çalışmanın tek grup ön test-son test tasarımında uygulanmış olması ve bir kontrol grubunun olmamasıdır. İkinci bir kısıtlılık ise çalışmanın kısa ve uzun dönem takip sonuçlarının olmamasıdır. Öğrencilerin eğitim sonrası flört şiddetine ilişkin

tutumları tekrar sorgulanmış ancak eğitimin uzun dönem sonuçları sorgulanmamıştır. Üçüncü bir kısıtlılık ise çalışma tek bir üniversitenin tek bir fakültesinde uygulanmıştır. Bundan sonraki çalışmalarda hem deney hem kontrol gruplarını içeren, farklı üniversitelerde farklı fakültelerin öğrencilerini de kapsayan ve eğitimlerin uzun dönem etkinliğini de inceleyen çalışmalara ihtiyaç vardır.

SONUÇ

İnteraktif online flört şiddeti eğitimiyle genç bireylerin flört şiddeti konusundaki farkındalığını arttırmayı, güvenli ve sağlıklı ilişkiler konusunda onları bilgilendirmeyi amaçladığımız çalışmamızın sonucunda flört şiddeti eğitiminin katılımcıların flört şiddeti tutumlarında değişiklik yaratmada etkili olabildiği görülmektedir. Bu çalışma, Türkiye’de flört şiddetini önleme konusunda interaktif online bir eğitim ortaya koyan ve eğitimin etkinliğini inceleyen ilk çalışmalardan biri olma özelliğini taşıdığı için ulusal literatüre katkı sağlamaktadır.

Yapılan eğitimde kız öğrencilerin flört şiddetine ilişkin tutumlarında daha yüksek oranda değişim olduğundan bu alanda yapılacak çalışmalarda erkek öğrenciler için farkındalığı daha çok arttıracak uygulamalar yapılabilir.

Çıkar Çatışması

Yazarlar, herhangi bir potansiyel çıkar çatışması beyan etmemektedir.

Yazar Katkıları

Plan, tasarım: EG, DAD; **Gereç, yöntem ve veri toplama:** EG, DAD; **Veri analizi ve yorumlar:** EG; **Yazım ve eleştirel değerlendirme:** EG.

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Frequency of Vitamin B12 Deficiency and Associated Findings in Children

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ABSTRACT

Objective: The purpose of this study was to determine clinical findings associated with vitamin B12 deficiency in patients presenting to the pediatric neurology department with various different symptoms and to discuss these in the light of the current literature. **Materials and Methods:** Children aged between one month and 18 years presenting to the Balıkesir University Medical Faculty pediatric neurology clinic, Turkey, between 01.08.2019-01.08.2021 and with vitamin B12 levels lower than 250 ng/mL were included in this retrospective, cross-sectional study. **Results:** One hundred sixty-one cases were included in the study 63 (39.1%) female and 98 (60.9%) male. The patients' mean age was 7.43±5.77 (0-17) years. The majority of patients of both genders were in the 0-6 age group. Neurological findings were present in 81.98% of patients with Vitamin B12 deficiency, and non-neurological findings in 18.02%. The most frequent neurological finding was neuromotor retardation (N=73, 45.34%), followed by seizures (N=33, 20.50%). The mean Vitamin B12 level among all the patients was 167.70±45.17 (58-250) pg/ml. No statistically significant difference was determined between boys and girls in terms of Vitamin B12 and ferritin levels or complete blood count parameters [Hb, Htc, and MCV] (respectively p=0.428, p=0.646, p=0.110, p=0.128, p=0.864). **Conclusions:** This study provides a general evaluation for health professionals regarding clinical presentations encountered by us in association with the disease.

Keywords: Vitamin B12, Children, Treatment, Deficiency, Sign.

Çocuklarda B12 Vitamini Eksikliği ve İlişkili Bulguların Sıklığı

ÖZ

Amaç: Bu çalışmanın amacı, çocuk nörolojisi polikliniğine çeşitli semptomlarla başvuran hastalarda vitamin B12 eksikliği ile ilişkili klinik bulguları belirlemek ve güncel literatür ışığında tartışmaktır. **Gereç ve Yöntem:** Bu retrospektif kesitsel çalışmaya 01.08.2019-01.08.2021 tarihleri arasında Balıkesir Üniversitesi Tıp Fakültesi Çocuk Nöroloji Polikliniğine başvuran ve B12 vitamini düzeyi 250 ng/mL'nin altında olan 1 ay-18 yaş arası çocuklar dahil edildi. **Bulgular:** Çalışmaya 63 (%39.1) kadın ve 98 (%60.9) erkek 161 olgu dahil edildi. Hastaların yaş ortalaması 7.43±5.77 (0-17) yılı idi. Her iki cinsiyetteki hastaların büyük çoğunluğu 0-6 yaş grubundaydı. Vitamin B12 eksikliği olan hastaların %81.98'inde nörolojik bulgular, %18.02'sinde nörolojik olmayan bulgular mevcuttu. En sık görülen nörolojik bulgu nöromotor retardasyon (N=73, %45.34), ardından nöbetler (N=33, %20.50) idi. Tüm hastalarda ortalama B12 vitamini düzeyi 167.70±45.17 (58-250) pg/ml idi. B12 vitamini ve ferritin düzeyleri veya tam kan sayımı parametreleri [Hb, Htc ve MCV] açısından erkek ve kız çocukları arasında istatistiksel olarak anlamlı fark saptanmadı (sırasıyla p=0.428, p=0.646, p=0.110, p=0.128, p=0.864). **Sonuç:** Bu çalışma, sağlık çalışanları için hastalıkla ilişkili olarak karşılaştığımız klinik tablolar hakkında genel bir değerlendirme sunmaktadır.

Anahtar Kelimeler: B12 Vitamini, Çocuk, Eksiklik, Bulgu, Tedavi.

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INTRODUCTION

Vitamin B12 plays a role in several systems in the body, and in cell division and differentiation, by taking part, together with folic acid, in DNA synthesis (Dogan & Demirci, 2010). Vitamin B12 is not synthesized in the human body, and therefore needs to be absorbed with diet. As in the rest of the world, Vitamin B12 deficiency is also a frequently encountered problem in Turkey (Hafizoglu, 2020). The most frequent clinical symptoms in vitamin B12 deficiency are delayed growth and development, gastrointestinal motility disorders, hyperpigmentation, stomatitis, glossitis, lethargy, ataxia, hyporeflexia, tremor, seizures, movement disorders, retardation in acquired motor skills, abnormal mental states, and coma (Graham, Arvela & Wise, 1992). Vitamin B12 deficiency can also emerge with non-specific symptoms and findings. Neurological findings develop in 80-90% of patients with vitamin B12 deficiency. These may be peripheral or central in nature (Healton, Savage, Brust, Garrett, & Lindenbaum, 1991). The onset of peripheral symptoms typically takes the form of symmetrical paresthesia in the extremities and gait ataxia. Memory disorder, altered personality, and even psychosis have been described as central and psychiatric symptoms associated with vitamin B12 deficiency in the literature (McCaddon, 2013). No link has been reported between the presence of nervous system findings in patients with vitamin B12 deficiency and the extent of vitamin deficiency (McCaddon, 2013).

The purpose of this study was to determine neurological and non-neurological manifestations in patients presenting to the pediatric neurology department with various different symptoms and to discuss these in the light of the current literature.

MATERIALS AND METHODS

Study group

Children aged between one month and 18 years presenting to the Balıkesir University Medical Faculty paediatric neurology clinic, Turkey, between

01.08.2019 and 01.08.2021 and with Vitamin B12 levels lower than 250 ng/mL were included in this retrospective, cross-sectional study. Demographic data and clinical and laboratory findings (vitamin B12, complete blood count, and ferritin) were recorded from the patient files.

Patients with structural anomalies, folic acid deficiency, a history of gastrointestinal surgery and drug use, or with acute infection findings, patients with autoimmune, metabolic, or genetic disorders, or chronic neurological disease, and patients whose clinical findings contracted with Vitamin B12 therapy were excluded from the study.

Statistical analysis

Statistical analysis was performed by using SPSS 15 package program. Descriptive statistics were presented as mean, standard deviation, minimum, and maximum values for measurement variables, and as number (n) and percentage (%) for qualitative variables. Normality of distribution of numerical variables was evaluated using the Shapiro-Wilk test. The Mann-Whitney U test was applied in the comparison by gender of non-normally distributed numerical data, and Student's t test was used for independent groups exhibiting normal distribution. p values <0.05 were regarded as statistically significant.

Ethical considerations

Ethical approval for this study was obtained from the local ethical committee before the start of the study (no. 2021/224 dated 06.10.2021).

RESULTS

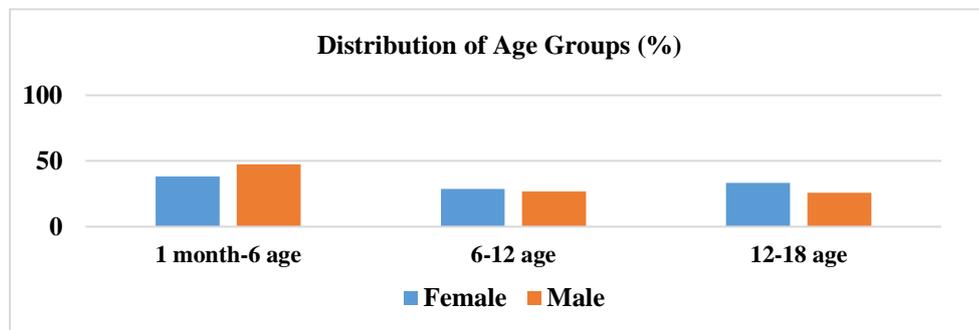
One hundred sixty-one cases were included in the study 63 (39.1%) female and 98 (60.9%) male. The patients' mean age was 7.43 ± 5.77 (0-17) years. Seventy (43.5%) cases were in the 0-6-year age group, 44 (27.3%) in the 6-12 age group, and 47 (29.2%) in the 12-18 age group. Mean ages were 7.36 ± 5.58 (0-17) years for boys and 7.54 ± 6.11 (1-17) for girls. The majority of patients of both genders were in the 0-6 age group (Table 1, Graphic 1).

Table 1. Patients' symptoms and signs.

Gender n (%)	
Girl	63 (39.1%)
Boy	98 (60.9%)
Average age	7.43±5.77 (0-17) year
Age groups n (%)	
0-6 years	70 (43.5%)
6-12 years	44 (27.3%)
12-18 years	47 (29.2%)
Vitamin B12 levels	
<150 pg/ml	64 (39.8%)
150-190 pg/ml	44 (27.3%)
>190 pg/ml	53 (32.9%)

Table 1. (Continue) Patients' symptoms and signs.

Symptoms	n (%)
Neuromotor retardation	73 (45.3%)
Hypotonia	7 (4.3%)
Skin hyperpigmentation	11 (6.8%)
Paleness	1 (0.6%)
Apathy	1 (0.6%)
Vomiting	15 (9.3%)
irritability	3 (1.9%)
Athetoid head movements	4 (2.5%)
Seizure	33 (20.5%)
Loss of appetite	1 (0.6%)
Sparse and depigmented hair	1 (0.6%)
Tremor	3 (1.9%)
Syncope	5 (3.1%)
Headache	2 (1.2%)
Dizziness/Vertigo	1 (0.6%)

**Graphic 1. Distribution of age groups between gender.**

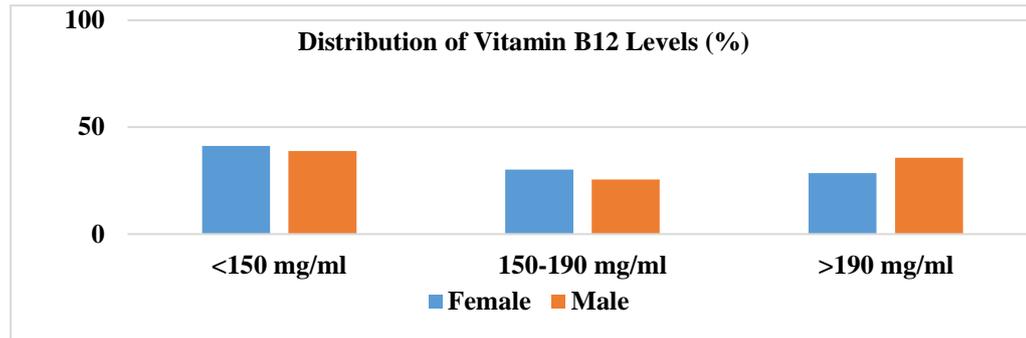
Neurological findings were present in 81.98% of patients with Vitamin B12 deficiency, and non-neurological findings in 18.02%. The most frequent neurological finding was neuromotor retardation (N=73, 45.34%), followed by seizures (N=33, 20.50%). Gastrointestinal motility disorders (N=15, 9.30%) were the most common non-neurological finding. The least frequent findings were vertigo, apathy, loss of appetite, and depigmented hair (N=1, 0.6%). The most frequent reason for presentation was neuromotor retardation in both boys and girls with Vitamin B12 deficiency (N=55, 56.1% and N=18, 28.57%). The mean vitamin B12 level among all the patients was 167.70±45.17 (58-250) pg/ml. Mean vitamin B12 levels were 169.90±44.09 (N=98) pg/ml

in boys and 164.27±46.95 (N=63) pg/ml in girls. Vitamin B12 levels were <150 ng/ml in 64 cases (39.8%), 150-190 pg/ml in 44 (27.3%), and >190 pg/ml in 53 (32.9%). Among boys, vitamin B12 levels were <150 pg/ml in 38 (38.8%) cases, 150-190 pg/ml in 25 cases (25.5%), and >190 pg/ml in 35 (35.7%). Among the girls, vitamin B12 levels were <150 pg/ml in 26 cases (41.3%), 150-190 pg/ml in 19 (30.20%), and >190 pg/ml in 18 (28.60%) (Graphic 2). The cases' mean hemoglobin (Hb) level was 12.73±1.42 (7.5-16.4) g/dl, mean hematocrit (Htc) level 38.03±4.22 (29.1-49.9), mean erythrocyte volume (MCV) 79.79±6.32 (58.2-97.4) fL, and mean ferritin level 26.71±42.80 (2.2-470.9) ml/ng (Table 2).

Table 2. Laboratory characteristics of the patients.

	All patients	Girls	Boys
Hemoglobin (g/dL)	12.73±1.42 (7.5-16.4)	12.52±1.10 (10.2-14.6)	12.87±1.58 (7.5-16.4)
Hematocrit (%)	38.03±4.22 (29.1-49.9)	37.42±3.52 (30.1-44.0)	38.43±4.60 (29.1-49.9)
MCV (fL)	79.79±6.32 (58.2-97.4)	79.90±6.66 (61.4-92.5)	79.72±6.12 (58.2-97.4)
WBC/mm ³	8.57±3.18 (3.8-28.9)	8.48±2.45 (3.8-15.8)	8.63±3.59 (4.0-28.9)
Platelet/mm ³	340.41±101.67 (166-761)	347.03±110.50 (166-761)	336.02±95.74 (177-653)
Vitamin B12 (pg/mL)	167.70±45.17 (58-250)	164.27 ±46.95 (58-248)	169.90±44.09 (76-250)
Ferritin (ml/ng)	26.71±42.80(2.2-470.9)	19.01±20.48 (2.2-134.0)	31.66±51.86 (2.9-470.9)

WBC: White Blood Cell. MCV: Mean Corpuscular Volume.



Graphic 2. Distribution of vitamin B12 levels.

No statistically significant difference was determined between boys and girls in terms of vitamin B12 and ferritin levels or complete blood count parameters [Hb, Htc, and MCV] (respectively $p=0.428$, $p=0.646$, $p=0.110$, $p=0.128$, $p=0.864$).

DISCUSSION

Vitamin B12 deficiency is a widespread problem in Turkey and worldwide, the prevalence of which is known to vary from one country to another. While the prevalence increases in elderly patients, deficiency can also be seen in childhood (Koç, Kocyigit, Soran, Demir, Sevinc, Erel, & Mil, 2006). Findings associated with vitamin B12 deficiency are generally chronic and progressive, and clinical findings linked to dietary deficiency may take 3-18 months to emerge (Koç, Kocyigit, Soran, Demir, Sevinc, Erel, & Mil, 2006; Hector & Burton, 1988). Neurological symptoms such as neuromotor retardation and seizures were frequently detected in the children with vitamin B12 deficiency in the present study.

In previous studies of children with vitamin B12 deficiency, Arslan et al reported a mean age of 13.62 years (min: six months, max: 18 years), and Demir et al. a mean age of 12.07 ± 3.85 months (Arslan, 2020; Demir, Koc, Ustyol, Peker, & Abuhandan, 2013). The mean age of the patients in the present study was 7.43 ± 5.77 years (0-17). Vitamin B12 deficiency was frequently observed in the 0-6-year age group in both sexes.

Demir et al. reported that vitamin B12 deficiency was more common in male gender (male/female:1.56), while Arslan described it as more common in girls (male/female:0,62) (Arslan, 2020; Demir, Koc, Ustyol, Peker, & Abuhandan, 2013). The majority of cases of vitamin B12 deficiency in the present study were male (N=98, 60.9%).

Demir et al. reported a mean vitamin B12 level of 139.7 ± 64.5 pg/ml, and Aslan one of 160.409 (min: 77 pg/mL, max: 263 pg/mL) (Arslan, 2020; Demir, Koc, Ustyol, Peker, & Abuhandan, 2013). The mean vitamin B12 level in the present study was 167.70 ± 45.17 (58-250) pg/ml, with mean values of 169.90 ± 44.09 (76-250) pg/ml in boys and

164.27 ± 46.95 (58-248) pg/ml in girls. Vitamin B12 levels were <150 ng/ml in the majority of cases in both sexes (N=64, 39.8%).

While B12 deficiency can affect several system functions, findings and symptoms such as feeding difficulties, seizures, delayed growth, megaloblastic anemia, developmental delay, hypotonia, microcephaly, lethargy, involuntary movements, irritability, and cerebral atrophy are frequently observed (Nawaz, Khattak, Khan, & Nangyal, 2020). Non-specific findings such as diarrhea, pallor, weakness, lack of appetite, vomiting, irritability, and tremor have also been determined in patients with vitamin B12 deficiency (Demir, Koc, Ustyol, Peker, & Abuhandan, 2013).

Together with folic acid, vitamin B12 plays a very important role in fetal brain development and in the myelination process in children up until puberty (Nawaz, Khattak, Khan, & Nangyal, 2020). The neurological symptoms and findings of vitamin B12 deficiency are generally mild, although clinical presentations are quite variable. Severe nervous system pathologies are seen in 30% of patients (Lee, 1993). Arslan described dizziness and/or fainting as the most frequently seen symptoms (61.7%) in patients with vitamin B12 deficiency (Arslan, 2020). That study also reported a history of concentration difficulty and forgetfulness and decreased academic performance in 18.3% of patients, numbness and/or tingling or a stinging sensation in 10.8%, congenital hypotonicity in 3.3%, and symptoms involving more than one system in 5.8% (Arslan, 2020). Demir et al. determined motor retardation, hypotonia, and pallor in all (100%) infant patients, followed by apathy, anorexia, and skin pigmentation (78%), and lack of eye contact and social retardation (68.3%) (Demir, Koc, Ustyol, Peker, & Abuhandan, 2013). Similarly, Taşkesen et al. also reported hypotonia (100%) as the most common neurological finding. Other neurological findings encountered by Taşkesen et al. included lack of appetite (92.8%), and neurodevelopmental (85.7%) and social (80.9%) retardation Taşkesen, Yaramış, Katar, Piriççioğlu, & Söker, (2011). The most frequently observed

neurological findings in another study of 27 infants with vitamin B12 deficiency were apathy, hypotonia (66.7%), motor retardation (59.3%), social retardation (51.9%), tremors (14.8%), and athetoid movements (3.6%) (Zengin, Sarper, & Kilic, 2009). The most common finding in the present study was neuromotor retardation (n=73, 45.34%), followed by seizures (N=33, 20.50%), and gastrointestinal motility disorders (N=15, 9.30%). Neuromotor retardation was the most frequent finding in both girls and boys (N=55, 56.1% and N=18, 28.57%, respectively). The least common findings in our patients were dizziness, loss of appetite, and depigmented hair (N=1, 0.6% each).

Epilepsy is a rare condition in children with vitamin B12 deficiency (Lee, Chang, Wu, Weng, & Chen, 200, Kumar, 2004). Demir et al. reported a prevalence of seizures of 14.6% in patients with vitamin B12 deficiency (Demir, Koc, Ustyol, Peker, & Abuhandan, 2013).

The literature contains case studies of seizures associated with vitamin B12 deficiency. Kirik and Çatak that hypotonia was the neurological finding most frequently accompanying seizures (Kirik, & Çatak, 2021). The mechanism involved in vitamin B12-related epilepsy is still unclear. However, it is thought that myelin damage occurs in neurons in associated with deficiency, and that this may be associated with central neurons being more susceptible to the stimulating effects of glutamate central neurons (Kumar, 2004). The prevalence of seizures in the present study was 20.50% (N=33).

The treatment of vitamin B12 deficiency involves replacing the missing vitamin, and improvement in clinical and imaging findings occurs with treatment in the majority of patients. Keskin determined atrophy in the cerebral cortex at magnetic resonance (MR) imaging in a 12-month-old girl with psychomotor retardation diagnosed with severe vitamin B12 deficiency and reported improvement of both the psychomotor retardation and cranial MR finding with replacement therapy (Keskin, 2016). We also observed improvement of clinical findings in the majority of patients started on vitamin B12 therapy. However, if diagnosis is delayed, deficiency can lead to irreversible neurological damage, for which reason many researchers have emphasized the importance of early diagnosis and treatment (Demir, Koc, Ustyol, Peker, & Abuhandan, 2013).

In that context, studies have stated that clinicians must take particular care not to miss the diagnosis, especially in infants, since the signs and symptoms are not specific (Zengin, Sarper, & Kilic, 2009).

CONCLUSION

The principal limitations of this study are its retrospective nature, the fact that development testing could not be performed due to the lack of a child development specialist, the inability to measure homocysteine in our center, and the fact that our is a

tertiary health institution in our region and that there is therefore a risk that the homogeneity of our patient population could not be established on account of the patients referred to us.

B12 deficiency is a health problem frequently encountered both in Turkey and worldwide, and early diagnosis and treatment are highly important. This study provides a general evaluation for health professionals regarding clinical presentations encountered by us in association with the disease. We think that our study will contribute to the literature both in Turkey and worldwide.

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

Author Contributions

Plan, design: HA, OK; **Material, methods and data collection:** HA, OK, EO; **Data analysis and comments:** HA, OK, EO; **Writing and corrections:** HA, OK, DC.

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Comparison of Genders in the Effectiveness of Acupuncture Therapy in Tension-Type Headache

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ABSTRACT

Objective: Tension-type headache (TTH) with an average lifetime prevalence of 46% (12-78%) in adults is the most common primary headache type. Acupuncture treatment is one of the adjunctive therapies in tension type headache so we wanted to evaluate the response difference of acupuncture between genders. **Materials and Methods:** In this retrospective study, the medical records of TTH patients, aged between 18 and 60, who did not receive conventional medical treatment (patient refusing medical treatment or intolerance due to drug side effects) and completed pre-determined sessions of acupuncture therapy in the outpatient clinics between 2017 and 2019 were evaluated. The monthly attack frequency, attack severity (Visual Analogue Scale), and the duration of attacks before and after acupuncture treatment between genders were documented. **Results:** A total of 17 patients meeting the study criteria were included in the study. No statistically significant difference in the frequency, the duration, and the severity of monthly attacks before and after the treatment were found between the genders. The monthly attack frequency and Visual Analogue Scale (VAS) score were found to be significantly decreased in women themselves before and after treatment in themselves. There was no statistically significant difference in the frequency, duration and severity of monthly attacks before and after the treatment among the men. **Conclusion:** Acupuncture should be considered as an alternative treatment option, especially in women with chronic TTH who do not want to use drugs or who have tolerability problems due to side effects or combination with medical therapy.

Keywords: Tension-Type Headache, Acupuncture, Gender Differences.

Gerilim Tipi Baş Ağrısında Akupunktur Tedavisinin Etkinliğinde Cinsiyetlerin Karşılaştırılması

ÖZ

Amaç: Gerilim tipi baş ağrısı (TTH), erişkinlerde yaşam boyu ortalama %46 prevalans (%12-78) ile en sık görülen baş ağrısı tipidir. Akupunktur tedavisi, gerilim tipi baş ağrısında yardımcı tedavilerden biri olduğundan, bu çalışmada akupunkturun cinsiyetler arasındaki yanıt farkını değerlendirmek istedik. **Gereç ve Yöntem:** Bu retrospektif çalışmada, poliklinikte konvansiyonel tıbbi tedavi almayan (tıbbi tedaviyi reddeden veya ilaç yan etkileri nedeniyle intoleransı olan) ve önceden belirlenmiş akupunktur tedavisi seanslarını tamamlayan 18-60 yaş arası TTH hastalarının 2017-2019 yılları arasındaki tıbbi kayıtları incelenmiştir. Cinsiyetler arasında aylık atak sıklığı, atak şiddeti (Görsel Analog Skala) ve akupunktur tedavisi öncesi ve sonrası atak süreleri kaydedildi. **Bulgular:** Kriterlere uygun toplam 17 hasta çalışmaya alındı. Cinsiyetler arası aylık atak sıklığı, atakların süre ve şiddetinde tedavi öncesi ve sonrası istatistiksel olarak anlamlı farklılık bulunmadı. Kadınlarda aylık atak sıklığı ve VAS (Visual Analog Skala) skoru tedavi öncesi ve sonrası istatistiksel olarak anlamlı şekilde azalmış bulundu. Erkeklerde ise tedavi öncesi ve sonrası aylık atak sıklığı, atakların süre ve şiddetinde istatistiksel açıdan farklılık bulunmadı. **Sonuç:** Akupunkturun kronik TTH tedavisinde ilaç kullanmak istemeyen veya yan etkileri nedeniyle tolerabilite problemi yaşayan özellikle kadınlarda alternatif tedavi seçeneği olarak değerlendirilmesi gerekir.

Anahtar Kelimeler: Gerilim Tipi Baş Ağrısı, Akupunktur, Cinsiyet Farklılığı.

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INTRODUCTION

Tension-type headache (TTH) is the most common primary headache in adults, with an average lifetime prevalence of 46% (ranging between 12% and 78%) according to the results of five population-based studies (Stovner et al., 2007). TTH is classified into three subtypes regarding their frequency: rare episodic (<1 day/month), frequent episodic (1-14 days/month), and chronic (15 days/month for ≥ 3 months) (IHS, 2018). In episodic TTH, which has a female-to-male ratio of approximately 5 to 4, the female-to-male ratio increases when the headache becomes chronic. The TTH, which usually develops before the age of 30, reaches its highest prevalence between the ages of 40 and 49 before a decrease with advancing age in both genders (Schwartz, 1998). The pathophysiology of TTH is not clear. Long-term potentiation of nociceptive neurons and reduced activity of the antinociceptive system can lead to chronic TTH. Secondary segmental central sensitization, disturbance of the supraspinal modulation of stimuli, and myofascial derived prolonged, painful stimuli are thought to be important factors for conversion to chronic form (Fumal & Schoenen, 2008).

Nonsteroidal anti-inflammatory drugs are used for treatment during attacks, while prophylaxis using serotonin-noradrenaline reuptake inhibitors, tricyclic antidepressants, and serotonin reuptake inhibitors is maintained for the chronic subtype. In addition to the medical treatment, acupuncture, relaxation techniques, and stress-fighting methods are among the options used in complementary medicine for TTH (Kaniecki, 2012). As far as we know, the number of studies on acupuncture for TTH is extremely rare, while there are no studies comparing the effectiveness of acupuncture between genders in the literature.

In this study, we aimed to evaluate the clinical features of the chronic TTH patients who did not receive conventional medical treatment (patient refusing medical treatment or intolerance due to drug side effects) and had acupuncture to assess the relationship efficacy of acupuncture treatment between genders.

MATERIALS AND METHODS

Study type

In this retrospective study, the neurology and acupuncture outpatient medical records of patients who received acupuncture for THH between 2017-2019 were evaluated. The inclusion criteria consisted of patients with THH who did not receive conventional medical treatment due to medical (intolerance to side effects of medications) or personal (resistance to drug use) reasons and were treated using acupuncture. The patients who did not complete the pre-determined acupuncture sessions, who had systemic disease including psychiatric comorbid conditions, and history of antidepressant,

anxiolytic, and chronic analgesic use >65 - <18 years were excluded from the study.

The demographics of patients and the clinical characteristics regarding the THH attacks (monthly frequency, duration, and severity using VAS) before and after the acupuncture treatment were documented. The duration of the acupuncture effect was recorded to assess long-term results.

In our acupuncture outpatient clinics, the acupuncture treatment protocol for THH patients consisted of two separate methods, body and ear applications. In body applications, a total of 15 body needles were placed horizontally at specific points on the nasal, frontal, parietal, temporal, and occipital regions in the head region. In addition, a total of 8 oblique needles were placed, one on the lateral corner points of both arm-forearm junction lines (base of cubital fossa), one between the thumb and index fingers on the back of both hands and two on the medial sides of both ankles. After the patient was allowed to rest for 40 minutes, all body needles were collected. Then, the second stage of ear acupuncture was started. A total of 8 ear needles, 4 in each, were placed on specific points on both auricles to stay in ear points for 5-7 days. This protocol was applied once a week for a total of 8 weeks. The acupuncture treatment of patients as body and auricular acupuncture applications were continued for two months, while the ear needles were replaced with new ones every week. Then, the acupuncture sessions of all patients were suspended for a month before, booster treatments using body and auricular acupuncture applications once a week for 4 weeks were given to all patients.

Statistical analysis

The mean and standard deviations of age, pre-acupuncture headache frequency, duration and attack severity, and post-acupuncture headache frequency, duration, and attack severity values were calculated. Independent Samples t-test and Mann Whitney U test were used to compare the differences in gender regarding the mentioned parameters. Comparison of gender was made with Wilcoxon test. $p < 0.05$ was accepted to be statistically significant.

Ethical considerations

Balikesir University Faculty of Medicine Clinical Research Ethics Committee (Date: March 13, 2019; Decision no: 2019/51) was obtained and the study participants provided informed consent.

RESULTS

The medical records of 17 patients (10 females, 7 males), who met the study inclusion criteria were reviewed. The mean age of the patients was 52.71 ± 14.71 years in males and 44.5 ± 17.26 years in females ($p=0.3$). There was no significant difference between the genders in the frequency, duration and severity of monthly attacks before and after acupuncture (Table 1).

Table 1. Demographic data of patients before treatment.

	Male (n=7)		Female (n=10)		Between genders
	Before acup.	After acup.	Before acup.	After acup.	p (before acup./after acup.)
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Headache attacks per month	22.86±9.58	12.14±13.27	26.8±7.32	12.1±10.84	0.3 / 0.7
Headache attack duration (h)	27.43±15.04	14.71±18.15	21.6±5.06	16.9±12.76	0.5 / 0.4
VAS	6.71±2.06	3.57±2.88	6.7±1.49	4.9±2.18	0.8 / 0.3

SD: Standard deviation.

The frequency of attacks in women significantly reduced after the acupuncture (pre-treatment 26.8±7.32 attacks/month and post-treatment 12.1±10.84 attacks/month, p=0.02) (Figure 1).

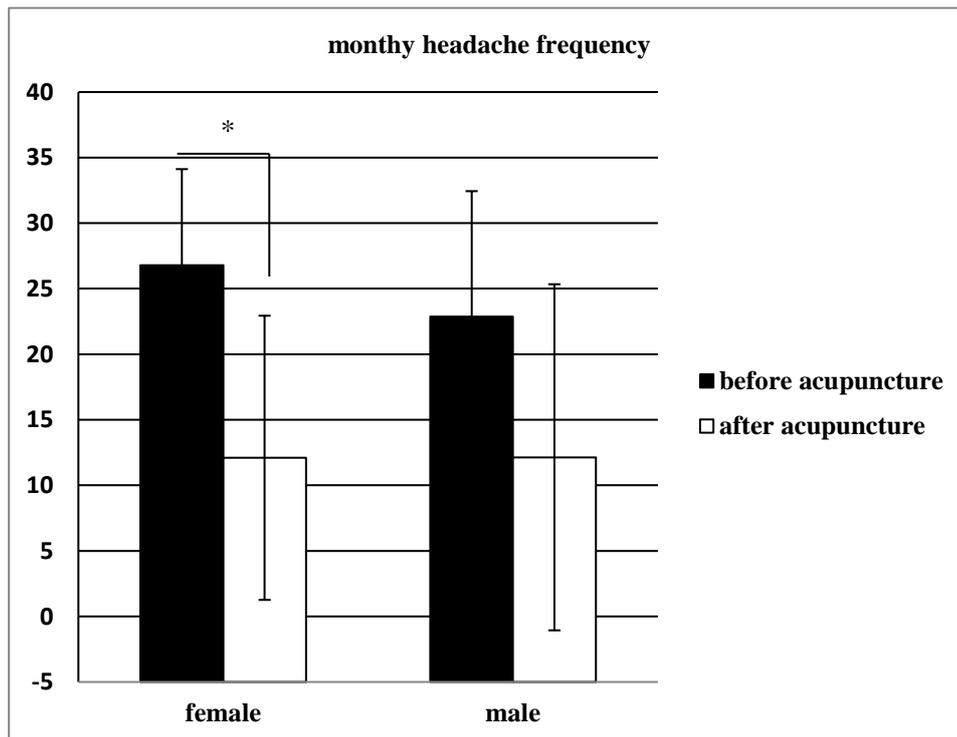


Figure 1. When comparing within genders, the decrease in monthly headache frequency in women after the treatment was found to be statistically significant, p=0.02.

The VAS score in women also statistically significantly decreased after the treatment (pre-treatment: 6.7 ± 1.49 and post-treatment: 4.9 ± 2.18 , $p=0.01$) (Figure 2).

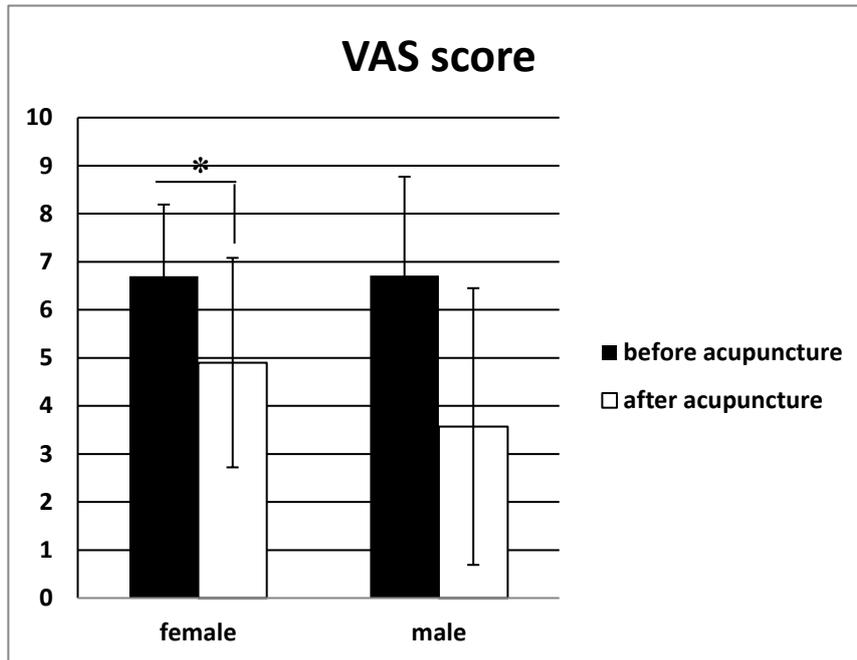


Figure 2. In the comparison of the VAS score before and after acupuncture between the genders, a significant decrease was found in the VAS score in women after the treatment. VAS (Visual Analogue Scale), $p=0.01$.

There were no statistically significant differences in the monthly attack frequency, duration, and severity among men before and after treatment (Table 2).

Table 2. Acupuncture long-term results.

Gender	Response
Female	no response
Female	no response
Female	no response
Female	1 month responsiveness
Female	2 month responsiveness
Female	stable for 9 months
Female	stable for 12 months
Female	stable for 12 months
Female	stable for 18 months
Female	stable for 18 months
Male	no response
Male	no response
Male	no response
Male	1 month responsiveness
Male	6 month responsiveness
Male	stable for 18 months
Male	stable for 18 months

While three out of ten female patients did not respond to acupuncture, well-being persisted for 1-2 months in two and > 6 months in five patients. No response to acupuncture was observed in three out of seven male patients, while one of the remaining continued to be well after a month, one for 6 months, and two for longer than 6 months. A state of well-being of more than 6 months was observed more frequently in women than in men.

DISCUSSION

The approach to TTH management includes a combination of lifestyle, physical and pharmacological measures. Although the scientific evidence is limited, non-pharmacological options should always be considered for treatment. Stress management techniques, passive physical manipulation, active cervical muscle stretching or exercise programs, and acupuncture therapy are among the most common options for non-pharmacological treatment. Acupuncture therapy in chronic TTH had been recommended in the National Institute for Health and Clinical Excellence clinical guidelines and the evaluation results of the Cochrane Library Systematic (National Clinical Guideline Centre, 2012; Linde et al., 2016).

In addition, acupuncture therapy has been recommended by the European Federation of Neurological Societies as a complementary treatment option for TTH (Bendtsen et al., 2010). Although the mechanism of acupuncture treatment is not fully understood, various theories suggest its serotonergic and anti-inflammatory effects on the control of the pain perception (Herman et al., 2005; Gaul et al., 2009).

Acupuncture appears to be an effective treatment for TTH. However, the methodological quality of the relevant studies is often low; thus, the reliability of results is limited. While several studies reported that acupuncture was superior to sham acupuncture in the treatment of TTH, no such significant difference was found by some others (Endres et al., 2007; Melchart et al., 2005). The effect of twenty sessions of acupuncture that lasted for an average of 20 weeks found in a recent randomized controlled study could be considered as strong evidence for a sustained effect after acupuncture treatment (Zhao et al., 2017). In a recent database study, systematic review or meta-analysis revealed that twenty-five of the studies conducted until 2019 (25/36, 69.4%) had low or very low evidence quality, four (4/36, 11.1%) had medium and seven (7/36, 19.4%) had high evidence quality. Descriptive analysis results showed that acupuncture treatment for TTH decreased the frequency and severity of the headaches (Huang et al., 2020).

Gender differences were observed in biopsychosocial factors associated with pain perception, brain structure development, function, and pain experience (Maurer et al., 2016; Ingalhalikar et al., 2014; Racine et al., 2012). In our study, no statistically significant

difference was observed in the monthly attack frequency, duration, and severity between the genders, while the monthly attack frequency and VAS score severity in women were found to be statistically significant after the treatment. The responsiveness of women to acupuncture was better than the men. Long-term results demonstrated that well-being state longer than 6 months was higher in women than men. The long-term effectiveness (longer than 6 months) of acupuncture therapy in seven of 17 patients also points to acupuncture as a complementary therapy option in patients receiving prophylactic treatment. Moreover, the combination of physiotherapy or psychotherapy methods and acupuncture treatment in patients who do not accept drug intake or cannot tolerate due to side effects might contribute to better results. Factors such as the perception of pain, serotonin levels, and the density of myofascial trigger points may also play a role in the acupuncture effectiveness, which seems to be more prominent in women. We suggest that acupuncture treatment should be prioritized, especially in female patients. In addition, booster sessions with eight-week sessions and four booster sessions after one month break in patients who responded to treatment might also contribute to responsiveness.

CONCLUSION

Acupuncture should be considered as an alternative treatment option, especially in women with chronic TTH patients who do not want to use drugs or who have tolerability problems due to side effects. In this patient group, in addition to acupuncture, yoga, pilates, psychotherapy, and relaxation exercises can be used to increase efficiency. Future studies conducted to include the above-mentioned combinations would possibly provide stronger support for our results and contribution to the growing body of literature.

Conflict of Interest

The author declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Author Contributions

Plan, design: BG, NT; **Material, methods and data collection:** BG, NT; **Data analysis and comments:** BG, NT; **Writing and corrections:** BG, NT.

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Nurse Collaboration and Work Stress in Nurses Working at A University Hospital

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ABSTRACT

Objective: This study was carried out as a descriptive study to evaluate the level of collaboration between nurses, the level of work stress and the factors affecting it in nurses working in a university hospital. **Materials Methods:** A sample of the study was composed of nurses working at a university hospital who wanted to participate in the study. The research was conducted between June-August 2020. The study was completed with 228 nurses who agreed to participate in the study. Data collection tools of the study were applied via Google form to participants using the "Introductory Information Form", "Work Stress Scale" and "Nurse-Nurse Collaboration Scale". **Results:** The average score of the nurse - nurse collaboration scale was 48.21±9.10; the average score of the work stress scale was 2.54±0.57. In addition, a moderately positive statistically significant association was found between the nurse-nurse collaboration scale score averages and the work stress scale score averages (Pearson correlation: 0.554, p=0.001). **Conclusion:** Today, with the increase in chronic diseases and the complexity of health care services, work and team cooperation have become important issues in the areas where health care services are provided. Excess work and lack of team collaboration are factors affecting both employee and patient satisfaction. Empowering nurses in the management of work stress and increasing cooperation between nurses; It is thought that it will help to manage the difficulties experienced in this regard more effectively.

Keywords: Collaboration, Work Stress, Nursing.

Bir Üniversite Hastanesinde Çalışan Hemşirelerde Hemşireler Arası İş Birliği ve İş Stresi

ÖZ

Amaç: Bu çalışmada bir üniversite hastanesinde çalışan hemşirelerde hemşireler arası iş birliği ve iş stresi düzeyleri ile bu düzeyleri etkileyen faktörlerin değerlendirilmesi amaçlanmıştır. **Yöntem:** Araştırmanın örneklemini bir üniversite hastanesinde çalışan hemşirelerden araştırmaya katılmak isteyenler oluşturmuştur. Araştırma Haziran-Ağustos 2020 tarihleri arasında yürütülmüştür. Çalışma araştırmaya katılmayı kabul eden 228 hemşire ile tamamlanmıştır. Araştırmanın veri toplama araçları, "Tanıtıcı Bilgi Formu" "İş Stresi Ölçeği", "Hemşire-Hemşire İş Birliği Ölçeği" kullanılarak katılımcılara Google form üzerinden uygulanmıştır. **Bulgular:** Hemşireler arası iş birliği ölçek toplam puan ortalamaları 48.21±9.10; iş stresi ölçek puan ortalamaları 2.54±0.57 olarak bulunmuştur. Ayrıca hemşire- hemşire iş birliği ölçek puan ortalamaları ile iş stresi ölçek puan ortalamaları arasında orta derecede pozitif yönde istatistiksel olarak anlamlı düzeyde ilişki tespit edilmiştir. **Sonuç:** Günümüzde kronik hastalıkların artışı, sağlık bakım hizmetlerinin daha kompleks hale gelmesi ile sağlık bakım hizmetlerinin verildiği alanlarda iş stresi ve ekip iş birliği önemli konular haline gelmiştir. İş stresinin fazlalığı ve ekip iş birliğinin azlığı hem çalışan hem de hasta memnuniyetini etkileyen faktörlerdendir. Hemşirelerin iş stresinin yönetimi konusunda güçlendirilmelerinin ve hemşireler arası iş birliğinin artırılmasının; bu konuda yaşanan güçlüklerin daha etkili bir şekilde yönetilmesine yardımcı olacağı düşünülmektedir. **Anahtar Kelimeler:** İş birliği, İş Stresi, Hemşirelik.

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INTRODUCTION

Nursing is a profession that develops from the past to the present and renews itself with the changing and undertakes the most important task in health care services. It is responsible for protecting and improving the health of the individual, family and community and improving health by providing optimal care in case of illness; consists of science and art (Karadağ, 2002; Tan et al., 2012; Yıldırım and Durmuş, 2018). Nurses, who have an important place in the health care team providing health care services, have the opportunity to interact with the patient 24 hours a day, 7 days a week, and they have intense relations with the patients they care for. Communications with patients and their relatives, as well as with the healthcare team, are extremely important. Employees working happily and peacefully in the workplace environment also increases the efficiency of the work done (Tınaz, 2011). The coronavirus (Covid-19) epidemic, which started in China in December 2019 and continues today, has affected health workers both psychologically and physiologically (Aslan, 2020; Bao et al., 2020). While the physical effects of pandemics are devastating and deadly, the psychological reactions that occur during the epidemic also have very long-lasting results (Taylor, 2019). The workload and work stress of health professionals are increasing significantly (Tuncay et al., 2020). Long working hours, worsening working conditions, stigmatization towards healthcare professionals, increase in the number of patients per nurse, shortage of materials and personnel, lack of social opportunities, responsibility to care for terminally ill patients, increase in society's expectations and concerns have also greatly affected the resilience of employees (Alçelik et al., 2005; Atasoy and Yorgun, 2013; Kırılmaz, 2016). The fear, anxiety, panic, insecurity and intense stress experienced in the COVID-19 pandemic affect employees badly; causes feelings such as burnout, depression, anxiety and helplessness (Tınaz, 2011; Wang, 2020). In addition to these, work-related stress decreases the job satisfaction by reducing the productivity and motivation of the employees (Sünter et al., 2006).

For this reason, cooperation in the nursing profession, which works with colleagues for a long time during the day before and after the pandemic, is an undeniable reality (Bott et al., 2015). The cooperation and teamwork of nurses is an important factor in the timely, on-site, sequential and safe delivery of care and treatment (Weinstein, 2003). Therefore, "Nurse-Nurse Collaboration" is very important to reduce medical errors, improve patient care, reduce work stress, and increase nurses' job satisfaction (Temuçin, et al., 2018; Yıldırım and Durmuş, 2018). Nurse-nurse cooperation; It is a very important determinant during patient handover or shift change, in the communication process and in times of crisis (Yıldırım and Durmuş, 2018). Work stress can affect nurses' relationships with each other, critical thinking and problem-solving skills, and job satisfaction (Sayılan and Boğa, 2018). In this direction, in this study, it was aimed to evaluate the cooperation between nurses and the level of work stress and the factors affecting it in nurses working in a university hospital.

Research questions

- What is the nurse-nurse collaboration level of nurses?
- What is the work stress level of nurses?
- What are the factors affecting the collaboration and work stress levels of nurses?

MATERIALS AND METHODS

This study was carried out as a descriptive study in order to evaluate the level of collaboration between nurses, the level of work stress and the factors affecting it in nurses working in a university hospital. The universe of the research consists of 890 nurses working in the hospital. The sample, on the other hand, was calculated as 208 nurses, with a 90% confidence interval and a 5% margin of error, with a known universe sampling method. The research was carried out between June and August 2020 and was completed with 228 nurses who wanted to participate in the research.

Data collection

The data collection tools of the research were applied to the participants online via Google form by the researchers.

Introductory information form: Introductory information form created by the researchers. It includes data such as gender, age, marital status, number of children, educational status, working status, working unit, years of work in the unit worked, the status of choosing the unit of work herself and satisfaction with the unit worked

Work stress scale (WSS): It is a 5-point Likert-type scale consisting of 15 questions included in the works of Baltaş and Baltaş (1992) (1 = never, 2 = rarely, 3 = sometimes, 4 = often, 5 = almost always). In the evaluation process of the scale, the total score is divided by 15 and the individual's scale score average is calculated (A=3,5-4,0; B=1,0-1,3; C=1,4-1,9; D=2,0-2,5; E=2,6-3,1; F=3,2-3,4). A, B, E and F are considered as stress levels that can affect productivity and threaten health. An increase in the scale score indicates an increase in work stress. The Cronbach Alpha value of the scale was found to be 0.84 by Baltaş and Baltaş (1992), and the Cronbach Alpha value was determined as 0.84 in this study.

Nurse-nurse collaboration scale (NNSC): The validity and reliability of the scale, which consists of 35 items and five sub-dimensions, was made by Temuçin et al. (2019). Subscales are management (7 items), communication (8 items), sharing process (8 items), coordination (5 items) and professionalism (7 items). The increase in the total score obtained from the scale, which is a four-point Likert type scale, indicates that the collaboration between nurses is good. The Cronbach Alpha reliability coefficient for the whole of the original scale was 0.89, and it was determined as 0.90 in this study.

Statistical analysis

SPSS 21.0 (SPSS Inc., Chicago, IL, USA) package program was used in the analysis of the data. Descriptive statistics are expressed as percentage, mean, standard deviation, and minimum-maximum values. The conformity of the data to the normal distribution was made with the Kolmogorov Smirnov test. Mann Whitney U test and Kruskal Wallis test were used for statistical analysis because the data did not conform to normal distribution.

“ $p<0.05$ ” was accepted as an indicator of statistical significance.

Ethical consideration

In order to carry out the research; Ethics Committee approval with the number 05 dated 09.05.2020 from the ethics committee of a university and institutional permission from the institution where the research will be applied was obtained and then the study started. In addition, informed consent forms were obtained from the nurses included in the study via the online Google form.

RESULTS

When the introductory data of the nurses were analyzed, 87.7% (n=200) were female, 58.8% (n=134) were between

the ages of 20-30, 50.9% (n=116) were single. 84.2% (n=192) of nurses have bachelor's degree. In addition, 43.4% (n=99) of the nurses were working as regular staff, 48.2% (n=110) were working as intensive care nurses, 51.8% (n=118) of them had worked in the unit between 0-3 years. 75% (n=171) did not choose the unit they worked for, and 75.4% (n=172) were satisfied with the unit they worked in (See Table 1.). NNSC total score averages were 48.21 ± 9.10 ; WSS total score averages were found to be 2.54 ± 0.57 (Table 2.)

Table 1. Nurses' introductory information (n=228).

Introductory Information		n	%
Gender	Woman	200	87.7
	Man	28	12.3
Age	20-30	134	58.8
	31-40	77	33.8
	41 and above	17	7.5
Marital status	Married	109	47.8
	Single	116	50.9
	Other	3	1.3
Number of children	0	142	62.3
	1	50	21.9
	2	32	14.0
	3	4	1.8
Educational status	Associate degree	6	2.6
	Bachelor's degree	192	84.2
	Graduate	30	13.2
Working status	Regular	99	43.4
	Contractual	129	56.6
Working unit	Clinical nurse	97	42.5
	Intensive care nurse	110	48.2
	Head nurse	21	9.2
Years of work in the unit worked	0-3 years	118	51.8
	4-7 years	32	14.0
	8 years and over	78	34.2
The status of choosing the unit of work herself	Yes	57	25.0
	No	171	75.0
Satisfaction with the unit worked	Yes	172	75.4
	No	56	24.6

Table 2. Nurse-nurse collaboration scale mean scores and work stress scale mean scores.

Scale and Subscales	Min	Max	Mean	Standard deviation
Conflict Management Subscale	4	10.00	6.06	1.31
Communication Subscale	5	13.00	8.84	1.34
Sharing Process Subscale	4	14.00	8.25	2.18
Coordination Subscale	5	17.00	10.01	2.65
Professionalism Subscale	8	27.00	15.03	4.40
Nurse-Nurse Collaboration Scale-Total Score	29	74.00	48.21	9.10
Work stress Scale Total-Score	1	3.87	2.54	0.57

In addition, a moderately positive and statistically significant relationship was found between the NNSC score averages and the WSS mean score (Spearman's rho:0.532, $p=0.001$). Table 3 shows the results of the comparison of NNSC and subscales mean scores and WSS mean scores with the descriptive data. It was determined that there was no statistically significant difference between the NNSC and its subscales according to the education level of the nurses, the number of children and the choice of the unit they worked for. ($p>0.05$). When the gender of the nurses and the NNSC score averages were compared; It was determined that female nurses' "Communication" subscale mean score was higher than that of men, and this difference was statistically significant ($p<0.05$). When the NNSC mean scores of the nurses were compared according to their marital status; It was seen that the "Conflict Management", "Coordination" and "Professionalism" subscale mean scores of those who were single were higher than those who were married, and this difference was statistically significant ($p<0.05$). It was determined that nurses' "Sharing Process", "Coordination", "Professionalism" subscale and NNSC score averages of nurses differed statistically significantly according to age ($p<0.05$). In the paired analyzes conducted to determine which group caused this difference, it was determined that the "Sharing Process" ($Z=-0.531$, $p=0.011$), "Coordination" subscales ($Z=-2.171$, $p=0.030$) and NNSC score averages of the nurses in the 20-30 age group ($Z=-2.686$, $p=0.007$) was found to be higher than that of nurses aged 41 and over ($p<0.05$). In addition, it was determined that the "Professionalism" ($Z=-2.464$, $p=0.014$) subscale and NNSC score averages ($Z=-2.230$, $p=0.026$) of nurses in the 20-30 age group were higher than those in the 31-40 age group ($p<0.05$). It was determined that the sub-dimensions of "Conflict", "Sharing Process", "Coordination", "Professionalism" and NNSC score averages of contracted nurses were higher than those of regular employees, and this difference was statistically significant ($p<0.05$). A statistically significant difference was found between nurses' "Sharing Process", "Coordination", "Professionalism" subscale and NNSC score averages according to the place of work. In the dual analyzes conducted to determine which group caused this difference, it was found that the "Sharing Process" ($Z=-3.325$, $p=0.001$; $Z=-3.637$, $p=0.001$), "Coordination" ($Z=-3.323$, $p=0.001$; $Z=-3.077$, $p=0.001$) subscales and

NNSC score averages of the nurses working as clinical nurses and intensive care nurses were higher than those of the nurses in charge; It was observed that the "Professionalism" ($Z=-3.325$, $p=0.011$) subscale mean scores of those working as intensive care nurses were higher than those of the nurses in charge. A statistically significant difference was found between nurses' "Sharing Process", "Coordination", "Professionalism" subscales and NNSC score averages according to the year of work in the unit. In the paired analyzes carried out to determine which group caused this difference, it was determined that the NNSC score averages of the nurses who worked for 0-3 years were higher than those of the nurses who worked for 8 years and above ($Z=-2.752$, $p=0.006$; $Z=-2.869$, $p=0.004$; $Z=-1.981$, $p=0.048$; $Z=-2.614$, $p=0.009$). It was determined that there was no statistically significant difference between the WSS according to the nurses' gender, number of children, education level, place of work, and whether they chose the unit they worked for ($p<0.05$). WSS mean score of single nurses was higher than that of married nurses; It has been determined that contracted nurses are higher than regular nurses, and nurses who are not satisfied with the unit they work are higher than those of dissatisfied nurses, and this difference is statistically significant ($p<0.05$). A statistically significant difference was found in the mean WSS scores of the nurses according to age. In the paired analyzes carried out to determine from which group this difference originated, it was determined that the mean WSS scores of the nurses in the 20-30 age group were higher than those of the nurses aged 41 and over, and this difference is statistically significant ($Z=-2.547$, $p=0.011$). A statistically significant difference was found between the WSS score averages of the nurses according to the working year in the unit they worked in ($p<0.05$).

Table 3. Comparison of nurse-nurse collaboration scale and subscale mean scores and work stress scale mean scores with descriptive data.

Introductory Information		Conflict Management Subscale	Communication Subscale	Sharing Process Subscale	Coordination Subscale	Professionalism Subscale	NNSC TOTAL	WSS TOTAL
Gender	Woman	Z=-0.791	Z=-2.359	Z=-0.034	Z=-0.592	Z=-0.995	Z=-0.908	Z=-0.791
	Man	p=0.429	p=0.018*	p=0.973	p=0.554	p=0.320	p=0.364	p=0.429
Age	20-30							X ² =12.6
	31-40	X ² =2.767	X ² =1.339	X ² =10.849	X ² =6.024	X ² =10.231	X ² =10.193	5
	41 and above	p=0.013*	p=0.720	p=0.013*	p=0.110	p=0.017*	p=0.017*	p=0.005*
Marital status	Married	Z=-1.625	Z=-1.273	Z=-0.79	Z=-3.201	Z=-2.301	Z=-3.214	Z=-2.010
	Single	p=0.044*	p=0.203	p=0.429	p=0.001**	p=0.021*	p=0.001**	p=0.044*
Number of children	0							X ² =4.73
	1	X ² =0.132	X ² =0.807	X ² =0.054	X ² =0.362	X ² =0.362	X ² =4.081	9
	2 and above	p=0.717	p=0.369	p=0.816	p=0.547	p=0.547	p=0.130	p=0.094
Educational status	Associate degree							X ² =0.49
	Bachelor's degree	X ² =0.001	X ² =0.001	X ² =0.782	X ² =0.007	X ² =0.085	X ² =0.064	3
	Graduate	p=0.970	p=0.976	p=0.377	p=0.933	p=0.771	p=0.800	p=0.482
Working status	Regular	Z=-3.299	Z=-1.754	Z=-4.683	Z=-3.715	Z=-4.094	Z=-4.796	Z=-3.188
	Contractual	p=0.001**	p=0.079	p=0.001**	p=0.001**	p=0.001**	p=0.001**	p=0.001**
Working unit	Clinical Nurse							X ² =5.54
	Intensive Care Nurse	X ² =3.216	X ² =4.827	X ² =13.404	X ² =11.242	X ² =6.030	X ² =10.886	0
	Head Nurse	p=0.200	p=0.089	p=0.001**	p=0.004*	p=0.049*	p=0.004**	p=0.063
Years of work in the unit worked	0-3 Years							X ² =5.54
	4-7 Years	X ² =3.216	X ² =4.827	X ² =13.404	X ² =11.242	X ² =6.030	X ² =10.886	0
	8 Years and over	p=0.084	p=0.089	p=0.001**	p=0.004**	p=0.049*	p=0.004**	p=0.063
The status of choosing the unit of work herself	Yes	Z=-0.131	Z=-1.682	Z=-0.015	Z=-1.704	Z=-1.141	Z=-1.210	Z=-0.338
	No	p=0.896	p=0.495	p=0.988	p=0.088	p=0.254	p=0.495	p=0.736
Satisfaction with the unit worked	Yes	Z=-0.288	Z=-3.718	Z=-7.234	Z=-5.030	Z=-7.894	Z=-7.569	Z=-6.975
	No	p=0.773	p=0.001**	p=0.001**	p=0.001**	p=0.001**	p=0.001**	p=0.001**

*p<0.05, **p<0.01

DISCUSSION

Nurses, who are one of the most important members of the healthcare team, can feel tired both physiologically and psychologically due to their intense work schedule. Nurses can feel stronger and more motivated by increasing cooperation among themselves, performing their duties and responsibilities more effectively, approaching the problems encountered with a solution-oriented approach and supporting each other in team cooperation (Yeşiltaş and Güven, 2016). However, it is known that the workload and work stress of healthcare workers are increasing day by day (Baltas and Baltas, 1992). It is thought that with the increase in professional collaboration, the team can work more efficiently and more effectively, and this will contribute to the quality of patient care and increase patient satisfaction. It was seen that the level of collaboration between nurses were above the medium

level, and their work stress levels were at the most favorable stress level in terms of health and productivity. In addition, a moderate positive relationship was found between the collaboration between nurses and work stress. In Uludağ's (2019) study investigating the relationship between work stress and motivation, it was determined that there was a significant and inverse relationship between work stress and motivation. In the study conducted by Çelik and Karaca (2017), the effect of the motivation level of nurses on teamwork was evaluated. According to the results of the study, it was determined that there was a significant relationship between the motivation level of nurses and teamwork. It is known that low-level stress increases work motivation and work efficiency, while high-level stress reduces an individual's work ability and motivation. It is seen that the work stress level of the nurses participating in the study is at a level that will increase their productive

work, and this strengthens the collaboration. It is thought that a good level of collaboration between It was determined that female nurses' communication scores were higher than male nurses. In the study of Ylitörmänen et al. (2019) conducted by Norwegian and Finnish nurses to compare the perceptions of care and collaboration between nurses, it was stated that male participants' communication scores were lower than female participants in both countries. In the study of Al-Hamdan et al. (2021), in which they examined the relationship between emotional intelligence and nurse-nurse collaboration, it was observed that male participants (female participant: 195 male participants: 116) had a higher communication score than female participant. It is thought that the low number of male nurses participating in the study in our study also affected this result. On the other hand, it is thought that the characteristics such as motherhood, collaboration, and being able to express their feelings and thoughts more easily due to being a woman may have contributed to their being higher than male nurses by reflecting on their communication scores.

It was observed that the nurses' collaboration scores in the 20-30 age group were higher than the nurses in the older age group. In a study similar to our findings, Adıgüzel et al. (2011) stated that the professionalism level of nurses in the 26-30 age group in business life was significantly higher than the professionalism level of the nurses in other groups. These data suggest that nurses who have recently graduated are younger than others, their knowledge is up-to-date, and therefore their motivation to work can increase the level of collaboration between nurses.

It was observed that the conflict management, collaboration and professionalism scores of the single nurses were higher than the married ones. Similar to our study findings, Göriş et al. (2014) stated in their study that the professional values scale scores of single nurses were higher than those of married ones. This finding shows that marital status can positively or negatively affect situations that are important in business life, such as conflict management, collaboration, and professionalism. It is thought that the responsibility and burden of being married and having children are reflected in areas such as crisis management in business life, professionalism and working in collaboration.

It was determined that the conflict, sharing process, collaboration, professionalism, and NTSC scores of the contracted nurses were higher than those of the regular employees. This finding suggests that since the job guarantee of contracted employee is lower than that of regular employees, they increase their performance in business life, and this may be reflected in conflict, sharing process, collaboration, professionalism and NNSC scores.

The sharing process, collaboration and NNSC scores of the nurses working as clinical nurses and intensive care nurses were higher than those of the nurses in charge; It was observed that the professionalism scores

nurses will positively affect both the quality of care and patient and employee satisfaction.

of those working as intensive care nurses were higher than those of the nurses in charge. Similar to this finding, in the study conducted by Durmuş et al. (2018), it was determined that the collaboration scores of intensive care nurses were higher than the nurses working in other units. Working as a clinical nurse and intensive care nurse requires mutual interaction, strong collaboration. Especially when the patient group and care process of intensive care nurses are evaluated, it is a desired and expected situation that the sharing process, collaboration and professionalism scores are high. It is thought that this result will positively affect the quality of care, the collaboration of the team and patient satisfaction. The fact that the sharing process, collaboration and NNSC scores of the head nurses are low compared to the others suggests that the number of head nurses participating in the research is less than the others and the difference brought about by the duties, authorities, and responsibilities of the head nurses.

It has been determined that the sharing process, collaboration, professionalism and NNSC scores of nurses working for 0-3 years are higher than those of 8 years and above. This finding is surprising, and the fact that new nurses are more collaborative; It is thought that their efforts to perform at a high level may have contributed to the motivation of being new to the profession and being up to date in their knowledge. In the study conducted by Ylitörmänen et al. (2019), it was stated that, like our findings, nurses with more than ten years of work experience had lower conflict management scores than nurses with less than four years of work experience, but higher collaboration and professionalism scores.

It was determined that the work stress scores of the single nurses participating in the study were higher than those of the married ones. Similarly, in the study of Çankaya (2020) in which she evaluated the effect of work stress on the intention to leave, it was stated that the work stress perceptions of single nurses were higher than those of married nurses. In this respect, considering that single nurses meet their vital responsibilities at an individual level, it is thought that the fact that they may be affected more negatively from job loss in financial terms may increase work stress.

It was determined that the work stress scores of nurses in the 20-30 age group were higher than those of the nurses aged 41 and over. Similarly, in the study of Tuna and Baykal (2013) in which they examined work stress and its affecting factors in oncology nurses, it was stated that the levels of work stress may be high due to the fact that nurses under the age of 24 have just entered the business life and have little experience. When the literature is examined, there are also studies showing that nurses under the age of 30 have high levels of work stress due to the fact that they have just started to work (Najimi et al., 2012; Yılmaz et al.,

2021). The fact that nurses who worked for 0-3 years in our study had higher work stress scores than those who worked for 8 years or more also supports this finding.

The work stress scores of the contracted nurses compared to the regular nurses; It was determined that the nurses who were not satisfied with the unit they worked in were higher than the nurses who were satisfied. Although contracted nurses have the same duties, authorities, and responsibilities as regular nurses, it is thought that their low financial and social rights may increase their work stress. It is thought that work stress may be high due to the decrease in the work motivation of the personnel who are not satisfied with the unit.

This study is single-center. Since the data collection tools applied to nurses represent nurses' self-reports, this study can only be generalized to this sample group.

CONCLUSION

There was no difference between the levels of collaboration among nurses according to the education level of the nurses, the number of children and the choice of the unit they worked for. It was determined that the level of collaboration between nurses was high among nurses who were female and in the age group of 20-30, working as clinical nurse and intensive care nurse, and working years between 0-3 years. In addition, conflict management, collaboration and professionalism scores of single nurses were higher than those of married ones. There was no difference in

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the levels of work stress of the nurses according to gender, number of children, education level, place of work and choosing the unit of work. On the other hand, it has been observed that the work stress levels of the nurses who are married, in the 20-30 age group, who work under contract and who are not satisfied with the unit they work in, are higher. Execution of health care services requires team collaboration, effective communication skills, control of work stress and a positive working atmosphere. In this direction, nurses should be strengthened to work in team collaboration, both in undergraduate education and in-service training. In addition, it is recommended to provide trainings that increase their awareness in terms of factors that determine cooperation such as effective communication skills, conflict management, professionalism, collaboration.

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Conflict of Interest

The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Author Contributions

Plan, design: GD, GS; **Material, methods and data collection:** GD, GS; **Data analysis and comments:** GD, GS; **Writing and corrections:** GD, GS.

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