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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Evaluation of Penetrating Keratoplasty Results: Initial Experiences

Penetran Keratoplasti Sonuçlarının Değerlendirilmesi: İlk Deneyimler

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ABSTRACT

Aim: *It is aimed to examine the indication distributions and clinical results of penetrating keratoplasties (PKP) performed with the first experience of surgeons in a center where PKP was started for the first time.*

Material and Method: *In this retrospective study, 64 eyes of 63 patients who underwent PKP at Van Training and Research Hospital between 2017 and 2020 were evaluated. Demographic characteristics, PKP indications, and additional pathologies of the patients were obtained from the files of the patients and their records in the Van Training and Research Hospital eye bank. In addition, preoperative, postoperative first, third, and sixth-month visual acuities of the patients were evaluated, and postoperative complications were examined. The surgeries were performed by three different surgeons (EM, MSD, YE) who had just started performing PKP.*

Results: *Penetrating keratoplasty indications are respectively pseudophakic bullous keratopathy (32.8%), keratoconus (17.1%), corneal scar due to previous herpes keratitis (14%), corneal dystrophy (10.9%), aphakic bullous keratopathy (9.3%), traumatic scar (9.3%), graft with rejection reaction (4.6%), and a corneal abscess that did not regress with medical treatment (1.5%). Postoperative complications were glaucoma (9.3%), rejection reaction (7.8%), cataract (6.2%), suture loosening (4.6%), keratitis (3.1%), traumatic perforation (1.5%) and endophthalmitis (1.5%). There was a statistically significant increase in visual acuity in the first, third, and sixth months compared to the preoperative value (for all; p<0.01). However, there was no significant increase in visual acuity in the sixth postoperative month compared to the third month (p>0.05).*

Conclusion: *The most common indication for PKP was pseudophakic bullous keratopathy. In this patient series, which consisted of the first experiences of surgeons, the postoperative complication rate was 34.3%. Despite this, a significant improvement in visual acuity and graft survival was achieved in 95.3% of patients in early postoperative follow-ups.*

Key words: *penetrating keratoplasty; indications; complications*

ÖZET

Amaç: *İlk defa penetran keratoplasti (PKP) yapılmaya başlanan bir merkezde cerrahların ilk deneyimleri ile yapılan PKP'lerin endikasyon dağılımlarının incelenmesi, klinik sonuçlarının sunulması amaçlanmıştır.*

Materyal ve Metot: *Bu retrospektif çalışmada Van Eğitim ve Araştırma Hastanesinde 2017–2020 yılları arasında PKP yapılan 63 hastanın 64 gözü değerlendirildi. Hastaların dosyaları ve Van Eğitim Araştırma Hastanesi göz bankasındaki kayıtlarından hastaların demografik özellikleri, PKP endikasyonları, ek patolojileri ile ilgili verileri elde edildi. Hastaların preoperatif, postoperatif birinci, üçüncü ve altıncı aydaki görme keskinlikleri değerlendirildi ve postoperatif komplikasyonları incelendi. Ameliyatlar yeni PKP yapmaya başlayan üç farklı cerrah tarafından (EM, MSD, YE) gerçekleştirildi.*

Bulgular: *PKP endikasyonları sırasıyla psödofakik büllöz keratopati (%32,8), keratokonus (%17,1), geçirilmiş herpes keratitine bağlı kornea skarı (%14), kornea distrofisi (%10,9), afakik büllöz keratopati (%9,3), travmatik skar (%9,3), red reaksiyonu gelişmiş grefon (%4,6) ve medikal tedavi ile gerilemeyen kornea apsesi (%1,5) idi. Postoperatif komplikasyonlar sırasıyla glokom (%9,3), red reaksiyonu (%7,8), katarakt (%6,2), sütür gevşemesi (%4,6), keratit (%3,1) travmatik perforasyon (%1,5) ve endoftalmi (%1,5) idi. Ameliyat öncesi değere göre birinci, üçüncü ve altıncı ayda görme keskinliğinde istatiksel olarak anlamlı bir artış olduğu görüldü (hepsi için; p<0,01). Postoperatif altıncı ayda üçüncü aya göre görme keskinliğinde anlamlı bir artış olmadı (p>0,05).*

Sonuç: *En sık PKP endikasyonunun psödofakik büllöz keratopati olduğu tespit edildi. Cerrahların ilk deneyimlerinden oluşan bu hasta serisinde postoperatif komplikasyon oranı %34,3 idi. Buna rağmen erken postoperatif takiplerde hastaların görme keskinliğinde anlamlı bir artış ve %95,3'sinde greft sağkalımı elde edilmiştir.*

Anahtar kelimeler: *penetran keratoplasti; endikasyonlar; komplikasyonlar*

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Introduction

Corneal diseases are the leading causes of vision loss, especially in developing countries[1]. Keratoplasty is a surgical method applied to ensure the transparency of the cornea or to correct its geometry. Various pathologies cause corneal deformity or irreversible clouding, making keratoplasty indispensable for visual rehabilitation. In our country and in the world, keratoplasty is performed with optical (nephelion or scarring of the cornea), therapeutic (corneal infection or inflammation resistant to medical treatment), tectonic (corneal perforation) and cosmetic (conditions where the corneal image deteriorates and vision increase is not expected) indications[2,3]. The fact that the cornea is an avascular tissue and the immunological privileges it provides eliminates the tissue compatibility requirement for other organ transplants in corneal transplants and allows higher success rates[4]. These advantages make corneal transplants the most common tissue transplant. Tissue rejection, loosening of the sutures and related suture reaction, high astigmatism, glaucoma, uveitis and synechia may occur after PKP[5]. These complications require careful follow-up of the patients and may overshadow the surgical success.

Although surgical techniques that enable lamellar keratoplasty have been developed today, the need for penetrating keratoplasty (PKP) still continues[2]. The frequency of lamellar and PKP surgeries may vary according to the socioeconomic status of the countries. In addition, the frequency and complication rates of surgeries may change over the years[3]. In fact, surgical indications, frequency of surgery, complication rates and, accordingly, success rates may differ in different clinics in the same country, depending on their technological infrastructure and surgical experience, which may change over the years[2,6–10]. Therefore, it is important to report surgical results in different clinics or regions. In this study, it was aimed to examine the first experiences of surgeons in a center where PKP was started for the first time, and to examine the indications in patients who underwent PKP, to present the clinical results and to compare them with the literature data.

Material and Method

In this retrospective study, 64 eyes of 63 patients who underwent PKP surgery in Van Training and Research Hospital between 2017 and 2020 were evaluated. Approval was obtained from the Van Training and Research Hospital Ethics Committee for the study, and the study was conducted in accordance with the Helsinki Declaration standards. Data on age and gender distribution, PKP indications, and additional pathologies were obtained from the files of the patients and their records in the Van Training and Research Hospital eye bank. The initial diagnosis of patients who underwent more than one PKP in our clinic was taken into account. If the first PKP in our clinic was performed due to graft failure, the diagnosis was evaluated as graft failure. The best corrected visual acuities of the patients preoperatively, postoperatively at first, third and sixth months were evaluated and postoperative complications were examined. The donor tissues used in the surgeries were obtained from the Van Training and Research Hospital eye bank and the Ankara eye bank corneal transplantation coordination center. Donor corneas obtained within six hours after death were stored in corneal storage solution (Optisol, Chiron Ophthalmics, Irvine, CA) at +4°C until transplantation.

Before the surgery, detailed histories of the patients were taken and the best corrected visual acuity, anterior segment examination, and retina and optic nerve examination were performed in possible patients, and keratoplasty indications were determined. B-mode ultrasonography was performed when it was not possible to examine the retina biomicroscopically. Written informed consent was obtained from all patients before surgery. Before starting the surgery, the graft was prepared by cutting the donor cornea with a vacuum punch (Katena Products Inc., Denville, NJ, USA) with a diameter of 0.25–0.50 mm larger than the recipient bed. After determining the appropriate diameter by marking the corneal center of the patient, the recipient bed was prepared by cutting with a vacuum trephine (Katena Products Inc., Denville, NJ, USA) and cleaning the remaining tissues with scissors. After the graft was fixed to the recipient bed with four main fixation sutures, it was sutured with continuous or 12 single 10/0 nylon sutures. After trepanization of the recipient cornea, patients with anterior or posterior synechiae underwent synechiotomy, patients with cataracts underwent extracapsular cataract extraction and intraocular lens placement, and patients who have vitreous in anterior chamber underwent anterior vitrectomy. In the evaluation of corneal graft transparency, the findings in the last examination of the patient were taken as basis. At the end of the operation, subconjunctival

moxifloxacin and dexamethasone injections were performed. The surgeries were performed by three surgeons (EM, MSD, YE) who were just beginning to perform PKP.

In the postoperative period, topical 1% prednisolone acetate (Pred Forte®, Allergan, Irvine, CA) was started eight times a day and then the dose was reduced. During follow-up, topical 1% prednisolone acetate was switched with 0.5% loteprednol etabonate (Lotemax, Bausch&Lomb). Topical 0.5% moxifloxacin (Vigamox, Alcon) was given for one week or until the epithelial defect healed. All patients were routinely fitted with therapeutic contact lenses in the postoperative period and preservative free topical artificial tear treatment was started. After the epithelium healed, the therapeutic contact lens was removed and the patient was followed up with artificial tear drops. Therapeutic contact lenses continued to be used in patients with delayed closure of the epithelial defect. The frequency of topical steroid application was decreased and the frequency of preservative free topical artificial tear treatment was increased, and the patient was followed closely. If the epithelial defect did not shrink, topical autologous serum was started. The amniotic membrane was covered in patients whose epithelial defect persisted despite these treatments. Preoperative prophylactic systemic acyclovir treatment was started for individuals with corneal opacity due to previous herpetic keratitis and this treatment was continued in the postoperative period. The suture removal procedure of the patients without suture complications was postponed after the sixth month.

In the controls performed on the first day, first week, first, third and sixth months postoperatively, the status of the graft, any signs of complications were evaluated by biomicroscopic examination, and intraocular pressure measurements were made. The best corrected visual acuity was obtained with eyeglasses correction. Correction with contact lens was not performed because refraction stabilization was expected after suture removal for correction with contact lens. Visual acuity determined by pinhole was accepted as the best corrected visual acuity in patients whose refraction values could not be measured. Complications that occurred were recorded in detail. Intraocular pressure measurements were made using applanation tonometry or pneumotonometry. Patients with irregular follow-up and missing information on the follow-up card were not included in the study.

Statistical analyzes were performed after the data were entered into the SPSS program. Quantitative data were presented as the mean standard deviation. Postoperative changes were evaluated using repeated-measures analysis of variance. Qualitative data were presented as a percentage. The relationship between the parameters was evaluated with Pearson correlation analysis. A p value less than 0.05 was considered statistically significant.

Results

Within the scope of the study, 64 eyes of 27 female (42.2%) and 36 male (56.3%) patients were evaluated. The mean age of the patients was 61.25±20.34 (min-max: 15–92) years. Penetrating keratoplasty indications, in descending order, were pseudophakic bullous keratopathy in 21 (32.8%) eyes, keratoconus in 11 (17.1%) eyes, corneal scarring due to previous herpes keratitis in nine (14%) eyes, corneal dystrophy in seven (10.9%) eyes , aphakic bullous keratopathy in six (9.3%) eyes, traumatic scar in six (9.3%) eyes, graft with rejection reaction in three (4.6%) eyes, and cornea abscess that did not regress with medical treatment in one (1.5%) eye. Five of the patients with corneal dystrophy had macular dystrophy and two had granular dystrophy. In terms of additional pathologies, four (6.2%) patients had amblyopia, two (3.1%) degenerative myopia, two (3.1%) optic atrophy, one (1.5%) retinitis pigmentosa, one (1.5%)) macular hole and one (1.5%) had senile macular degeneration (Table 1).

In addition, during surgery, in four (6.2%) patients cataract extraction and intraocular lens implantation, four (6.2%) synechiotomy, three (4.6%) pupilloplasty, one (1.5%) scleral fixation, one (1.5%) 1.5% anterior vitrectomy and one (1.5%) silicone removal were performed. Postoperative complications were observed in 22 (34.3%) patients during the six-month follow-up period which were glaucoma in six patients (9.3%), rejection reaction in five patients (7.8%), cataract in four patients (6.2%), suture loosening in three patients (4.6%), keratitis in two patients (3.1%) traumatic perforation in one patient (1.5%) and endophthalmitis in one patient (1.5%) (Table 2). The intraocular pressure of the patients who developed glaucoma was controlled with topical anti-glaucomatous agents and surgery was not needed. While two of the rejection reactions could be controlled with medical treatment, surgery was performed again in

Table 1. *Surgical indications of patients before surgery and the frequency of additional pathologies*

Surgical indications	Eye (%)	Additional pathologies	Eye (%)
Pseudophakic bullous keratopathy	21 (32.8)	Amblyopia	4 (6.2)
Keratoconus	11 (17.1)	Degenerative myopia	2 (3.1)
Herpes keratitis scar	9 (14)	Optic atrophy	2 (3.1)
Corneal dystrophy	7 (10.9)	Retinitis pigmentosa	1 (1.5)
Aphakic bullous keratopathy	6 (9.3)	Macular hole	1 (1.5)
Traumatic scar	6 (9.3)	Senile macular degeneration	1 (1.5)
Graft with rejection	3 (4.6)		
Corneal abscess	1 (1.5)		
Total	64 (100)		11 (17)

Table 2. *Frequency of additional surgeries and postoperative complications during penetrating keratoplasty*

Additional surgery	Eye (%)	Postoperative complications	Eye (%)
Cataract extraction and intraocular lens implantation	4 (6.2)	Glaucoma	6 (9.3)
Synechiotomy	4 (6.2)	Rejection reaction	5 (7.8)
Pupilloplasty	3 (4.6)	Cataract	4 (6.2)
Anterior vitrectomy	1 (1.5)	Suture loosening	3 (4.6)
Scleral fixation	1 (1.5)	Keratitis	2 (3.1)
Silicon removal	1 (1.5)	Endophthalmitis	1 (1.5)
		Traumatic perforation	1 (1.5)
Total	11 (17.1)		22 (34.3)

three of them. Indications for PKP in patients with rejection reaction that could be controlled with medical treatment were traumatic scar and pseudophakic bullous keratopathy. In those who developed permanent rejection reaction, the indications were herpetic keratitis scar in two and bullous keratopathy in one. Suture revision was performed in patients with suture loosening, and re-suture was required in patients with traumatic perforation. One of the three patients who developed cataracts underwent phacoemulsification and intraocular lens implantation. Keratitis was controlled with topical drugs and graft failure did not develop in these patients. The patient who developed endophthalmitis required vitrectomy and was referred to the upper center. Topical cyclosporine treatment was applied to seven patients in the postoperative period. These were patients with severe dry eye or patients at risk of rejection due to reasons such as corneal vascularization.

Mean visual acuity before surgery was 2.52±0.69 (min-max: 1.30–3.10) logMAR. Postoperative visual acuity according to logMAR was 1.10±0.52 at first month, 0.77±0.59 at third month and 0.77±94 at sixth month (Fig. 1). There was a statistically significant increase in visual acuity at the first, third and sixth months compared to the preoperative value (for all; $p<0.01$). When visual acuity was evaluated after surgery, it was found that visual acuity was significantly higher at the third and sixth months compared to the first month (for both; $p<0.01$). However, there was no statistically significant increase in visual acuity after the third postoperative month ($p>0.05$) (Fig. 1).

Discussion

Although the indications and frequencies of PKP may change over the years depending on the development of new surgical techniques, PKP is still a frequently applied surgical method today[11]. Penetrating keratoplasty indications may also vary according to the socioeconomic status and the location of the countries[12,13]. In our study, the three most common PKP indications were pseudophakic bullous keratopathy, keratoconus and herpes keratitis scar, respectively. In their study with 151 patients, Çubuk et al.[2] reported that the

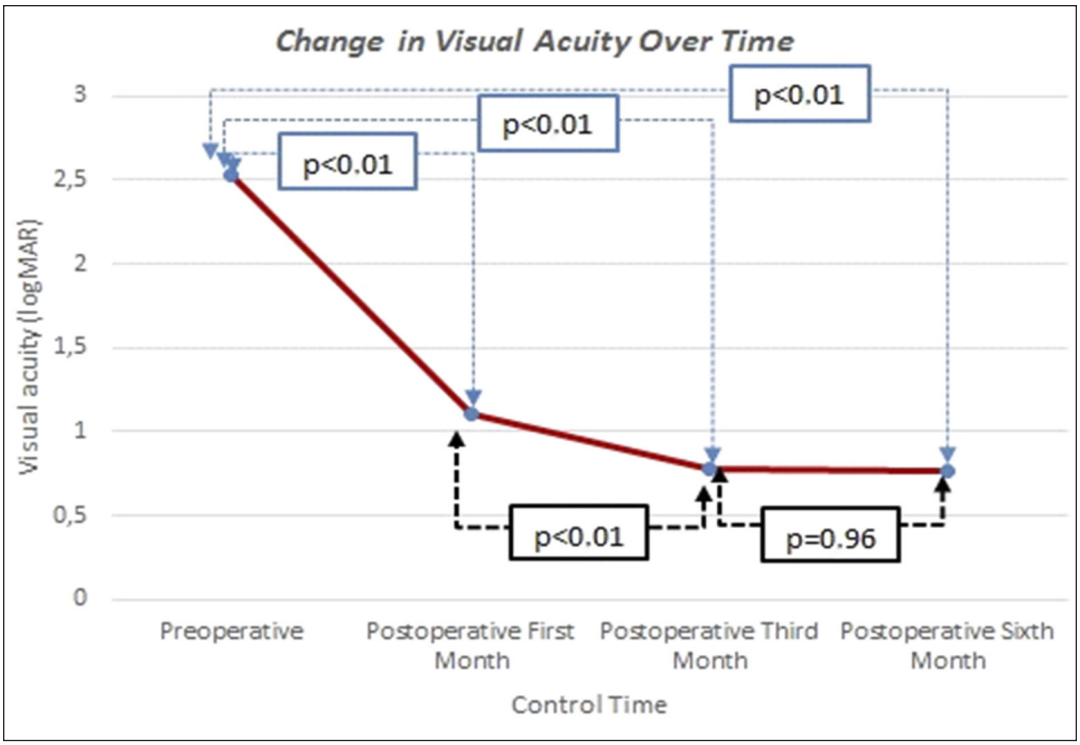

Figure 1. *Change in visual acuity over time.*

most common indications for PKP were graft failure, followed by pseudophakic bullous keratopathy and traumatic corneal scar. The reason why graft failure was not among the most common causes in our study may be that PKP surgery was just started in our center and therefore there were no long-term results. In a large series study conducted with 665 patients in 2009, Burcu Nurözler et al.[10] reported that the most common indications for PKP were keratoconus (33.2%), corneal leukoma (14.9%), and pseudophakic bullous keratopathy (13.0%). The fact that 32.8% of the indications in our study was pseudophakic bullous keratopathy may have resulted from the accumulated patients followed up in our center with this diagnosis, since keratoplasty was started for the first time in our center. In addition, there is approximately 10 years between our study and the study of Burcu Nurözler et al. We think that the rate of keratoconus in our study (17.1%) was lower than this study, since the progress of keratoconus to surgery could be prevented or delayed with the contact lenses developed in these 10 years and the widespread cross-linking applications[14].

It is also important to compare the results of keratoplasty performed in the eastern Anatolia region in the literature with our results, since the most common indication for keratoplasty may differ even between different regions in the same country[15,16]. In this context, in a study conducted by Öndaş et al.[6] at Erzurum Atatürk University, it was determined that the most common cause of keratoplasty was pseudophakic bullous keratopathy, as in our study, but corneal leukoma and scars followed it. Unlike our study, the reason why keratoconus was not among the most common indications in Öndaş et al.'s study is that they only evaluated patients over 65 years of age. In different studies in the literature, it has been reported that keratoconus is one of the most common indications of PKP[10,17]. However, we believe that keratoconus will lose its place among the most common causes of PKP in the coming years, as successful visual rehabilitation can be achieved without the need for surgery in keratoconus patients with the developments in recent years[18].

When evaluated in terms of preoperative best corrected visual acuity, the preoperative visual acuity was found to be 1.43±0.76 logMAR in Öndaş et al.'s study[6], and 2.53±0.69 logMAR in our study. The reason for the lower preoperative visual acuity of the patients in our

study may be that the surgeons preferred to operate on patients with lower visual acuity because they had their first keratoplasty experience.

When evaluated in terms of additional surgeries, it was seen that cataract extraction, intraocular lens implantation and synechiotomy were the most common procedures in our study. Similar to our study, it has been reported in the literature that the most common additional surgery is cataract extraction and intraocular lens implantation[2,6]. However, unlike our study, the second most common additional surgery was found to be anterior vitrectomy[2,6]. The reason for this may be that since keratoplasty was started for the first time in our center, it was avoided to operate on complicated cases. Because keratoplasty itself is already a major surgery. This may have caused surgeons new to surgery to avoid complicated cases, as additional pathologies may obscure surgical success.

In our study, the three most common complications were glaucoma, rejection reaction, and cataract development. In a study by Çubuk et al.[2] reported that complications developed in 23% of patients after PKP, the three most common complications were listed as graft rejection, suture loosening, and glaucoma. Although keratoplasty is performed for the first time in our center, the most common complications are mostly consistent with the literature. In a study conducted in our country in 1995, endophthalmitis developed in one of the 42 eyes with PKP and evisceration was applied to the patient[19]. In our study, endophthalmitis developed in only one patient and the patient was referred to the upper center to have vitrectomy. This result shows that the approach to endophthalmitis, which is a catastrophic complication after PKP, can change depending on the changing experience and technological support over the years.

It has been reported that graft transparency is an indicator of the success of kertoplasty[10,20]. Although donor-related factors may affect the transparency of the graft, the indication for PKP and the additional pathologies found in the patient are the main factors in maintaining the transparency of the graft[20,21]. In our study, rejection reaction developed in 5 (7.8%) eyes, graft transparency was lost in 3 (4.6%) eyes, and the need for rekeratoplasty arose. In a study conducted in our country in recent years, the rate of graft rejection was found to be 7%, similar to our study[2]. However, in our study, we think that this rejection rate will increase even more over time. Because, as the follow-up period increases, the graft survival rate has been found to decrease[22]. It has been reported in the literature that approximately half of the cases that developed rejection returned with medical treatment[10]. In our study, in accordance with these results, the reaction was controlled with medical treatment in 40% of the patients who developed a rejection reaction.

Physicians who will perform keratoplasty should be aware that additional surgeries such as cataract extraction and intraocular lens implantation, synechiotomy, pupilloplasty and rarely anterior vitrectomy and scleral fixation may be needed during surgery. They should not forget that postoperative follow-up is also very important in addition to a successful surgery. For this reason, surgeons should closely monitor their patients in terms of glaucoma, rejection reaction, cataract development, suture status and infective signs during the postoperative follow-up period. In addition, we think that these physicians should have sufficient knowledge and skills in the diagnosis, follow-up and treatment of these complications.

Our study is of great importance in terms of evaluating the results of surgeons' first PKP experiences. Despite this, the lack of long-term results and the small number of patients are limitations of our study. For this reason, there is a need for studies with longer follow-ups and larger series in which the results of physicians who have just started keratoplasty surgery are monitored.

Conclusion

The most common indication for PKP in our study was pseudophakic bullous keratopathy. In this patient series consisting of the first experiences of surgeons, the postoperative complication rate was 34.3%. Despite this, a significant improvement in visual acuity and graft survival was achieved in 95.3% of patients in early postoperative follow-ups.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Examination of Primary School Students' Knowledge of the Flu and the Factors Affecting Their Decision to Get Flu Vaccines

İlköğretim Öğrencilerinin Gribe Yönelik Bilgileri ve Öğrencilerin Grip Aşısı Yaptırma Durumlarını Etkileyen Faktörlerin İncelenmesi

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ABSTRACT

Aim: *Influenza, also known as flu, is a viral disease with high mortality and morbidity. Although every age group is at risk, people aged six months to 18 years are at high risk of developing serious flu-related complications. This descriptive study had two objectives: 1) determining primary school student's level of knowledge about the flu, and 2) examining the factors affecting their decision to get flu shots.*

Material and Method: *The sample consisted of 670 sixth-, seventh-, and eighth-grade students from three public and three private schools. Data were presented as percentiles, ratios, chi-squares, and means (± standard deviation). The data were analyzed at a significance level of 0.05.*

Results: *Less than a quarter of the participants defined the flu as a viral disease (23.7%). More than half the participants stated that the flu was contagious (65.5%). More than a quarter of the participants noted that the flu was transmitted through aerosols containing the virus, direct contact, or contact with contaminated surfaces (35.5%). More than half the participants said it was necessary to get flu shots (71.3%). Eight in ten of those participants had flu shots (82.8%). More than half of mothers with high school or higher degrees had their children vaccinated against the flu (56.7%). Most fathers with high school or higher degrees had their children vaccinated against the flu (78.4%) (p=0.030). Almost all parents with health insurance had their children vaccinated against the flu (p<0.001).*

Conclusion: *Students do not know much about the flu and flu shot. Therefore, children and parents should be informed about the effects of the flu, how to avoid getting it, and what the flu vaccine is.*

Key words: *human flu; students; influenza; flu vaccines*

ÖZET

Amaç: *Grip olarak da bilinen influenza, mortalite ve morbiditesi yüksek viral bir hastalıktır. Her yaş grubu risk altında olmasına rağmen, özellikle altı aydan 18 yaşına kadar olan kişiler, griple ilgili ciddi komplikasyonlar geliştirme riski altındadır. Bu tanımlayıcı çalışmanın iki amacı vardı: 1) ilkokul öğrencilerinin grip hakkındaki bilgi düzeylerini belirlemek ve 2) grip aşısı olma kararlarını etkileyen faktörleri incelemek.*

Materyal ve Metot: *Örneklemi üç resmi ve üç özel okuldan 670 altıncı, yedinci ve sekizinci sınıf öğrencisi oluşturmuştur. Veriler yüzdelikler, oranlar, ki-kareler ve ortalamalar (± standart sapma) olarak sunuldu. Veriler 0,05 anlamlılık düzeyinde analiz edildi.*

Bulgular: *Katılımcıların dörtte birinden azı gribi viral bir hastalık olarak tanımladı (%23,7). Katılımcıların yarısından fazlası gribin bulaşıcı olduğunu belirtmiştir (%65,5). Katılımcıların dörtte birinden fazlası, gribin virüs içeren aerosoller yoluyla, doğrudan temas yoluyla veya kontamine yüzeylerle temas yoluyla (%35,5) bulaştığını belirtti. Katılımcıların yarısından fazlası grip aşısı olmak gerektiğini belirtmiştir (%71,3). Bu katılımcıların sekizinde (%82,8) grip aşısı vardı. Lise ve üzeri eğitimli annelerin yarısından fazlası çocuklarına (%56,7) grip aşısı yaptırmıştır. Lise ve üzeri eğitimli babaların çoğu, çocuklarına grip aşısı yaptırmıştır (%78,4) (p=0,030). Sağlık güvencesi olan ebeveynlerin hemen hemen tamamı çocuklarını grip aşısı yaptırmıştır (p<0,001).*

Sonuç: *Öğrenciler grip ve grip aşısı hakkında pek bilgi sahibi değiller. Bu nedenle çocuklar ve ebeveynler gribin etkileri, nasıl önlenebileceği ve grip aşısının ne olduğu konusunda bilgilendirilmelidir.*

Anahtar kelimeler: *insan gribi; öğrenciler; grip; grip aşıları*

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Introduction

The flu virus has caused epidemics and pandemics throughout history[1]. According to the Center for Disease Control and Prevention (CDC) flu burden report, between 2010 and 2020, the flu caused 9–41 million symptomatic illnesses, 140–710 thousand hospitalizations, and 12–52 thousand deaths per year[2]. In European Union countries, children constitute 10–30% of the general population with influenza each year. It is an acute viral infectious disease characterized by fever, cough, headache, fatigue, and myalgia lasting 7–10 days[3,4]. However, this period may be prolonged in risk groups with viral or bacterial pneumonia. Flu type B virus may cause Reye's Syndrome in children on aspirin therapy for acute rheumatic fever[5]. Myocarditis, pericarditis, myositis, and encephalopathy are other complications of the flu[6,7].

Healthy people should avoid having close contact with those infected with the flu virus and wash their hands frequently[8,9]. In addition, they should eat healthy food, get enough sleep, drink enough water, do sports regularly, and avoid too much stress and heat[9,10]. There are two ways to protect infected people from getting sick. First, we should vaccinate them to make them resistant to the virus[6]. Second, we should give them antiviral medication right after they are infected or in the early stage of the disease (chemoprophylaxis) to prevent the infection from spreading and causing disease[4].

Vaccination is the primary important intervention for influenza prevention and control[1]. The effectiveness of the seasonal vaccine varies between 19% and 60%[1]. Vaccination can also reduce flu-related complications (e.g., otitis media and pneumonia) and mortality rates in developing countries[11,12]. Priority risk groups for flu vaccination are persons over 65 years of age, those with chronic medical conditions (asthma, chronic lung diseases, diabetes, or chronic cardiovascular diseases), those aged six months to 18 years who are on long-term aspirin therapy, immunocompromised persons, those with morbid obesity (Body Mass Index >40), and the elderly living in long term care facilities[6]. Another target group for flu vaccination includes the family members of high-risk groups, healthcare professionals, persons working in long-term care facilities, and those aged 50–64 years[13]. Special target groups for flu vaccination include women at four months of pregnancy, HIV-infected people, frequent travelers, those who want to be protected from the adverse medical and economic effects of the flu, business people, athletes, etc.[5] Vaccination is recommended because it reduces childhood hospitalizations in developed countries. In the systematic review and meta-analysis study, it was revealed that influenza vaccine plays a role in preventing hospitalizations in children between 40.8% and 74.1%[11]. This study sought answers to two questions: 1) how much do primary school students know about the flu? and 2) what factors affect their decision to get flu shots?

Materials and Method

1. Research Type:

A descriptive study

2. Research Setting and Time Frame:

The study was conducted in three public and three private schools in the spring semester of the 2014–2015 academic year.

3. Population and Sample

The sample size was determined using the formula for a known population[14–16].

N=Total population=1577

n=Desired minimum sample p=Frequency (probability) of occurrence of an event=0.5

q=Frequency of absence of the event (1-p)=0.5

t=Level of significance, that is, type 1 (alpha) error level=1.96

d=Standard error=0.05

$$\tilde{n} = \frac{1577.(0,5).(0,5).(1,96).(1,96)}{1576.(0,05).(0,05) + (1,96).(1,96)(0,5).(0,5)} = \frac{1.514,5508}{3,94 + 0,9604} = 309$$

The formula revealed that a sample size of 309 students from six schools would be large enough to detect significant differences. Six hundred and seventy students were recruited to avoid missing data.

4. Data Collection:

The data were collected using a survey. Each grade filled out the survey collectively. Data collection took 20–30 minutes.

5. Survey:

The survey was developed by the researcher based on a literature review and expert opinion. The instrument consisted of 28 items divided into two parts. The first part consisted of 13 items on sociodemographic characteristics (age, grade level, chronic disease, etc.) The second part consisted of 15 items on the flu (having the flu, knowing the ways to protect from the flu, having gotten a flu shot, etc.). A pilot test was conducted with ten sixth-grade students from the Kafkas University Kalkinma Vakfi Private Primary and Secondary School.

6. Data Analysis:

The data were analyzed using the Statistical Package for Social Sciences (SPSS, v. 20) at a significance level of 0.05. Mean and percentage were used for descriptive analysis. Significance was determined using the chi-square test. Chi-square was used for multi-group analysis.

7. Variables:

The independent variables were sociodemographic characteristics. The dependent variables were "having the flu in the past year" and "getting a flu shot in the past year."

8. Ethical Considerations:

The study was approved by the Ethics Committee of the Faculty of Medicine of Kafkas University (No: 80576354-050-99/05). Permission was obtained from the schools. All students were informed about the research purpose and procedure. They were also informed that the data would be used only for scientific purposes and would not be shared with third parties and that they could withdraw from the study at any stage. Participation was voluntary.

9. Limitations and Generalizability:

The study had one limitation. The sample consisted only of students from three public and three private schools in Kars, Turkey. Therefore, the results are sample-specific and cannot be generalized to the whole population.

Results

Participants had a mean age of 12.70±0.03 years. Average age of the mothers of the participants is 45.15±19.37; father's mean age is 42.65±15.05 years. More than half of the participants were girls (57%). Less than half of the participants were sixth graders (37.5%). More than half of the participants went to public schools (56.1%). Most participants had siblings (91.8%). The majority of the participants had no chronic diseases (93.3%). Less than half of the mothers were between the ages of 36 to 41 years (46.1%). The majority of the mothers were housewives (72.2%). Half of the fathers were older than 41 (48.8%). Half of the fathers had freelance jobs (49.9%). More than half of the families had a neutral income (66.3%). Seven in ten participants had the flu in the past year (72.4%). The majority had the flu 1–3 times in the past year (89.3%), while the half had the seasonal flu in the winter (49.6%). Almost half of the participants had had flu shots before (47.6%). Eight in ten participants did not get flu shots in the past year (82.7%).

A quarter of the participants described the flu as a viral disease (23.7%). More than half of the participants stated that the flu was contagious (65.5%). Less than half of the participants noted that the flu was transmitted through aerosols containing the virus, by direct contact, or through contact with contaminated surfaces (35.5%). Four in ten participants remarked that the flu symptoms were fever, joint and muscle pain, dry and hacking cough, and fatigue (39%). Almost half of the participants expressed that one should have a balanced diet and enough sleep to protect from the flu (44.9%). More than half of the participants stated that they visited their doctors when they had the flu (61%). Seven in ten participants believed that it was necessary to get flu shots (71.3%). Nearly half of the participants have heard of the flu vaccine (48.6%). Six in ten participants had heard of flu shots from their parents (61.9%). More than a quarter of the participants stated that they had not gotten flu shots due to negligence (27.9%) (Table 1).

The variables "gender," "school," "having siblings," "having chronic diseases," "parents' age," "parents' employment status," and "family income" did not affect flu vaccination rates ($p>0.05$). However, sixth graders got significantly more flu shots than seventh and eighth graders ($p=0.019$) ($p=0.019$)

There was a significant difference between parents' education and participants' flu vaccination status. More than half of the mothers with high school or higher degrees had their children vaccinated against the flu (56.7%). Most fathers with high school or higher

Table 1. *Students' knowledge levels on seasonal influenza and flu vaccine (n=670)*

Features		Number	%
Influenza effect	Viral	159	23.7
	Bacterial	511	76.3
Influenza contagious situation	Yes	439	65.5
	No	231	34.5
Transmission line (n=439)	Contact person	156	35.5
	By way of respiration	151	34.4
	Infected with contaminated material	20	4.6
	All	112	25.5
Influenza symptoms	Fever	52	7.8
	Joint and muscle pain	30	4.4
	Dry and hacking cough	114	17.0
	Fatigue	213	31.8
	All	261	39.0
Influenza protection shape	Balanced diet and regular sleep	301	44.9
	Drink plenty of water and regular sports	186	27.8
	Stress control and shelter protection	111	16.6
	All [1]	29	4.3
	I do not pay much attention	43	6.4
Behavioral shape in flu experience[2]	Applying to a doctor	409	61.0
	Use of antibiotics	178	26.6
	Mint-lemon consumption	352	52.5
	Rest	321	47.9
	Continue everyday life	92	13.7
Requirement of flu vaccine	Yes	478	71.3
	No	192	28.7
Hearing influenza status	Yes	326	48.6
	No	334	49.4
From who or where he heard of the flu vaccine[2] (n=326)	Parent	202	61.9
	Health personel	55	16.8
	Social media resources	87	26.6
Reasons for not getting a flu vaccine	Hearing flu vaccine	121	18.1
	Do not need to	343	51.2
	Negligence	187	27.9
	Financial situation	19	2.8

[1] All of them contain the first three predecessors.
[2] This question has been answered more than once.

degrees had their children vaccinated against the flu (78.4%) (p=0.040). There was a significant difference between parents' health insurance status and participants' flu vaccination status. 90.3% of participants with health insurance had flu vaccine. In other words, the majority of the participants with health insurance got flu shots (p=0.004; Table 2).

The relationship between the thought that flu vaccine is necessary and the status of being vaccinated was statistically significant (p<0.001). Most of the children (82.8%) who think that flu vaccine is necessary are those who have been vaccinated against the flu. The relationship between not getting the flu vaccine and being vaccinated against the flu was found to be statistically significant (p<0.001). Contrary to this situation, more than half (56.4%) of the children who stated that the reason for not getting the flu vaccine was because they didn't need it, did not get the flu vaccine (Table 3).

Discussion

This study investigated how much primary school students knew about the flu and what factors affected their decision to get flu shots.

Table 2. *The relationship of descriptive variables with influenza vaccination status (n=670)*

		Influenza vaccination status					
		Yes		No		Total	
Descriptive properties		n	%	n	%	n	%
Gender	Girl	138	43.3	177	50.4	315	47.0
	Male	181	56.7	174	49.6	355	53.0
	Test and p value	X^2=3.446 *p=0.063*					
Class	6th grade	129	40.4	122	34.8	251	37.5
	7th grade	106	33.2	101	28.8	207	30.9
	8th grade	84	26.3	128	36.5	212	31.6
	Test and p value	X^2=7.938 ***p=0.019***					
School status	State school	178	55.8	198	56.4	376	56.1
	Private school	141	44.2	153	43.6	294	43.9
	Test and p value	X^2=0.025 *p=0.874*					
Brotherhood	Yes	289	90.6	326	92.9	615	8.2
	No	30	9.4	25	7.1	55	91.8
	Test and p value	X^2=1.155 *p=0.283*					
Chronic illness	There is	21	6.6	24	6.8	45	6.7
	No	298	93.4	327	93.2	625	93.3
	Test and p value	X^2=0.017 *p=0.895*					
Mother age	26–30 age	18	5.6	27	7.7	45	6.8
	31–35 age	92	28.7	99	28.4	191	28.5
	36–40 age	147	45.8	140	40.1	287	42.8
	41 and over age	63	19.9	83	23.8	147	21.9
	Test and p value	X^2=3.519 *p=0.318*					
Father age	26–30 age	4	1.2	1	0.3	5	0.7
	31–35 age	26	8.1	17	4.9	43	6.4
	36–40 age	118	36.8	139	39.8	257	38.4
	41 and over age	173	53.9	192	55.0	365	54.5
	Test and p value	X^2=5.228 *p=0.156*					
Mother education	Not literate	17	5.3	36	10.3	53	7.9
	Primary education	121	37.9	135	38.5	256	38.2
	High school and over	181	56.7	180	51.3	361	53.9
	Test and p value	X^2=6.065 ***p=0.040***					
Father education	Not literate	6	1.9	18	5.1	24	3.6
	Primary education	63	19.7	97	27.6	160	23.9
	High school and over	250	78.4	236	67.3	486	72.5
	Test and p value	X^2=12.128 ***p=0.030***					
Mother's profession	Housewife	230	72.1	254	72.4	484	72.2
	Worker	24	7.5	32	9.1	56	8.6
	Officer	65	20.4	65	18.5	130	19.4
	Test and p value	X^2=0.806 *p=0.668*					
Father's profession	Officer	115	36.1	115	32.8	230	34.3
	Worker	47	14.7	59	16.8	106	15.8
	Self-employment	157	49.2	177	50.4	334	49.9
	Test and p value	X^2=1.030 *p=0.597*					
Social security status	No	31	9.7	38	10.8	69	10.3
	Yes	288	90.3	313	89.2	601	89.7
	Test and p value	X^2=11.300 ***p=0.004***					
Economic status of the family	Income less than expenses	29	9.1	41	11.7	70	10.4
	Income equal to expenses	211	66.1	66.4	52.5	444	66.3
	Income more than expenses	79	24.8	77	21.9	156	23.3
	Test and p value	X^2=1.648 p=**0.439**					

Table 3. *Influenza vaccination according to students' influenza vaccination examining attitudes (n=670)*

		Influenza vaccination status					
		Yes		No		Total	
Descriptive properties		n	%	n	%	n	%
Requirement of flu vaccine	Yes	264	82.8	214	61.0	478	71.3
	No	55	17.2	137	39.0	192	28.7
	Test and p value	X^2=38.811 ***p<0.001***					
Not getting a flu vaccine reason	Hearing flu vaccine	33	10.3	88	25.1	121	18.1
	Do not need to	145	45.5	198	56.4	343	51.2
	Negligence	132	41.4	55	15.7	187	27.9
	Financial situation	9	2.8	10	2.8	19	2.8
	Test and p value	X^2=63.565 ***p<0.001***					
Influenza status (last 1 year)	Yes	220	69.0	265	75.5	485	72.4
	No	99	31.0	86	24.5	185	27.6
	Test and p value	X^2=3.56 *p=0.117*					
Number of influenza (last 1 year)	1–3	291	91.2	307	87.5	598	89.3
	4 and up	28	8.8	44	13.5	72	10.7
	Test and p value	X^2=2.961 *p=0.316*					

Influenza is associated with 10% of respiratory hospitalizations in children under 18 years of age worldwide. Children under 6 months of age constitute 5% of hospitalizations and children aged 5–17 years constitute 16%[17]. A randomized controlled study revealed that 31% of hospitalized children had influenza-like illness and an average of 12% had an attack[18]. At the same time, The risk of the child infecting other family members in the house is high and may cause absenteeism and additional burden to parents and caregivers[19]. It is known that vaccination reduces the risk of flu-related hospitalizations, school and work days, visits to a doctor and antibiotic use, as well as reducing the risk of flu[20]. Most importantly, the vaccine has been shown to be life-saving in children[21]. In the study, when a child in each household is vaccinated, the risk of getting sick from other unvaccinated family members of unvaccinated children is twice as low[22]. For these reasons; In the CDC update report for 2019–2020 flu vaccines, in cooperation with the Advisory Committee on Immunization Practices Advisory Committee on Immunization Practices (ACIP), recommends that everyone 6 months of age and older without any contraindications get the flu vaccine every year and as soon as possible[5].

In this study, half of the participants (52.4%) had never had a flu vaccine before. In Yildirim's study with children under the age of 18, only 4 (5.6%) of the patients had influenza vaccination this season, and 3 (4.2%) the previous season[23]. In a study conducted within the scope of The Global Influenza Hospital Surveillance Network, it was found that the rate of vaccination in patients under the age of 5 who were found to be positive for influenza was 2.1%[24]. In a study conducted in our country during the 2014–2015 seasonal influenza season, using laboratory-confirmed influenza data in the Turkish population; Among 2561 patients, the vaccination rate was found to be 2.6% in individuals with influenza positive, and the rate of vaccination among an influenza-negative control was found to be 4.2%[25]. There was no significant difference in vaccination rates between male and female participants (p>0.05, Table 2), which has also been reported by earlier studies[23–25].

There were significantly more six graders who got flu shots than seven and eighth graders (p=0.019; Table 2).–There are studies emphasizing that the highest influenza vaccination rate is in children aged 6–17 years[26,27] and that vaccination is important for this age group[28–31]. Our participants had a mean age of 12.70±0.03. Therefore, there must be some factors other than age affecting our results.

There was no significant association between having a chronic disease and getting a flu shot. Only forty-four students with chronic diseases got flu shots (p>0.05; Table 2). Yanik ve Şahin also did not find an association between having chronic diseases and getting flu shots[32]. In the Yildirim study, it was reported that none of the children with chronic diseases were vaccinated[23].

Based on these results, we can state that there is an increasing number of children with chronic diseases who miss vaccinations because of sheer negligence on the part of their parents despite the fact they are more likely to develop flu-related complications than their healthy counterparts.

There was a significant relationship between parents' education and participants' vaccination status (p=0.040; Table 2). More than half of the mothers with high school or higher degrees had their children vaccinated against the flu (56.7%). Most fathers with high school or higher degrees had their children vaccinated against the flu (78.4%) (Table 2), which has also been reported by earlier studies[33,34]. It has also been demonstrated with different sample groups that the increase in education level has a positive effect on vaccine intake[35,36]. This result indicates that children of more educated parents are more likely to have complete or timely vaccinations because such parents are more aware of the danger of the flu and the importance of vaccinations.

There was no significant relationship between family income and vaccination rates (p=0.439; Table 2). However, almost all participants with health insurance got flu shots (90%.3) (p=0.004*;* Table 2*)*. The studies also show a positive correlation between socioeconomic status (SES) and vaccination rates[36,37]. This is probably because health insurance systems in Turkey do not cover flu vaccination, and therefore, low-SES parents cannot afford it.

Eight in ten participants who believed it was necessary to get flu shots got flu shots (p<0.001; Table 3). Almost half the participants stated that they had not gotten flu shots because they believed it was unnecessary (45.5%). They also failed to answer six out of ten questions about flu vaccination. These results indicate that those children do not know enough about the effectiveness and significance of flu shots. In academic studies, the lack of general knowledge about influenza and vaccination is seen as an obstacle to vaccination[38,39] and is among the factors affecting vaccination[40].

Nearly half of the participants (48.6%) heard of the flu vaccine. 61.9% of the participants heard about the flu vaccine from their parents and 26.6% from social media (Table 2). Research shows that two in ten children hear of flu shots from the media[41,42]. Topaloğlu et al. reported that seven in ten people did not get flu shots because of the misinformation in the media[42]. These results indicate that we should use the media wisely to raise public awareness of the importance and effectiveness of flu shots.

Less than a quarter of the participants defined the flu as a viral disease (23.7%). More than half of the participants stated that the flu was contagious (65.5%). More than a quarter of the participants noted that the flu was transmitted through aerosols containing the virus, by direct contact, or through contact with contaminated surfaces (35.5%). More than half of the participants knew that the flu was contagious, but only 34.4% stated that the flu was transmitted through aerosols containing the virus. Almost half of the participants expressed that one should have a balanced diet and regular sleep to protect oneself from the flu (44.9%) (Table 1). These results show that children do not know enough about what the flu is, how it is transmitted, and how to protect themselves from it.

The following are results:

1. The average age of the students is 12.70±0.03.
2. Almost half the children have gotten flu shots before (47.6%). The majority of the children did not get flu shots in the past year (82.7%).
3. The variables "gender," "school," "having siblings," "having chronic diseases," "parents' age," "parents' employment status," and "family income" have no effect on flu vaccination rates.
4. More educated parents are more likely to get their children vaccinated against the flu. More than half of the mothers with high school or higher degrees have had their children vaccinated against the flu (56.7%). Most fathers with high school or higher degrees have had their children vaccinated against the flu (78.4%) (p=0.030).
5. The majority of the children with health insurance have gotten flu shots (90.3 %) (p=0.004)
6. Most children who believe it is necessary to get flu shots have been vaccinated against the flu (82.8%) (p<0.001).
7. Almost half the children (48.6%) have heard of flu shots from their parents (61.9%) or the media (26.6%).
8. More than half of the children believe that the flu is contagious (65.5%). Only three in ten children believe that it is transmitted through aerosols containing the virus (34%.4).

9. These results show that children do not know enough about what the flu is, how it is transmitted, and how to protect themselves from it.

The recommendations section:

1. Both students (primary and middle school) and their parents should be provided with training on the seasonal flu, ways of protection, and the effectiveness and significance of flu shots.
2. Schools, parents, and healthcare institutions should collaborate and launch media campaigns to increase vaccination rates.
3. Schools and healthcare institutions should identify high-risk groups (e.g., children with chronic diseases) and inform their parents about the complications of the flu and the effectiveness of flu shots.
4. The Ministry of Health should design initiatives and projects to increase flu vaccination rates.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

The Antibacterial and Antibiofilm Activities of Resveratrol on Gram-positive and Gram-negative Bacteria

Resveratrol'ün Gram-pozitif ve Gram-negatif Bakteriler Üzerindeki Antibakteriyel ve Antibiyofilm Aktiviteleri

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ABSTRACT

Aim: *Resveratrol (3,5,4'-trihydroxystilbene) shows antimicrobial activity against many pathogens. It has been detected that subinhibitory concentrations can reduce bacterial motility and interference with quorum sensing, lead to reduced bacterial toxin production, and inhibit biofilm formation. In this study, we aimed to investigate resveratrol's antibacterial and antibiofilm activities on some Gram-positive and Gram-negative bacteria.*

Material and Method: *The bacterial strains, including Staphylococcus aureus (ATCC-29213), Bacillus subtilis (ATCC-6051), Escherichia coli (ATCC-25923), and Pseudomonas aeruginosa (ATCC-27853) were grown overnight in LB broth at 37°C in a humidified chamber. The resveratrol was dissolved in 1.5% of dimethyl sulfoxide (DMSO). Serial two-fold dilutions of the resveratrol, ranging from 16 to 0.5 mg/ml, were prepared in a 96-well plate. The microdilution method determined the minimum inhibitory concentration (MIC) for the resveratrol. Bacterial biofilm formation was assessed using the crystal violet assay. The agar gel diffusion assay was also performed to determine the antimicrobial activity.*

Results: *In these assays, the resveratrol inhibited the growth of both Gram-positive and Gram-negative bacteria strains tested, with inhibition zone diameters ranging from 19.8 to 22 mm and MIC values of 4 mg/ml, confirming its antimicrobial properties. Concerning the effect of resveratrol on biofilm formation, an inhibition ranging from 24% to 99% on the total biofilm mass was achieved for all bacteria strains (Fig. 2 and Fig. 3). 16 mg/ml of resveratrol is the most effective dose for antibiofilm activity.*

Conclusion: *Resveratrol has gained significant scientific and public attention not only for being a possible natural antimicrobial but also for its potential functional and therapeutic applications. Further studies should be planned to understand the molecular mechanism underlying resveratrol's inhibitory effect, investigate the synergistic effects of resveratrol with antibiotics, and apply it in clinical practice.*

Key words: *antibacterial activity; antibiofilm activity; minimal inhibitory concentration; resveratrol; gel diffusion*

ÖZET

Amaç: *Resveratrol (3,5,4'-trihidroksistilben), birçok patojenlere karşı antimikrobiyal aktivite gösterir. İnhibe edici konsantrasyonlarda, bakteri hareketliliğini ve haberleşme ağını azaltabileceği, bakteri toksin üretiminin azalmasına yol açabileceği ve biyofilm oluşumunu engelleyebileceği saptanmıştır. Bu çalışmada, resveratrol'ün bazı Gram-pozitif ve Gram-negatif bakteriler üzerine antibakteriyel ve antibiyofilm aktivitelerini araştırmayı amaçladık.*

Materyal ve Metot: *Staphylococcus aureus (ATCC-29213), Bacillus subtilis (ATCC-6051), Escherichia coli (ATCC-25923) ve Pseudomonas aeruginosa (ATCC-27853) bakteri suşları, 37°C'de LB sıvı besiyerinde bir gece inkübasyon sonrası üretildi. Resveratrol, %1,5 dimetil sülfoksit (DMSO) içinde çözüldü. Doksan altı kuyucuklu bir plakada 16 ila 0,5 mg/ml arasında değişen resveratrol dozları ile seri dilüsyonlar hazırlandı. Resveratrol için minimum inhibitör konsantrasyonu (MİK), mikrodilüsyon yöntemiyle belirlendi. Bakteri suşlarının biyofilm oluşturma yetenekleri, kristal viyole testi ile değerlendirildi. Bunlara ilave olarak, antimikrobiyal aktiviteyi belirlemek için agar jel difüzyon testi yapıldı.*

Bulgular: *Bu testlerde resveratrol, 19,8 ila 22 mm arasında değişen inhibisyon çapları ve 4 mg/ml MİK değerleri ile hem Gram-pozitif hem de Gram-negatif bakteri suşlarının üremesini inhibe ederek antimikrobiyal özelliklerini ortaya koymuştur. Resveratrol'ün biyofilm oluşumu üzerindeki etkisi ile ilgili olarak, tüm bakteri suşları için toplam biyofilm kütlesi üzerinde %24 ila %99 arasında değişen bir inhibisyon elde edilmiştir. On altı mg/ml konsantreli resveratrol uygulaması, antibiyofilm aktivitesi için en etkili dozdur.*

Sonuç: *Resveratrol, yalnızca doğal bir antimikrobiyal olduğu için değil, aynı zamanda fonksiyonel ve terapötik uygulamalar için potansiyel bir ajan olduğu içinde bilimsel alanda dikkat çekmiştir. Resveratrolün inhibitör etkisinin altında yatan moleküler mekanizmayı anlamak, antibiyotiklerle sinerjistik etkilerini araştırmak ve klinik pratikte uygulamak için yeni çalışmalar planlanmalıdır.*

Anahtar kelimeler: *antibakteriyel aktivite; antibiyofilm aktivite; jel difüzyon; minimal inhibitör konsantrasyon; resveratrol*

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Introduction

In order to prevent and control bacterial infections, broad-spectrum antibiotics and chemical bactericides are used to temporarily counter bacterial colonization and remove their biofilms[1–3]. However, many of the currently available oral antibiotics have side effects such as nausea, diarrhea, and vomiting[4]. At that point, the discovery and use of natural compounds that can be effective for bacteria activities and biofilm formations are inevitable.

Resveratrol is present in numerous plants such as peanuts (*Arachis hypogea*), blueberries and cranberries (*Vaccinium* spp.), Japanese knotweed (*Polygonum cuspidatum*) a traditional Asian herbal medicine, and most importantly as a natural source for human consumption in grape wines (*Vitis vinifera*)[5]. Resveratrol, a natural phytochemical found at high levels in especially red wine and grapes, has been extensively studied for a variety of different kinds of health-beneficial effects, including anti-aging, antioxidant, anti-inflammation, anti-carcinogenesis, anti-proliferative, cardiovascular protection and apoptotic effects. It has also an antimicrobial activity against a wide range of bacterial pathogens. It has been studied that even at subinhibitory concentrations, it can reduce bacterial motility and interference with quorum sensing, lead to reduced bacterial toxin production, and inhibit biofilm formation[6–12].

Current literature supported that the resveratrol has an antibacterial activity by effecting different mechanisms such as inactivating the efflux pump systems[13], binding reversibly to the ATP synthase, partially inhibiting both ATP hydrolysis and ATP synthesis functions of the ATP synthase[14], inhibiting biofilm formation of bacteria[15].

In this study, our aim was to investigate the possible anti-bacterial and anti-biofilm activities of resveratrol on Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

Materials and Methods

Preparation of Resveratrol and Bacterial Strains:

The bacterial strains used in this study were *S. aureus* (ATCC-29213), *B. subtilis* (ATCC-6051), *E. coli* (ATCC-25923), and *P. aeruginosa* (ATCC-27853). All bacteria strains were growth overnight in Luria-Bertani (LB) broth at 37°C and 100 µl of bacterial cells with a concentration of 10^8 cfu/ml were added into 96-well plates. The resveratrol was commercially purchased (CAS Number: 501–36–0, Sigma Aldrich, Taufkirchen, Germany). The resveratrol was prepared as a stock solution of 16 mg/ml and then was sub-diluted as following concentrations (16, 8, 4, 2, 1 and 0.5 mg/ml).

Agar Gel Diffusion Test:

Mueller-Hinton Agar (MHA) agar plate surface is inoculated by spreading 100 µl of the bacterial inoculum over the entire agar surface and let to dry 10 min at room temperature. Wells were drilled out on seeded MHA agar plates (6 mm in diameter) with a sterile cork borer. Then, 16 mg/ml stock and sub-dilutions of the resveratrol was introduced into the drilled well. Then, MHA agar plates were left to incubate at 37°C for 24 hours in a flat position. At the end of the incubation the bacterial inhibition zone diameters were measured[16].

Crystal Violet (CV) Test:

A hundred µl Mueller-Hinton Broth (MHB) and 100 µl of bacterial cells suspension (10^8 cfu/ml) were added to polystyrene 96-well plates and left to static incubation at 37°C for 48 hours. At the end of the period, all the wells were washed 3 times with Phosphate Buffered Saline (PBS). 0.5% CV dye was applied to wells for 20 minutes for staining attached cells. The residual dye was cleaned with tap water. After fixation with 30% acetic acid, measurement was taken at 590 nm by Multiskan™ GO UV/Vis microplate spectrophotometer (Thermo Scientific, Schwerte, Germany)[17].

Determination of Minimum Inhibitory Concentration (MIC):

We performed the MIC assay to determine the lowest concentration of an resveratrol that prevents visible growth of a microorganism. For this reason, "European Antimicrobial Susceptibility Test" (EUCAST)[18] protocols have been applied for determining the MIC values. Briefly, 100 µl of bacterial cells with a concentration of 10^8 cfu/ml and resveratrol with the specific concentrations (0.5; 1; 2; 4; 8; 16 mg/ml) were added into 96-well plates. After 24 hours of incubation at 37°C, 100 µl of samples were taken from the wells and inoculated on MHA agar plate by the spread

plate method. After 24 hours of incubation of these plates at 37°C, the MIC is the lowest concentration of antimicrobial agent that completely inhibits colony formation.

Determination of Minimum Biofilm Inhibitory Concentration (MBIC):

Inhibitory effects on biofilm formation are commonly assessed by the MBIC, which is the lowest concentration of an antimicrobial substance at which there is no time-dependent increase in the mean number of biofilm viable cells. For this reason, MBIC value was determined by CV test. Briefly, 100 µl of the bacterial cell (10^8 cfu/ml) concentration was added to each well of the 96-well plate. Then, resveratrol at specific concentrations (0.5; 1; 2; 4; 8; 16 mg/ml) was added. Total volume was completed to 150 µl with MHB medium. The plate was left to incubate statically at 37°C for 48 hours. At the end of the period, all contents in the wells were discarded and washed 3 times with PBS. 200 µl of 0.5% CV dye was added to wells and incubated in the dark condition for 20 minutes. At the end of the incubation, the wells were washed and fixed with 30% acetic acid. Measurement was taken at 590 nm via a spectrophotometer[19].

Results

After the Agar Gel Diffusion test, it was seen that the resveratrol inhibited the growth of both Gram-positive and Gram-negative bacteria stains which have been tested in our study with inhibition diameters ranging from 19.8 to 22 mm (Fig. 1).

On the other hand, antibiofilm activity of resveratrol were determined in a dose dependent manner on both Gram-positive and Gram-negative bacteria stains which were tested in our study. As seen in Fig. 2, an inhibition ranging from 61% to 99% on the total biofilm mass was achieved for *B. subtilis* and *S. aureus* strains. 16 mg/ml resveratrol application was the most effective dose for preventing the biofilm formation in tested Gram-positive bacteria.

Additionally, an inhibition ranging from 24% to 99% on the total biofilm mass was achieved for *E. coli* and *P. aeruginosa* strains. 16 mg/ml of the resveratrol was also the most effective dose for preventing the biofilm formation in tested Gram-negative bacteria. The MIC values of all groups were detected as 4 mg/ml dose of resveratrol (Fig. 3).

Discussion

In this study, we evaluated the possible antibacterial and antibiofilm activities of resveratrol on some Gram-positive and Gram-negative bacteria such as *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa*. For this reason, we used a catalogued resveratrol and standard bacterial strains obtained from ATCC.

After the agar diffusion test and microdilution assay, it was detected that the resveratrol inhibited the growth

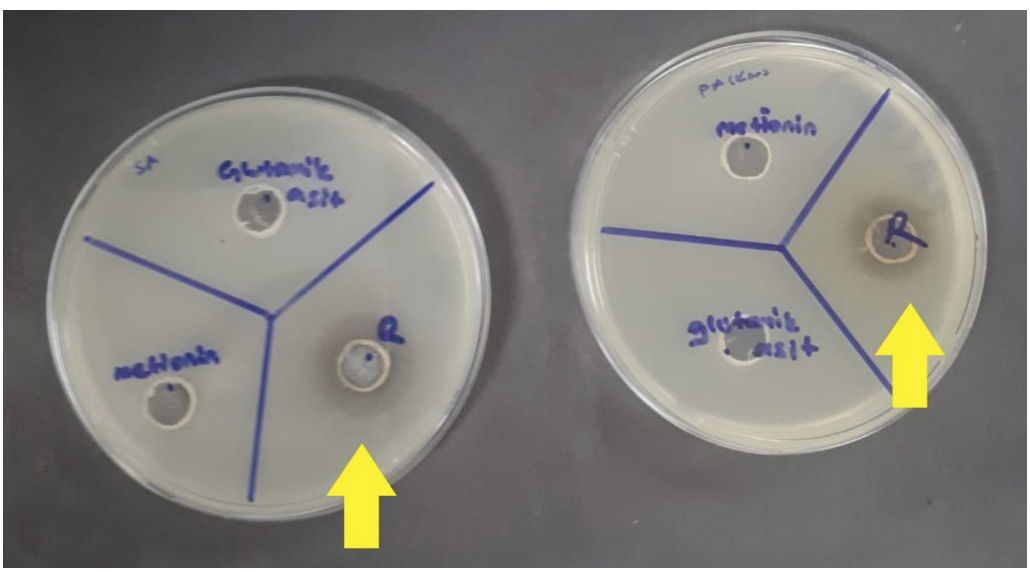

Figure 1. *Agar gel diffusion assay results for resveratrol. The markings (R) show the resveratrol gel diffusion test on agar plate.*

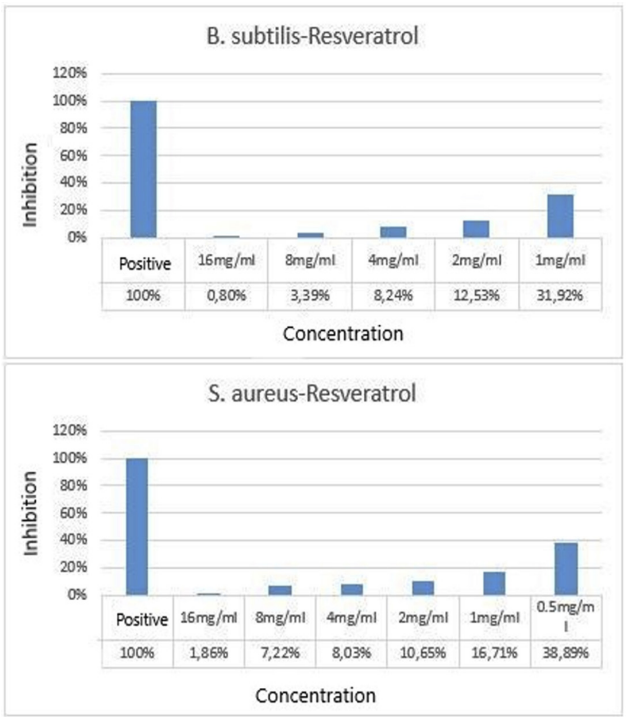

Figure 2. *The antibiofilm effect of resveratrol on gram positive bacteria.*

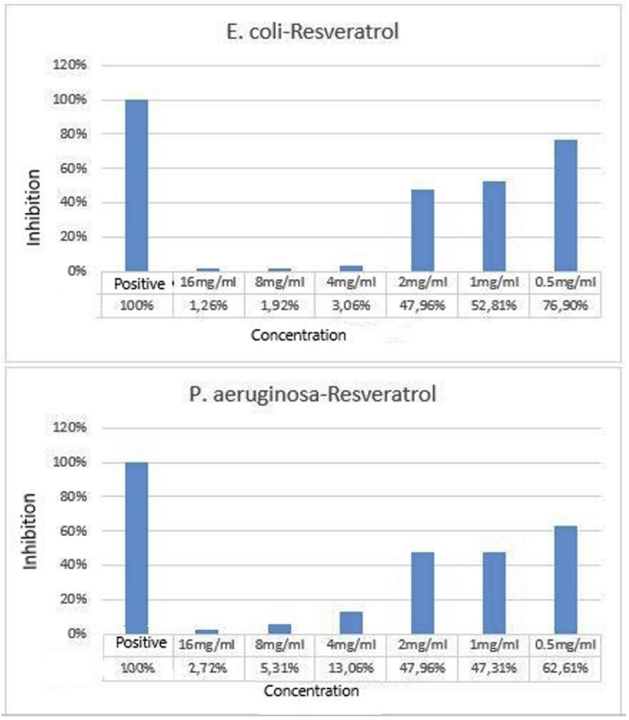

Figure 3. *The antibiofilm effect of resveratrol on gram negative bacteria.*

of both Gram-positive and Gram-negative bacteria strains, with inhibition diameters ranging from 19.8 to 22 mm and MIC values of 16 mg/ml, confirming its antimicrobial properties. Concerning the effect of the resveratrol on biofilm formation, an inhibition ranging from 24% to 99% on the total biofilm mass was achieved for all bacteria strains. The 16 mg/ml application of resveratrol is the most effective dose for antibiofilm activity.

Several studies in current literature[11,20] have demonstrated that the resveratrol has antibacterial, antifungal, antiviral, and antibiofilm effects. These studies have also investigated the therapeutic application of the resveratrol against infectious diseases. The resveratrol inhibits growth of most of pathogens in different concentrations such as *Bacillus cereus* (MIC=50 µg/mL), *Mycobacterium smegmatis* (MIC=64 µg/mL), *Helicobacter pylori* (MIC=25 µg/mL), *Arcobacter cryaerophilus* (MIC=50 µg/mL), *Campylobacter coli* (MIC=64 µg/mL), *Vibrio cholera* (MIC=60 µg/mL), *Neisseria gonorrhoeae* (MIC=75 µg/mL), *Mycobacterium tuberculosis* (MIC=100 µg/mL), *Staphylococcus aureus* (MIC=10 µg/mL), *Enterococcus faecalis* (MIC=20 µg/mL), *Escherichia coli* (MIC >200 µg/mL), *Klebsiella pneumoniae* (MIC >200 µg/mL), and *Pseudomonas aeruginosa* (MIC >200 µg/mL)[21–28]. Our results were closely parallel with the current literature (MIC=16 µg/mL); however, we understand that we should have needed to perform more concentrations of resveratrol on bacterial strains. Several studies have reported lower susceptibilities for several Gram-negative pathogens compared with Gram-positive bacteria. This may be the result of active extrusion of the resveratrol by efflux pump systems[29].

On the other hand, bacteria can live as planktonic cells or in aggregates attached to surfaces, referred to as biofilms. The formation of bacterial biofilms must, necessarily, begin with the adhesion of a small number of bacterial cells to a surface[30]. There are advantages of biofilm formation such as providing phagocytosis and antimicrobial agents and biofilm formation is crucial for bacteria and clinically important in chronic and recurrent infections[31].

The resveratrol has been studied on various bacterial pathogens for its ability to reduce biofilm formation. In our study, 16 µg/mL resveratrol application inhibited the biofilm formation ranging from 24% to 99% on the total biofilm mass was achieved for *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa*. A study reported that resveratrol inhibits biofilm formation at concentrations

of 4–6-fold below the MIC value on some Gram-negative anaerobic bacteria[32]. Furthermore, the resveratrol demonstrated its antibiofilm properties against Gram-negative bacteria including *E. coli* at concentrations of 2–6-fold below the MIC value [24] and Gram-positive bacteria including *Propionibacterium acnes* and *S. aureus* at concentrations of 3–4-fold below the MIC value[33,34]. Same studies claimed that the resveratrol inhibited the biofilm formation in especially *E. coli* by reducing expression of csgA and csgB genes[35].

The resveratrol has gained significant scientific and public attention not only for being a possible natural antimicrobial but also for its potential functional and therapeutic applications. Further studies should be planned to understand the molecular mechanism underlying the inhibitory effect of resveratrol, to investigate the synergistic effects of resveratrol with antibiotics, and to apply in clinical practice.

Our study has some limitations. First, the concentrations of resveratrol should have been in a wider range. Second, more microorganisms should have been tested in this study.

Conflict of interest

The authors have no conflict of interest.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Determining the Relationship Between Coronavirus Anxiety and Attitudes Towards the COVID-19 Vaccination in Students from Vocational School of Health Services

Sağlık Hizmetleri Meslek Yüksekokulu Öğrencilerinin Koronavirüs Anksiyetesi ve Covid-19 Aşısına Yönelik Tutumları Arasındaki İlişkinin Belirlenmesi

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ABSTRACT

Aim: *This research was aimed to determine the relationship between coronavirus anxiety and attitudes toward the COVID-19 vaccination in students from the vocational school of health services.*

Material and Method: *This cross-sectional study was completed with 700 students attending the Vocational School of Health Services at a university in eastern Turkey in the 2020–2021 academic year. The data were collected through an online survey via the Social media platform (Whatsapp) between 15–31 January 2021. The data were collected Student Information Form, Coronavirus Anxiety Scale, and Attitude towards the COVID-19 Vaccine. The data were evaluated using the SPSS 22.0 program with standard deviation, mean, percentage, number, t-test in independent groups, one-way analysis of variance, and Spearman rho correlation analysis. The statistical significance level was taken as p<0.05.*

Results: *Attitudes Towards the COVID-19 Vaccine Scale positive attitude subscale mean score of the students was moderate (11.07±4.03), and their Attitudes Towards the COVID-19 Vaccine Scale negative attitude subscale mean score (15.37±3.64) was moderate. The Coronavirus Anxiety Scale mean score (6.81±3.42) was low. It was found that the students who suffered from a chronic disease, had family members with a chronic disease, had family members infected with COVID-19, and lost a family member due to COVID-19 had higher Coronavirus Anxiety Scale mean scores compared to the other students and this was statistically significant.*

Conclusion: *These results highlight that training should be planned to increase students' positive attitudes toward the COVID-19 vaccine.*

Key words: *anxiety; Covid-19 vaccine; student*

ÖZET

Amaç: *Bu araştırma COVID-19 pandemisi sürecinde sağlık hizmetleri meslek yüksekokulu öğrencilerinin Koronavirüs anksiyetesi ve COVID-19 aşısına yönelik tutumları arasındaki ilişkinin belirlenmesi amacıyla yapıldı.*

Materyal ve Metot: *Kesitsel türde yapılan bu araştırma Türkiye'nin doğusunda bulunan bir üniversitenin Sağlık Hizmetleri Meslek Yüksekokulu'nda 2020–2021 eğitim-öğretim yılında derslere devam eden 700 öğrenci ile tamamlandı. Veriler 15–31 Ocak 2021 tarihleri arasında sosyal medya platformu (Whatsapp) aracılığıyla çevrimiçi anket yoluyla, Öğrenci Tanıtım Formu, Koronavirüs Anksiyete Ölçeği ve COVID-19 Aşısına Yönelik Tutumlar Ölçeği kullanılarak toplanmıştır. Verilerin değerlendirilmesinde SPSS 22.0 programında standart sapma, ortalama, yüzde, sayı, bağımsız gruplarda t-testi, tek yönlü varyans analizi ve Spearman rho korelasyon analizi kullanıldı.*

Bulgular: *Öğrencilerin COVID-19 Aşısına Yönelik Tutumlar Ölçeği Olumlu Tutum alt boyut puan ortalaması orta seviye (11,07±4,03), Olumsuz Tutum alt boyut puan ortalaması orta seviye (15,37±3,64) ve Koronavirüs Anksiyete Ölçeği puan ortalaması düşük seviye, (6,81±3,42) olarak belirlendi. Kronik hastalığa sahip, aile bireylerinde kronik hastalık bulunan, aile bireyleri COVID-19 enfeksiyonu geçiren ve COVID-19 enfeksiyonu nedeniyle aile bireyi vefat eden öğrencilerin, Koronavirüs Anksiyete Ölçeği puan ortalamaları diğer öğrencilere göre daha yüksek ve anlamlı bulundu.*

Sonuç: *Araştırma sonuçları öğrencilerin COVID-19 aşısına yönelik olumlu tutumlarını artırmak amacıyla eğitimlerin planlanması gerektiğini vurgulamaktadır.*

Anahtar kelimeler: *anksiyete; Covid-19 aşısı; öğrenci*

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Introduction

The restriction measures taken for the COVID-19 pandemic have negatively affected all areas and have started a difficult process especially in the education system[1]. In Turkey students started to receive online education as of 26 March 2020 but this has caused university students to suffer from social isolation, fail to receive hands-on training for professional competence,[2] and experience inequality of opportunity in technological dimension[3]. University students currently studying in the field of health have a high level of anxiety due to some reasons such as the pressure of academic achievement, the difficulties of the clinical environment, and working with patients with chronic and terminal diseases[4]. Unexpected sudden changes in the education system with the COVID-19 pandemic and health threats associated with infection have led students to feel increasingly anxiety / fear and concern[5–7]. Anxiety can have a negative effect on students' quality of life, academic achievement, and family and social relations and can lead them to experience serious problems such as deterioration of their mental health and school dropout[8,9].

Increased level of fear/anxiety individuals feel due to uncertainty during epidemics also influences their thoughts and tendencies about treatment methods and basic preventive practices such as vaccines[10,11]. Therapeutic approaches are significant; however, the most important factor in preventing the spread of infection is vaccination. The key to the vaccination is the positive attitude of the society towards the vaccine[12]. In 2019, the World Health Organization[13] reported that vaccine refusal is one of ten global problems and similarly, rate of vaccine refusal has increased in Turkey in recent years. The positive expectations of the societies for the development of vaccines in the fight against coronavirus at the beginning of the pandemic have been replaced by the problems of doubt, hesitation and vaccine rejection regarding the COVID-19 vaccine today[14].

COVID-19 vaccine hesitancy has been commonly encountered in numerous countries[11]. In a study conducted with 3936 people regarding attitudes towards the COVID-19 vaccine in Turkey, it was determined that 31% of the participants were hesitant about the COVID-19 vaccine and 3% refused to be vaccinated[15]. In their study, Köse et al.[16], determined that 68.6% of the healthcare professionals (n=1138) requested to be vaccinated. In a study conducted by the American Nurses Foundation (ANF) with 13,000 nurses, approximately one-third (36%) of the nurses stated that they did not want to be volunteers to get vaccinated against COVID-19 and approximately one-third (31%) expressed that they were undecided about getting vaccinated. In addition, in the same study, 65% of the nurses stated that they provided care for confirmed or suspected COVID-19 cases[17].

It is important to evaluate the views and expectations of all healthcare professionals and the society about vaccination in order to achieve COVID-19 vaccination programs[18]. Healthcare professionals are the most effective and reliable consultants for people to exhibit vaccine acceptance and a positive attitude towards vaccination[19]. Students studying in the field of health are expected to take part in the COVID-19 pandemic or other epidemics in the near future and consult the society about vaccination. Given the guidance offered by students in the field of health protection and promotion, it is of primary importance to determine their anxieties about the COVID-19 pandemic and their attitudes towards the COVID-19 vaccine.

The aim of this study, which was conducted at the beginning of the COVID-19 vaccination in Turkey, was to determine the relationship between the anxieties of the students, who represent an important group in the field of health, about the COVID-19 infection and their attitudes towards the COVID-19 vaccine.

Research Questions

- How is the Coronavirus Anxiety Scale (CAS) score of students from Vocational School of Health Services (VSHS) during the pandemic?
- How is the Attitudes Towards the COVID-19 Vaccine (ATV-COVID-19) Scale score of VSHS students during the pandemic?
- Is there any correlation between the CAS score and ATV-COVID-19 Scale score and some sociodemographic characteristics of VSHS students?
- Is there any correlation between the Coronavirus Anxiety score and the ATV-COVID-19 score of VSHS students?

Material and Method

Type of the study: The study is cross-sectional.

Period and setting of the study: The study was conducted in a vocational school of health services between 15 and 31 January 2021.

Population and sample: The population of the study consisted of a total of 1730 first– and second-year students studying in the departments of Anaesthesia, First and Emergency Aid, Medical Imaging Techniques, Medical Laboratory Techniques, Home Patient Care, Elderly Care, Medical Documentation and Secretarial, and Medical Promotion and Marketing in the Vocational School of Health Services. No sample selection was applied in the study and the sample consisted of 700 (response rate=40%) students who actively joined in classes in the 2020–2021 academic year, agreed to participate in the study, and filled out the measurement tools completely. Students who didn't actively participate in the classes in the 2020–2021 academic year, didn't accept to participate in the research and filled the measurement tools incompletely were excluded from the study.

Data collection tools and method: Data collection tools include three parts; student information form, Coronavirus Anxiety Scale (CAS) and Attitudes Towards the COVID-19 Vaccine (ATV-COVID-19) Scale.

Student information form: This form was prepared by the researcher upon the literature review[20,21]. The form includes the questions about socio-demographic characteristics of the students such as gender, age, department, marital status, education level, income status, status of suffering from a chronic disease and having a parent with a chronic disease as well as questions related to COVID-19 such as being diagnosed with COVID-19 and losing a relative due to COVID-19.

Coronavirus Anxiety Scale (CAS): The Coronavirus Anxiety Scale (CAS) was developed by Lee in 2020 to identify possible dysfunctional anxiety cases associated with the COVID-19 crisis and its Turkish validity and reliability study was conducted by Biçer et al.[20]. This 5-point Likert scale consists of 5 questions and a single dimension. The scale is scored as "0": "never", "1": "Rarely, less than one or two days", "2": "A few days", "3": "more than seven days", and "4": "almost every day in the last two weeks". The Cronbach's Alpha reliability coefficient was calculated as 0.832. In this study, Cronbach's Alpha coefficient was determined as 0.875.

Attitudes Towards the COVID-19 Vaccine (ATV-COVID-19) Scale: The scale was developed by Geniş et al.[21], and consists of 9 items and two subscales (positive and negative attitude). The items are rated as "Strongly disagree (1)", "Disagree (2)", "Undecided (3)", "Agree (4)", and "Strongly agree (5)". The items in the negative attitude subscale (items 5, 6, 7, 8 and 9) are scored reversely. High scores obtained from the positive attitude subscale (items 1, 2, 3, and 4) indicate that the attitude towards the vaccine is positive. In the negative attitude subscale, high scores signify that negative attitudes towards the vaccine are less. Reverse items are coded as 1 → 5; 2 → 4; 3 → 3; 4 → 2; and 5 → 1.

Data collection method: The survey link created via Google Form was sent to the students via the social media platform (Whatsapp) to collect the data and they were asked to answer it in the electronic environment.

Data assessment: SPSS 22.0 packaged software was used for the statistical analysis of the data and descriptive statistics (mean, standard deviation, number and percentage) were calculated. Parametric test assumptions were checked before data analysis. Independent samples t-test or one-way analysis of variance was used to compare the groups. The correlation between numerical variables was determined using the Spearman rho correlation coefficient. The value of $p<0.05$ was taken as statistical significance level.

Ethical considerations: The study was approved by the Republic of Turkey Ministry of Health Scientific Research Platform (2020-12-30T00_28_45) and the Non-Invasive Research Ethics Committee of the Faculty of Health Sciences of Kafkas University (05.01.2021/136). In order to carry out the study, permission to use scales was taken from their authors and institutional permission was obtained. Before completing the online survey, voluntary participants were asked to click the checkbox stating that they agree to participate. They were informed that the survey did not contain any personal information and the answers would be collected anonymously in order to prevent any coercion.

Results

The average age of the participants was 20.43±1.85 (18–35) years. Table 1 shows the distribution of some socio-demographic characteristics of the students and ATV-COVID19 and CAS mean scores. It was determined that anxiety scores and scores of positive

attitude towards the vaccine were higher and statistically significant in female students than male students (p=0.001).

When examining mean scores of the ATV-COVID-19 positive attitude subscale, these mean scores were higher in those residing in the Eastern Anatolia region than those residing in the Mediterranean region and this was statistically significant (p=0.002; Table 1). Scores of positive attitude towards the vaccine were lower and in students who were infected with COVID-19 than those who were not (p=0.003) and in students with low level of income than those with the other income levels (p<0.001) and this was statistically significant (Table 1).

The ATV-COVID-19 negative attitude subscale mean scores were lower in students residing in the Black Sea region compared to those residing in the Aegean region; in students with low level of income than those with the other income level; and in students who were infected with COVID-19 than those who were not and this was statistically significant (p<0.001). The ATV-COVID-19 negative attitude subscale mean scores were higher in those residing in the village than those residing in the province and this was statistically significant (p=0.001; Table 1).

When the anxiety scores of the students were examined, it was found that the students who suffered from a chronic disease, had family members with a chronic disease and lost a family member due to COVID-19 had higher CAS mean scores compared to the other students and this was statistically significant (p<0.001). Additionally, it was found that the students who infected with COVID-19 (p=0.008) and had family members infected with COVID-19 (p=0.001) had higher CAS mean scores compared to the other students and this was statistically significant (Table 1).

Table 2 shows ATV-COVID-19 Scale subscale and CAS total mean scores. ATV-COVID-19 positive attitude subscale mean score of the participants was moderate (11.07±4.03), and their ATV-COVID-19 negative attitude subscale mean score (15.37±3.64) was moderate. The CAS mean score (6.81±3.42) was low.

Table 3 shows the Spearman correlation between ATV-COVID-19 subscales scores and CAS total mean scores. There was no correlation between the CAS mean score and the ATV-COVID-19 positive attitude subscale (p=0.128) and ATV-COVID-19 negative attitude subscale (p=0.740) mean scores.

Discussion

The present study is believed to be important since such study has not been conducted with these students and this study examines the correlation between the coronavirus anxiety score and the score of attitude towards the COVID-19 vaccine in the VSHS students.

In the study, the students' CAS mean score was found to be low. However, studies conducted on students have revealed that restrictive measures for COVID-19 infection have led them to experience high levels of anxiety[22–25]. Low anxiety score found in the present study is thought to be associated with the fact that the students have been adapted to the online education process of about 10 months, the home quarantine process was over, they have regained the opportunity to meet with their peers outside, and hospital processes are carried out online.

In the study, it was determined that the positive attitude scores of the students towards the COVID-19 vaccine were moderate. In the literature, there are numerous studies indicating that the positive attitude towards the vaccine in the field of health is moderate[7,26,27]. In one study, it was reported that only 45% of students studying in health-related departments wanted to be vaccinated with the COVID-19 vaccine and the most important reason for their drawbacks about the vaccination was vaccine safety and its side effects[26]. In a study conducted with medical students from the USA, about three-quarters (75%) of the students stated that they wanted to be vaccinated and one-fourth (25%) were hesitant about the vaccine[27,]

When the findings were evaluated in terms of demographic variables, it was found that the anxiety scores of female students were higher than those of male students and this was statistically significant (p=0, 001). Likewise, the studies conducted with students on coronavirus anxiety have revealed that the anxiety scores of female students were higher compared to their male counterparts[22,23,28]. In the study conducted by Özdin and Özdin[28] in Turkish society, they reported that the anxiety scores of women were higher than the anxiety scores of men in a statistically significant manner during the COVID-19. It is known that women generally have a higher level of anxiety than men due to their gender characteristics, and cultural situations, social expectations and experiences play a role in increasing this anxiety[29]. The fact that the anxiety scores were higher in female gender than males in the present study was thought to be associated with academic

Table 1. *Some socio-demographic characteristics of the students and ATV-COVID-19 and CAS mean scores*

Variables		n	%	ATV-COVID-19 subscales: Positive attitude Mean ± SD	ATV-COVID-19 subscales: Negative attitude Mean ± SD	CAS total score Mean ± SD
Gender	Female	491	70.1	11.38±3.83	15.41±3.45	7.01±3.60
	Male	209	29.9	10.33±4.38	15.26±4.07	6.33±2.90
				p=0.001	*p=0.277*	***p=0.001***
Marital status	Single	685	97.9	11.07±4.02	15.35±3.65	6.83±3.44
	Married	15	2.1	12.00±4.74	16.00±3.21	5.00±1.53
				p=0.730	*p=0.628*	*p=0.111*
Department	Anaesthesia	70	10.0	11.47±3.20	15.20±3.36	6.90±3.72
	First and Emergency Aid	153	21.9	11.23±3.87	15.22±3.48	6.50±3.25
	Home Patient Care	95	13.6	10.53±4.04	14.75±3.64	6.98±3.21
	Medical Imaging Techniques	128	18.3	11.32±4.40	15.78±3.61	6.78±3.12
	Medical Laboratory Techniques.	56	8.0	11.17±3.53	14.83±2.49	7.25±4.05
	Medical Documentation and Secretarial	100	14.3	10.60±4.08	15.01±3.88	6.64±2.96
	Medical Promotion and Marketing	27	3.9	11.07±4.72	16.96±3.36	6.03±1.65
	Elderly Care	71	10.1	11.15±4.45	16.22±4.50	7.38±4.62
				p=0.790	*p=0.089*	*p=0.487*
Place of residence	Cities	325	46.4	10.92±3.95	15.19±3.50	6.84±3.24
	District	214	30.6	11.00±4.05	15.04±3.86	6.57±3.10
	Village	161	23.0	11.45±4.15	16.16±3.52	7.06±4.10
				p=0.380	***p=0.001***	*p=0.275*
Geographical region they resided in	The Eastern Anatolia	348	49.7	11.65±4.09	15.85±3.53	6.76±2.99
	The South Eastern Anatolia	197	28.1	10.47±3.76	15.10±3.63	6.90±3.66
	The Central Anatolia	27	3.9	11.22±4.62	15.22±4.43	8.14±6.21
	The Black Sea	27	3.9	10.55±3.60	14.18±3.22	5.66±1.54
	The Marmara	28	4.0	9.46±4.76	13.64±5.05	6.71±4.22
	Mediterranean	56	8.0	9.98±3.70	14.35±3.02	6.94±3.65
	The Aegean	17	2.4	12.82±2.72	16.88±2.17	6.00±2.09
				p=0.002	***p<0.001***	*p=0.203*
Employment status	Unemplayment	606	86.6	11.15±4.02	15.48±3.49	6.74±3.38
	Working in healthcare field	21	3.0	10.61±3.99	14.00±4.30	7.00±4.42
	Working outside in healtcare field	73	10.4	10.54±4.12	14.83±4.50	7.31±3.42
				p=0.419	*p=0.215*	*p=0.076*
Income status	High	63	9.0	11.60±4.32	15.85±4.47	6.55±3.24
	Middle	511	73.0	11.32±3.92	15.56±3.41	6.57±3.02
	Low	109	15.6	10.21±3.98	14.73±3.85	7.89±4.67
	Very low	17	2.4	7.00±3.93	11.82±3.50	7.88±4.44
				p<0.001	***p<0.001***	*p=0.079*
Infected with COVID-19	Yes	112	16.0	9.56±4.23	13.87±3.88	8.59±5.64
	No	588	84.0	11.22±3.98	15.52±3.58	6.62±3.05
				p=0.003	***p<0.001***	***p=0.008***
Chronic disease	Yes	66	9.4	11.09±3.97	15.17±3.66	7.38±4.07
	No	634	90.6	11.04±4.11	15.61±3.61	6.09±2.17
				p=0.761	*p=0.169*	***p<0.001***
Having a parent with a chronic disease	Yes	389	55.6	11.09±3.97	15.17±3.66	7.38±4.07
	No	311	44.4	11.04±4.11	15.61±3.61	6.09±2.17
				p=0.761	*p=0.169*	***p<0.001***
Had family members infected with COVID-19	Yes	246	35.1	11.32±4.03	15.35±3.70	7.26±3.80
	No	454	64.9	10.93±4.03	15.37±3.61	6.56±3.17
				p=0.16	*p=0.347*	***p=0.001***
Losing a relative due to COVID-19.	Yes	61	8.7	10.91±4.03	14.81±3.84	8.95±5.49
	No	639	91.3	11.08±4.03	15.42±3.62	6.60±3.08
				p=0.647	*p=0.061*	***p<0.001***

ATV-COVID-19:Attitudes Towards the COVID-19 Vaccine; CAS: Coranavirus Anxiety Scale

Table 2. *ATV-COVID-19 Scale subscales and CAS total mean scores*

	N	Ortalama ± SS	Min-Max
ATV-COVID-19 positive attitude	700	11.07±4.03	4.00–20.00
ATV-COVID-19 negative attitude	700	15.37±3.64	5.00–25.00
Coronavirus anxiety scale	700	6.81±3.42	5.00–25.00

ATV-COVID-19:Attitudes Towards the COVID-19 Vaccine; CAS: Coranavirus Anxiety Scale

Table 3. *The Spearman correlation between ATV-COVID-19 subscales scores and CAS total mean scores*

Spearman's rho	ATV-COVID-19 negative attitude	Coronavirus anxiety scale
Positive attitude correlation coefficient	0.654*	0.058
P value	***<0.001***	0.128
Negative attitude correlation coefficient		-0.013
P value		0.740

p* <0.01; ATV-COVID-19: Attitudes Towards the COVID-19; CAS: Coronavirus Anxiety Scale

achievement as well as obligations such as traditionally doing household chores and helping family members due to staying at home.

In this study, anxiety scores were found to be higher and statistically significant in students who had a chronic disease in themselves and their family members, were infected with COVID-19 or lost a family member due to COVID-19 infection ($p<0.05$). In a study conducted in Jordan during the pandemic, it was determined that university students who suffered from a chronic disease and had family members with the chronic disease had higher levels of anxiety than the other students and this was statistically significant[23]. A study conducted with nursing students reported that the students with family members/relatives who tested positive for the COVID-19 had higher anxiety scores than other students[22]. In another study conducted with medical students actively working in the clinics, the students who were infected with COVID-19 were found to have higher levels of anxiety than those who were not[30]. Chronic diseases are life-threatening and can cause people to experience anxiety and depression especially during the pandemic[31]. The constant mentioning of topics related to the susceptibility to COVID-19 infection in the media and society, especially in those with chronic diseases, is effective in increasing anxiety[32].

When examining the relationship between the attitude towards the COVID-19 vaccine and demographic data of the participants, it was concluded that gender, geographical region they resided in, and income status affected their attitude towards the vaccine ($p<0.05$). In the study, it was determined that the positive attitude towards the vaccine was higher and statistically significant in females than males. However, contrary to the results of the study, there are studies in the literature demonstrating that men are more willing to get vaccinated than women[26,28]. In this study, it was determined that the score of positive attitude towards the vaccine was low in families with low level of income. High-income and educated individuals are more likely to have the COVID-19 vaccine. Unemployment can also reduce the positive attitude towards the vaccine. Low-income families are often literate at low rates[33]. It is considered that the low literacy rate may cause individuals to be more affected by environmental discourses and family members under the same roof to affect each other, resulting in a negative attitude towards the vaccine.

In this study, it was concluded that being infected with COVID-19 reduced the positive attitude towards the vaccine and this was statistically significant ($p<0.05$). This result was due to the insufficient level of knowledge of the students about the COVID-19 infection and the newly applied coronavirus vaccination and it was interpreted as the fact that that the students thought that being infected with COVID-19 can eliminate the need for vaccination.

When the positive attitude subscale was examined, it was determined that those residing in the Black Sea and Eastern Anatolia Regions exhibited positive attitudes towards the vaccine and the regional difference was statistically significant. Considering that the climatic conditions of these regions are harsh, it is thought that individuals living in these regions prefer to be vaccinated more in diseases such as COVID-19 that require medical assistance and care. Although the scores of positive attitude towards the vaccine were high in the Eastern Anatolia Region in this study, the vaccination rate was low according to the vaccination data of the Ministry of Health[34]. It is claimed that the slow progress of vaccination in the region is due to the fact that people who are not illiterate and do not speak Turkish have trouble in hospital appointment system[35].

No correlation was found between the CAS mean score and the ATV-COVID-19 positive and negative attitude subscale mean score in the study. Unlike this study, there are studies in the literature showing that high anxiety is associated with high vaccine acceptance[15,36]. In a study conducted with a total of 5024 people from the United Kingdom (n=1088) and Turkey (n=3936), it was determined that the vaccine acceptance rates of the participants with high COVID-19 anxiety scores were also high[36]. Similarly, in their study, Bandeu et al.[14], concluded that individuals with high anxiety were highly willing to be vaccinated.

In this study, it was determined that the coronavirus anxiety score of the students was low and ther attitude score towards the vaccine was moderate. For this reason, it is thought that it would be beneficial for the educators to identify the students who are hesitant about the vaccine in particular and to improve the education method to increase compliance with the vaccination protocols in order to increase the positive attitude of the students towards the COVID-19 vaccine in the related Vocational School. In addition, it is recommended to conduct comparative studies with students studying in other departments or in different provinces.

Limitations

The limitation of the study are the research is single-centered and the low response rate (40%) to online surveys since the study was conducted during the online education period.

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Conflict of Interest

There is no conflict of interest related to this study.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Analysis of PD-L1 Immunohistochemistry Results for Different Sampling Procedures of Non-small Cell Lung Carcinoma. Can We Use Cell Blocks for Evaluation of PD-L1?

Küçük hücre Dışı Akciğer Karsinomlarında Tümörün Farklı Örnekleme Prosedürlerine Göre PD-L1 Immünohistokimyasal Sonuçlarının Değerlendirilmesi. Hücre Blokları PD-L1 Değerlendirilmesinde Kullanılabilir mi?

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ABSTRACT

Aim: *Current guidelines recommend evaluating the expression of PD-L1 immunohistochemically in most tumors, especially lung cancer. However, it is not easy to evaluate the immunohistochemical staining of PD-L1 because of the tumor heterogeneity. Therefore, we aim to evaluate the different patterns of PD-L1 in the lung's non-small cell carcinoma and the relationship with clinicopathological features. We also wanted to see if cell blocks obtained from cytology materials can be an alternative for PD-L1 immunohistochemistry.*

Material and Method: *We retrospectively analyzed the immunohistochemical patterns of PD-L1 performed between 2018 and 2019. Biopsy/aspiration procedure of tumors, tumor type, patient's age, and gender were reviewed. Positive tumor cells (percentage) were categorized according to density and distribution as negative (<1%), low expression (1–49%), and high expression (>50%).*

Results: *Material adequacy was found to be satisfactory in evaluating PD-L1 in the cases of cell blocks. Positive staining with PD-L1 was detected even with a small number of tumor cells in the cell block. For optimal evaluation of PD-L1 expression in the cell block, the tumor cell ratio of 10% is significant (p=0.002). The cases with negative PD-L1 expression mostly belonged to small biopsy samples (48.3%). However, the difference in PD-L1 expression in histological and cytological samples was insignificant (p=0.79). Besides this, expression of PD-L1 was negative in almost half of the cases (48.3%) diagnosed with squamous cell carcinoma. In adenocarcinoma cases, the PD-L1 expression rate was between 1–49 % in more than half (51.8%) of them. The difference in histological subtype was not significant in PD-L1 staining (p=0.009).*

Conclusion: *In conclusion, we can use cell blocks for immunohistochemical evaluation of PD-L1 expression. Analysis of PD-L1 staining in cytological and histological tissue samples may be a guide for other studies.*

Key words: *PD-L1; tumor; lung carcinoma; cell block*

ÖZET

Amaç: *Güncel kılavuzlar, akciğer kanserleri başta olmak üzere çoğu tümörde PD-L1 ekspresyonunun immünohistokimyasal olarak değerlendirilmesini önermektedir. Ancak, tümör heterojenitesi nedeniyle PD-L1'in değerlendirilmesi kolay değildir. Çalışmamız, akciğerin küçük hücre dışı karsinomlarda PD-L1'in farklı boyanma paternlerini ve bu ekspresyonun klinikopatolojik özelliklerle ilişkisini değerlendirmeyi amaçlamaktadır.*

Materyal ve Metot: *Bu amaçla 2018–2019 yıllarında immünohistokimyasal olarak çalışılan PD-L1'in ekspresyon paternleri retrospektif olarak analiz edildi. Yapılan işlemin prosedürü (biyopsi/aspirasyon), tümör tipi, hastanın yaşı, cinsiyeti gözden geçirildi. PD-L1 pozitif tümör hücreleri, yoğunluk ve dağılım baz alınarak yüzdelerine göre negatif (<%1), düşük ekspresyon (%1–49) ve yüksek ekspresyon (>%50) olarak kategorize edildi.*

Bulgular: *Çalışmamızda, hücre bloğunda PD-L1'in değerlendirmesi için gereken hücresellik tatmin edici bulundu. Hücre bloğunda az sayıda hücre bile olsa PD-L1 ile pozitif boyama elde edilebildi. Hücre bloğunda PD-L1 ekspresyonunun optimal değerlendirilmesi için tümör hücre oranı %10 sınırı anlamlı bulundu (p=0,002). PD-L1 ekspresyonu negatif olan olgular çoğunlukla küçük biyopsi örneklerine (%48,3) aitti. Ancak histolojik ve sitolojik örneklerdeki PD-L1 ekspresyonu farkı anlamlı değildi (p=0,79). Bunun yanında skuamöz hücreli karsinom tanısı alan olguların neredeyse yarısında (%48,3) PD-L1 ekspresyonu negatif olarak saptandı. Adenokarsinom olgularının yarısından fazlasında (%51,8) PD-L1 ekspresyon oranı %1–49 arasındaydı. Histolojik alt tip PD-L1 boyanmasında anlamlı farklılık göstermedi (p=0,009).*

Sonuç: *Analizimize göre, PD-L1 ekspresyonunun immünohistokimyasal olarak değerlendirilmesinde hücre blokları kullanılabilir. Hem sitolojik ve hem de histolojik doku örneklerinde PD-L1 ekspresyonlarının analizi diğer çalışmalar için yol gösterici olabilir.*

Anahtar kelimeler: *PD-L1; tümör; akciğer kanseri; hücre bloğu*

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Introduction

The pathway of PD-1/PD-L1 plays an important role in immune regulation by balancing T cell activation, tolerance and immune-mediated tissue damage. Binding PD-L1 to PD-1 causes the formation of PD-1/ TCR. This formation suppresses T cell activation by increasing apoptosis, inhibing proliferation and decreasing cytokine secretion[1]. The formation of this complex causes depletion of effector T cells and thus tumor cells escape from the immune system. PD-L1 also transmit the anti-apoptotic signal to tumor cells. So tumor cells can be protected from apoptosis. Also according to Shi et al., PD-L1 may act as an oncogenic molecule in colorectal carcinogenesis[2]. Mechanism of PD-1/ PD-L1 binding on the tumor development has been illustrated in Fig. 1.

PD-L1 is not expressed on the surfaces of normal tissues. Therefore, antibodies targeting PD-1 and PDL-1 is novel therapeutic option in cancer treatment. Monoclonal antibodies targeting the PD-L1 or PD-1 receptor prevent the suppressive effects of this pathway on T cells, thereby increasing T cell function. Clinical studies have demonstrated that these antibodies leads to impressive outcomes in many cancer types such as renal cell carcinoma, bladder, melanoma and non-small cell lung cancers[3,4].

Besides oncogenic potential, PD-L1 has different prognostic effects in different malignancies. It is associated with poor prognosis in cancers of stomach, esophagus, pancreas, ovarian, bladder, kidney and liver. Rarely, the high rate of PD-L1 expression in tumor cells is associated with a better clinical course. Patients with breast cancer and Merkel cell carcinoma expressing PD-L1 have longer disease-free survival times. The prognostic value of PD-L1 for lung cancer, colorectal carcinoma and melanoma is controversial[1].

Evaluation of tumor PD-L1 expression (PD-L1 exp.) has become a predictive biomarker for selecting patients for immunotherapy. However, it is difficult to evaluation of PD-L1 by immunohistochemistry. PD-L1 shows heterogeneity within the tumor. This makes immunohistochemical evaluation difficult. Therefore, evaluation of PD-L1 exp. can be much more difficult in small tissue samples. This situation causes problems especially in lung cancers which have typically limited samples. Current guidelines recommend determining

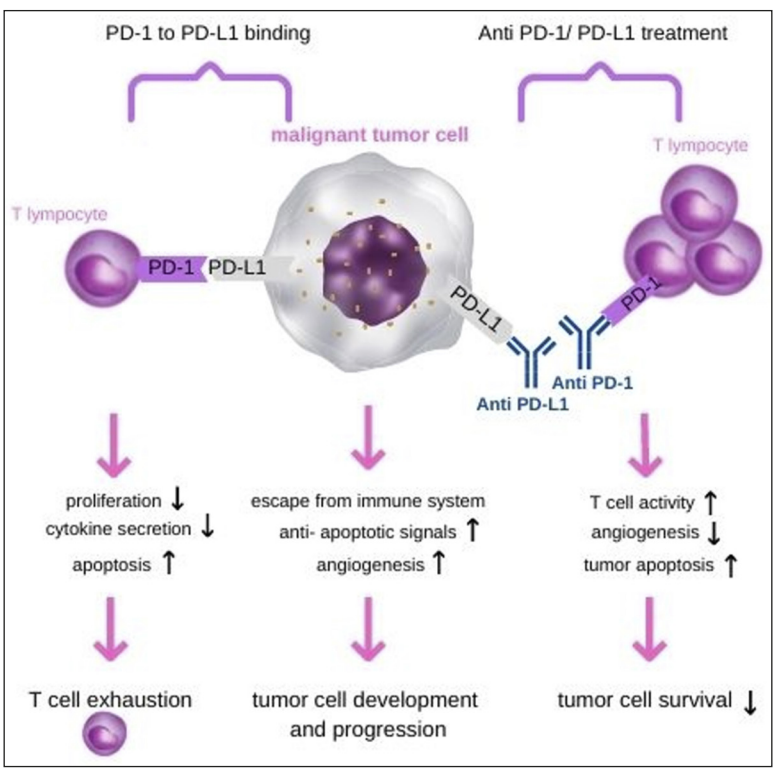

Figure 1. *Mechanism of PD-1/ PD-L1 pathway and anti PD-1/PD-L1 treatment. (Image was prepared with Canva for Education Program by Esin Kaymaz)*

PD-L1 exp. levels with immunohistochemistry in advanced lung cancers[5]. The diagnosis of lung tumors is mostly based on endobronchial / transbronchial aspiration biopsies or pleural effusion materials. Cell blocks obtained from cytological materials has recently been proposed as an alternative way of evaluating PD-L1 exp.

Current study aims to identify clinicopathologic features and expression patterns of PD-L1 in primer and metastatic non-small cell lung tumors. Also we investigated the usability of the cell block for PD-L1 immunohistochemistry.

Materials and Methods

We retrospectively reviewed the data of PD-L1 immunohistochemical analysis of patients between 2018 and 2019 in the Pathology Laboratory. PD-L1 immunohistochemical stain (Dako Clone 22 c3) were applied to tissue samples or cell blocks. The procedure of sampling (biopsy / aspiration), tumor type, gender and also the percentages tumor cells with PD-L1 positivity which were categorized according to density and distribution were recorded. A total of 89 cases between these years have been documented, regardless tumor localization. Here, we wanted to see the distribution of PD-L1 studied cases. But the evaluation and statistical analysis were performed just for tumors localized to lung.

Formalin fixed paraffin embedded tissue blocks have been prepared for all cases. 4 micrometer thick sections were cut and stained with Hematoxylin and Eosin for evaluation. Also cell block was prepared from effusion or aspiration samples. Effusion samples were centrifuged, and the supernatant was tossed away. The sediment was placed on a slide and mixed with 4–5 drops of plasma and 4–5 drops of thromboplastin. The sample was centrifuged again and topped with 10% formalin. The clumped sample is then placed into a cassette, processed through the routine processing protocol. For consultation cases, each block were analyzed for if they have sufficient tumor cell.

PD-L1 was performed if the sample had more than 100 tumor cells. In cases with sufficient tumor immunohistochemical staining were performed with PD-L1 antibody, Dako Clone 22 c3 in dense 1/50.

The percentage of positive staining was determined by an experienced pathologist. Membrane staining (local/global) at any intensity greater than background staining was evaluated, and only viable tumor cells were scored. For non-small cell lung carcinoma, PD-L1 expression was divided into three categories according to the percentage of staining: <1% (negative), 1–49% (low expression) and ≥50% (high expression)[6]. An example for each category has demonstrated in Fig. 2.

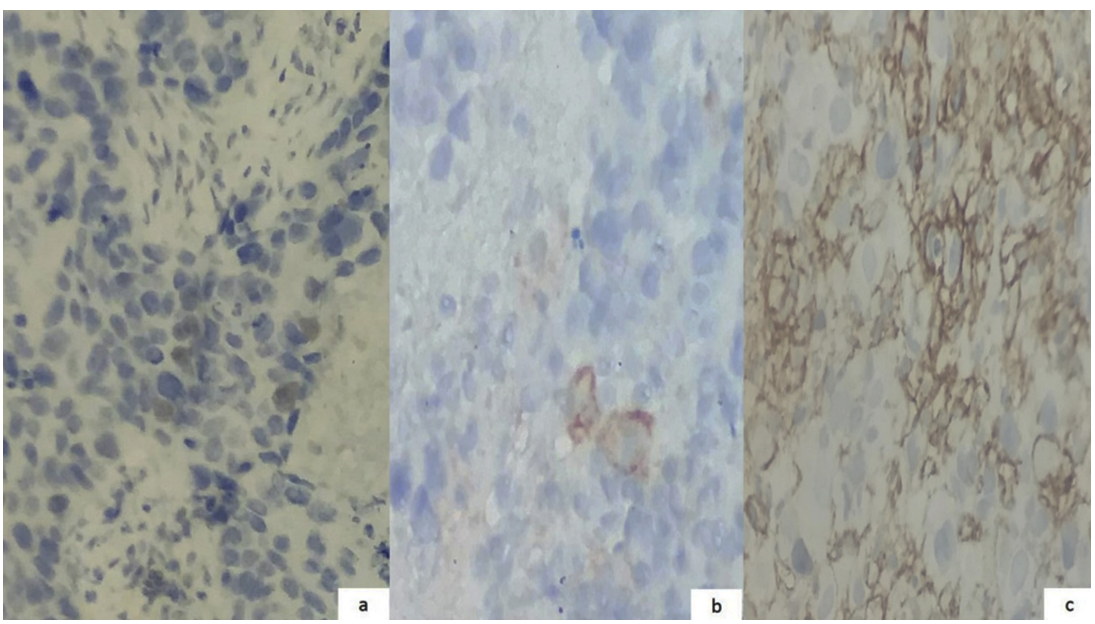

Figure 2. *PD-L1 expression according to percentage of staining: a) <1% (negative) (×400), b) 1–49% (low expression) (×400) and c) ≥50% (high expression) (×400) (Dako Clone 22 c3) (1/50 dilution).*

For the statistical evaluation we considered PD-L1 expression in two groups as negative (<1%) and positive (≥1%). In other word we grouped score 2 and 3 as just positive.

Evaluating the difference of the present/absent or the weak/intense staining of PD-L1 among specimen types was one of the main points we focused on in our study. For statistical analysis, we classified the specimen types into two groups as cytology materials (cell block) and histology materials (biopsy, resection).

We used JASP Team (2022) (Version 0.16.1) program for statistical evaluation. Categorical variables were compared using Chi-Square test. Duo to the small number of cases, comparison between groups were made using Fisher's exact analysis. P value of less than 0.05 was considered statistically significant for all tests.

Results

There were 89 cases in which PD-L1 immunohistochemical staining was studied between 2018 and 2019 in our department.

While 83 of these cases were lung tumors, 2 of them were colon, 2 of them among stomach, 1 of them was belonging to breast and 1 of them to the nasopharynx. Colon and stomach samples were in the form of small biopsies and the pathological diagnosis of them were adenocarcinoma. The diagnosis of the mastectomy material and the biopsy of nasopharynx were as follows; invasive breast carcinoma and malignant melanoma. As can be seen, most of the cases studied with PD-L1 belong to the lung.

We evaluated the tumors of lung. Among them 18 were female and 70 were male. Of the 83 cases belonging to lung, 60 were tissue samples and 23 were cell blocks prepared from aspiration / pleural effusion. Of the 60 tissue samples, 27 were in the form of wedge/segmental resection or lobectomy, and 33 were transbronchial biopsies, transbronchial or small biopsies of metastatic lesion. Regardless of the sampling method, it was determined that 29 cases were reported as squamous cell carcinoma and 54 cases as adenocarcinoma. The majority of squamous cell carcinomas showed moderate differentiation. Acinar pattern was dominant in samples diagnosed with adenocarcinoma. Also 76 of the cases were diagnosed with lung samples, while 7 were samples from the brain, lymph node or bone tissues metastases of lung cancer. However, the location of the metastatic tumor was not of significance for the study. The main thing here was that the tumor was of lung origin.

We evaluated PD-L1 exp. in 3 categories. In 29 of the cases diagnosed with non-small cell lung cancer, either no or <1% staining was observed with PD-L1. These cases were evaluated as negative by PD-L1. Staining was low (1–49%) with PD-L1 in 41 cases while it was high in 13 cases. In Fig. 3a, a squamous cell carcinoma with score 3 of PD-L1 (80% percentage) is demonstrated. In Fig. 3b, we can see a strong expression pattern in an adenocarcinoma.

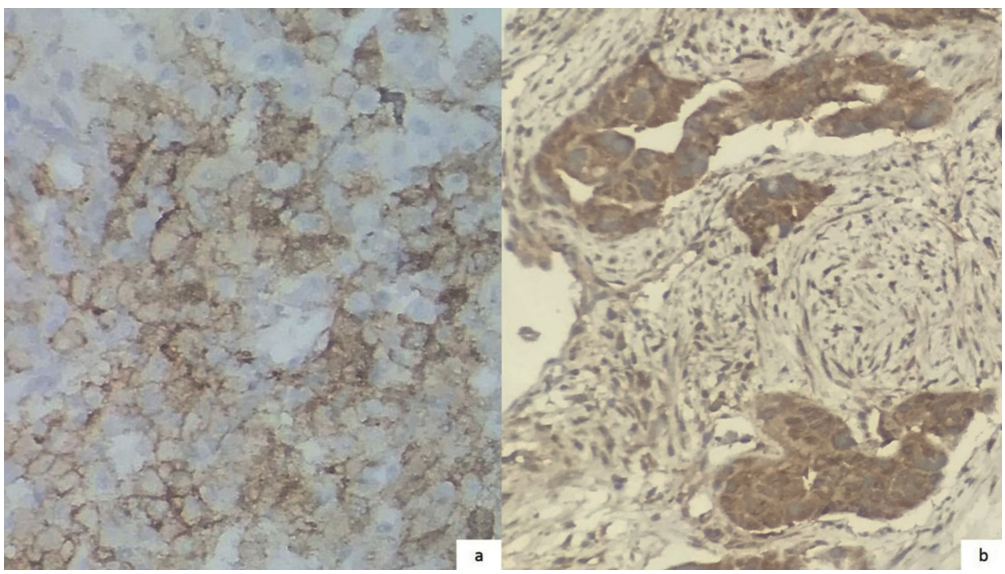

Figure 3. *a) Squamous cell carcinoma of lung with 80% PD-L1 staining (×200) b) PD-L1 staining strong in an adenocarcinoma with score 3 (×200) (Dako Clone 22 c3) (1/50 dilution).*

Evaluation results of PD-L1 exp. according to patient gender, primary or metastatic tumor and the subtype of tumor diagnosis are summarized in Table 1. PD-L1 exp. was negative in almost half of the cases (48.3%) diagnosed with squamous cell carcinoma. In the cases of adenocarcinoma PD-L1 exp. rate was between 1–49 % in more than half (51.8%) of them.

In this study, we performed a statistical analysis of PD-L1 expression in primary and metastatic tumors of the lung, excluding 6 tumors localized in other organs. We evaluated PD-L1 expression statistically between primary and metastatic tumors and also between two histological subtypes (squamous cell carcinoma and adenocarcinoma).

PD-L1 positivity or negativity was not statistically significant between primary and metastatic tumors (p=1). Same way it was not significant between squamous cell carcinoma and adenocarcinoma (p=0, 09). For histological subtypes we also evaluated the difference of low or high expression. The p value was determined as 0.76 for this analysis. So we can say that different histological subtypes did not affect the expression density.

Sampling type and PD-L1 exp. results in cases diagnosed with non-small cell lung carcinoma are summarized in Table 2. The case distributions were similar, if not equal. Also low and high PD-L1 rates were similar in different sampling procedures. When we compared PD-L1 expression as present/absent or low/strong for cytology and histology samples, no significant difference was found between the groups (p=0.79, p=0.74 respectively). We subdivided the histology materials into two main groups as small biopsy materials and large resection materials. We have analyzed that the cases with negative PD-L1 exp. mostly belonged to small biopsy samples (48.3%). But it was not significant with statistically when we compared these groups with PD-L1 expression status. We determined that small or large material does not affect the positivity or density of PD-L1. (p=0.42, p=1).

Material adequacy was found to be satisfactory in the cases of cell blocks obtained from the fluid. Sufficient numbers of tumor cells were observed in the samples to assess PD-L1 expression. The tumor cell rate varied between 5 and 80% in these cases. Positive staining with PD-L1 was detected even with a small number of tumor cells in the cell block (Fig. 4a, b). Figure 4c belongs to an adenocarcinoma of lung in cell block with score 3 of PD-L1. We have found similar proportions of tumor cells in small biopsy samples.

Table 1. *PDL-1 expression and clinicopathological parameters*

	Tumor proportion score of PD-L1					
Specimen type	<1%, n (%) (negative)	1–49%, n (%)	≥50%, n (%)	≥1%, n (%) (positive)	Total (n)	P*
Cell block of pleural or bronchial fluid	7 (30.4)	12 (52.2)	4 (17.4)	16 (69.6)	23	0.79
Histology sample	22 (36.7)	29 (48.3)	9 (15)	38 (63.3)	60	
– Large biopsy (resection)	8 (29.6)	15 (55.6)	4 (14.8)	19 (70.4)	27	0.42
– Small biopsy	14 (42.4)	14 (42.4)	5 (15.2)	19 (57.6)	33	

* P value of less than 0.05 was considered significant

Table 2. *PDL-1 expression in cell blocks and histology samples that belong to non-small cell lung carcinoma*

	Tumor proportion score of PD-L1					
Parameters	<1%, n (%) (negative)	1–49%, n (%)	≥50%, n (%)	≥1%, n (%) (positive)	Total (n)	P*
Diagnosis						
SCC	14 (48.3)	10 (34.5)	5 (17.2)	15 (51.7)	29	0.09
Adenocarcinoma	15 (27.8)	31 (57.4)	8 (14.8)	39 (72.3)	54	
Sex						
Male	23 (35.4)	32 (49.2)	10 (15.4)	42 (64,6)	65	1
Female	6 (33.3)	9 (50)	3 (16.7)	12 (66,7)	18	
Origin						
Primary	27 (35.5)	38 (50)	11 (14.5)	49 (54.5)	76	1
Metastatic	2 (28.6)	3 (42.8)	2 (28.6)	5 (71.4)	7	
Total	29 (34.9)	41 (49.4)	13 (15.7)	54 (65.1)	83	

* P value of less than 0.05 was considered significant

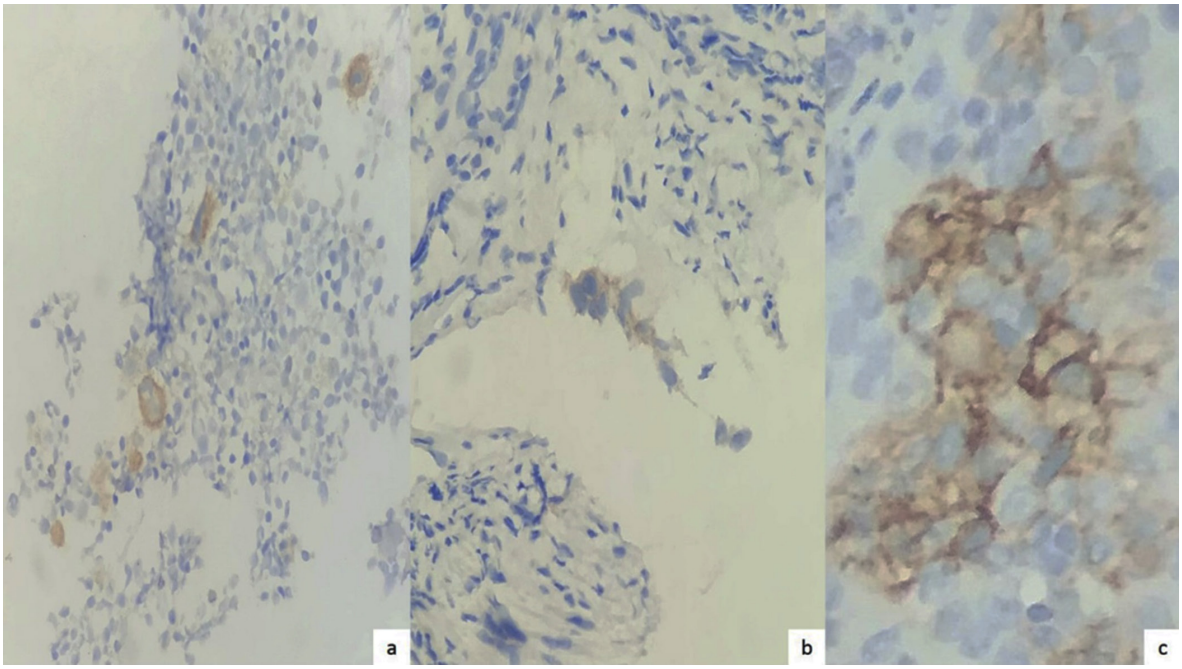

Figure 4. *Tumor cells with positive PD– L1 staining in the cell block. a, b) Low PD-L1 staining with a ratio 1–2% (×100) c) PD-L1 staining with a ratio 20% in adenocarcinoma (×200) (Dako Clone 22 c3) (1/50 dilution).*

We evaluated statistically the effect of cellularity in the cell block on PD-L1 expression status. We separately compared the presence or absence of PD-L1 according to whether the cell block contains more than 10% tumor cells or more than 50% tumor cells. If tumor cells constituted more than 10% of the material, PD-L1 expression was significantly different as negative or positive (p=0.02). The 50% rate of tumor cell amount was not statistically significant for PD-L1 (p=0.5). These datas are summarized in Table 3.

In addition the cellularity in the PD-L1 negative group belonging all specimen types was not lower from the other groups, it was similar.

Discussion

Bubendorf et al. compared PD-L1 exp. in small biopsy specimens and resection specimens and they have found much lower PD-L1 in small biopsy specimens. In our study although it is not significant statistically, half of the PD-L1 negative cases belonged to small biopsy samples, similarly. Bubendorf et al. suggested that this difference may be due to the heterogeneity and the sampling procedure as well as the scoring algorithm and even inter-observer variability[7]. Also different antibody clones may explain the reason for the low PD-L1 expression in some studies[1].

Table 3. *PDL-1 expression in cell blocks according to cellularity*

	Tumor proportion score of PD-L1			
Proportion of cells in a cell block	<1%, n (%) (negative)	≥1%, n (%) (positive)	Total (n)	P*
<10% cells	***6 (28.5)***	***4 (19.1)***	***21***	***0.02***
≥10% cells	***1 (4.8)***	***10 (47.6)***		
<50% cells	6 (28.5)	13 (61.9)	21	0.5
≥50% cells	0 (0)	2 (9.6)		

* P value of less than 0.05 was considered significant

Scoring systems and cut-off values used for PD-L1 evaluation also vary for different types of tumors. The triple scoring system currently accepted for lung cancer is such as <1%, 1–49% and >50%. A cut-off value of 10% is used for pancreatic, esophageal and stomach cancer[1].

Aspiration, a noninvasive procedure, is preferred for diagnosis in many tumors, especially for lung tumors. Cytological preparations allow for definitive diagnosis as well as many molecular tests. It is known that cytological samples are used for PD-L1 immunohistochemical evaluation. Studies demonstrating the usability of the cell block for this purpose are limited. However, according to current data, PD-L1 exp. in cell block and biopsy samples overlaps.

Zou et al. have suggested to prefer cell blocks for the assessment of PD-L1 exp. instead of small biopsy samples[6]. Considering the intra-tumor heterogeneity, it is not surprising to observe lower PD-L1 exp. in small biopsy samples[8,9]. They have considered that cytology samples have stronger PD-L1 exp. However, the reason for this is not very clear. One of the reason according to Zou et al. is decrease of tumor heterogeneity due to the distribution of tumor cells in cytology samples[6]. Another hypothesis for strong expression is the close contact between tumor cells and the immune microenvironment in pleural effusions. So, it is believed that T lymphocytes and macrophages in this environment increase PD-L1 exp. on the tumor cell surface[10].

In our study, cell block samples with high level of positive PD-L1 staining were higher than small biopsy samples. The statistical inconsistency in our study may be to the small number of cases. However, in general, we can say that cell blocks obtained from aspiration material may be a good idea to evaluate PD-L1. Here, the main point to be considered in order for optimal is that the number of cells, and therefore the tumor cell, is sufficient. For this, evaluation of PD-L1 expression in a cell block containing 10% tumor cells may be satisfactory. In this case, the need for a small biopsy and also for an interventional procedure to the patient may be eliminated.

Zou et al. also evaluated many factors that may be effect the strong PD-L1 exp. in the cell block[6]. They suggested that the fixation period (15–20 minutes for cell block, 2–24 hours for histological samples) may be a factor that may affect PD-L1 staining. Pinar et al., on the other hand, considered that the preservative solution used for cell block can affect the PD-L1 exp. in these samples. In their study, they compared the distribution and intensity of PD-L1 expression in the cell blocks formed with different preservative solutions. They concluded that –direct application of formalin with dripping 96% alcohol have the best results[11].

There are also some difficulties in the evaluation of immunohistochemical staining in the cell block. It can be difficult to determine the rate of positive tumor cell, especially if the tumor cells are dispersed as single. In addition, false positivity may occur in histiocytes and it is not easy to distinguish between histiocytes and single tumor cells in the cell block[12]. This evaluation should be done by an experienced pathologist to avoid from misleading[12]. Dual immunohistochemical staining (CD68 for histiocytes or TTF-1 for tumor cells) with PD-L1 may also help for this discrimination[1].

There are also studies investigating clinicopathologic data that may affect PD-L1 expression. However, no definite results could be obtained in this regard. A meta-analysis study evaluating PD-L1 in patients with lung cancer showed that PD-L1 expression was stronger in adenocarcinoma than in squamous cell carcinoma[13]. However, East Asian data differ from these results[14]. We could not have any significant difference between PD-L1 expression and clinicopathological datas such as gender, histological subtypes of the tumor and the status of primer or metastatic.

Our study has some limitations. One of them is the retrospectivity of the study. Another limitation was that the cytology and biopsy materials in this study belonged to different cases. So we could not perform cytology– histology comparison on the same tissue. However, we believe that our study will provide an idea about PD-L1 expression in cell blocks.

As we know cell blocks contain a limited number of cells. Finding the maximum number of cells to be obtained in the cell block should be the point to be noted. According to our study, it is more meaningful to evaluate PD-L1 expression in cell blocks containing tumor cells in at least 10% of the material. In other words, with the maximum number of cells that can be obtained, we can reach more optimal results in the PD-L1 evaluation.

As conclusion; in this retrospective analysis samples consisted of small biopsy samples and cell blocks prepared from cytological samples as well as resection materials with similar ratio. According to our analysis,

cell blocks prepared from cytological samples may be a good alternative for immunohistochemical evaluation of PD-L1 exp. When we evaluated the results of PD-L1 exp. of cell blocks, it was not at all different from the resection materials. As we know cell blocks contain a limited number of cells. Finding the maximum number of cells to be obtained in the cell block should be the point to be noted. According to our study, it is more meaningful to evaluate PD-L1 expression in cell blocks containing tumor cells in at least 10% of the material. In other words, with the maximum number of cells that can be obtained, we can reach more optimal results in the PD-L1 evaluation. We think that revealing the data on PD-L1 exp. in cytological and histological tissue samples is valuable and also may be a guide for other studies.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Do Social Determinants of Health Affect Discretionary Abortion? A Hospital Focused Cross-sectional Study

Sağlığın Sosyal Belirleyicileri İsteğe Bağlı Düşüğü Etkiliyor mu? Hastane Odaklı Kesitsel Bir Çalışma

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ABSTRACT

Aim: *It was aimed to determine the level of voluntary abortions and related factors in women over the age of 49 who applied to the obstetrics and gynecology outpatient clinic in a secondary public hospital and were examined.*

Material and Method: *Patients who applied to the secondary care state hospital between January 15 and March 15, 2021, were asked whether they had an optional abortion. In addition, the patients' socio-demographic, bio-demographic, and socio-economic characteristics were recorded by face-to-face interview technique.*

Results: *When women living in villages are taken as reference, optional abortions are 7,954 (CI: 3,625–17,449) times for women living in the city center, 2,990 times for those living in a nuclear family when living in an extended family is taken as reference, 7,719 times for those who have social health insurance as a reference (CI: 3,614–16,487) times, when the total income level of people entering the house is taken as reference, it is 6,637 (CI: 3,059–14,401) times more for those who are inadequate.*

Conclusion: *Social service policies should be arranged to increase access to health services and prevention methods in the eastern regions of Turkey.*

Key words: *voluntary abortion; abortion; sociocultural factors*

ÖZET

Amaç: *İkinci basamak kamu hastanesinde kadın doğum polikliniğine müracaat edip muayene olan 49 yaş üzerindeki kadınlarda isteğe bağlı düşüklerin düzeyi ve ilişkili faktörleri belirlemek amaçlanmıştır.*

Materyal ve Metot: *İkinci basamak devlet hastanesinde 15 Ocak-15 Mart 2021 tarihleri arasında başvuran hastalara isteğe bağlı düşük yapıp yapmadıkları sorulmuştur. Ayrıca bu hastaların sosyo-demografik, biyo-demografik ve sosyo-ekonomik özellikleri yüz yüze görüşme tekniği ile kayıt edilmiştir.*

Bulgular: *Köyde yaşayan kadınlar referans alındığında şehir merkezinde yaşayan kadınlarda isteğe bağlı kürtaj 7.954 (CI: 3.625–17.449) kat, geniş ailede yaşamak referans alındığında çekirdek ailede yaşayanlarda 2.990 (CI: 1.270–7.038) kat, sosyal sağlık güvencesi olanlar referans alındığında olmayanlarda 7.719 (CI: 3.614–16.487) kat, eve giren toplam gelir düzeyi yeterli olanlar referans alındığında yetersiz olanlarda 6.637 (CI: 3.059–14.401) kat daha fazladır.*

Sonuç: *Türkiye'nin doğu bölgelerinde sağlık hizmetleri ve korunma yöntemlerine ulaşımı artıracak sosyal hizmet politikaları düzenlenmelidir.*

Anahtar kelimeler: *isteğe bağlı düşük; kürtaj; sosyokültürel faktörler*

Introduction

On-demand abortion (miscarriage) is the process of terminating a pregnancy that has settled in the uterus of the woman's own will, without gaining the ability to live outside the uterus[1]. It is estimated that 25 million unsafe optional abortions are performed every year in the world. When maternal deaths occurring within a year are examined, it is estimated that between 4.7% and 13.2% of these deaths are due to optional abortions that are not performed safely[2].

Worldwide, there are more than 80 million unwanted pregnancies, and 42 million of these pregnancies end with abortion[3]. In Turkey, the level of optional abortion in married women is 15.0%[4].

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While voluntary abortions were used as a family planning method in previous years, they are not used for this purpose today[2].

It was aimed to determine the level and related factors of voluntary abortions in women over the age of 49 who applied to the obstetrics and gynecology outpatient clinic in a secondary level public hospital.

Material Methods

The research was carried out in the 2nd Stage public hospital of Kars province, which is located in the 30th health region, whose main source of livelihood is agriculture and animal husbandry, and which is below the average of Turkey in terms of socio-economic development. When the sociodemographic characteristics of the region are examined, illiterate people constitute 11.7% of the total population of the region, and those who have never received any education constitute 13.5%[5]. The region is below Turkey's average in terms of health personnel per thousand people. Respectively, infant mortality rates are 11.2 per thousand, and maternal deaths are above Turkey's average with 24.5 per hundred thousand (Turkey averages are 6.8 per thousand infant deaths and 14.6 maternal deaths per hundred thousand, respectively)[6].

Type of study: It is a hospital-focused cross-sectional study.

The universe of the study: The number of women who completed their reproductive period, who applied to the obstetrics and gynecology outpatient clinic in 2019, was used to determine the population. Accordingly, 2763 women applied to the polyclinic.

The sample of the study: sample: since the universe of the study is known, the number of women to be sampled was calculated with the formula n=Nt 2 pq/d 2 (N-1) + t 2 PQ (N, number of individuals in the population; n, number of individuals to be sampled; p, examined incidence (probability) of the event; q, the frequency (probability) of the event under investigation; t, the theoretical value found in the t table at a certain degree of freedom and the detected error level; d, the desired ± deviation according to the incidence of the event. 9 Accordingly, p=0.15 When; q=0.85, t=1.96, d=0.01, the sample size was determined as 1766 women.

Creating the data collection form: The data collection form was prepared by the researchers by scanning the literature[4,7,8].

Definitions of the research variables and variables:

Dependent variable: whether they had induced abortion before or not was considered as the dependent variable.

Independent variables: Sociodemographic, biodemographic, and socioeconomic characteristics of the women included in the study were taken as independent variables.

Ethics committee approval and verbal consent of the study: Ethics committee approval of the study was obtained from the local committee with the number 818295502.9037/98. In addition, written and verbal consent was obtained from the participants in the study.

Data collection: The data of the study were collected by the researcher using the face-to-face interview technique in the obstetrics and gynecology outpatient clinic between 15 January and 15 March 2021, after obtaining written and verbal consent from the women.

A preliminary trial of the study: It was conducted with 13 women aged 50 and over who applied to the outpatient clinic. The missing parts in the data collection form were determined and necessary corrections were made.

There is no conflict of interest between the authors in the study. In addition, ethical consent was obtained and research and publication ethics were complied with.

Statistical Analysis

SPSS 21 package was used to analyze the data. Chi-square analysis was performed in pairwise comparisons. Variables that were significant in chi-square analyzes were included in the Backward LR logistic regression analysis and risk factors for voluntary abortion were determined.

Results

In our study, the rate of optional abortion was determined as 9.3%. In the study, 6% of women who had voluntary abortions were under the age of 19 and 66.5% were over the age of 35.

When the factors affecting voluntary abortion were examined in the study with binary analysis, the place of residence (p<0.001), family type (p<0.001), social security status (p=0.05), completion of formal education (p=0.028), It was found statistically significant that the total income entering the house was sufficient

(p<0.001), the number of children in the house being 4 or more (p<0.001), and having a boy living at home (p<0.001) (Table 1). However, when these variables are examined by logistic regression analysis when women living in villages are taken as reference, optional abortions are 7,954 (CI: 3,625–17,449) times for women living in the city center, and 2,990 (CI: 1,270–7,038) times for those living in a nuclear family when living in an extended family is taken as reference. When those who have security are taken as reference, it is 7,719 (CI: 3,614–16,487) times higher for those who do not, and 6,637 (CI: 3,059–14,401) times for those who have insufficient income when the total income level is taken as reference (Table 2).

Table 1. *Factors affecting having an optional abortion*

		Dependent variables			
Independent variables		Women with an optional abortion (%)	Women without an optional abortion (%)	Total (%)	P
Place of residence	Village/town	61 (%5,8)	995 (%94,2)	1056 (%59,8)	<0,001
	City/district center	103 (%14,5)	607 (%85,5)	710 (%40,2)	
Family type	Large	29 (%6,3)	428 (%93,7)	457 (%25,9)	0,006
	Nuclear	135 (%1,3)	1174 (%89,7)	1309 (%74,1)	
Marital status	Married	150 (%9,5)	1433 (%90,5)	1583 (%89,6)	0,256
	Not married	14 (%7,7)	169 (%92,3)	183 (%10,4)	
Health insurance	No	66 (%7,5)	818 (%92,5)	884 (% 50,1)	0,005
	Yes	98 (%11,1)	784 (%88,9)	882 (%49,9)	
Education	≥9 years	86 (%9,7)	792 (%90,3)	878 (%49,7)	0,283
	≤8 years	79 (%8,9)	810 (%91,1)	888 (%50,3)	
Partner education	≥9 years	94 (%8,3)	1045 (%91,7)	1139 (%64,5)	0,028
	≤8 years	70 (%11,2)	557 (%88,8)	628 (%35,5)	
Household income	Sufficient	80 (%7,1)	1052 (%92,9)	1132 (%64,1)	<0,001
	Insufficient	84 (%13,2)	550 (%86,8)	634 (%35,9)	
Total number of pregnancies	≤4	145 (%10,6)	1226 (%89,4)	1371 (%77,6)	<0,001
	≥5	19 (%4,8)	376 (%95,2)	395 (%22,4)	
Boy at home	Yes	120 (%15,6)	650 (%84,4)	770 (%43,6)	<0,001
	No	44 (%4,4)	952 (%95,6)	996 (%56,4)	
Woman employment	Yes	39 (%7,7)	465 (%92,3)	504 (%28,5)	0,084
	No	125 (%9,9)	1137 (%99,1)	1262 (%71,5)	
Man employment	Yes	36 (%8)	414 (%92)	450 (%25,5)	0,194
	No	128 (%9,7)	1188 (%90,3)	1316 (%74,5)	

Table 2. *Logistic regression analysis of socio-cultural factors affecting having a voluntary*

Dependent variable: availability of optional abortion			
Independent variables		OR	%95 Confidence interval (minimum value-maximum value)
Place of residence	Village/town	7,954	3,625-17,449
	City/district center		1(reference)
Family type	Large	2,990	1,270-7,038
	Nuclear		1(reference)
Health insurance	No	7,719	3,614-16,487
	Yes		1(reference)
Household income	Insufficient	6,637	3,059-14,401
	Sufficient		1(reference)

When the women who had a voluntary abortion were evaluated within themselves, it was seen that 28.7% of these women used contraception before the abortion, while 71.3% did not use any contraception method. Conservation method preferences were determined as 28% withdrawal, 13.4% oral contraceptive, 11.6% intrauterine device, and 9.1% condom, respectively.

Discussion

When the TNSA 2018 data is analyzed, the rate of optional abortion in Turkey is 15%. However, this rate decreased to 10% in eastern Turkey[4]. In our study, the rate of optional abortion was found to be 9.3%, in line with the TNSA 2018 data.

In the study, 6% of women who had optional abortions were under the age of 19, 32.9% were between the ages of 20–35 and 66.5% were over the age of 35. By both TNSA 2018 and other studies in the literature, it has been shown in our study that the rate of voluntary abortion increases with the age of the woman. It is estimated that the reason for this situation is that the older woman does not want more children because she has reached a sufficient number of children[9].

When women living in rural areas are taken as a reference, voluntary abortion increases 7,954 times (CI: 3,625–17,449) among women living in the city center. Although logistic regression analysis is not performed in the studies in the literature, it is seen that there are more voluntary abortions in urban areas than in rural areas[7,10]. The probable reason for this situation is that women living in the city center have easier access to health services[4].

In our study, when those who had voluntary abortion social health insurance were taken as the reference, it was 7,719 (CI: 3,614–16,487) times higher for those who did not, and 6,637 (CI: 3,059–14,401) times for those who had insufficient income when the total income level of their households was sufficient as the reference. When the previous studies were examined, it was seen that in cases where the economic level of the woman was insufficient, optional abortion was more common with the thought of not being able to have another child[4,11].

In our study, when the women who had a voluntary abortion were evaluated within themselves, although it was insignificant in the dual analysis, in accordance with the literature data, it was seen that 28.7% of these women used contraception before the abortion, while 71.3% did not use any contraception. Conservation method preferences were determined as 28% withdrawal, 13.4% oral contraceptive, 11.6% intrauterine device, and 9.1% condom[4].

Due to cultural influences, voluntary abortions are less common in the region and unwanted pregnancies are increasing. As a result, in order to prevent unwanted pregnancies, all individuals in the society, health officials working in family medicine should be informed about family planning methods, and social service policies should be arranged to increase access to health services and prevention methods in the eastern regions of Turkey, where the socioeconomic level is lower.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

The Role of Endosonography in Patients With Moderate and High Probability of Choledocholithiasis

Orta ve Yüksek Olasılıklı Koledokolitiazis Hastalarında Endosonografinin Rolü

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ABSTRACT

Aim: *In this study, we aimed to investigate the diagnostic efficiency and place of EUS in clinical practice in patients with moderate to a high probability of choledocholithiasis according to their ASGE score.*

Material and Method: *This study includes patients with moderate to high risk of CBDSs who were admitted to the Department of Gastroenterology between August 2015-August 2016. The results of patients undergoing EUS and ERCP for suspected choledocholithiasis were retrospectively reviewed from the hospital registry.*

Results: *Two hundred and twenty nine patients were included in the present study and 56.3% of the patients (n=129) were female, and the average age of the patients was 62.8±18.3 (20–91). The sensitivity of EUS was found to be 89.2%. The specificity was 94.6%, the positive predictive value was 95.6%, and the negative predictive value was 86.9%. In addition, the choledochal diameter measured in AUS and EUS was found to have diagnostic values in predicting the CBDSs [AUC (95% GA p); respectively, 0.617 (0.409–0.825) p=0.310 and 0.765 (0.619–0.915) 0.020].*

Conclusion: *Endosonography is both a high-diagnostic and a low-invasive diagnostic method, so it is increasingly used in patients with suspected CBDSs. Referral of suspected patients with CBDSs to a center with EUS and an experienced endoscopist will ensure that the patient receives the correct diagnosis and is not subjected to unnecessary invasive procedures.*

Key words: *EUS; ERCP; choledocholitiasis*

ÖZET

Amaç: *Bu çalışmada ASGE skoruna göre orta-yüksek olasılıklı koledokolitiazis hastalarında EUS'nin tanısal etkinliğini ve klinik pratikteki yerini araştırmayı amaçladık.*

Materyal ve Metot: *Bu çalışma Ağustos 2015-Ağustos 2016 tarihleri arasında Gastroenteroloji kliniğine başvuran orta ve yüksek olasılıklı koledokolitiazis hastalarını içermektedir. Koledokolitiazis şüphesiyle EUS ve/veya ERCP yapılan hastaların sonuçları hastane kayıtlarından retrospektif olarak tarandı.*

Bulgular: *Çalışmaya 229 hasta dâhil edildi ve bunların %56,3 (n=129)'ü kadın ve hastaların yaş ortalaması 62,8±18,3 (20–91) idi. "Endosonography"nin duyarlılığı % 89,2, özgüllüğü %94,6, pozitif prediktif değeri %95,6, negative prediktif değeri % 86,9 olarak bulundu. "Abdominal ultrasonography" ve EUS'de ölçülen koledok çapının CBDSs'leri öngörmede tanısal değerlere sahip olduğu bulundu [AUC (% 95 GA p); sırasıyla, 0,617 (0,409–0,825) p=0,310 ve 0,765 (0,619–0,915) 0,020].*

Sonuç: *Endosonography hem yüksek tanısal hem de düşük invaziv bir prosedür tanı yöntemi olduğundan CBDSs şüphesi olan hastalarda giderek daha fazla kullanılmaktadır. "Common bile duct stone" şüphesi olan hastaların EUS ve deneyimli bir endoscopist olan bir merkeze yönlendirilmesi hastanın doğru tanı almasını ve gereksiz invaziv müdahalelere maruz kalmamasını sağlayacaktır.*

Anahtar kelimeler: *EUS; ERCP; koledokolitiazis*

Introduction

Common bile duct stones (CBDSs) are a common clinical condition in daily practice. The migration of gallstones to the main bile duct causes patients with symptoms to apply frequently to the clinic. In addition, CBDSs can cause serious complications such as pancreatitis, cholangitis, obstructive jaundice, and secondary biliary cirrhosis[1]. Therefore, early diagnosis and treatment of gallstonesis vital.

Abdominal ultrasonography (AUS) is recommended for patients with choledocholithiasis based on the clinical and laboratory findings. However, AUS is relatively insufficient in diagnosis, and additional diagnostic methods are needed[2]. Diagnosis of choledocholithiasis includes multiple images such as endosonography (EUS), Endoscopic retrograde cholangiopancreatography

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(ERCP), magnetic resonance cholangiopancreatography (MRCP), Computed Tomography (CT), and intraoperative cholangiography. In previous years, ERCP was preferred as both a diagnostic and therapeutic method. The sensitivity of ERCP in patients with CBDSs was found to vary between 80–93% and the specificity was reported to be 99–100%[3,4]. The ERCP application, which is used as a very important therapeutic method, is an invasive procedure that requires experience and can lead to some complications. In addition, patients are exposed to radiation during this process. Endoscopic retrograde cholangiopancreatography related complications such as pancreatitis, cholangitis, bleeding, and perforation may occur[5].

The aforementioned information shows that AUS is non-invasive but has a low diagnostic value and that ERCP is the gold standard in diagnosis and is a therapeutic method since it is an invasive procedure[6]. However, neither method is considered ideal in the diagnosis of CBDSs. Patients with suspected CBDSs need a diagnostic method that has a high diagnostic specificity and a lower risk of complications.

In 2010, the American Society for Gastrointestinal Endoscopy (ASGE) divided patients with suspected choledocholithiasis into three groups as high, moderate, and low probability. According to the ASGE score, preoperative ERCP is recommended because patients with a high risk of choledochal stones are above 50% clinically. Intraoperative cholangiography is another option for these patients. All other patients in the medium risk group were offered EUS, MRCP, intraoperative cholangiography, or laparoscopic ultrasonography before cholecystectomy, depending on cost effectivity[2].

In recent years, EUS has become widely used due to its less invasiveness and high sensitivity in the diagnosis of choledocholithiasis[7]. In particular, there is a widespread belief that EUS reduces unnecessary ERCP and complications associated with this procedure[8]. In this study, we aimed to investigate the diagnostic efficiency and place of EUS in clinical practice in patients with moderate to high probability of choledocholithiasis according to their ASGE score.

Materials and Methods

Patients and Study Design

This study includes 229 patients with moderate to high risk of CBDSs who were hospitalized to Dişkapi Yildirim Beyazit Education and Research Hospital Department of Gastroenterology in Ankara, Turkey, between August 2015-August 2016. The patients' clinical risk of CBDSs was assessed according to the ASGE 2010 guidelines. Based on these criteria, patients were categorized as having a low (<10%), moderate (10% to 50 %), or high (>50%) probability of CBDSs, using their age, liver function test results, and transabdominal ultrasonography findings. We excluded patients who had a history of malignant disease, had previouly undergone endoscopic procedures for biliary reasons, or who were <18 years old. The results of patients undergoing EUS and/or ERCP for suspected choledocholithiasis were retrospectively reviewed from the hospital registry. Preoperative complaints, abdominal USG data, WBC, hemoglobin, platelet, INR, total bilirubin, direct bilirubin, ALT, GGT, amylase, and lipase values were recorded. Dilated CBD was defined as a CBD when diameter was above 6 mm or 10 mm in cases of cholecystectomy on AUS[9]. The results of the EUS and ERCP procedures, complications related to the procedures, and laboratory values obtained 6 months later were retrospectively reviewed.

EUS/ERCP

A radial ultrasonic echoendoscope (model of CLV-180-Aloka; Olympus, Tokyo, Japan) was used for EUS examinations. Endosonography was performed within 24 hours after admission by an experienced endoscopist. Common bile duct stones were positively defined by the observation of a hyperechoic focus within the common biliary duct with or without an acoustic shadow. The widest diameter of the CBD was measured.

Endoscopic retrograde cholangiopancreatography was performed with a lateral scope (Fujinon EPX-4400) when CBDSs were detected on EUS or for patients with a high probability CBDSs according to the ASGE criteria. The patients were treated after at least 12 hours of fasting. The patients were sedated with Midazolam (mean 5–10 mg, maximum 40 mg) and propofol (mean 10–20 mg) after they were informed about the procedure. After selective biliary cannulation, the biliary tract was visualized by contrast agent administration, and sphincterotomy was performed when indicated. If a filling defect or stones were present in the CBD, the stone extraction was performed using a balloon and/or basket. After the procedure, the patients were observed in the hospital for at least 24 hours, and biochemical tests were recorded 6 hours and 24 hours post procedure. Endoscopic retrograde cholangiopancreatography related complications were

defined and graded according to standardized criteria in a consensus panel[5].

Statistical Analysis

All data were evaluated using SPSS 22.0 (Statistical Package for Social Sciences, SPSS Inc, Chicago, IL) for Windows. The suitability of the variables to normal distribution was examined using visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov Test). Descriptive statistics were expressed as mean, median (minimum-maximum), frequency distribution, and percentage. Pearson chi-square tests were used for the evaluation of categorical variables. The diagnostic predictivity of the CBD diameter measured by USG and EUS in predicting CBDSs were analyzed by Receiver Operating Characteristics (ROC) curve analysis. Sensitivity, specificity, and the positive and negative predictive values of these limits were calculated in the presence of significant limit values. Statistical significance level was accepted as $p<0.05$.

Results

56.3% of the patients (n=129) were female, and the average age of the patients was 62.8±18.3 (20–91). 91.7% of patients (n=210) had symptoms of abdominal pain. 66.4% (n=152) of the patients were in the high probability group according to their ASGE scores. 18.8% (n=43) had a history of cholecystectomy. 66.8% (n=153) of the patients were diagnosed with gallstones in AUS, while 24.9% (n=57) had choledochal stones in AUS and 79.0% (n=181) were found to have choledochal dilatation in AUS (Table 1). The mean values of the laboratory data pertaining to the patients are presented in Table 2.

According to the patients' ASGE scores, ERCP was performed on 84 patients in the high-probability choledocholithiasis group, while EUS was performed on 77 patients with moderate probability. Only 68 patients with high-probability comorbid diseases (e.g., advanced cerebral insufficiency, chronic obstructive pulmonary disease, etc.) or with anticoagulant-antiplatelet use were assessed with EUS. Endoscopic retrograde cholangiopancreatography was performed in the same session on 69 patients with choledochal stone in EUS, and follow up visits were conducted with 61 patients. In seven of these patients, choledochal stones were found in three patients who underwent ERCP due to elevated cholestasis enzymes and suspected CBDS. Of the 54 patients who received clinical follow-up, five patients were discharged; however, a few days after discharge, ERCP was performed on these patients due to recurrent cholestasis enzymes and epigastric pain. The remaining 49 patients did not have any clinical or biochemical pathology in their 6-month follow-up (Fig. 1).

Choledocholithiasis was detected by ERCP in 95.7% (n=66) of patients who were suspected of having choledochal stones with EUS. In 13.1% (n=8) of patients who were detected to have choledochal stones without EUS, choledocholithiasis was diagnosed in clinical follow-up (5 patients) and with ERCP (3 patients). Statistically significant differences were found between choledochal stones detection using EUS and the presence of choledocholithiasis ($p<0.001$) (Table 3).

Table 1. *Clinical and demographic data of patients*

Patients	(n=229)
Gender (Female/male) n (%)	129 (56.3) / 100 (43.7)
Age (years) Mean ± SD	62.8±18.3
After cholecystectomy n (%)	43 (18.8)
High probability CBDSs n (%)	152 (66.4)
Moderate probability CBDSs n (%)	77 (33.6)
Symptom n (%)	
Abdominal pain	210 (91.7)
Jaundice	81 (35.4)
Fever	21 (9.2)
AUS findings n (%)	
Stone / sludge in the gallbladder (yes / no)	153 (66.8) / 76 (33.2)
Choledochal dilatation (yes / no)	181 (79.0) / 48 (21.0)
Choledochal stones/ sludge (yes / no)	57 (24.9) / 172 (75.1)

CBDSs: Common bile duct stones; AUS: Abdominal ultrasonography.

Table 2. *Laboratory data of patients*

Patients (n=229)	$\overline{X}$±SD
WBC	9.6±4.7
HB	13.0±1.8
PLT	234.1±82.0
GGT	366.1±363.8
ALT	230.0±218.2
Amylase	421.2±943.6
Lipase	1173.3±3612.6
T. bilirubin	4.5±3.8
D. bilirubin	2.8±3.9

WBC: White blood cells; HB: Hemoglobin; PLT: Platelet; GGT: Gama glutamyl transferase; ALT: Alanine transferase.

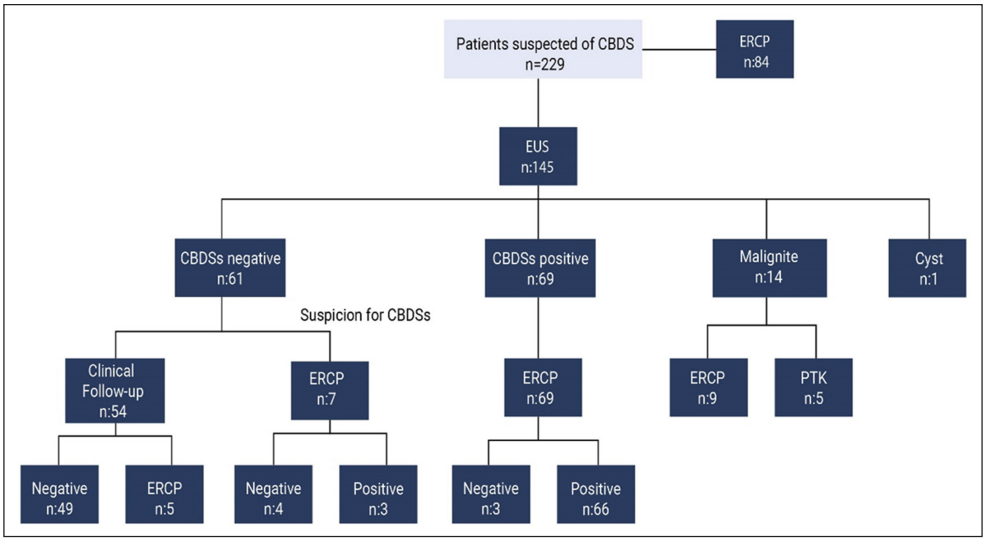

Figure 1. *Flowchart representing patient groups.*

The sensitivity of EUS was found to be 66/74×100=89.2; the specificity was 53/56×100=94.6; positive predictive value was 66/69×100=95.6; and negative predictive value was found as 53/61×100=86.9.

The diagnostic predictivity of the CBD, measured in AUS and EUS, were evaluated by ROC curve analysis in predicting the CBDSs. Accordingly, the choledochal diameter measured in AUS and EUS was found to have diagnostic values in predicting the CBDSs [AUC (% 95 GA p); respectively, 0,617 (0.409–0.825) p=0.310 and 0,765 (0.619–0.915) 0.020] (Fig. 2). Sensitivity, specificity, and the positive and negative predictive values are presented in Table 4 according to different cut-off values of measurement values.

Table 3. *Distribution of choledochal stone presence in EUS according to the presence of choledocholithiasis in participants*

	CBDS		
EUS CBDS	Negative n (%)	Positive n (%)	p
Negative	53 (86,9)	8 (13,1)	**<0.001**
Positive	3 (4,3)	66 (95,7)	

CBDSs: Common bile duct stones; EUS: Endoscopic ultrasonography.

Discussion

This study showed that EUS had high diagnostic efficiency in patients with suspected CBDSs and prevented patients from being exposed to unnecessary ERCP. No pathology was observed in most patients without choledochal stones in the EUS. Furthermore, this study revealed that patients who did not have a choledochal stone in the EUS could be followed up with after they were discharged.

Biliary pain and related complications occur in 10–25% of patients with gallstones[10,11], and major complications in 1–2%[12]. Symptoms often occur due to stones in the main bile duct. This condition can sometimes lead to life-threatening clinical signs. For this reason, the diagnosis and treatment of the main bile duct stones should not be delayed. In a recent guide, stone extraction treatment was recommended for the patients who had main bile duct stones with and without symptoms and who could tolerate the treatment[13].

The first imaging modality to be performed in patients with suspected choledocholithiasis is AUS[2]. Another

Table 4. *The diagnostic value of choledochal diameter measured in AUS and EUS in predicting choledochal stone*

	Choledochal diameter cut-off	Sensitivity	Specificity	PPD	NPD
AUS	8.25	72.8	57.1	94.4	17.3
EUS	7.1	76.8	71.4	96.3	23.8

EUS: Endoscopic ultrasonography; AUS: Abdominal ultrasonography.

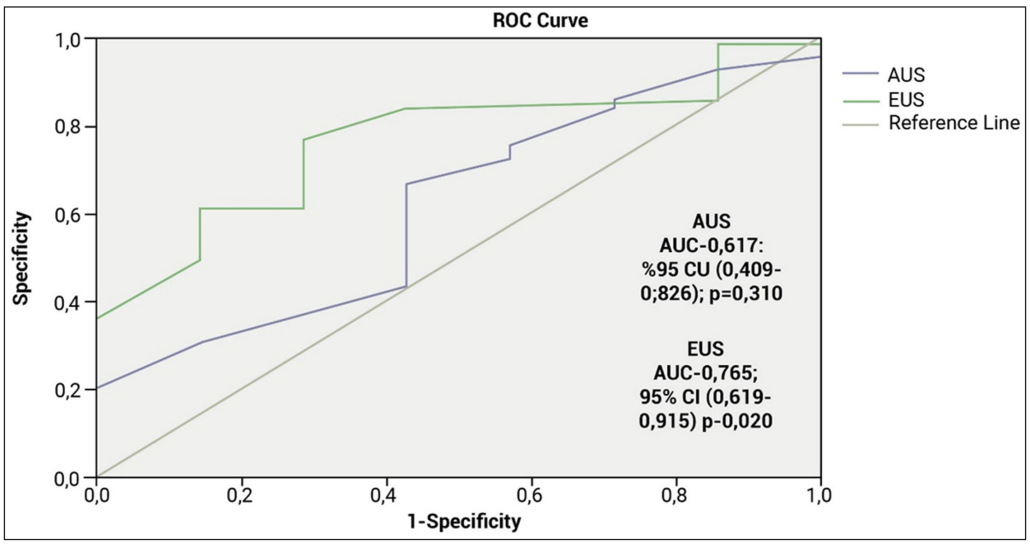

Figure 2. *Diagnostic predictivity of choledochal diameter measured in AUS and EUS in predicting choledochal stones.*

recommended first approach in patients with suspected CBDSs is liver function tests and AUS[9]. While AUS is a successful imaging method in showing gallstones, it is less successful in diagnosing CBDS. Abdominal ultrasonography has a sensitivity of 22–55% in detecting choledochal stones[14,15]. Distal choledochal stones are not often seen in AUS. The experience of a person who performs the ultrasonography, gastric bypass, and obesity are considered as factors restricting the use of AUS[14,16]. The sensitivity of the AUS is only 77–87%[3], even when evaluated in line with another parameter such as choledochal diameter dilatation. In our study, ROC analysis was performed at AUS on the of choledochal diameter to predict choledochal stones. The results showed that when the choledochal diameter is above 8.25 mm, the sensitivity is 72.8% and the PPD is 94.4%. Previous studies revealed that a diameter value above 8 mm strongly predicts biliary obstruction[17]. This study found the sensitivity of the AUS to be consistent with the literature. However, the results suggest that AUS does not have sufficient diagnostic efficacy in patients with choledocholithiasis.

In its 2010 guide, the ASGE recommended the application of ERCP for high-risk CDBS patients, while the application of MRCP or EUS methods were recommended for patients in the medium-risk group[2]. In another study, it was suggested that patients in the high-risk group might be exposed to unnecessary ERCP, and that for this reason, these patients should also first be evaluated with EUS[18].

Several studies were conducted on the diagnostic efficiency of EUS in patients suspected of having CBDSs. In a study that included 93 patients and was conducted to demonstrate the diagnostic efficiency of EUS in CBDSs, EUS was found to have a sensitivity of 100%, a specificity of 80%, a positive predictivity of 96.55%, and a negative predictivity of 100%[19]. In another study involving 62 patients, EUS sensitivity was found to be 96%, specificity was found to be 57%, positive predictivity was 88%, and negative predictivity was 80%[20]. In their study of 78 patients conducted in 2017, Patel et al.[18] found EUS sensitivity to be 93.9%, specificity was 97.3%, positive predictivity was 96.6%, and negative predictivity 94.7%. In a study involving a total of 200 patients at moderate and high risk for choledocholithiasis, Jeon et al.[21] found EUS sensitivity to be 97.5%, specificity to be 79.5%, positive predictivity to be 95.2%, and negative predictivity to be 88.6%.

In our study, the sensitivity of EUS to CBDSs was 89.2%, the specificity was 94.6%, the positive predictivity value was 95.6%, and the negative predictivity value was 86.9%. Subsequent 6-month clinical and biochemical follow-ups of patients who were not detected to have stones using EUS were included in the calculation of these values. In 49 of the 54 patients who received clinical follow-up, no pathology was found in their follow-ups. These findings suggest that EUS can significantly reduce unnecessary ERCP applications. In addition, no EUS-related complications were reported. Patients detected with choledochal stones in EUS were taken to

ERCP in the same session. A recent study has reported that performing EUS and ERCP in the same session is both safer and less expensive[22]. In our study, the predictive feature of the evaluation of the choledochal diameter with EUS in predicting the choledochal stones was detected by ROC analysis. According to this analysis, the sensitivity was found to be 76.8% and the specificity was 71.4% when the choledochal diameter cut off value was at 7.1 mm in EUS.

Since EUS is both a high-diagnostic and a low-invasive diagnostic method, it is increasingly used in patients with suspected CBDSs. One of the most important contributions of the EUS is undoubtedly the prevention of unnecessary ERCPs. In this study, we aimed to investigate the role of EUS in patients with moderate to high probability of CBDSs. Referral of suspected patients with CBDSs to a center with EUS and an experienced endoscopist will ensure that the patient receives the correct diagnosis and is not subjected to unnecessary invasive procedures.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

The Importance of Intense Pain Management for the Treatment Success of Extracorporeal Shock Wave Lithotripsy

Ekstrakorporeal Şok Dalga Litotripsinin Tedavi Başarısında Yoğun Ağrı Yönetiminin Önemi

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ABSTRACT

Aim: *Shockwave lithotripsy is a painful procedure and requires adequate analgesia. This study aimed to determine the effect of effective pain management in extracorporeal shock wave lithotripsy on objective parameters such as radiation dose and operation time.*

Material and Method: *A total of 202 patients were included in the study. The patients were divided into two groups. The first group was administered a triple analgesia combination (hyoscine N-butylbromide, metamizole, pethidine) for adequate pain control. The second group was administered diclofenac sodium alone.*

Results: *There were 100 patients in group 1 and 102 in group 2. There was a statistically significant difference between the groups in the duration of treatment, lithotripsy period, frequency of shock waves, and radiation doses used (p<0.05).*

Conclusion: *Effective pain management in ESWL reduces the procedure time, period, and radiation dose, thus increasing the success of the treatment.*

Key words: *ESWL; renal stone; lithotripsy*

ÖZET

Amaç: *Şok dalgası litotripsi ağrılı bir işlemdir ve yeterli analjezi gerektirir. Bu çalışmada ekstrakorporeal şok dalga litotripsisinde etkin ağrı yönetiminin radyasyon dozu ve ameliyat süresi gibi objektif parametrelere etkisinin belirlenmesi amaçlanmıştır.*

Materyal ve Metot: *Çalışmaya toplam 202 hasta dâhil edildi. Hastalar iki gruba ayrıldı. Birinci gruba etkili ağrı kontrolü için üçlü analjezi kombinasyonu (hiyosin N-bütilbromür, metamizol, petidin) uygulandı. İkinci gruba tek başına diklofenak sodyum verildi.*

Bulgular: *Grup 1'de 100, grup 2'de 102 hasta vardı. Tedavi süresi, litotripsi süresi, şok dalgalarının sıklığı ve kullanılan radyasyon dozları açısından gruplar arasında istatistiksel olarak anlamlı fark vardı (p<0,05).*

Sonuç: *ESWL'de etkin ağrı yönetimi işlem süresini, süresini ve radyasyon dozunu azaltarak tedavinin başarısını artırmaktadır.*

Anahtar kelimeler: *ESWL; böbrek taşı; litotripsi*

Introduction

Urolithiasis is a common pathology and its highest incidence occurs between 30 and 40 years of age. Approximately 84% of renal calculus are calcium oxalate and phosphate calculus, but uric acid and infection calculus are rare[1]. Approximately 11.1% of urinary stones are encountered between the 10th and 70th decades[2].

Extracorporeal Shock Wave Lithotripsy *(ESWL)* is an effective method with low complication rate that is frequently used in the treatment of urolithiasis. However, since it is a painful procedure, it requires pain management. Inadequate pain control reduces the effectiveness of the procedure. In pain control, non-steroidal anti-inflammatory drugs *(NSAIDs),* analgesics or opioid agents can be administered locally, intravenously *(IV),* and orally. Opioid agents may not be the first choice because of longer follow-up, side effects such as nausea, hypotension, respiratory depression, and vomiting[3].

The power of the energy used in ESWL is important in breaking the urinary system stones. In the use of strong energy, the pain threshold of the patients should be increased with medication. This prevents patient movements and provides an effective treatment with high energy and in a short time[4].

In ESWL, patient-related variables can affect pain. Patients with younger age *(adolescents),* gender *(female),* depression and anxiety, and patients with low body mass index are more sensitive to pain. Extracorporeal Shock Wave Lithotripsy related

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variables can be listed as the type of shock wave, energy power, entry location, location and size of the stone[5].

Uninterrupted transmission of high energy is effective in the success of ESWL, and for this, the patient's immobility plays an important role. Intense analgesia reduces patient movement. It is also important that a new generation of lithotripsy devices that cause less pain have been developed[6]. This has led to the use of topical drugs and local anesthetics, oral and intravenous sedatives. In addition, transcutaneous electrical nerve stimulation, anesthetic local administration, intravenous administration, inhaled administration can be used.

Opioid agents may not be the first choice because of their side effects and costs. These agents can be preferred in selected patients. General or spinal anesthesia may be preferred in selected patients and children[4].

Despite developing technology, ESWL is still a painful treatment and requires adequate analgesia. The aim of this research is to manage effective pain; To investigate the effectiveness of ESWL through objective parameters such as radiation duration and dose, energy amount and duration, number of shocks, and procedure time.

Materials and Method

This study adhered to the tenets of Helsinki Declaration of the World Medical Association, and received full approval from Scientific Research Ethics Committee of Agri Ibrahim Cecen University (2022–73). A total of 202 patients with urinary system stones were included in the study. Two groups were created before ESWL application. In the first group, triple analgesia combination consisting of hyoscine N-butylbromide, metamizole and pethidine was applied for effective pain control. The second group was given diclofenac sodium for analgesia. Objective parameters *(ESWL duration, frequency, radiation dose, amount and duration of shock wave energy used)* of the procedures applied to the groups were recorded retrospectively.

Patients with known drug hypersensitivity, coagulation disorders or anticoagulants, uncontrolled hypertension, pregnant women, urinary system infection, abdominal aneurysm, distal ureteral stones, obesity and skeletal disorders were not included in the study. In addition, patients who left the ESWL procedure unfinished were also excluded from the study. Extracorporeal Shock Wave Lithotripsy procedure was performed by a single expert with a single device.

Data were analyzed with SPSS *(Statistical Package for the Social Sciences)* version 21.0 program. Mann Whitney U-Test and Chi-square Test for categorical variables were used for group comparisons. A value of $p<0.05$ was defined as statistical significance.

Results

There were 100 patients in the Group 1 that were given triple drug combination for effective analgesia. The Group 2 individuals were given only diclofenac sodium consisted of 102 patients. Half an hour before the ESWL procedure, pethidine was administered intramuscularly at a dose of 0.8 mg/kg. Diclofenac sodium was administered intramuscularly at a dose of 75 mg half an hour before the procedure. Other drugs were injected in hydrochloride saline (100 cc).

Demographic characteristics and objective parameters of the study patients are given in Table 1. There was no statistically significant difference between the two groups in terms of gender, age, location of urinary system stones and the size of the stones ($p>0.05$).

Considering the objective parametric data, treatment duration and period were shorter in Group 1 than in Group 2, and a statistically significant difference was found ($p=0.002$).

When we compared the frequency of shock waves used in ESWL, a statistically significant difference was found in Group 1 compared to Group 2 ($p<0.0001$).

Considering the applied energy doses, the applied energy dose in Group 1 was more effective than Group 2 and a statistically significant difference was found ($p<0.0001$).

The radiation range and dose used to locate the stones were also lower in Group 1 compared to Group 2, and a statistically significant difference was found ($p<0.0001$).

Discussion

General anesthesia was used for pain control in the early years of ESWL. With the development of lithotriptors, general anesthesia has been replaced by less invasive anesthetic methods today, except for selected patients. However, ESWL is still a painful procedure today. Shock waves can cause pain by various mechanisms as they pass through the skin, muscles, kidney capsule and deeper internal structures. Accompanying body movements with pain causes difficulties in focusing the stones[7,8].

Table 1. *The demographic and clinical features of patients*

		Total N=202	Grup 1 N=100	Grup 2 N=102	p-value
Gender (N (%))	Male	144 (71,3)	**63 (63,0)**	**81 (79,4)**	**0.013**
	Female	58 (28,7)	**37 (37,0)**	**21 (20,6)**	
Age (year, median and interquartile range (IQR*))		46 (34–57)	**43 (33–52)**	**47 (37–60)**	**0.026**
Localisation (N (%))	Right	78 (38,6)	36 (36,0)	42 (41,2)	0.497
	Left	124 (61,4)	64 (64,0)	60 (58,8)	0.719
Stone localisation (N (%))	Renal Upper Clayx	17 (8,4)	6 (6,0)	11 (10,8)	0.225
	Renal Medium Clayx	43 (21,3)	25 (25,0)	18 (17,6)	0.286
	Renal Pelvis	75 (37,1)	38 (38,0)	37 (36,3)	0.908
	Renal Lower Clayx	14 (6,9)	6 (6,0)	8 (7,8)	0.593
	Ureter Upper End	37 (18,3)	19 (19,0)	18 (17,6)	0.869
	Ureter Medium	16 (7,9)	6 (6,0)	10 (9,8)	0.317
Dimensions of stone (N (%))	5×10	95 (47,0)	40 (40,0)	55 (53,9)	0.124
	10×15	98 (48,5)	55 (55,0)	43 (42,2)	0.225
	15×20	9 (4,5)	5 (5,0)	4 (3,9)	0.739
Treatmenttime (minute, medianand IQR)		33 (30–36)	**32 (30–34)**	**34 (30–41)**	**0.002**
X-Ray Application (second, medyan median and IQR)		33 (24–46)	**29 (20–40)**	**37 (29–54)**	**<0.0001**
X-Ray Dose (cyG/cm^2, median and IQR)		116.57 (90,39–173,51)	**91.13 (58,81–102,76)**	**169.46 (133,95–251,86)**	**<0.0001**
ScWCount (medianand IQR)		2817 (2500–2965)	**2941 (2800–2988)**	**2548 (2334–2881)**	**<0.0001**
EW Frequency (P/minute, median and IQR)		120 (120–120)	120 (120–120)	120 (120–120)	1.000
EW Time (minute, median and IQR)		23 (21–25)	23 (21–25)	24 (21–25)	0.109
EW Energy (J., median and IQR)		87.07 (71,37–96,34)	**95.58 (85,98–103,63)**	**72.45 (51,12–87,71)**	**<0.0001**
M. Energy (J., median and IQR)		4.0 (3,0–4,7)	**4.5 (4,0–5,05)**	**3.2 (2,7–4,0)**	**<0.0001**
A. Energy (J., median and IQR)		2.0 (1,6–2,4)	**2.3 (2,0–2,6)**	**1.8 (1,3–2,0)**	**<0.0001**

* IQR: Interquartile Range.

Stone size is one of the most important factors determining the effectiveness of ESWL, and the best clinical results are achieved in calculus smaller than 2 cm. Considering the stone structures, cystine calculus are resistant to ESWL. Extracorporeal Shock Wave Lithotripsy is contraindicated in conditions such as pregnancy, uncontrolled hypertension, severe sepsis, obesity. Pain mechanisms in ESWL are not clear, nociceptor inductions by microbubbles can cause visceral and peritoneal pain. The pain threshold is personal, and the patient's compliance and perception of anxiety affect the success of ESWL[9].

Local anesthesia can be used in ESWL. The pain is felt on the skin surface and gives signs of erythema and petechiae[10]. Akçali et al.[11] compared paracetamol, lornoxicam and tramadol and found no significant difference in clinical efficacy, but reported that they provided adequate analgesia in ESWL. In another study; they found no significant difference between the efficacy of NSAIDs and opioids. However, opioids were reported to be effective in the first 10 minutes. It has been shown to provide adequate analgesia for both and the mean pain scores are similar[1].

Combination treatments of local anesthetics and parenteral drugs are preferred for pain control in ESWL[12]. Pentazosin + Lorazepam combination was found to be more effective in the comparison of pentazocine/morphine/lorazepam[13]. In another study, it was shown that intramuscular sodium diclofenac is more effective than local anesthetics and its combination has no superiority[14].

Effective analgesia provided positive benefits in all these parameters when the criteria for radiation duration, radiation dose, energy application time and amount, total number of shocks were evaluated according to the study plan. Extracorporeal Shock Wave

Lithotripsy period and frequency were significantly lower in effective analgesia. Significant results were also found in the applied energy doses. Maximum energy doses administered to patients in effective analgesia provided effective treatment. The radiation range and dose used during the ESWL procedure were also reduced with effective analgesia.

It is evident that pain management in ESWL is directly related to the success of the procedure as well as patient comfort. The lack of pain scoring in our study was a missing aspect of the study.

Conclusion

Effective analgesia management in ESWL reduces the procedure time, period and radiation dose. It is expected to increase the relative effectiveness of the treatment, as it facilitates the effective transfer of the energy used and the focusing process in the stone localization.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Knowledge of Health Professionals on Blood Transfusion Practices, Storage, Clinical Use and Reactions

Sağlık Profesyonellerinin Kan Transfüzyon Uygulamaları, Saklanması, Klinik Kullanımı ve Reaksiyonları Hakkında Bilgi Düzeyi

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ABSTRACT

Aim: *Blood transfusion is also a tissue transplant and continues to be applied in many areas. Blood Transfusion training should be given to all healthcare professionals, and information should be updated in light of new developments. In our study, we aimed to evaluate the knowledge of physicians and nurses about the clinical use and transfusion of blood products.*

Material and Method: *A questionnaire consisting of 16 questions was applied to 147 doctors and nurses working in the surgical and coronary intensive care units of XXXX Hospital between 01.10.2021 and 15.10.2021 to determine and compare their knowledge levels on blood and blood product transfusion. Questionnaire questions were prepared under four main headings: Basic transfusion information, clinical use of blood products, transfusion reactions, and storage of blood products.*

Results: *65.3% (n: 96) of the participants were female, and the mean age was 29.6±6.5. The mean age of the doctors and nurses, respectively (35.3±8.1 vs. 27.4±4.0 <0.001), was statistically significant. No employee could answer all questions correctly, and only four physicians answered 15 questions correctly. Nurses could answer a maximum of 13 questions (n: 3). While physicians gave statistically significant answers to 4 questions in total, it was determined that other healthcare professionals gave statistically better answers than physicians in only one question (Question 14). All employees' average correct answers were 9 (range 7–10). Physicians gave correct answers to 10 (9–12) questions and other healthcare professionals 9 (7–10) questions, and it was not statistically significant.*

Conclusion: *It is essential to maintain the knowledge level of physicians and health workers in transfusion practices after graduation. It should be ensured that they benefit from scientific meetings and in-service training regarding current developments.*

Key words: *blood transfusion; health care professionals; hemovigilance; blood supply safeties*

ÖZET

Amaç: *Kan transfüzyonu da bir doku naklidir ve birçok alanda uygulanmaya devam edilmektedir. Transfüzyon eğitimi tüm sağlık çalışanlarına verilmeli ve yeni gelişmeler ışığında bilgiler güncellenmelidir. Çalışmamızda hekim ve hemşirelerin kan ürünlerinin klinik kullanımı ve transfüzyonu hakkındaki bilgilerini değerlendirmeyi amaçladık.*

Materyal ve Metot: *Kan ve kan ürünleri transfüzyonu konusunda bilgi düzeylerinin belirlenmesi ve karşılaştırılması amacıyla xxxxx Hastanesi cerrahi ve koroner yoğun bakımları ile servislerinde çalışan 147 doktor ve hemşireye 01,10,2021–15,10,2021 tarihleri arasında toplam 16 sorudan oluşan bir anket uygulanmıştır. Anket soruları Temel transfüzyon bilgileri, Kan ürünlerinin klinik kullanımı, Transfüzyon reaksiyonları, Kan ürünlerinin saklanması konularında dört ana başlık olarak hazırlanmıştır.*

Bulgular: *Katılımcıların %65,3 (n: 96) kadın idi ve yaş ortalaması 29,6±6,5 olarak saptandı. Doktorların ve hemşireleri yaş ortalaması sırasıyla (35,3±8,1 vs. 27,4±4,0<0,001) istatistiksel olarak anlamlı bulundu. Hiçbir çalışan tüm sorulara doğru yanıt veremedi ve sadece dört hekim 15 soruya doğru yanıt verdi. Hemşireler en fazla 13 soruya cevap verebildi (n: 3). Toplamda dört soruda hekimler istatistiksel olarak anlamlı yanıt verirken, diğer sağlık çalışanları yalnız bir soruda (soru 14) hekimlerden istatistiksel olarak daha iyi yanıt verdikleri belirlendi. Toplamda tüm çalışanların doğru cevap verme ortalaması 9'du (7–10 aralığı). Hekimler 10 (9–12), diğer sağlık çalışanları 9 (7–10) soruya doğru yanıt vermişlerdi ve istatistiksel olarak anlamlı saptanmadı.*

Sonuç: *Hekim ve sağlık çalışanlarının transfüzyon uygulamalarındaki bilgi düzeylerinin mezuniyet sonrası da devam ettirilmesi önemlidir. Güncel gelişmeler ile ilgili olarak hizmet içi eğitimlerin yansıra bilimsel toplantılardan da yararlanmaları sağlanmalıdır.*

Anahtar kelimeler: *kan transfüzyonu; sağlık profesyonelleri; hemovijilans; kan ürün güvenliği*

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Introduction

Blood is known as a tissue with many functions consisting of cells and structures. Therefore, blood transfusion is also a tissue transplant[1]. It has many indications such as acute bleeding, surgical operations, increasing the hemoglobin level in anemia of serious chronic diseases, and correcting bleeding disorders. The basic rule in transfusion is to safely supply, transport, store and transfuse the blood components required for the patient[2].

In 2020, the need for blood donation was 2,704,636 units, and the Turkish Red Crescent met 90% of them. A total of 229,132 units of blood were used in 13 centers serving as Temporary Regional Blood Centers, 29% of which was provided by the Turkish Red Crescent. Turkish Red Crescent met 95% of the need for 2,448,445 units of blood of 1,131 hospitals with the status of Transfusion Center[3].

Although transfusion applications save lives when necessary, they can also cause life-threatening complications if appropriate conditions are not provided. Transfusion-related complications may occur due to erroneous practices such as not recording the patient's personal information correctly, using the wrong tube, inconsistency, not paying attention to the administration time, and giving it with inappropriate fluids[4]. According to the British Serious Hazard of Transfusion (SHOT) data, the most important cause of transfusion-related deaths is ABO incompatibility, and 19 ABO-incompatible transfusions and 1495 near-miss events were reported between 2016 and 2020. Again, in the same reporting system, 39 transfusion-related deaths were reported in 2020, and 14 (35.9%) of them were found to be preventable[5].

It is important for healthcare professionals to have sufficient knowledge and skills in order to perform safe blood transfusion. Persons who put and apply transfusion indications should have sufficient knowledge and skills about giving the right blood to the right patient, informing the patient about transfusion, keeping the blood appropriately and observing the patient for signs of reaction during transfusion, preventing possible complications and what to do when complications develop. In our study, we aimed to evaluate the knowledge of physicians and nurses about the clinical use and transfusion of blood products.

Material and Methods

In order to determine and compare their knowledge levels on blood and blood product transfusion, a questionnaire consisting of 16 questions was applied to 147 doctors and nurses working in the surgery and cardiology intensive care units of our center between 01.10.2021 and 15.10.2021.

In the questionnaire titled "Investigation of Knowledge Levels of Health Care Professionals on Blood Transfusion and Reactions", eight questions were asked including demographic information such as age and gender, as well as professional information such as duration of work, transfusion practices in their clinics, and whether they participated in in-service training on blood transfusion. Questionnaire was prepared under four main headings (Table 1):

1. Basic transfusion information
2. Clinical use of blood and blood products
3. Transfusion reactions
4. Storage of blood products

Continuous variables were expressed as mean and standard deviation, and categorical variables were expressed as percentages. Student-t test was used to compare data with normal distribution, and Mann-Whitney U test or Chi-square (X^2) test was used to evaluate data that did not show normal distribution. p value <0.05 was considered statistically significant.

Results

0%.65 (n: 97) of the participants were female and the mean age was 29.6±6.5. The mean age of the doctors and nurses respectively (35.3±8.1 vs. 27.4±4.0<0.001) was found to be statistically significant compared to other healthcare professionals. The number of employees in surgical departments was 109 (74.1%). A statistically significant difference was found among those who worked for 0–5, 5–10, 10 years or more according to the working year. 57.8% (n: 85) of the participants had been working for less than 5 years and 66.7% (n: 98) stated that they had received transfusion training. In the question about how the transfusion decision was made, the number of employees who knew the decision correctly according to the hemoglobin and hematocrit values was found to be 112 (76.2%), and no statistical significance was found (Table 2).

Table 1. *Survey questions*

1. Age:
2. Title:
3. Department:
4. Years in profession?
5. Transfusion frequency in your inpatient clinic?
6. Who makes the transfusion decision?
7. Did you join any training, course or seminar etc. related to blood transfusion?
8. Which of the following is taken into consideration when planning a blood transfusion decision in your clinic?

Basic transfusion information

1. Which of the following is false regarding the transfusion of blood products?
2. Which is wrong about the identity verification process of the recipient before the transfusion?
3. Which of the procedures to be done after the blood product is taken from the transfusion center and brought to the service is incorrect?
4. Which of the applications in the blood product request is wrong?

Clinical use of blood products

1. Which of the following is false?
2. Which of the following is not one of the transfusion transmitted diseases should be screened in our country?
3. Which of the following is false regarding transfusion in emergencies?

Transfusion reactions

1. Which of the following is not a cause of transfusion errors?
2. Which of the following is not a manifestation of acute hemolytic transfusion reaction?
3. Which of the following is not one of the things that should be done in an acute hemolytic transfusion reaction?
4. Which of the following is not a finding seen in transfusion-related acute lung injury?
5. Which of the following is not a suspicious finding of allergic reaction?
6. It is not one of the characteristics of transfusion-related circulatory overload (TACO)?

Storage of blood products

1. Which of the following is false regarding the storage and transfer of blood products?
2. Which of the following is false about massive transfusion?
3. Which of the following is incorrect regarding the duration of use of blood products?

Table 2. *The general characteristics of the participanst and their approach to transfusion decisions according to their educational information*

Variables	All group n: 147 (%)	Doctors n: 41(%)	Other healthcare professionals n: 106 (%)	P value
Age, mean (SD)	29.6±6.5	35.3±8.1	27.4±4.0	**<0.001**
Department (surgical)	109(74.1)	28 (68.3)	81 (76.4)	0.31
Profession years				
0–5	85 (57.8)	20 (48.8)	65 (61.3)	**<0.001**
5–10	29 (19.7)	–	29 (27.4)	
10 and above	33 (22.4)	21 (51.2)	12 (11.3)	
Transfusion frequency				
Seldom	10 (6.8)	2 (4.9)	8 (7.5)	0.72
Often	137 (93.2)	39 (95.1)	98 (92.5)	
Transfusion training	98 (66.7)	16 (39)	82 (77.4)	**<0.001**
Transfusion decision accuracy	112 (76.2)	29 (70.7)	83 (78.3)	0.33

In total, 16 multiple choice questions were asked to all healthcare professionals (Table 1). No employee could answer all the questions correctly, and only 4 physicians answered 15 questions correctly. Nurses were able to answer a maximum of 13 questions (n: 3). The least correct answer number was 7 (7.5%). The most correct answer by the participants was question 1 (98%). It was found that other healthcare professionals responded better than doctors to questions asked about procedures such as questions 3 and 14. It was observed that physicians responded significantly better than other healthcare personnel, especially to the questions of blood transfusion reactions. While physicians gave statistically significant answers to 4 questions in total, it was determined that other healthcare professionals gave statistically better answers than physicians in only one question (Question 14). Table 3 shows the response rates to the questions.

Table 3. *Comparison of questions between physicians and other healthcare professionals*

n (%)	All group n: 147	Doctors n: 41	Other healthcare professionals n: 106	P value
Q 1	144 (98)	41 (100)	103 (97.2)	0.56
Q 2	64 (43.5)	16 (39)	48 (45.3)	0.49
Q 3	90 (61.2)	19 (46.3)	71 (67)	0.02
Q 4	120 (81.6)	36 (87.8)	84 (78.2)	0.34
Q 5	108 (73.5)	31 (75.6)	77 (72.6)	0.71
Q 6	98 (66.7)	38 (92.7)	60 (56.6)	**<0.001**
Q 7	11 (7.5)	3 (7.3)	8 (7.5)	0.99
Q 8	130 (88.4)	37 (90.2)	93 (87.2)	0.78
Q 9	43 (29.3)	29 (70.7)	14 (13.2)	**<0.001**
Q 10	64 (43.59	16 (39)	48 (45.3)	0.49
Q 11	79 (53.7)	32 (78)	47 (44.3)	**<0.001**
Q 12	32 (21.8)	14 (34.1)	18 (17)	0.02
Q 13	87 (59.2)	32 (78)	55 (52)	**0.004**
Q 14	61 (41.5)	10 (24.4)	51 (48.1)	**0.009**
Q 15	91 (61.9)	27 (65.9)	64 (60.4)	0.54
Q 16	78 (53.1)	28 (68.3)	50 (47.2)	0.02

Q: Question.

Table 4. *Comparison of physicians and other health workers according to survey sections*

	All group n: 147	Doctors n: 41	Other healthcare professionals n: 106	P value
Basic transfusion informations	3 (2–4)	2 (2–3)	3 (2–4)	0.18
Clinical use of blood products	2 (1–2)	2 (2–2)	1 (1–2)	0.003
Transfusion reactions	3 (2–4)	4 (3–4)	3 (2–3)	**<0.001**
Storage of blood products	2 (1–2)	2 (1–2)	2 (1–2)	0.81
Total	9 (7–10)	10 (9–12)	9 (7–10)	0.005

The average of correct answers of all employees was 9 (range 7–10). Physicians gave correct answers to 10 (9–12) questions and other healthcare professionals 9 (7–10) and it was not statistically significant. The general average of transfusion reactions questions among all question groups was found to be significantly higher in the physician group (Table 4).

Discussion

In this study, the knowledge of age, professional years and transfusion training of physicians and nurses working in our hospital on blood and blood product use and reaction was made. Both groups were compared in terms of basic transfusion information, clinical use of blood products, transfusion reactions, and storage of blood products, and it was determined that physicians were better than nurses in terms of use and reaction.

Blood transfusion is a procedure that is performed in emergencies, inpatient clinics, post-traumatic, intraoperative and many other fields of medicine. It is a complex, multidisciplinary and multi-stage process, although it is thought of as a simple process because we use it frequently in our daily practice. Each step must be performed according to the written procedure, as any incorrect or incomplete step can lead to a fatal outcome. Blood transfusion is now considered a tissue transplant, and procedures are emerging in relation to the subject. Pre-transfusion verifications are essential for patient and employee safety. Although it is tried to close the deficiencies in blood transfusion with in-service trainings after university education, this process should be learned by all authorized health professionals. We measured the level of knowledge of physicians and nurses working in areas where blood transfusion is

common in our hospital, by asking questions about the basic knowledge of blood transfusion, its clinical use, reactions and storage.

In our study, it was seen that the level of knowledge of physicians about transfusion reactions requiring medical knowledge was higher than that of other healthcare professionals, and this was statistically significant. There was a significant difference between the two groups according to their transfusion education status. In a survey conducted between public and private health centers in Pakistan, it was shown that as the years of service increase, the knowledge and experience of transfusion increases[6]. In another study to investigate awareness and perceptions of blood safety and blood donation among healthcare professionals in a teaching hospital in Kolkata, only 69.7% of resident physicians, 43.3% of other healthcare professionals, 23.3% of physicians and 8% of nurses. It has been shown that 1% do not know the mandatory tests required in transfusion[7]. In our study, 92% of physicians and 56.6% of other healthcare professionals answered the question of mandatory tests (question 6) that should be done in transfusion correctly, and the difference between them was found to be statistically significant (38 (92.7%) vs. 60 (56.6%) p<0.001).

Among the necessary steps for safe transfusion, the correct identification of the patient and blood sample is very important. It is essential that the patient's identification number (ID) bracelet matches the ID barcode of the blood or blood product. In the study of Hiji et al. with 49 nurses, it was reported that 29% of the nurses confirmed the ABO compatibility of the blood bag and ID bracelet, while 4% checked the patient identification wristband, blood bag, blood request form and the information recorded in the patient observation form[8]. In our study, the correct response rates of physicians regarding the approach to transfusion reactions were found to be significantly higher than those of other healthcare professionals.

Limitations of the study are the data of this survey are limited to the responses given by healthcare professionals working at a training and research hospital so, the results should not be generalized and the number of participants were less than as expected, more participation could be achieved.

Conclusion

In our center doctors and other healthcare professionals have enough exposure to blood transfusions but there is a need to further enhance their knowledge regarding complications of transfusions and rational clinical use of blood and blood products. In addition, it should be encouraged by the country's health authority to provide transfusion training and to ensure that repeated at certain periods.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Relation Between Obstructive Sleep Apnea Syndrome and 25-Hydroxyvitamin D Levels in Patients at High Altitude

Yüksek Rakımda Obstruktif Uyku Apnesi Sendromu ile 25-Hidroksivitamin D Arasındaki İlişkinin Değerlendirilmesi

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ABSTRACT

Aim: *Present study's objective is to investigate the relationship between serum 25-hydroxyvitamin D and the severity of sleep apnea at altitude.*

Material and Method: *Fifty obstructive sleep apnea syndrome patients and 18 persons without apnea participated in this study. Apnea Hypopnea Index scores were measured with polysomnography during the night and classified as AHI<15:control, 15≥AHI<30 moderate, and AHI≥30 severe obstructive sleep apnea. In addition, the serum 25-hydroxyvitamin D levels of the patients were also measured.*

Results: *In the logistic regression analysis, it was found that there was an independent correlation between the apnea-hypopnea index and 25-hydroxyvitamin D (AUROC=0.658, p=0.028). We found a significant difference between the control and obstructive sleep apnea groups for 25-hydroxyvitamin D (p<0.05). A significant relationship has been found between moderate and severe obstructive sleep apnea groups for vitamin D deficiency and average vitamin D (p<0.0001).*

Conclusion: *We found an independent association between 25-hydroxyvitamin D and obstructive sleep apnea severity at high altitudes. An interesting point of our study is that our study has been conducted at a high altitude region with an elevation of more than 1768 meters. Therefore, effective vitamin D management may help prevent obstructive sleep apnea syndrome development, and patients' medical status can be improved.*

Key words: *25-Hydroxyvitamin D; polysomnography; obstructive sleep apnea syndrome; altitude*

ÖZET

Amaç: *Mevcut çalışmada, yüksek rakımlı coğrafi bölgede yaşayan hastalarda obstrüktif uyku apne sendromunun şiddeti ile serum 25-hidroksivitamin D düzeyleri arasındaki ilişkinin araştırılması amaçlandı.*

Materyal ve Metot: *Çalışmaya obstrüktif uyku apne sendromu olan 50 hasta ve 18 sağlıklı birey katıldı. Apne Hipopne İndeksi skorları tüm gece polisomnografisi ile ölçüldü ve AHI<15:kontrol, 15≥AHI<30 orta derecede obstrüktif uyku apnesi ve AHI≥30 şiddetli obstrüktif uyku apnesi olarak sınıflandırıldı. Hastaların serum 25-hidroksivitamin D düzeyleri ölçüldü.*

Bulgular: *Lojistik regresyon analizinde apne hipopne indeksi ile 25-hidroksivitamin D düzeyleri arasında bağımsız korelasyon görüldü (AUROC=0,658, p=0,028). Kontrol grubu ile obstrüktif uyku apnesi grubu arasında 25-hidroksivitamin D düzeyleri ile istatistiksel olarak anlamlı fark vardı (p<0,05). D vitamini eksikliği ve normal D vitamini düzeyleri için orta ve şiddetli obstrüktif uyku apnesi grupları arasında anlamlı ilişki gözlendi (p<0,0001).*

Sonuç: *Yüksek rakımda yapılan mevcut çalışmada 25-hidroksivitamin D düzeyleri ile obstrüktif uyku apnesi şiddeti arasında bağımsız bir ilişki olduğunu gözlemlendi. Çalışmamız rakımı 1768 metreden fazla olan yüksek rakımlı bölgede yapılmıştır. Vitamin D düzeylerinin dikkatli yönetimi, obstrüktif uyku apne sendromu gelişiminin önlenmesinde tıbbi durumlarının iyileştirilmesinde yardımcı role sahip olabilir.*

Anahtar kelimeler: *25-hidroksivitamin D; polisomnografi; obstrüktif uyku apne sendromu; yüksek rakım*

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Introduction

Obstructive sleep apnea syndrome (OSA) is a combination of unexplained severe daytime sleepiness with at least five respiratory events (apnea or hypopnea) per sleep hour. Apnea is defined as a respiratory arrest for at least 10 seconds in adults during sleep, while hypopnea is defined as a reduction of ventilation for at least 50% compared to the baseline value for a minimum of 10 seconds. The evaluation of the diagnosis and the severity is measured with apnea hypopnea index (AHI) which consists average apnea and hypopnea count during sleep[1]. Gominak et al.[2] suggested that the sleep disorder epidemic in the world may be associated with vitamin D and there are potential epidemiological, anatomical connections between sleep disorders and vitamin D.

Discovery of receptors for vitamin D in brainstem areas responsible for sleep maintenance suggests that sleep management is affected by vitamin D. Immunological mechanisms overlapping with OSA and vitamin D make it necessary to address this issue[3]. Current study intended to investigate the relationship between vitamin D levels and sleep apnea severity in patients living in high altitude conditions.

Material and Methods

Sixty-eight patients who underwent nocturnal polysomnography in Kafkas University central sleep laboratory were included in the study. All patients were residing in Kars region for at least 24 months. All participants have given written consent. Ethics commission confirmation from Kafkas University Faculty of Medicine with Session No: 08, Approval No: 163 were obtained. This study was conducted at high altitude Kars region, Turkey which has an altitude more than 1768 meters.

According to recods during nocturnal poysomnography; apnea hypopnea index results were classified as following; AHI<15 as control group, 15≥AHI<30 as moderate OSA group and AHI≥30 as severe OSA group. 25-hydroxyvitamin D (25(OH)D) measurements were obtained from all participants and were classified as following; <20 ng/ml defined as vitamin D deficiency, 20–29 ng/ml defined as vitamin D insufficiency and ≥30 ng/ml defined as normal.

Patients with kidney failure, malignancy, active infection, rheumatoid arthritis, malabsorption, ankylosing spondylitis, collagen tissue disease, sarcoidosis, acute myocardial infarction, thyroid and parathyroid disease, bone metabolism disease, hepatic dysfunction, celiac disease were excluded from the study. Patients using calcium preparations, vitamin D, bisphosphonate, calcitonin, antiepileptic and steroid were omitted.

All participants underwent physical examination, height and body weight measurement. Body mass index (BMI) were defined as dividing weight (kilograms) and square of height (meters) (kg/m^2).

Serum 25-hydroxyvitamin D level was measured with analyzer Cobas e 411 (Roche Diagnostics GmbH, Mannheim, Germany). Polysomnography measurements were made at night time in the Kafkas University Medical School Center of Sleep Disorders, which is accredited by the Turkish Association of Sleep Medicine. Polysomnography device Embla N7000 system (Medcare; Reykjavik, Iceland) was used. During the polysomnography session; measurements of electroencephalography, body position measurements, electrocardiography, electrooculography, thoracoabdominal movements, submental muscle electromyography, nasal pressure, finger-tip oxygen saturation with finger oximeter, anterior tibialis electromyography, oronasal airflow measurement via thermal sensor were performed. Patient information, gender, height and body weight of the patients were recorded by the technician before the polysomnography.

Statistical Analysis

Statistical analysis of the study was obtained using the IBM SPSS 20.0 (IBM Inc. Chicago, IL.) Program. "Kolmogorov-Smirnov normality test" was used to analyze whether the distributions were normal or not, and "Levene test" was used to determine the homogeneous variance equality. Continuous variables were expressed as mean ± standard deviation or median, and categorical variables were expressed as patient count and percentages. In normal distribution data; the chi-square test was used in the analysis of categorical variables and the Independent t-test was used in the analysis of numerical variables. In comparing the variables; when hypotheses for continuous variables were confirmed One-Way ANOVA was used and when it was not provided Kruskal-Wallis H Test and Mann-Whitney U test was used.

Evaluating for different results from One-Way ANOVA, Tamhane Test was used in case of non-homogenous condition and Tukey Test was used in case of the homogenous condition. Paired-Samples' t-test was used to investigate the binary correlation of the variables. The Spearman's Degree Correlation test was used when the variables were homogeneous in the correlation analysis, and the Kendall correlation test was used when the variables were not homogeneous. In all comparisons, $p<0.05$ was considered significant and assessed at 95% confidence level.

Results

Forty-one of the patients were male (60.29%) and 27 (39.21%) were female. According to the polysomnographic results, 34 patients had severe OSA, 16 patients had moderate OSA and 18 of the patients were classified as the healthy control group. The distribution of the groups according to gender was given in Table 1.

In terms of variables such as age, sex, height, weight and BMI; age and weight showed a significant difference. There was no difference in height and BMI. Mean and standard deviation values of the age in moderate and severe apnea groups were found to be similar. In all OSA groups, the mean values of the height were not statistically significant and close to each other. The distribution of groups according to demographic variables was given in Table 2.

There was not a relation between the apnea hypopnea index and BMI ($p>0.05$). There was nor an association between vitamin D and BMI.

Statistically significant difference was found between moderate and severe OSA groups for vitamin D deficiency and normal 25(OH)D levels ($p<0.0001$). Significant difference was found between the control group and OSA group for 25(OH)D levels ($p<0.05$). The mean 25(OH)D was found to be highest in the control group (Table 3).

In the logistic regression analysis, it is found that there was an independent correlation between apnea hypopnea index and 25(OH)D (AUROC=0.658, $p=0.028$). ROC curve analysis of 25(OH)D is shown in Fig. 1.

Table 1. *Distribution of the patients according to the gender*

	Control	Moderate OSAHS	Severe OSAHS	Total
Male	9 (21.95%)	12 (29.26%)	20 (48.78%)	41 (60.29%)
Female	9 (33.33%)	4 (14.81%)	14 (51.85%)	27 (39.70%)
Total	18 (26.47%)	16 (23.52%)	34 (50.00%)	68 (100%)

Table 2. *Distribution of demographic characteristics of the patients according to the groups*

Variables	AHI <15 (N=18)	15≤AHI <30 (N=16)	30≤AHI (N=34)	p value
Age (Years)	41.1±12.5	52.8±11.7	53.5±11.2	<0.0001*
Gender (F/M)	9/9	4/12	14/20	1.000
Height (cm)	168.8±9.88	171.5±8.0	163.0±20.8	0.401
Weight (kg)	80.4±18.5	91.0±11.7	100.6±15.1	<0.0001*
BMI (kg/m^2)	28.2±6.67	31.1±5.45	36.8±7.47	0.241

AHI: Apnea Hypopnea Index.

Table 3. *Relation of groups to 25-Hydroxyvitamin D (ng/ml)*

	Control	Moderate OSA	Severe OSA	p value	P
Vitamin D deficiency	10.3±3.63	13.7±3.68	11.5±4.17	<0.0001*	
Vitamin D insufficiency	23.5±2.15	23.7±3.93	22.9±1.77	0.2329	<0.0001*
Normal vitamin D	72.75±0.00a	33.5±0.00a	44.4±19.0	<0.05*	

OSA: Obstructive Sleep Apnea Syndrome.

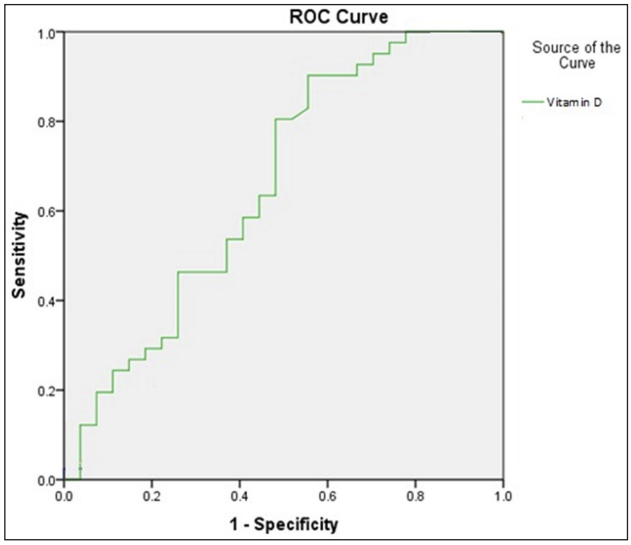

Figure 1. *ROC curve analysis of 25-Hydroxyvitamin D (AUC=0.658, 95% CI = 0.519–0.797, p=0.028)*

Discussion

In our study relation of apnea hypopnea index and serum 25-OH vitamin D in sleep apnea patient group and healthy controls were analyzed. For 25(OH) D levels, significant statistical difference was found between the control group and obstructive sleep apnea patients ($p<0.05$). For vitamin D deficiency and normal 25(OH)D levels we found significant difference between moderate and severe OSA groups ($p<0.0001$). In the logistic regression analysis, it is found that there was an independent association between apnea hypopnea index and 25(OH)D (AUROC=0.658, p=0.028).

Obstructive sleep apnea syndrome is a prominent threat for mortality and can be cause of morbidity. Risk factors include obesity and the winter season[4–7]. Metabolic disorders such as systemic inflammation, hyperglycemia, hyperlipidemia, increased bone density loss may also be associated with sleep apnea[8–10]. Lower vitamin D level is also related to the winter season, obesity, systemic inflammation and metabolic disorders[11,12]. 25(OH)D receptor existence in the brain regions responsible for sleep control has potential implications for obstructive sleep apnea syndrome etiology[13–16]. Presentation and severity of obstructive sleep apnea syndrome may be affected by vitamin D.

Lin et al.[8] have reported that high-sensitivity C reactive protein, one of the inflammation markers, is related to the severity of OSA, independent of other factors such as obesity. Vitamin D is also reported to be associated with systemic inflammation[17,18]. The transcription factor NF-κB, and tumor necrosis factor-alpha and interleukin-8 expression are increased in relation to sleep hypoxia[17]. NF-κB has an enhancing effect on the inflammatory cascade by upregulating interleukin-8, tumor necrosis factor-alpha cytokines[18]. Vitamin D is also known to have effects on these transcription factors and cytokines[19,20]. These overlapping features of OSA and vitamin D in the inflammatory processes suggest that the relationship between these two entities should be investigated[21].

At high altitudes, changes in breathing patterns are observed during sleep which comprises apnea intervals. Studies investigating between the high altitude and the severity of obstructive sleep apnea have been performed in only small patient groups[22]. Researchers reported that in OSA patients hypoxemia deteriorates at altitudes up to 2590 meters[23]. However, optimal treatment approaches and effects of high altitude on the severity of the disease in OSA patients living at high altitudes are largely unknown[24].

In another study 25(OH)D was found reduced in severe OSA patients[25]. A study reported that compared to control group, 25(OH)D were lower in the OSA patients[26]. Our research results are consistent with the studies conducted in Turkey, although our study was performed at a higher altitude. Our study is conducted at Kars, Turkey which has an elevation of more than 1768 meters. In a recent study conducted at a lower altitude than our study, it is reported that severe OSA patients had a higher prevalence of vitamin D deficiency[27]. Reports of research found that vitamin D deficiency in the severe OSA group was found to be highest compared to moderate and non-OSA groups[4]. Another study reported that decreased vitamin D was related with risk of AHI meausurement 30 or more[28]. The significant decrease of 25(OH)D in the severe obstructive sleep apnea group in our study was consistent with the literature.

In our study we found independent association between 25-hydroxyvitamin D and obstructive sleep apnea severity at high altitude. An interesting part of our study is the independent association between AHI and 25(OH)D levels (AUROC=0.658, p=0.028). Our findings may indicate that 25(OH)D may be attributed to obstructive sleep apnea etiology and the disease severity. An interesting point of our study is

that our study has been conducted at a high altitude region, which has an elevation of more than 1768 meters. Measurement of 25(OH)D levels in obstructive sleep apnea patients and, if necessary, adding 25(OH) D into the treatment may be considered as an appropriate option.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Does Subclinical Hypothyroidism Alter the Axis of QRS and P Waves?

Subklinik Hipotiroidi QRS ve P Dalgası Aksını Değiştirir mi?

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ABSTRACT

Aim: *What changes subclinical hypothyroidism (SCH) causes on the 12-lead surface electrocardiogram (ECG) has remained elusive. We examined the relationship between subclinical hypothyroidism and cardiac electromechanics, including P wave and QRS axes on ECG and cardiac functions by 2D speckle-tracking echocardiography (2D-STE).*

Material and Method: *This cross-sectional study included 109 SCH patients who presented to the internal disease outpatient clinic between November 10, 2018, and January 30, 2019. ECG, 2D-STE images, and laboratory findings at admission were recorded for all patients. Findings were compared with a sex and age-matched control group of 74 healthy adults.*

Results: *The median age of the patients was 41 (IQR, 34–50) years, and 76.1% were female. QTc interval was significantly longer in the patient group than in the control group.[435 ms (IQR, 421–457) vs. 424 ms (IQR, 412–438), p=0.001]. The remaining ECG features, including P wave and QRS axes, were similar between the patient and control groups. There were no significant differences between the patients and control group regarding laboratory and echocardiography findings, including left ventricle global longitudinal strain.*

Conclusion: *According to our findings, individuals with SCH exhibited no change in myocardial mobility as measured by strain echocardiography. In addition, SCH may not cause significant ECG changes, except that these patients have a longer QTc interval than subjects with euthyroidism.*

Key words: *ECG; electrocardiogram; QRS axis; QTc interval; speckle-tracking echocardiography; subclinical hypothyroidism*

ÖZET

Amaç: *Subklinik hipotiroidi (SCH)'nin yüzeyel 12-lead'li elektrokardiyografi (EKG)'de yaptığı değişikler tam olarak açıklığa kavuşmamıştır. SCH ile EKG'deki P dalgası ve QRS eksenleri ve 2D speckle-tracking ekokardiyografi (2D-STE) ile incelenen kardiyak fonksiyonlar dâhil kardiyak elektromekanik arasındaki ilişkiyi araştırmayı amaçladık.*

Materyal ve Metot: *Bu kesitsel çalışmaya 10 Kasım 2018 ile 30 Ocak 2019 tarihleri arasında dâhiliye polikliniğine başvuran 109 SCH hastası dâhil edildi. Tüm hastaların başvuru anındaki EKG, 2D-STE görüntüleri ve laboratuvar bulguları kaydedildi. Bulgular, 74 sağlıklı yetişkinden oluşan cinsiyet ve yaş uyumlu kontrol grubuyla karşılaştırıldı.*

Bulgular: *Hastaların ortanca yaşı 41 (IQR, 34–50) yıl ve %76,1'i kadındı. QTc süresi hasta grubunda kontrol grubuna göre anlamlı olarak daha uzundu.[435 ms (IQR, 421–457) vs. 424 ms (IQR, 412–438), p=0,001]. P dalgası ve QRS eksenleri dâhil kalan EKG özellikleri hasta ve kontrol grupları arasında benzerdi. Sol ventrikül global longitudinal strain dâhil ekokardiyografi ve laboratuvar bulguları açısından hastalar ve kontrol grubu arasında anlamlı fark yoktu.*

Sonuç: *Çalışmamıza göre, 2D-STE ile incelendiğinde SCH hastalarının miyokard hareketinde değişiklik olmadığını görüldü. Ayrıca, bu hastaların ötiroid bireylerden daha uzun QTc süresine sahip olması dışında, önemli EKG değişikliklerine neden olmadığı ortaya kondu.*

Anahtar kelimeler: *subklinik hipotiroidi; EKG; elektrokardiyogram; speckle-tracking ekokardiyografi; QTc süresi; QRS aksı*

Introduction

Thyroid dysfunction, often observed in the general population, is closely linked with the cardiovascular system[1]. It influences the hemodynamics, cardiac mass, cardiac contractility, and autonomic cardiovascular system control via the thyroid hormone receptors, which are abundantly available in the heart[2]. Moreover, thyroid dysfunction has been widely associated with various cardiovascular disorders. It is linked with increased cardiovascular and all-cause mortality[3].

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Subclinical hypothyroidism (SCH) is characterized by elevated thyroid-stimulating hormone (TSH) without clinical alterations and with thyroxine (T4) and triiodothyronine (T3) levels within the normal range[4]. Subclinical hypothyroidism might affect up to 20% of the population, and rates vary depending on sex, age, iodine deficiency, and the TSH reference values used in each study[5,6].

12-lead surface electrocardiogram (ECG) provides crucial data related to cardiac functions[7]. The relationship between the QRS axis and many diseases such as hypertension,[8] sickle cell anemia,[9] chronic kidney disease (CKD),[10] pulmonary hypertension (PH),[11] and chronic obstructive pulmonary disease (COPD)[12] was widely revealed. Also, it was shown that an abnormal P-wave axis could be regarded as an indicator of clinical and subclinical atrial pathology, including atrial inflammation and fibrosis[13].

Thyroid disorders exert a significant impact on ECG. Reports suggest that thyroid dysfunction can lead to ECG changes and arrhythmias, including sinus bradycardia, atrioventricular blocks, atrial arrhythmias, prolonged QTc, and severe ventricular arrhythmias[3,14–16]. However, no study is available investigating the relationship between SCH and P wave and QRS axes; both could indicate some cardiac abnormalities. Thus, our purpose was to analyze the association of SCH with cardiac electromechanics, including P wave and QRS axes on ECG and cardiac functions with 2D speckle-tracking echocardiography (2D-STE). We hypothesized that SCH might alter the P wave and QRS axes on ECG.

Material and Method

Patients with SCH who presented to the internal disease outpatient clinic between November 10, 2018, and January 30, 2019, were included in this cross-sectional study. Patients withhistory of thyroid diseases, heart failure (left ventricle ejection fraction below 50%), acute coronary syndrome, renal failure (eGFR <30 ml/min/1.73 m^2), atrial fibrillation, severe cardiac valve disorders, renal failure (eGFR <30 ml/min/1.73 m^2), chronic obstructive pulmonary disease (COPD), and being under 18 years old were specified as exclusion criteria. Cases with erroneous or artifact ECG and inadequate echocardiographic images for STE analysis were also excluded.

Demographics, ECG, TTE, and laboratory findings at admission were recorded, including TSH, free T3, and T4. Electrocardiogram records were performed using Cardio-M Plus ECG device (medicalECONET GmbH, Im Erlengrund 20, D46149 Oberhausen, Germany). Electrocardiogram analysis was executed by a blinded cardiologist (M. K) using a standardized comprehensive ECG reading protocol[17]. It comprised intervals, rate, P wave, QRS axes and morphology, premature atrial and ventricular contract, and T-wave abnormalities. The corrected QT interval (QTc) was estimated using the Fridericia formula[18].

Echocardiographic images were acquired utilizing Philips Epiq7 (Philips Healthcare, Inc., Andover, MA, USA) and recorded by standard techniques per the guidelines from American Echocardiography Association[19]. For TTE, Left ventricular end-systole and end-diastolic diameters, left atrium diameter, interventricular septum thickness, left ventricular posterior wall thickness, and right atrium and ventricle diameters were recorded. Mitral valve peak early (E-wave) and late (A-wave) diastolic filling velocities were measured. E/A ratio was also calculated. Early diastolic mitral velocities peak early (E-wave) and late (A-wave) diastolic filling velocities from septal and lateral annulus (e') were calculated in the apical four-chamber view[20]. Left ventricular ejection fraction (LVEF) was calculated operating the modified Simpson's rule[21].

Left ventricular global longitudinal strain (LVGLS) was studied by an experienced cardiologist (D. I.), who was blinded to the study, using the Qlab13 (Philips Healthcare, Andover, Massachusetts) software. While the end-diastole is considered the peak R wave of the ECG, end-systole was evaluated as aortic valve closure. Left ventricular global longitudinal strain was estimated by averaging the peak longitudinal strain values of apical two-chamber, apical three-chamber, and apical four-chamber images. Automatic endocardial margins were detected at the end-systole. Manual modifications were made to ensure accurate tracking and fit left ventricle wall thickness when required. The speckle-tracking examination was conducted per the Consensus Document of the EACVI/ASE/Industry Task Force to Standardize right ventricle and LV myocardial Deformation Imaging[22,23].

Kafkas University Ethics Committee approved the study protocol (Date: January 30, 2019; Number 80576354–050–99/27)

Statistical Analysis

SPSS program (Version 20.0, SPSS, Inc., Chicago, IL, institutionally registered software) was utilized for statistical examination. The Kolmogorov– Smirnov test was conducted for the normality test. Continuous variables showing normal distribution were expressed as mean ± standard deviation. Variables with no normal distribution are represented as median (IQR).

Categorical variables were defined as a percentage. An independent t-test was used to analyze continuous data exhibiting normal distribution, Mann-Whitney U test was employed to examine variables not showing normal distribution. A p value of <0.05 was regarded as statistically significant.

Results

Demographic, laboratory, ECG, and TTE data of 119 patients were recorded. Ten cases were excluded due to exclusion criteria. The final patient group included 109 subjects [median age 41 (IQR, 34–50) years, 76.1% female]. Findings were compared with a sex and age-matched [median age 37 (IQR, 34–44) years, 68.9% female] healthy control group consisting of 74 adults. Baseline data of the study population are summarized in Table 1. Thyroid-stimulating hormone level was significantly higher in the patient group than in the Control group [5.32 (IQR, 4.98–7) vs. 2.25 (IQR, 1–3.1), p<0.001]. The levels of fT3 and fT4 were similar between the groups. Other laboratory results were similar, except for the C-reactive protein (CRP) level, which was within normal clinically (reference range, 0–5 mg/L) (Table 1).

There were no significant differences between the patients and the control group regarding demographic characteristics and comorbidities, including BMI, hypertension , diabetes (DM), smoking, and hyperlipidemia (HPL) (Table 1).

For ECG, the QTc interval was significantly higher in the patient group than in the control group.[435 (IQR, 421–457) vs. 424 (IQR, 412–438), p=0.001]. The remaining ECG features, including P wave and QRS axes, were similar between the patient and control groups (Table 2).

Regarding echocardiographic characteristics, all parameters, including left and right ventricular functions and left ventricle global longitudinal strain (LVGLS), were similar between the two groups (Table 2).

Discussion

This cross-sectional analysis of 109 patients found no association of SCH with cardiac electromechanics, which suggests no influence of SCH on axes, rhythm, conduction, and systolic and diastolic functions of the heart. The only association found was a longer QTc interval in SCH patients than in the control group.

The association of the QRS axis with diseases such as HT, sickle cell anemia, CKD, PH, and COPD was documented[8–12]. It has been suggested that the change in the axis of QRS is due to the change in the geometry of the heart. There is no study investigating the relationship between SCH and P wave and QRS axes. Our study demonstrated echocardiographically that the geometry of the heart and systolic and diastolic functions did not change in these patients. Moreover, QRS and P axis did not alter either, supporting this.

Previous reports speculated that hypothyroidism might cause PR, QRS, and QTc interval alterations[24,25]. It is yet unclear what changes SCH causes on the ECG. Some large-scale works announced conflicting data. According to a report including 132,707 participants, like our study, patients with SCH had significantly longer QTc interval than subjects with euthyroidism[25]. Another cross-sectional analysis of 13,341 individuals in a Brazilian cohort found no association of SCH with HR, rhythm alterations, or conduction disorders[26]. Further, considering the 11,795 ECGs analysis in the same study, no abnormality was associated with SCH, even in the subgroup of older adults or those with extreme TSH values.

Strain measurement using speckle-tracking echocardiography (STE) is a recently developed technique to assess cardiac function[27]. Compared with conventional echocardiography measurement, this method is a more sensitive, reliable, and reproducible modality for assessing left ventricle systolic function, particularly deducing subtle left ventricle dysfunction in the early stage of the diseases[28]. Although a few small-scale types of research showed impaired LVGLS in patients with SCH, the effect of SCH on the left ventricle has remained elusive[29,30]. We found no evidence of left ventricle dysfunction in the current research according to strain measurements and conventional TTE. This outcome showed that the impact of SCH on myocardial functions was below the limits detectable with strain measurements.

Thus, our study provided valuable data for the literature as it showed that SCH does not cause consequential deterioration of left ventricular systolic function.

The main limitations of the current study were as follows; 1) a relatively small number of patients in our study might represent a significant limitation. 2) Because this is an observational study, it is impossible to draw direct conclusions on causality regarding the findings. More studies with many patients in this field are required to reveal the relationship between SCH and cardiac electromechanics.

In conclusion, our study showed that patients with SCH had no change in myocardial movement determined by 2D-STE. In addition, SCH may not cause significant ECG changes, except that these patients have a longer QTc than subjects with euthyroidism.

Table 1. *Demographic, clinical and laboratory characteristics of the participants*

	Overall (n=183)	Patient (n=109)	Control (n=74)	P-value
Female sex, n (%)	134 (73.2)	83 (76.1)	51 (68.9)	0.278
Age (years), median [IQR]	39 [34–48]	41 [34–50]	37 [34–44]	0.113
BMI (kg/m^2) median [IQR]	26.5 [24–29.4]	27.2 [24–30]	26.2 [24–28.5]	0.351
Laboratory				
TSH (μU/mL), median [IQR]	4.85 [2.8–5.47]	5.32 [4.98–7]	2.25 [1–3.1]	**<0.001**
fT3 (ng/dl), median [IQR]	2.93 [2.48–3.2]	2.93 [2.48–3.13]	2.89 [2.48–3.2]	0.730
fT4(ng/dl), median [IQR]	1.3 [1–1.68]	1.32 [1.02–1.68]	1.3 [0.98–1.68]	0.933
Hgb (g/dL), median [IQR]	14.2 [13.5–15.2]	14.2 [13.5–15]	14.35 [13.5–15.3]	0.237
WBC (× 103/μL), median [IQR]	7.1 [6.1–8.19]	7.1 [6.15–8.21]	7.17 [6.1–8.12]	0.980
Lymphocyte (× 103/μL), median [IQR]	2.26 [1.89–2.71]	2.28 [1.85–2.73]	2.24 [1.9–2.7]	0.825
Neutrophil (× 103/μL), median [IQR]	4.2 [3.44–4.9]	4.38 [3.5–4.8]	4.11 [3.44–4.96]	0.751
PLT (× 103/μL), median [IQR]	275 [250–304]	285 [244–321]	269 [256–292]	0.143
Glucose mg/dL median [IQR]	90 [86–98]	91 [87–98]	89 [85–96]	0.198
AST	20 [17–23]	20 [17–23]	20 [17–24]	0.537
ALT	20 [16–26]	19 [16–26]	21 [17–26]	0.601
Creatinine (mg/dL), mean ± SD	0.75 [0.65–0.85]	0.7 [0.65–0.85]	0.78 [0.63–0.9]	0.286
CRP (mg/L), median [IQR]	2.8 [1.04–4.0]	3 [1.57–4.2]	2.3 [0.84–4]	**0.006**
Comorbidities				
Hypertension, n (%)	26 (14.2)	19 (17.4)	7 (9.5)	0.130
Diabetes, n (%)	17 (9.3)	11 (10.1)	6 (8.1)	0.650
Smoking, n (%)	34 (18.6)	17 (15.6)	17 (23)	0.208
Hyperlipidemia, n (%)	16 (8.7)	13 (11.9)	3 (4.1)	0.064

ALT: alanine transaminase; AST: aspartate transaminase; BMI: body mass index; CRP: C-reactive protein; fT3: free thyroxine 3; fT4: free thyroxine 4; Hgb: hemoglobin; PLT: platelet; WBC: white blood count

Table 2. *Electrocardiographic and echocardiographic characteristics of the participants*

	Overall (n=183)	Patient (n=109)	Control (n=74)	P-value
Electrocardiogram features				
Heart rate (b.p.m), median [IQR]	75 [71–84]	78 [72–85]	75 [70–80]	0.132
P wave axis (°), median [IQR]	50 [19–58]	49 [20–58]	50 [19–58]	0.995
QRS axis (°), median [IQR]	50 [28–73]	46 [28–73]	53 [28–73]	0.653
PR interval (msec) median [IQR]	144 [132–158]	144 [130–156]	144 [132–160]	0.497
QRS interval (msec) median [IQR]	92 [88–100]	90 [88–8]	94 [88–100]	0.063
QTc (msec), median [IQR]	430 [416–448]	435 [421–457)	424 [412–438]	**0.001**
T-wave change, n (%)	11 (6)	5 (4.6)	6 (8.1)	0.325
fragmented QRS, n (%)	12 (6.6)	5 (4.6)	7 (9.5)	0.191
Premature atrial/ventricular contraction, n (%)	6 (3.3)	3 (2.8)	3 (4.1)	0.627
Echocardiography features				
LVDD (mm), median [IQR]	46 [44–48]	46 [44–48]	46 [44–48]	0.860
LVSD (mm), median [IQR]	30 [28–34]	30 [28–34]	30.5 [28–34]	0.522
IVS (mm), median [IQR]	8 [8–9]	8 [8–9]	8.5 [8–9]	0.502
PW (mm), median [IQR]	8 [7–8]	7.5 [7–8]	8 [7–8]	0.207
LA (mm), median [IQR]	32 [30–34]	32 [30–34]	32 [30–34]	0.656
RV (mm), median [IQR]	32 [30–34]	32 [30–34]	32 [29–34]	0.402
RA (mm), median [IQR]	32 [31–35]	32 [31–35]	33 [31–36]	0.384
Ejection Fraction (%), median [IQR]	65 [61–67]	65 [60–66]	65 [62–68]	0.365
E/A, median [IQR]	1.2 [1–1.4]	1.14 [0.9–1.4]	1.2 [1.1–1.4]	0.158
E/E', median [IQR]	6.3 [5.3–7.6]	6.23 [5.25–7.08]	6.55 [5.6–8.2]	0.140
TAPSE, median [IQR]	21 [20–23]	21 [20–23]	21 [20–22]	0.216
R', median [IQR]	12.8 [12.1–13.8]	12.8 [12.3–13.6]	12.8 [12–14.3]	0.875
PASB, median [IQR]	9 [8–12]	9 [8–12]	8 [8–12]	0.428
LVGLS, median [IQR]	-20.1 [-21.7- -18.1]	-20.5 [-21.8– -17.9]	-19.7 [-21.7– -18.9]	0.651

A: late diastolic filling mitral velocity; E: early diastolic filling mitral velocity; LVDD: left ventricle end-diastolic diameter; LVSD left ventricle end-systolic diameter; IVS: interventricular septum thickness; LA: left atrium diameter; PASP: pulmonary arterial systolic pressure; PW: left ventricular posterior wall thickness; QTc: Corrected QT; RV: right ventricle diameter; RA: right atrium diameter; TAPSE: tricuspid annular plane systolic excursion; LVGLS: left ventricle global longitudinal strain; E': the peak early diastolic velocity of the septal mitral annulus (tissue Doppler);

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Online Tools and Instructional Methods Used in Distance Learning on the Motivation and Attitudes of Nursing Students During COVID-19 Pandemic: A Cross-sectional Study

COVID-19 Pandemisi Sırasında Uzaktan Eğitimde Kullanılan Çevrimiçi Araçlar ve Öğretim Yöntemlerinde Hemşirelik Öğrencilerinin Motivasyon ve Tutumları: Kesitsel Bir Çalışma

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ABSTRACT

Aim: *This study aims to determine the effects of online tools and instructional methods used in distance education on the motivation and attitudes of nursing students.*

Material and Method: *This descriptive and cross-sectional study was carried out with 280 students studying in the nursing department of a university in Turkey. Data were collected between March and June 2021 through Google docs, including the Demographic form, The Instructional Materials Motivation Survey, and The Attitude Scale towards Distance Education.*

Results: *It was found that the satisfaction of nursing students from distance education (5±2.42), their motivation for instructional materials (81.50±15.92), and their attitudes towards distance education (98.96±20.32) were at a moderate level. Among instructional methods of distance education, video assignment was found to have a significant relationship with motivation for instructional materials (t=2.534, p=0.012), and case study was found to be significantly correlated with the attitude towards distance education (z=-2.262, p=0.024). The attitudes of students towards distance education were found to be positively correlated with their motivation for instructional materials (r=0.521, p=0.000), age (r=0.158, p=0.008), satisfaction (r=0.665, p=0.000) and grade (r=0.154, p=0.010). They were found to be negatively correlated with online class hours (r=-0.129, p=0.031).*

Conclusion: *The results of this study showed that the students' attitudes towards distance education were more positive as their motivation for instructional materials, age, satisfaction, and grades increased, and their attitudes were more negative as their online class hours were prolonged.*

Key words: *attitude; distance education; motivation; nursing student; online tools*

ÖZET

Amaç: *Bu çalışmanın amacı, uzaktan eğitimde kullanılan çevrimiçi araç ve öğretim yöntemlerinin hemşirelik öğrencilerinin motivasyon ve tutumlarına etkisini belirlemektir.*

Materyal ve Metot: *Tanımlayıcı ve kesitsel tipteki bu araştırma, Türkiye'de bir üniversitenin hemşirelik bölümünde öğrenim gören 280 öğrenci ile gerçekleştirilmiştir. Veriler, Demografik form, Öğretim Materyalleri Motivasyon Anketi ve Uzaktan Eğitime Yönelik Tutum Ölçeği ile Google dokümanları aracılığıyla Mart ve Haziran 2021 arasında toplanmıştır.*

Bulgular: *Hemşirelik öğrencilerinin uzaktan eğitimden memnuniyetlerinin (5±2,42), öğretim materyallerine yönelik motivasyonlarının (81,50±15,92) ve uzaktan eğitime yönelik tutumlarının (98,96±20,32) orta düzeyde olduğu belirlenmiştir. Uzaktan eğitim öğretim yöntemlerinden video çekiminin öğretim materyallerine yönelik motivasyon ile (t=2,534, p=0,012) ve vaka çalışmasının uzaktan eğitime yönelik tutumla (z=-2,262, p=0,024) anlamlı şekilde ilişkili olduğu bulunmuştur. Öğrencilerin uzaktan eğitime yönelik tutumları ile öğretim materyallerine yönelik motivasyon (r=0,521, p=0,000), yaş (r=0,158, p=0,008), memnuniyet (r=0,665, p=0,000), sınıfları (r=0,154, p=0,010) arasında pozitif yönde, çevrimiçi ders saatleri (r=-0,129, p=0,031) ile negatif yönde istatistiksel olarak anlamlı bir ilişkili bulunmuştur.*

Sonuç: *Bu çalışmanın sonuçları, öğrencilerin öğretim materyallerine yönelik motivasyon, yaş, memnuniyet ve notları arttıkça uzaktan eğitime yönelik tutumlarının daha olumlu olduğunu ve çevrimiçi ders saatleri arttıkça tutumlarının daha olumsuz olduğunu göstermiştir.*

Anahtar kelimeler: *tutum; uzaktan eğitim; motivasyon; hemşirelik öğrencisi; çevrimiçi araçlar*

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Introduction

COVID-19 pandemic has been characterized by severe acute respiratory failure and quickly spread to many parts of the world[1]. During this period, several restrictions that limit daily life such as lockdown have been implemented all over the world to alleviate the spread of disease. The universities have interrupted their face-to-face activities in the scope of these precautions and distance e-learning strategies have become the most accessible tool for education[2]. Also in our country, Yüksek Öğretim Kurumu (Council of Higher Education) announced that spring term of 2020 would be completely carried out through distance education to manage pandemic period effectively and to prevent victimization of the students such as getting unable to graduate and losing time[3]. The universities were not prepared for this period that was developed suddenly and very quickly due to COVID-19 and shifted to distance education from face-to-face education without distinction between theory and practice[4].

Distance education includes all learning activities that are carried out to promote communication and interaction between learners, teachers and learning sources at various locations and times through information and communication technologies[5]. During this process, universities have continued their education as online/synchronous, online/asynchronous or hybrid to the extent possible[4]. Educators have managed distance education by using global learning platforms such as Microsoft Teams, Zoom and Google Classroom or e-learning platforms of their own universities[6–7]. In nursing education, quarantine restrictions due to COVID-19 have denied the students' access to the patients and medical staff[8]. In this context, transition to distance education has created a difficulty for nursing education since it includes traditional classroom and clinical practice[9]. Designing and assessing distance education in a good way is very crucial for the success and stability of the system. This is associated with educators' opportunities to interact with the students, learning styles of the students, effective use of materials and environments by the educators and students and the needs, interest, attitudes and motivation of the educators and students[10]. Technologies with various functions have been described as the factors that may promote motivation of the students[11]. It has been considered that a distant learner, who is or has been motivated, may catch up with the opportunity to overcome place and time limitations quickly by distance education and to become successful by acquiring targeted learning achievements[12]. The motivation of students in learning may affect online learning experiences and persistence[13]. Even though the components such as the teachers creating distance education environment, the environment itself and content are developed as ensuring all standards, the most important factor in success is the attitudes and approaches of the learners towards these environments[14]. The attitude of the individual towards distance education and technologies may be directly associated with individual's learning. From this perspective, it is highly important to know the thoughts and feelings of the students regarding distance education environments to improve their attitudes towards these[15]. In a study, the attitudes of the nursing students towards distance education they had during COVID-19 pandemic were found to be at a moderate level. The students stated that they felt safe and experienced less stress since they were not in class environment and they did not have an internship during the pandemic[16].

However, in some other studies, it was indicated that most of the students experienced negative feelings such as anxiety and stress due to the pandemic and they had difficulty in coping with these emotional states; and thus, they had difficulty in following the lessons[17–19].

Motivating students and keeping this motivation alive is an issue that needs attention for teachers in distance education. There are too many online tools and instructional methods that may be used for teaching-learning process; however, less is known about the preferences of the students regarding these tools and methods. It is highly important to include successful features of online education in the education planning and to identify teaching tools that improve motivation and attitudes of the students during this period.

The aim of this study was to determine the effects of online tools and instructional methods used in distance education on the motivation and attitudes of nursing students. In this study, the following questions were addressed:

1) What is the level of satisfaction, attitude, and motivation for instructional materials among the nursing students during the pandemic?
2) Is there a relationship between sociodemographic data of the nursing students and their attitudes towards distance education and motivation for instructional materials?

Material and Methods

Study Design

A descriptive and cross-sectional design was used. STROBE Statement checklist was used for assuring the rigour of research methodology[20].

Setting and Participants

The study was conducted with the students who were studying in the nursing department of a university in Turkey. During the lockdown, university and undergraduate nursing program were carried out as online/asynchronous. Video recordings were prepared about the content/subjects of the courses and they were shared by the students by uploading to online system. Students were asked to watch course presentations and videos uploaded on the system before online/synchronous course and to attend the course as ready. A course legally lasts forty-five minutes. Online tools such as the university's live virtual classroom system (BigBlueButton), Zoom and Microsoft Teams were used for online courses. All courses including clinical ones were held by using methods such as video watching, powerpoint presentations, case discussions and homework preparation. While 1 st, 2nd and 3rd graders carried out clinical practices online, 4th graders (interns) had face-to-face clinical practices.

The universe of the study was composed of 802 nursing students who were studying in a nursing department during the spring term of 2020–2021 academic year. Inclusion criteria of the study were being a nursing student and being volunteer to participate in the study. A confidence interval of 95% and an error rate of 5% were used to determine sample size, and it was aimed to attain 260 nursing students. The study was conducted with 280 students who approved to participate in the study and data obtained from these students were analyzed.

Data Collection

The study, which was designed as an online survey, was conducted between March and June 2021. After obtaining ethics committee approval and institutional permission, the links of the questionnaires were sent to all students through e-mail. Participants completed the survey via Google Forms, which is a safe online survey platform. Each participant has one right to fill the survey. It took approximately 15–20 minute to complete the questionnaires.

Data Collection Tools

The data were collected with the Demographic Form, The Instructional Materials Motivation Survey and The Attitude Scale towards Distance Education.

Demographic form: The demographic form included sociodemographic data of the students (such as age, sex, working status, grade, grade point average (GPA), online class hours), satisfaction level during/from distance learning (1–10) and the questions for identifying online tools used in creating a virtual classroom and instructional methods used in distance education.

The Attitude Scale towards Distance Education: This 5-point Likert type scale was developed by Kişla (2016) and included 35 items and one dimension[21]. It was aimed to identify the attitudes of participants towards distance education. The total score that can be taken from the scale was between 35–175. High scores indicated more positive attitudes towards distance education. In reliability analysis of the scale, internal consistency coefficient (Cronbach's Alpha) was determined as 0.89; and it was found to be 0.88 in this study.

The Instructional Materials Motivation Survey (IMMS): It was developed by Keller in 1987 and it was adapted to Turkish by Kutu and Sözbilir (2011)[22]. Rather than determining motivation levels of the students, the scale aims to identify how much instructional materials used motivate students for the course. It is composed of 2 subscales including Attention-Relevance (11 items) and Confidence-Satisfaction (13 items) and 24 items. The scale is graded as 5-point likert type. Cronbach Alpha reliability coefficient of the scale was determined as 0.83, and it was found to be 0.92 in this study.

Data Analysis

Data were analyzed by using SPSS 25.0 (IBM SPSS Statistics 25 software (Armonk, NY: IBM Corp.)) package program. Continuous variables were given as mean (± standard deviation), and categorical variables were expressed as number and percentages. When parametric test assumptions were ensured, groups were compared by Independent Samples T-test and the comparison was made by Mann-Whitney U test when parametric test assumptions were not met. Repeated Measures Analysis of Variance was used for the comparison of parametric data and Friedman Test was used for comparing nonparametric ones. Moreover, the correlations between continuous variables were tested by Spearman or Pearson correlation analyses and the

differences between categorical variables were analyzed by Chi-Square test.

Ethical Considerations

An ethics committee approval was taken from Pamukkale University Non-interventional Clinical Trials Ethics committee to conduct the study (Date: 03.16.2021, No: 2021/06). The students who approved to participate in the study were informed about the aim of the study and that the study was on a voluntary basis and all personal data would be confidential; and the study was carried out in compliance with the principles of Helsinki Declaration. Participation in the study was on a voluntary basis and written consents were obtained from all participants prior to enrollment. A permission to use was taken from the authors of all data collection tools used in the study.

Results

Most of the students in the study were females (81.80%); and 8.20% were working outside school hours. A comparable number of students from each grade (1, 2, 3, 4) participated in the study. Grade point average of more than half of the participants (68.20%) was between 3.49–2.75 and 53.6% of them took online courses between 16–30 hours per week. During distance education period, the most used online tools for virtual classroom was Microsoft teams (67.90%); and the most frequently used instructional methods were homework preparation (92.90%), question-answer method (83.20%) and powerpoint presentation (82.10%) (Table 1). At the end of this study, it was found that students' satisfaction from distance education (5±2.42), their motivation for instructional materials (81.50±15.92) and their attitudes towards distance education (98.96±20.32) were at a moderate level (Table 2). No statistically significant

Table 1. *Personal and distance education characteristics of the participants (n=280)*

Variables	N	%
Age group		
18–20	127	45.4
21–34	153	54.6
Sex		
Female	229	81.80
Male	51	18.20
Working		
Yes	23	8.20
No	257	91.80
Class/year		
1st grade	70	25.00
2nd grade	80	28.60
3rd grade	68	24.30
4th grade	62	22.10
Grade point average		
3.50–4.00	54	19.30
3.49–2.75	191	68.20
2.74 and below	35	12.50
Online class hours		
1–15	86	30.70
16–30	150	53.60
31 and above	44	15.70
Online tools*		
Microsoft teams	190	67.90
University's live virtual classroom system (BigBlueButton)	70	25.00
Google classroom	23	8.20
Moodle	16	5.70
Zoom	20	7.10
Blackboard	14	5.00
Instructional method*		
Question-answer	233	83.20
Brainstorming	118	42.10
Video watching	145	51.80
Video assignment	111	39.60
Case study	172	61.40
Powerpoint presentation	230	82.10
Homework	260	92.90

* Participants gave more than one answer.

Table 2. *Participants' Satisfaction with Distance Education, The Instructional Materials Motivation Survey and The Attitude Scale towards Distance Education scores*

Scales	Mean ± standard deviation	Minimum value	Maximum value
Satisfaction level during/from distance learning (1–10)	5±2.42	1	10
The Instructional Materials Motivation Survey	81.50±15.92	32	116
The Attitude Scale towards Distance Education	98.96±20.32	61	151

differences were found between instructional materials motivation survey and the attitude scale towards distance education based on online virtual classroom tools (Microsoft Teams, BigBlueButton, Google Classroom, Moodle, Zoom, Blackboard) used in this study. There are statistically significant differences were found between instructional materials motivation survey and the attitude scale towards distance education based on video assignment, case study (p>0.05). In terms of instructional methods, the mean IMMS score of the students using video assignments method was found to be lower than those who were not using this method (t=2.534, p=0.012). Moreover, the mean score of the students using case discussion method from the attitude scale towards distance education was found to be higher than the ones who were not (z=-2.262, p=0.024) (Table 3). A positive correlation was found between mean score of the students from the attitude scale towards distance education and their mean score from IMMS (r=0.521, p=0.000). Attitude score towards distance education was found to be increased as age (r=0.158, p=0.008), satisfaction (r=0.665, p=0.000) and grade (r=0.154, p=0.010) increased and decreased as online class hours increased (r=-0.129, p=0.031). As online class hours increased (r=-0.147, p=0.014), mean score from IMMS was also found to be decreased (Table 4).

Table 3. *Comparison of the scores obtained from The Instructional Materials Motivation Survey and The Attitude Scale towards Distance Education according to online tools and teaching methods used in distance education*

Scales	The Instructional Materials Motivation Survey			The Attitude Scale towards Distance Education		
Online tools	Yes Mean ± SD	No Mean ± SD	p value (test value)	Yes Mean ± SD	No Mean ± SD	p value (test value)
Microsoft teams	80.37±16.67	83.88±14.02	0.085 (t=1.728)	98.72±20.90	99.49±19.12	0.687 (z=-0.403)
University's live virtual classroom system (BigBlueButton)	80.53±14.92	81.82±16.27	0.558 (t=0.586)	94.51±18.11	100.45±20.83	0.071 (z=-1.803)
Google classroom	82.35±17.09	81.42±15.85	0.79 (t=-0.267)	99.30±20.02	98.93±20.38	0.891 (z=-0.137)
Moodle	88.06±10.27	81.10±16.13	0.089 (t=-1.704)	103.94±21.04	98.66±20.28	0.352 (z=-0.93)
Zoom	80.80±15.76	81.55±15.97	0.84 (t=0.203)	103±22.68	98.65±20.14	0.444 (z=-0.765)
Blackboard	86.43±14.90	81.24±15.96	0.235 (t=-1.19)	105.93±19.75	98.60±20.32	0.189 (t=-1.318)
Instructional method						
Question-answer	82.15±16.01	78.26±15.26	0.126 (t=-1.533)	98.97±20.88	98.94±17.46	0.992 (t=-0.01)
Brainstorming	83.21±16.04	80.25±15.77	0.124 (t=-1.542)	100.40±19.07	97.92±21.18	0.314 (t=-1.008)
Video watching	81.86±15.97	81.86±15.97	0.959 (z=-0.052)	99.79±20.26	98.08±20.42	0.478 (z=-0.709)
Video assignment	78.55±15.32	83.43±16.06	0.012 (t=2.534)	96.42±20.01	100.63±20.4	0.115 (z=-1.578)
Case study	81.92±15.68	80.80±16.35	0.867 (z=-0.167)	100.88±19.51	95.91±21.29	0.024 (z=-2.262)
Powerpoint presentation	81.70±15.39	80.56±18.32	0.647 (t=-0.458)	98.55±19.03	100.88±25.56	1 (z=0)
Homework	81.45±15.85	81.45±15.85	0.872 (t=0.161)	98.55±19.91	104.35±25.05	0.219 (t=1.231)

Table 4. *Correlation of the scores obtained from The Instructional Materials Motivation Survey and The Attitude Scale towards Distance Education and some variables (n=280)*

Scales	The Instructional Materials Motivation Survey		The Attitude Scale towards Distance Education	
Variables	r	p	r	p
The Attitude Scale towards Distance Education	0.521**	0.000	-	-
Age	-0.021	0.721	0.158**	0.008
Grade point average	0.101	0.092	0.006	0.924
Online class hour	-0.147*	0.014	-0.129*	0.031
Satisfaction level during/from distance learning (1–10)	0.570**	0.000	0.665**	0.000
Grade	-0.006	0.914	0.154**	0.010

Discussion

Determination of the attitudes of nursing students towards distance education and their motivation for instructional materials used in distance education during COVID-19 pandemic will allow the identification of the strategies required for more efficient and effective conduction of education and the use of instructional methods with proven efficacy and easy-to-use/access technological tools more actively. Again, it may allow more efficient use of constantly evolving and changing technology within instructional activities by enabling the correct positioning of its place in educational activities.

The study was carried out during the pandemic period; and data were collected while students were having distance education actively. Distance education was carried out through online tools. Besides the virtual classroom included in the own information system of the university, several free web tools (MS Teams, Google Classroom, Moodle, Zoom, etc.) were used in the school where study was conducted. Students' attitudes and satisfaction for the instructional methods and online virtual classroom tools used in distance education and their motivation for instructional materials were found to be at a moderate level. In a study, it was indicated that students were generally satisfied with distance education during the pandemic but there were some problems such as lack of announcement of the instructional materials by the educators beforehand and deficiencies in internet access and online system, [23] results of another study showed that nursing students had some sort of positive attitudes towards e-learning as well as they were willing to have e-learning[24]. In some other studies, it was reported that nursing or medical faculty students had negative perception and attitudes towards e-learning during COVID-19 pandemic[25]. The students also stated that online learning environment decreased motivation and the most common problem in managing individual learning process was attention deficit[26]. In a study evaluating the effect of teaching through an online tool (Zoom) on the perception of students, participants reported that these online sessions disturbed monotonous routine and they felt themselves motivated for reading the topics[27]. In another study, students reported the use of several strategies including the recording of powerpoint presentations uploaded to the platforms such as Moodle, Zoom and Microsoft Teams during online education and indicated that online learning was not easy; but motivated them to improve their thinking and problem-solving skills[7].

Lack of other options than using web tools to continue education during the pandemic might have accelerated the students' adaptation in this study. No statistically significant differences were found between instructional materials motivation survey and the attitude scale towards distance education based on online virtual classroom tools used in this study.

In nursing education, various instructional methods are used alone or together at each stage of teaching. More than one method was used together in the university where study was conducted to make distance education more effective during the pandemic. These methods were question-answer, brain storming, video watching, case study, video assignments and homework preparation. Different from these, demonstration is actively used during normal education process. All these methods are used actively in the theoretical courses held in the classroom, in skill laboratories and during clinical practice. While theoretical courses have been carried out easily in the classrooms created by web tools during the pandemic, occupational skill laboratory and clinical practices were also performed through web tools. Teaching skills requires to make learner and educator work together in the same environment. In this context, students were asked to make a video showing that they could learn the skill and perform it. This method was used actively in some courses. The motivation of the students, who video assignments within the scope of the course, was found to be higher than those who did not. In a scoping review examining the effect of Video Making for Situated Learning, most of the participants stated that they welcomed the opportunity to make skill videos and rewatch them; and they found it as a dynamic tool for feedback. However, some participants stated that the use of video feature of smartphones to teach and learn a skill had difficiulties in terms of sustaining access, ethics, and privacy, and trying to learn how to use video feature of smartphone effectively created a distressful situation[28]. In a study investigating the effects of video assignments by yourself during COVID-19 pandemic, students reported that they experienced less stress since they had the opportunity to replay the recording before the final submission; but they felt a sort of or excess pressure to "do the right thing"[29]. At this point, being in an environment

different from skill laboratory and far from reality and, the obligation to perform repeated and time-consuming practices such as video making for many courses might have decreased the motivation of the students.

Case study is used actively in the planning, implementation, and teaching of nursing care. It is also actively used in teaching care during the pandemic period as in normal education process. Mean scores of the students, who were using case study method, were found to be more positive for the attitudes towards distance education compared to those who were not using that. In a qualitative study, Family Nursing Practitioner students stated that they had a more realistic view regarding disease and conditions following education given by experiencing case-based discussions[30]. Although there are many approaches for online learning, using case presentation method may provide students different social and cultural environments for challenge. Case study discussion is an effective teaching method to involve students in a challenging environment[31].

A positive correlation was found between instructional materials motivation survey and the attitude scale towards distance education. The more the instructional materials motivate the students for the course, the more the attitudes towards distance education enhance. Students with low motivation are not prepared for learning[32]. Learning motivation is one of the predictors of academic success and well-being[32,33].

Again, attitude scores of students towards distance education were found to increase as their ages increased. At this point, working in a job outside school hours might be a factor. There are also students coming to nursing department from other graduate programs, and they work at various fields in line with their graduation.

The grade of the students also affected their attitudes towards distance education. The satisfaction from distance education increased as they approached to last grade. The presence of a smaller number of courses and more clinical practice hours at last year and the continuation of clinical practice in line with pandemic conditions during the dates when data were collected might be an important fact at this point.

In the study, it was also found that the attitude score towards distance education and motivation for instructional materials decreased as online class hours increased. In a study examining students' views on distance education in the COVID-19 pandemic, 73% of the participants stated that they wanted a maximum of 50 minutes of asynchronous video recordings and synchronous course duration of 30–40 minutes[34]. Another study examines the relationship between distance education students' cognitive flexibility levels and their distance education motivations, it has been stated that the time spent in the distance education environment and the frequency of participation in synchronized classes are factors affecting motivation[35]. Being continuously online, class attendance responsibility and pandemic-associated conditional and environmental problems (such as lockdown of all family members due to the restrictions, noise, housework, inadequacy in the number of technological devices and internet access problems) might have led to this situation[36].

Study Limitations and Strengths

There were some limitations in the study. Since the participants of the study were the students studying in the Nursing Department of a Faculty of Health Sciences within a state university in Turkey, the results can not be generalized to whole universe. For this reason, there is a need for new studies that will include a greater number of participants from various institutions and countries.

Conclusions

At the end of this study, nursing students' satisfaction by distance education, their motivation for instructional materials and their attitudes towards distance education were found to be at a moderate level. The instructional method of video assignments was found to be significantly correlated with instructional materials motivation; and case discussion was found to be significantly correlated with the attitude towards distance education. As students' motivation for instructional materials, age, satisfaction, and grades increased, their attitudes towards distance education were found to be more positive; but their attitudes were more negative as online class hours increased. This was a descriptive and cross-sectional study. Considering that distance education has been used for limited number of courses in the nursing curricula until now, it was used actively during the pandemic and will be used at certain conditions later on, this study is suggested to guide promotion and development studies that will be carried out for distance learning to be used in nursing education which focus on people and their care.

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Declaration of Interest

None

Ethical Considerations

The research protocol has been approved by the ethics committee of Pamukkale University Medical Ethics Committee (Date: 03.16.2021, No: 2021/06). Permission has been obtained from the nursing school where the study was planned to be conducted. Written consent has been obtained from the participants who voluntarily accepted to participate in the study. The study was conducted according to Helsinki Declaration to protect participants. Permission was obtained from the scale authors for the use of the scales. No ethical issues arose in the study.

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Author Contributions

Arife Sanlialp Zeyrek: Conceptualization, Methodology, Software, Formal analysis, Data curation, Writing - Original draft preparation, Project administration.

Ozlem Fidan: Conceptualization, Methodology, Data curation, Writing - Original draft preparation, Writing - Reviewing and Editing.

Sumeyye Arslan: Conceptualization, Supervision, Visualization, Writing - Original draft preparation, Writing - Reviewing and Editing.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Evaluation of the Relationship Between Mitral Annular Calcification and CRP/albumin Ratio

Mitral Anüler Kalsifikasyon ile CRP/albümin Oranı Arasındaki Ilişkinin Değerlendirilmesi

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ABSTRACT

Aim: *The relationship between mitral annular calcification (MAC) and atherosclerotic diseases is known. The CRP/albumin ratio (CAR) is one of the indicators of inflammation of the atherosclerotic process. We aim to examine the relationship between MAC and CAR values.*

Material and Method: *The study included 197 patients with MAC and a control group of 200 retrospectively between January 2021 and December 2021. We analyzed the relationship between CAR and MAC according to the hospital records, including laboratory findings, echocardiography reports, and patient characteristics.*

Results: *We found higher CAR values in patients with MAC compared to the control group (p<0.001). In addition, CAR was predictive for MAC determined by regression analysis (OR: 52.37, 95% CI: 7.37–372.06, p<0.001).*

Conclusion: *CRP/albumin ratio values, essential indicators of inflammation, were higher in the patient population with MAC. This finding may reveal that inflammation is also effective in the pathogenesis of MAC.*

Key words: *CRP/albumin ratio; inflammation; valvular calcification*

ÖZET

Amaç: *Mitral anüler kalsifikasyon (MAK) ile aterosklerotik hastalıklar arasındaki ilişki bilinmektedir. CRP/albümin oranı (CAO), aterosklerotik sürecin iltihaplanmasının göstergelerinden biridir. Amacımız MAK ve CAO değerleri arasındaki ilişkiyi incelemektir.*

Materyal ve Metot: *Ocak 2021 ile Aralık 2021 tarihleri arasında geriye dönük olarak 197 MAK'li hasta ve 200 kişilik bir kontrol grubu çalışmaya alındı. CRP/albümin oranı ve MAK arasındaki ilişkiyi laboratuvar bulguları, ekokardiyografi raporları ve hastaların demografik özelliklerini içeren hastane kayıtlarına göre analiz ettik.*

Bulgular: *Kontrol grubuna göre MAK'li hastalarda daha yüksek CAO değerleri bulduk (p<0,001). Ayrıca, CAO, yapılan regresyon analizine göre MAK için öngördürücüydü (OR: 52,37, %95 GA: 7,37–372,06, p<0,001).*

Sonuç: *Enflamasyonun önemli göstergelerinden olan CAO değerleri, MAK'li hasta popülasyonunda daha yüksekti. Bu bulgu, enflamasyonun MAK patogenezinde de etkili olduğunu ortaya koyabilir.*

Anahtar kelimeler: *CRP/albümin oranı; enflamasyon; kapak kalsifikasyonu*

Introduction

Mitral annular calcification (MAC) describes a chronic calcium deposition that occurs in the fibrous portion of the mitral valve and is usually localized to the posterior annulus. In this process, the role of endothelial damage leading to lipid accumulation and the formation of calcium deposits is emphasized[1,2]. A close relationship with atherosclerotic heart disease risk factors such as hyperlipidemia, obesity, type II diabetes and hypertension has been demonstrated[3,4]. While it was formerly thought to be a passive process caused by calcium deposition that increases with age, later research has pointed out a role for lipoproteins and chronic inflammation similar to that in atherosclerosis[5–7].

On the other hand, the ratio of rapidly rising C-reactive protein (CRP) in inflammation and albumin, which is a negative acute phase reactant, is known as one of the predictive parameters of inflammation in the atherosclerotic process, as shown in previous studies[8–10].

Studies examining the relationship of MAC with inflammation revealed conflicting results[11–13]. To answer questions in this regard, we designed this study examining the relationship between MAC and CRP/albumin ratio (CAR).

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Material and Methods

Study Population

In this retrospective study, we concluded 197 patients with MAC and 200 age and sex matched patients without MAC who admitted to our cardiology outpatient clinic between January 2021 and December 2021. Our inclusion criteria were as follows: age between 18–100, proper echocardiography report which marks if the patient has MAC. Patients with any known acute or chronic inflammatory disease, active infection, medication including steroids, severe heart valve disease, history of acute rheumatic fever, prosthetic valve, decompensated heart failure, malignancy, kidney or liver dysfunction, hematological disease, and chronic obstructive pulmonary disease were excluded from the study. The study was approved from the Institutional Ethics Committee and conducted in accordance with the Helsinki declaration.

Clinical and Laboratory Data

Clinical and demographic data were obtained from hospital medical records. Hypertension and diabetes were defined as stated by the guidelines[14,15]. Laboratory parameters including CRP and albumin were recorded in the blood samples given by the patients after 8 hours of fasting.

Echocardiography

A complete transthoracic echocardiogram was performed by the same cardiologist using a 3.5 MHz transducer and VIVID 7 Dimension Cardiovascular Ultrasound System (Vingmed-General Electric, Horten, Norway). Mitral annulus calcification was defined as a highly dense echocardiographic structure with highly reflective features localized at the junction of the atrioventricular groove and the anterior or posterior leaflet of the mitral valve in the parasternal long or short axis, apical four– or two-chamber views[16].

Statistical Analysis

Since there was no data investigating the relationship between CAR and MAC, pre-analysis was performed with 15 patients with MAC and without MAC. When the sample size was calculated based on 95% power by statistical method, it was planned to include 200 patients with MAC and 200 patients without MAC by performing a power analysis.

Statistical analyzes were performed using the SPSS 20.0 (Statistical Package for Windows, Chicago, Illinois, USA) program. Kolmogorov-Smirnov test was used to examine the normal distribution of the data. Among the numerical variables, those with normal distribution (parametric) were expressed as mean, standard deviation, those that did not exhibit normal distribution (non-parametric) were expressed as median, median value (with interquartile range) and categorical variables as percentages. Student-t test or Mann-Whitney U-test was used for numerical variables, and chi-square test was used for analysis of categorical variables. Parameters predicting the presence of mitral annulus calcification were evaluated with multivariate logistic regression analysis.

Results

Baseline characteristics and laboratory parameters of the study groups are shown in Table 1. Mean age of control group and MAC group were 71.5±8.5 and 70.8±8.7 (p=0.39). There was no difference between the groups in terms of age, gender, comorbidities, smoking status and laboratory parameters except CRP, albumin, and CAR values. The CRP, albumin and CAR values of the MAC group were significantly higher than the control group [0.95 (0.50–2.13) vs. 0.67 (0.49–1.04), respectively, (p<0.001)].

Univariate and multivariate logistic regression analysis revealed a positive and independent relationship between CAR and MAC (OR: 52.37, 95% CI: 7.37–372.06, p<0.001) (Table 2). C-reactive protein was found to be an independent predictor for MAC.

Discussion

In this study, we showed that CAR levels were significantly associated with MAC and were also predictive of MAC. These findings are the first to show that inflammation is closely related to MAC besides other factors as mentioned earlier[3,4].

Studies examining the relationship between mitral annular calcification and inflammation have produced conflicting results. In a study examining the relationship between epicardial fat thickness and MAC, it was stated that adipose tissue may contribute to the formation of MAC through inflammatory cytokines[17]. In another study examining the relationship of inflammatory cytokines (C-reactive protein, intercellular

Table 1. *Baseline characteristic and laboratory parameters of patients*

	Control (n: 200)	MAC (n: 197)	P value
Age	71.5±8.5	70.8±8.7	0.39
Gender (Male)	69 (34.5%)	53 (26.9%)	0.81
Hypertension, n (%)	129 (64.5)	113 (57.3)	0.151
Diabetes Mellitus, n (%)	57 (28.5)	58 (29.4)	0.741
Smoking, n (%)	32 (16)	40 (20.3)	0.242
EF	56.2±7.0	56.9±5.5	0.295
Hemoglobin, g/dl	12.5±1.7	12.8±1.6	0.122
Glucose, mg/dl	122.4±52.2	119.7±50.9	0.601
Platelet, $10^3/mm^3$	293±101	290±91	0.783
WBC, $10^3/mm^3$	7.7±2.4	7.4±1.6	0.284
Albumin, mg/dl	4.4±0.3	4.3±3.7	0.004
Creatinin, mg/dl	0.83±0.19	0.85±0.18	0.141
Total cholesterol, mg/dl	200.4±40.1	208.4±34.6	0.474
HDL – cholesterol, mg/dl	42.2±11.5	43.2±11.1	0.200
LDL – cholesterol, mg/dl	120.7±25.2	123.6±24.7	0.177
Trigliseride, mg/dl	135 (95–85)	125 (91–184)	0.196
CRP	3.0 (2.1–4.8)	4.2 (2.2–9.4)	<0.001
CRP/Albumin ratio	0.67 (0.49–1.04)	0.95 (0.50–2.13)	<0.001

EF: Ejection Fraction; WBC: White Blood Cell; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; CRP: C-reactive Protein.

Table 2. *Univariate and multivariate regression analyses for Mitral Annuler Calcification*

Univariate regression analyses	OR	p
Age	0.990 (0.968–1.013)	0.390
Gender	0.699 (0.455–1.073)	0.102
Hypertension	0.740 (0.494–1.110)	0.145
Hemoglobin	1.094 (0.976–1.227)	0.123
CRP/Albumin	52.37 (7.37 – 372.06)	<0.001

CRP: C-reactive Protein.

adhesion molecule-1, interleukin-6, and monocyte chemoattractant protein-1) with valve calcification, increased values of these cytokines were found in patients with valve calcification[13]. They concluded that this close relationship was due to shared risk factors of both entities. Also, in a study of HIV-infected patients, infection was shown to be a predictor of mitral and aortic valve calcification[11]. However, unlike our study, they showed no association between inflammatory biomarkers and valve calcification. We would like to underline that the CRP/albumin ratio was not used in this study. In a genome-wide association study, two loci near the proinflammatory IL1F9 gene were found to be associated with MAC, although they were not replicated constantly[18]. In prospective study with 27 years follow-up period, examining the long-term consequences of exposure to atherosclerotic risk factors, CRP levels were shown to be associated with mitral annular calcification, similar to our study[19]. In a cross-sectional study, red blood cell distribution width (RDW) was found to be higher in patients with MAC, and it was stated that this could be an indicator of ongoing inflammation[20]. In contrast, a study in hemodialysis patients showed that valve calcification is a non-inflammatory process and is mostly associated with hyperparathyroidism[21].

Recent studies have shown that CAR was a more accurate sign of inflammation in atherosclerotic process than CRP or albumin alone[8,9,22]. Studies showing how high CRP and low albumin levels play a role in the atherosclerotic process at the cellular level reveal the importance of CAR[23–27]. Our study is important in that it shows that CAR values, which are indirect indicators of the atherosclerotic process, and the presence of MAC are closely related.

The limitations of our study should be mentioned. First, due to the retrospective and cross-sectional nature of the study, it is not possible to establish a causal relationship between MAC and CAR values. Prospective and follow-up studies are needed. It should also be noted that the presence of MAC is a finding that can be missed in routine echocardiography reports. But this may have prevented possible bias. In addition, we remind that all echocardiography reports were reviewed within the specified date range and all eligible patients were included in the study. In addition, the fact that echo parameters are limited to only ejection fraction and mitral annular calcification presence can be seen as another limitation of the study. Reliability of patient information is another limitation of our study, as whether patients have an inflammatory disease or not is defined only based on hospital registry data.

Conclusion

In conclusion, in this study, CAR values were found to be significantly higher in the patient group with MAC than in the control group. Therefore, it draws attention to the importance of inflammation, which is still a controversial issue in the pathogenesis of MAC. Future studies are needed to test the accuracy of this data with a larger patient population, a prospective design, and different markers of inflammation.

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OLGU SUNUMU / *CASE REPORT*

A Serious Complication of Liver Hydatid Cyst: Intra-abdominal Rupture

Karaciğer Hidatik Kistinin Ciddi Bir Komplikasyonu: Karın İçi Rüptür

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ABSTRACT

Intra-abdominal rupture is a severe and rare complication of a hydatid cyst. This case report is aimed to present the diagnosis and treatment algorithm for spontaneous intra-abdominal rupture of liver hydatid cyst. A 46-year-old lady was followed up in the gastroenterology service with a cholangitis diagnosis. Endoscopic retrograde cholangiopancreatography (ERCP) was applied to the patient. The patient's abdominal pain and vital signs worsened on the first day after ERCP. After the patient was hemodynamically stabilized, intravenous contrast-enhanced computed tomography (CT) was performed. On CT, there was a 130 mm diameter air-containing hydatid cyst in the liver dome accompanied by intraperitoneal free air. Liver hydatid cyst perforation was considered in the patient, and the patient was taken for an emergency laparotomy. Cystotomy and unroofing were performed with cholecystectomy. Control thorax CT was performed on the patient due to low saturation (90% under nasal oxygen support) on the sixth day of follow-up. Due to pleural effusion in the right hemithorax on the CT scan, thoracentesis was performed on the patient, and 500 cc of seropurulent fluid was aspirated. On the 14th postoperative day, the patient was discharged without any problem and was prescribed 10 mg/kg/day of albendazole. No pathology was detected on the control CT in the first-month follow-up.

Key words: *albendazole; anaphylactic reaction; echinococcosis; spontaneous rupture*

ÖZET

Karın içi rüptür hidatik kistin ciddi ve nadir bir komplikasyonudur. Bu vaka raporunun amacı, karaciğer hidatik kist spontan intraabdominal rüptürünün tanı ve tedavi algoritmasını sunmaktır. Kırk altı yaşında bayan hasta kolanjit tanısı ile gastroenteroloji servisinde takibe alındı. Hastaya endoskopik retrograd kolanjiyopankreatografi (ERCP) uygulandı. Endoskopik retrograd kolanjiyopankreatografi sonrası 1. günde hastanın karın ağrısı ve vital bulguları kötüleşti. Hasta hemodinamik olarak stabilize edildikten sonra intravenöz kontrastlı bilgisayarlı tomografi (BT) çekildi. Bilgisayarlı tomografide karaciğer kubbesinde intraperitoneal serbest havanın eşlik ettiği 130 mm çapında hava içerikli hidatik kist mevcuttu. Hastada karaciğer hidatik kist perforasyonu düşünüldü ve hasta acil laparotomiye alındı. Kolesistektomi ile kistotomi ve unroofing yapıldı. Hastaya takibin 6. gününde satürasyonun düşük olması (%90 nazal oksijen desteği altında) nedeniyle kontrol toraks BT çekildi. Bilgisayarlı tomografide sağ hemitoraksta plevral efüzyon nedeniyle hastaya torasentez yapıldı ve 500 cc seropürülan sıvı aspire edildi. Postoperatif 14. günde hasta komplikasyonsuz taburcu edildi ve 10 mg/kg/gün albendazol tedavisi verildi. Birinci ay takibinde kontrol BT'de patoloji saptanmadı.

Anahtar kelimeler: *albendazol; anafilaktik reaksiyon; ekinokokkoz; spontan rüptür*

Introduction

Hydatid disease, also known as cystic echinococcosis, is a zoonotic disease with an annual incidence rate of 1–200 per 100,000[1]. Cystic echinococcosis causes significant public health problems and severe economic losses in Turkey[2]. It is endemically seen in the Eastern and South-Eastern Anatolia regions. The causative agent of this disease is a parasite called Echinococcus granulosus[3]. The primary source of this parasite is meat-eating animals such as dogs, cats, wolves, and foxes. However, often the etiological source is dogs. The parasite lives in the small intestines of dogs. The disease is transmitted to humans by eggs excreted in dog faeces[4].

Cysts are most commonly seen in the liver and lungs[5]. They can also be located in the spleen, peritoneum, kidney, bone, eye, brain, heart, and ovaries. While hydatid cysts localized to the liver may progress

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asymptomatically, symptoms such as pain in the upper right of the abdomen, nausea, vomiting, and sometimes itching and jaundice are observed in symptomatic cases[1]. Surgeons should know that hydatid cysts can spontaneously rupture after surgery or trauma. Although hepatic hydatid cysts most commonly rupture into the biliary system, they can also rupture into the peritoneal cavity, bronchi, or blood vessels. Spontaneous intra-abdominal rupture of a hydatid cyst is a rare (1–8%) and life-threatening condition[6]. Patients with hydatid cyst rupture into the peritoneal cavity often present to the emergency department with acute abdomen. In addition, patients can apply to the emergency service with anaphylactic or allergic reactions up to 25%[7].

This case report is aimed to present the diagnosis and treatment algorithm for spontaneous intra-abdominal rupture of liver hydatid cyst.

Case Report

A 46-year-old lady was admitted to the Gastroenterology Clinic of Erzurum Regional Training and Research Hospital in January 2022 with complaints of right upper quadrant pain, fever, and chills lasting for about three days. She had no other disease or history of abdominal surgery. She was normotensive (blood pressure 134/72 mmHg), tachycardia (pulse 108/min), and febrile (body temperature 38.2°C). Her oxygen saturation in room air was 96%. On abdominal physical examination, the patient had abdominal pain in deep palpation in the right upper quadrant. Other system examinations, including digital rectal examination, were routine.

The patient's pathological laboratory parameters were as follows: alanine aminotransaminase (ALT): 119 U/L [7–40], aspartate aminotransaminase (AST): 108 U/L [13–40], alkaline phosphatase (ALP): 637 U/L [46–116], gamma-glutamyl transferase (GGT): >1200 U/L [0–38], total bilirubin (TB): 1.92 mg/dL [0.3–1.2], direct bilirubin (DB): 1.64 mg/dL [0–0.3], and c-reactive protein (CRP): 120 mg/L [0–5]. Other laboratory parameters were not remarkable, including tumor markers. On magnetic resonance cholangiopancreatography (MRCP) scan, condensed bile content was observed in the gallbladder. In addition, the liver had a 90 mm diameter hydatid cyst (Fig. 1). Endoscopic retrograde cholangiopancreatography (ERCP) was applied to the patient. During ERCP, it was observed that there was an occlusive stone distal to the common bile duct. After stone removal by sphincterotomy, a stent was placed in the common bile duct. The stent was removed on the normalization of control liver function tests.

The patient was admitted to the gastroenterology clinic in February 2022 with similar symptoms and physical examination findings. The patient's pathological laboratory parameters were as follows: alanine aminotransaminase (ALT): 49 U/L [7–40], aspartate aminotransaminase (AST): 42 U/L [13–40], alkaline phosphatase (ALP): 556 U/L [46–116], gamma-glutamyl transferase (GGT): 835 U/L [0–38], total bilirubin (TB): 2.10 mg/dL [0.3–1.2], direct bilirubin (DB): 1.58 mg/dL [0–0.3], and c-reactive protein (CRP): 323 mg/L [0–5]. Other laboratory parameters were not remarkable. Endoscopic retrograde cholangiopancreatography (ERCP) was applied to the patient. During ERCP, common bile duct and intrahepatic bile ducts were observed as dilated. Bile sludge and pus were extracted from the common bile duct with a balloon. On the 1st day after ERCP, the patient's abdominal pain and vital signs worsened. The patient was consulted at the general surgery clinic. She was hypotensive (blood pressure: 94/42 mm Hg), tachycardia (pulse rate: 118 beats per minute), and febrile (body temperature: 38.5°C). Her oxygen saturation on room air was 94%; on abdominal physical examination, she had defense and rebound on deep palpation in all quadrants. Her vital signs were stabilized with aggressive fluid resuscitation. Intravenous steroid (hydrocortisone 200 mg) and antihistamine (pheniramine 45.5 mg/2 mL) treatments were administered to resolve the allergic reaction. After the patient was hemodynamically stabilized, intravenous contrast-enhanced computed tomography (CT) was performed. A 130 mm diameter hydatid cyst was on a CT scan with air in the liver dome with intraperitoneal free air (Fig. 2 and 3). Liver hydatid cyst perforation was considered in the patient, and the patient was taken for an emergency laparotomy.

A ruptured liver hydatid cyst was observed during laparotomy (Fig. 4). In addition, free infective germinative membranes with massive purulent fluid were observed in the intra-abdominal cavity (Fig. 5). Cystotomy and unroofing were performed with cholecystectomy. Three bile duct repairs were performed due to the biliary fistula. The abdominal cavity was washed and cleaned with warm saline. One drain was placed in the cyst pouch, and the other was placed in the pelvis. The patient was taken to the intensive care unit for follow-up.

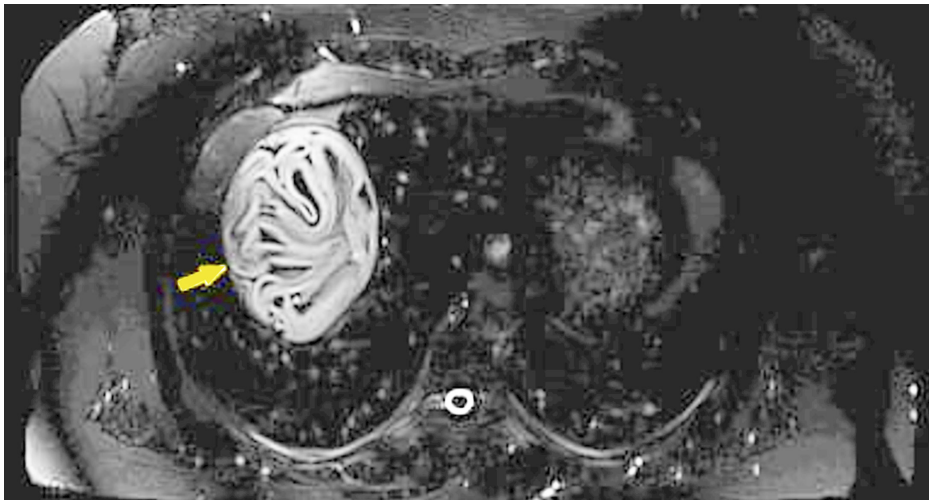

Figure 1. *Magnetic resonance imaging of liver hydatid cyst at first cholangitis attack (shown by a yellow arrow).*

Figure 2. *Preoperative computed tomography image of ruptured liver hydatid cyst: black arrows show ruptured hydatid cyst, and yellow arrows show intraperitoneal free air (axial image).*

Figure 3. *Preoperative computed tomography image of ruptured liver hydatid cyst: yellow arrow shows intraperitoneal free air due to perforation (sagittal image).*

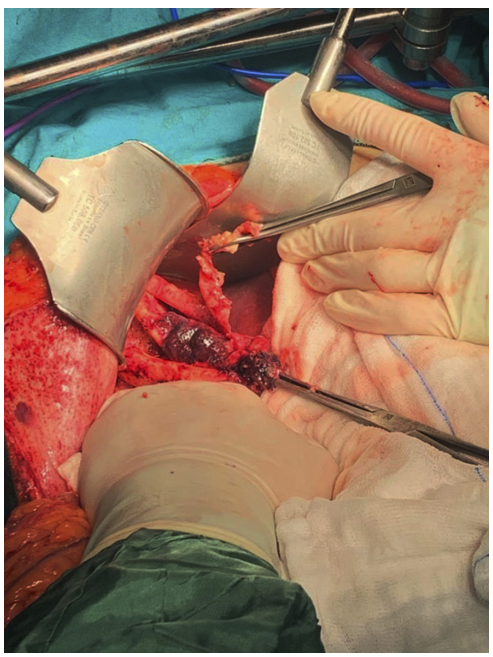

Figure 4. *Intraoperative view of ruptured liver hydatid cyst.*

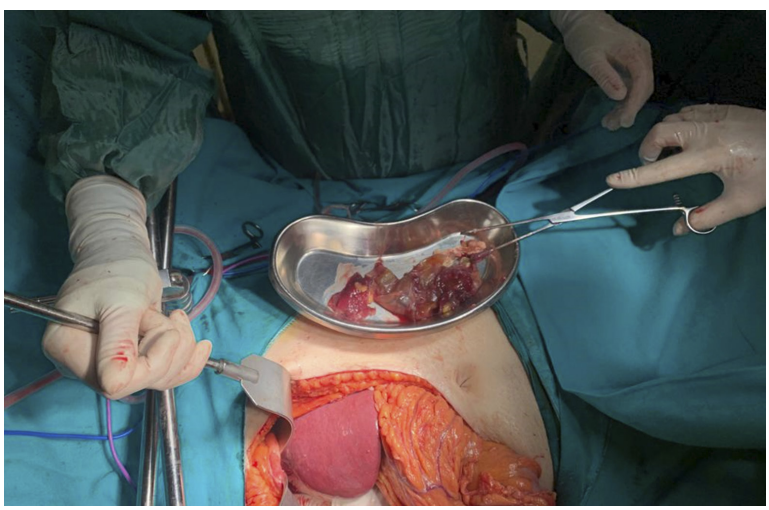

Figure 5. *Free infective germinative membranes.*

Piperacillin/Tazobactam (4.5 g vial every 6 hours), meropenem (1 g vial every 8 hours), and amikacin (500 mg vial every 12 hours) were started. In addition, treatment was administered with intravenous steroid (methylprednisolone 40 mg vial every 12 hours) and antihistamine (pheniramine 45.5 mg/2 mL vial every 12 hours). Control thorax CT was performed on the patient due to low saturation (90% under nasal oxygen support) on the 6th day of follow-up. Due to pleural effusion in the right hemithorax on the CT scan, thoracentesis was performed on the patient, and 500 cc of seropurulent fluid was aspirated. Both drains were removed on the 12 th postoperative day. On the 14th postoperative day, the patient was discharged without any problem and was prescribed 10 mg/kg/day of albendazole. No pathology was detected on CT in the first-month follow-up.

Discussion

Echinococcosis, also called hydatidosis, is a zoonotic infection that infects humans worldwide[8]. Hydatidosis results from an infection of the tapeworm *Echinococcus granulosus*. The disease characteristically shows hydatid cyst (metacestode) growth in the internal organs of intermediate hosts, including humans. The definitive hosts of the cestode are carnivores such as dogs. Humans and other hosts cause infection by ingesting eggs or pregnant proglottids excreted in the feces of the definitive host[9].

Liver hydatid cysts may be asymptomatic for many years and are often an incidental finding on ultrasound performed for another reason[10]. Although the diagnosis is often made when complications arise, jaundice is usually the first symptom, and rupture or secondary infection occurs as the first clinical presentation. The hydatid cyst rupture can occur in the biliary tract and the intra-abdominal cavity. When the cyst ruptures into the biliary tract, patients present with an attack of cholangitis. Intra-abdominal rupture, another condition, can cause life-threatening problems up to anaphylactic shock. The clinical picture was diffuse abdominal pain and mild anaphylactic shock findings in the presented case.

In laboratory evaluation, liver function tests are not specific in determining the severity of the disease, and pathological laboratory findings are seen only in approximately 40% of the cases. AST, ALT, and bilirubin levels typically remain within the normal range, while ALP is typically elevated. Eosinophilia may be seen in the complete blood cell count[11]. Detection of Echinococcus antibodies by ELISA is a standard method used for diagnosis. However, since this immune response depends on host immunity, positivity is not detected in every case[12]. In our case, there was an elevation in liver function tests, mainly due to a cholangitis attack. In addition, eosinophilia was not detected in the complete blood count, and the level of the Echinococcal antigen could not be determined due to the need for emergency surgery.

The most commonly used technique for diagnosis in radiology is USG. USG is the preferred method for cyst examination in elective conditions. On the other hand, CT imaging plays a vital role in situations where ultrasonography is difficult (e.g., obese patients) and is very important in the perioperative period. It can detect complications such as cyst rupture, underlying infection, and biliary or vascular involvement[13]. In the presented case, CT imaging was used as the first diagnostic tool and helped to establish the definitive diagnosis. CT scan revealed a ruptured hydatid cyst in the liver dome accompanied by intraperitoneal free air.

In treating a hydatid cyst, the stage of the cyst, the size of the cyst, and the presence of complications are important factors in making the treatment decision. Watch and wait, medical treatment with benzimidazoles, PAIR (puncture, aspiration, injection, re-aspiration), and surgery are the options for treatment. Surgery is the modality of choice for complicated cysts. Surgery is required for large cysts, superficial cysts prone to rupture, infected cysts associated with the biliary tract, and cysts pressing on adjacent organs[14]. Surgery was the first-line treatment because of intra-abdominal rupture and less severe allergic reaction findings.

Anaphylaxis due to intra-abdominal rupture is a rare and life-threatening condition. While there is an incidence of anaphylaxis up to 10 percent in the literature, less severe allergic reactions can be seen up to 25 percent[7,10]. Stabilization of vital signs should be prioritized in anaphylactic reactions affecting vital signs. It is essential to keep the airway open to ensure respiration continuity and protect cardiac functions. 0.01 mg/kg/dose of 1/1000 adrenaline solution (maximum dose=0.5 mg) should be applied to the anterolateral region of the middle part of the thigh in severe anaphylactic reactions[15]. However, intravenous steroid and antihistamine treatment is the first choice in cases with less severe allergic reactions. After stabilizing the patient's vital signs, the necessary surgical

intervention should be performed quickly. After discharge, all patients should be treated with albendazole for 2 to 3 months to decrease recurrences and followed with serologic and imaging tests for at least six months[16]. In this case, the patient had a less severe allergic reaction. She was operated on for rupture of the hydatid cyst after appropriate treatment for the allergic reaction, and albendazole was prescribed after discharge.

Conclusion

Intra-abdominal rupture is a severe and rare complication of a hydatid cyst and should be kept in mind in patients with a history of hydatid cysts. The patient's vital signs should be reviewed quickly, and appropriate resuscitation therapy should be applied during anaphylactic shock. After the patients are stabilized, a diagnosis should be made quickly, and surgical treatment appropriate for the diagnosis should be performed as soon as possible.

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DERLEME / *REVIEW*

The Cognitive Benefits of Playing Volleyball: A Systematic Review

Voleybol Oynamanın Bilişsel Yararları: Sistematik bir Derleme

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ABSTRACT

This systematic review examines the effects of playing volleyball, an open-skill sport, on cognition. Four hundred seventeen studies were accessed with specified search criteria, and 21 studies containing neurophysiological outcomes were found eligible for evaluation. Most studies reported cognitive improvement in volleyball players compared to control groups. Fewer studies demonstrated superior effects of playing volleyball over other sports types. Results indicate that playing volleyball has an improving effect on cognition, mainly executive functions.

Key words: *cognition; executive functions; open-skill exercise; volleyball*

ÖZET

Bu sistematik derleme, açık beceri sporu olan voleybolun biliş üzerindeki etkilerini incelemeyi hedeflemiştir. Belirlenen araştırma kriterleri ile 417 çalışmaya erişilmiş ve nörofizyolojik bulguları içeren 21 çalışma değerlendirmeye uygun bulunmuştur. Çalışmaların çoğunluğu, voleybolcularda kontrol gruplarına kıyasla bilişsel iyileşme bildirmiştir. Daha az sayıda çalışma, voleybol oynamanın diğer spor türlerine göre daha üstün etkileri olduğunu göstermiştir. Bulgular, voleybol oynamanın biliş üzerinde, özellikle yürütücü işlevlerde geliştirici bir etkiye sahip olduğunu göstermiştir.

Anahtar kelimeler: *açık beceri egzersizi; biliş; voleybol; yürütücü işlevler*

Introduction

A large number of studies demonstrated that physical activity creates structural and functional changes in the brain that promote cognitive functions[1,2]. Some studies suggest that different exercise types exert different effects on cognition[3]. A growing body of literature suggests that the effects of physical exercise on cognitive functions might be related to the exercise types[4,5].

Sport types are divided into two groups based on the predictability and consistency of the performing environment; open and closed skill sports[6]. Open-skill sports (e.g., volleyball, tennis, football, etc.) are externally paced activities performed in a dynamic, unpredictable environment, whereas closed-skill sports (e.g., running, swimming, archery, etc.) are internally paced and performed in a static and predictable environment. Within this scope, as an interactive and strategic sport, volleyball is an open-skill sport. The volleyball requires active decision-making and ongoing adaptability to randomly occurring external stimuli. The player's task involves the simultaneous processing of a significant amount of knowledge, such as teammates, opponents, field positions, and balls. The volleyball player must update the location of teammates/opponents, execute tactics, and follow the rules during the game[7]. Some studies demonstrate that open-skill athletes outperform the closed-skill athletes in visual attention, decision-making, action execution, and inhibitory control tasks[3,8,9]. For these reasons, volleyball players may be more cognitively flexible than closed-skill athletes in task-switching. A recent study showed that team sport athletes performed better in sustained attention and processing speed than recreational athletes[10]. As a team sport, volleyball might be more improving for some aspect of cognitive skills.

Additionally, volleyball is one of the sports with the lowest incidence of concussion[11]. Considering the cognitive functions such as attention, cognitive processing speed, and working memory are susceptible to the

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effects of sports-related concussion,[12] examining volleyball seems safer to understand the long-term effects of open-skill sports participation on cognition.

Therefore, based on the previous literature and focusing on neurophysiological outcomes, we did a systematic literature search to understand the effects of playing volleyball on cognition. We will present and discuss our findings to better understand how playing volleyball affects the brain and what the potential neurobiological mechanisms underlying are.

Materials and Methods

Search Strategy

PRISMA guideline[13] is used for the procedure of search. An electronic search was undertaken by two independent researchers between October 2020 and June 2021 in the Cochrane Library, PscyINFO and Pubmed databases. The last update for searching took place on June 24, 2021. We limited the search with papers published in English or Turkish. We used "AND" and "OR" operators to connect our search terms. The following search string has been used for each database: (volleyball) AND (cogniti* OR executive OR attention OR memory OR verbal OR working memory OR dual-task OR reaction time OR processing speed OR perceptual speed).

Selection Process and Data Extraction

We included studies published in peer-reviewed journals which recruited children and healthy adults investigating the effects of playing volleyball without any other intervention (e.g., further medicine and training prescription, or dietary). Multidomain interventions were excluded (e.g., volleyball plus lifestyle intervention). There was no restriction for participants' age range. Studies were eligible if at least there was a volleyball group that performed multiple weeks of training. Both intervention and cross-sectional studies were included. At least one of the following domains had to be represented in outcome measures: i) cognitive functions, ii) structural or functional brain data. Dissertations, conference papers, case studies, or studies that did not include any outcomes of interest were excluded.

Duplicates were eliminated and MESH terms, titles and abstracts were reviewed intensively. Two separate researchers evaluated the relevance of possible studies based on our inclusion and exclusion criteria. The remaining studies were read for the final selection in terms of their eligibility. In case of contradictory commentaries between two main reviewers, a third independent reviewer was consulted. Further studies found in the screened studies' reference lists were also evaluated for eligibility. All included studies were presented according to main study characteristics (First Author, Sample, Study Design, Procedure, Outcome Measures, Results, and Risk of Bias) (Table 1).

Methodological Quality of Included Studies

Methodological quality was assessed independently by two authors. Three different tools[14–16] were used to score the methodological quality of cross-sectional, intervention, and longitudinal studies (Supplementary Table I-II-III for details). The evaluation tool for cross-sectional studies consists of five components and 12 items in total. The maximum point can be obtained from was 12. The intervention study was assessed using the Physiotherapy Evidence Database (PEDro) scale, which consists of 11 items. The maximum point is obtained from was 11. The quality of studies are classified into three categories as follows; (<6 points = low, 6–9 points = moderate, ≥10 points = high). The quality of the longitudinal study was assessed by "The critical appraisal skill program" (CSAP) which consists of 12 item and three categories as follows; "low, moderate, high". The rating scores are presented in Table 2.

Results

Search Results

In the following section, we present the study characteristics details of the included studies.

Study Design and Participant Characteristics

Self-reports had reported participants' volleyball background in cross-sectional studies. All included 21 studies were published between 1998 and 2019 and conducted in 14 different countries (Italy=5, Brazil=2, Germany=2, Greece=2, Taiwan=2, Belgium=1, Canada=1, China=1, Iran=1, Israel=1, Japan=1, Poland=1, Spain=1, USA=1). Nineteen studies included control groups, seven of them had a passive control group that received no intervention (volleyball or any other sports activity). Six studies had only active control groups, and six studies administered active

and passive control groups. Twenty of the studies were cross-sectional studies, and one was a randomized control study.

Participants were recruited from national sports teams, universities, volleyball courts. Participants' maximum mean age was 33.9, but the minimum mean age was not specified (in a study, there was a group under 14 years old.)

Among the 21 studies, 1438 participants were recruited, of which 967 were volleyball players and 471 were control groups. Group sizes ranged from 7 to 274 participants.

Methodological Quality of Included Studies

Based on this 12-item assessment tool, the average score of the methodological quality of the 19 studies was 7.9 with scores ranging from 5 to 10. Seventeen of the observational studies were found to be of "moderate quality", one study was found to be "low quality", and one study was found to be "high quality". According to the PEDro scale, the methodological quality score of one intervention study was 5 which means "low quality". Lastly, the methodological quality score of only one longitidunal study was "moderate". The rating scores are presented in Table 2.

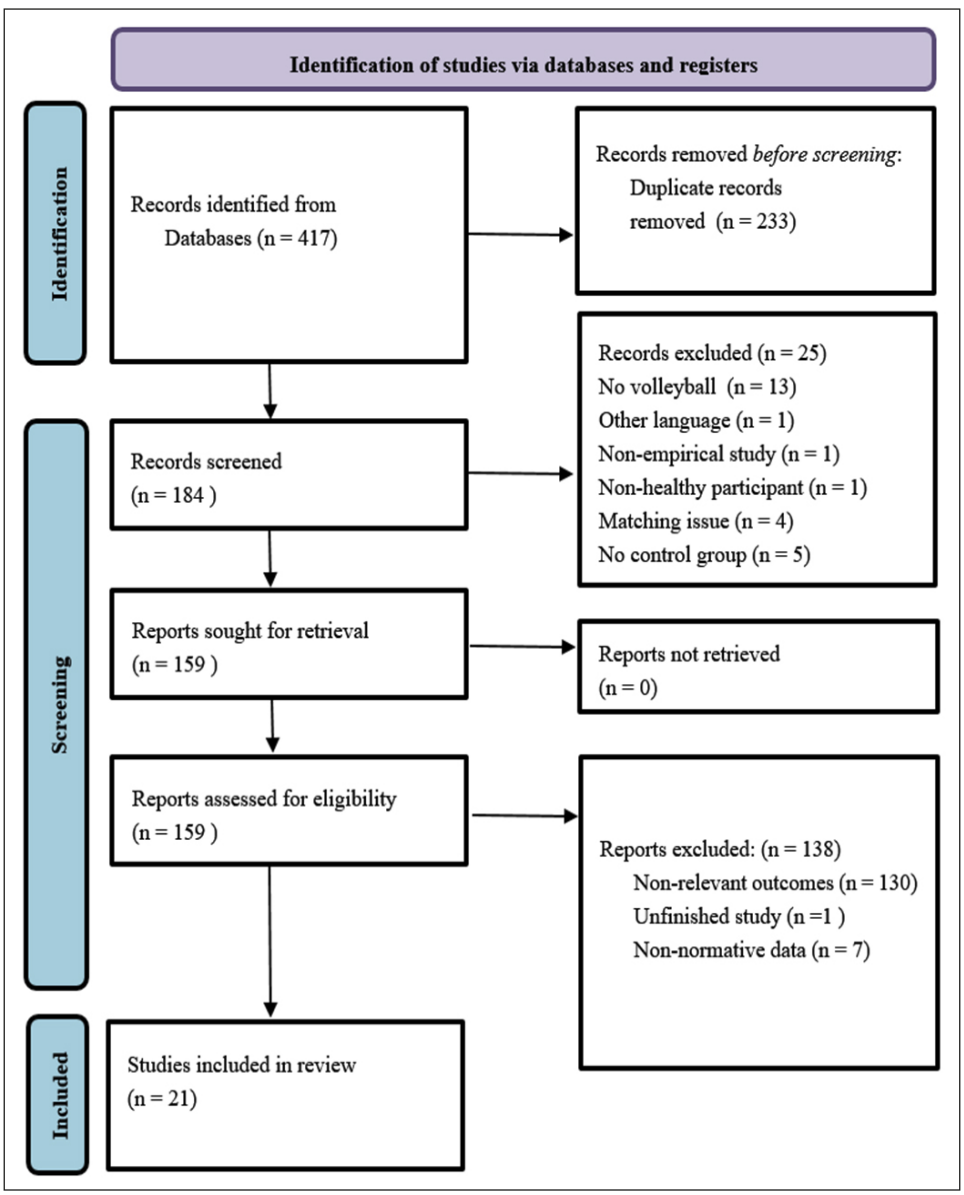

Table 1. *Prisma flow chart*

Table 2. Study characteristics

1st author; Quality score	Sample	Study design	Procedure	Outcome Measures	Results
Alves et al. (2013); (8)	Athlete group Va: n=30 Age=(F: 20.55±1.23, M: 24.85±4.40) Vj: n=57 Age=(F: 16.27±1.06, M: 17.58±0.92) Control group Ca: n=27 Age=(F: 21.55±1.50, M: 23.33±3.04) Cj: n=40 Age=(F: 16.45±1.5, M: 17.33±1.13)	USA, Brazil Two main groups and four subgroups (gender based)	Athlete group: A Control group: C Va: Adult volleybal player (F: 9.66±1.5, M: 11.61±4.75 training years) Vj: Junior volleyball player (F: 5.43±1.94 M: 5.25±2.43 training years) Ca: Adult control group (no training) Cj: Junior control group (no training)	Useful Field of View Visual Short-Term Memory (VSTM) Stopping (stop/go) Flanker Change Detection	Task switching RT: C >A Go RT: A >C Stop RT: C >A Stop probability: A >C Change detection RT: C >A
Chiu et al. (2017); (8)	V: n=11 Age=23.36±0.53 E: n=12 Age=21.50±0.58 C: n=8 Age=21.75±0.70	Taiwan, Three groups	V: volleyball group E: running and swimming group C: no exercise training years: n. a.	Flanker test	Accuracy: V >C V >E (approached significance, p: 0.053)
Costa et al. (2018); (7)	n=34 Nvc: n=n. a Evc: n=n. a Age=32.5±9.4	Brazil, Two groups	Nvc: Novice voleyball coach (<10 years) Evc: Experienced volleyball coach (>10 years)	Decision making task fNIRS: HbO_2 level during EF task	fNIRS: Nvc >Evc (PFC activity)
Fontani et al. (2006); (7)	Vh: n=12 Age=28±5 Vl: n=12 Age=19±2 Kh: n=9 Age=31±5 Kl: n=9 Age=32±5	Italy, Four groups	Vh: Volleyball group, (14±4 years training) Vl: Volleyball group (6±2 years training) Kh: Karate group (13±3 years training) Kl: Karate group (6±3 years training)	Alert Go/No-Go Divided Attention Working Memory reaction	RT Alert, Go/No-Go, Working Memory: Vh >Vl, Kl >Vh Divided Attention: Vh >Vl, Kh >Kl Variability Index Working Memory, Divided Attention: (Vh >Vl) Alert: Kl >Vh Errors Divided Attention: Vl >Vh, Kh >Kl
Giglia et al. (2011); (9)	Vn: n=12 Age=26±4.3 Vr: n=11 Age=25.6±3.4 R: n=10 Age=19.2±4.0 C: n=23 Age=24.8±2.5	Italy, Four groups	Vn: Volleyball group (national) Vr: Volleyball group (regional) R: Rower group C: Control group	Landmark task	RT Vn, Vr <R, C Errors Vn <Vr, R, C (total response) Vn < Vr, R, C (left response)
Gil et al. (2012); (7)	U14:n=261 U16:n=274	Spain, Two groups	U14:under 14 years old group (3.26±1.62 training years) U16:between 14–16 years old group (4.32±1.51 training years)	Declarative Knowledge (DK) Questionnaire Procedural Knowledge (PK) Questionnaire	DK, PK scores: U16>U14
Kioumourtzoglou et al. (1998); (7)	V: n=13 Age=18.5 B: n=12 Age=20.7 WP: n=19 Age=18.3 C1:n=18 Age=22.6 C2:n=21 Age=19.6	Greece, Five groups (only V and C2 are compared)	V: Volleyball group B: Basketball group WP: Water polo group C1:Control group of B C2:Control group of V and WP	Perceptual Speed Focus Attention Prediction Estimation of speed and direction of a moving object	RT Perceptual speed: V <C2 Accuracy Prediction: V >C2
Kioumourtzoglou et al. (2000); (7)	V: n=12 Age=18.5 C: n=18 Age=20.5	Greece, Two groups	V: Volleyball group (at least 10 years training) C: Control group (no volleyball or ball games training)	Perception speed (PS) Prediction Focused Attention Estimation of Speed and Direction of a Moving Object Analytic Ability Grouping of Information Retention of Information (RI)	PS (RT): V <C Prediction (correct response number): V >C Estimation (RT): V <C RI (Error, nonsport-specific task): V <C RI (missed response, nonsport-specific task): V <C RI (missed response, sport-specific task): V <C
Kokubu et al. (2006); (9)	V: n=10 Age=20.1±0.9 C: n=10 Age=22.3±1.3	Japan, Two groups	V: Volleyball group (6.6±2.5 years training) C: Control group (no training)	Saccadic-task Key-press task Dual-task	RT V <C (Key-press task)

Table 2. Study characteristics *(continues)*

1st author; Quality score	Sample	Study design	Procedure	Outcome Measures	Results
Loffing et al. (2015); (9)	V: n=20 Age=24.80±4.01 C: n=31 Age=25.10±3.94	Germany, Two groups,	V: volleyball group (12.40±5.05, training years) C: control group (no training)	Video-based perceptual-cognitive task	Prediction accuracy (target trials; non-target trials): V >C; V >C Response time (target trials; non-target trials): V <C; V <C
McAuliffe. (2004); (5)	V: n=11 Age: n. a. C: n=11 Age: n. a	Canada, Two groups	V: Volleyball group (college volleyball players) C: Control group (no training)	Spatial cueing task	Cueing effect V >C (onset cue–onset target, color cue–color target)
Meng et al. (2019); (10)	VG: n=25 Age=23.6±2.8 BG: n=35 Age=22.7±3.4 CG: n=27 Age=22.8±3.2	Taiwan, Three groups	VG: Volleyball group (11.57±3.1 training years) BG: Badminton group (11.31±3.1 training years) CG: control group (no training)	Inhibition control (Stop signal task) (SST) Attentional shifting (Task-Switching task) (TSWT). Visual sensory memory (Iconic memory task) (ICMT) Visual-spatial attention (Change detection task) (CDT). Attentional processing (Attention networks task) (ANT).	SST (SSR): VG >CG, BG TSWT (Global and Local Cost RT): CG >VG, BG Iconic memory (accuracy): VG, BG >CG ANT (alertness): VG >BG, CG
Nuri et al. (2012); (7)	V: n=11 Age=21.64±1.12 S: n=11 Age=22.91±2.16	Iran, Two groups	V: volleyball group (4.31±1.45, training years) S: sprinter group (4.27±1.47 training years)	Visual choice RT Visual complex choice RT Auditory choice RT Auditory complex choice RT Anticipatory skill (high/low speed)	Both auditory RT: S <V Both anticipatory skill: V >S
Schorer et al. (2013); (9)	Ve: n=11 Age=17.0±2.0 Va: n=13 Age=23.7±1.9 Vn: n=16 Age=23.5±2.3	Germany, Three groups	Ve: Expert volleyball (8.5±2.8 training years) Va: Advanced volleyball (11.9±2.8 training years) Vn: Novice volleyball (no regular training)	Perceptual-cognitive task	prediction accuracy (temporal occlusion): Ve >Va, Vn Va >Vn prediction accuracy (spatial occlusion): Ve >Va, Vn Va, Vn
Tomasino et al. (2012); (9)	V: n=21 Age=26.2±4.9 F: n=21 Age=33.9±8.4 C: n=21 Age=25.61±7.77	Italy, Three groups	V: volleyball group (10.7±3.2 training years) F: fans (7.1±2.6 volleyball watching experience years) C: control group (no training or watching)	Categorization task (CT) (RT, accuracy)	CT (accuracy): V >F, C; F >C CT (RT) V <F, C
Tomasino et al. (2013); (9)	VG: n=10 Age=27±7.35 CG: n=10 Age=25±4.07	Italy, Two groups	VG: Volleyball group (15±2.1 training years) CG: control group (no training)	Categorization task (CT) (RT, accuracy) fMRI	CT (accuracy): VG >CG fMRI: VG <CG (left M1 and left PMC activity for the impossible actions presented as positive commands)
Urgesi et al. (2012); (9)	V: n=12 Age=24.33±5.3 W: n=12 Age=27.5±8.97 C: n=12 Age=29.42±3.5	Italy, Three groups	V: Volleyball group (14.58±4.3 years training) W: Volleyball watchers (at least 10 years) C: Control group (no training or watching)	Prediction *viewing perspective (back, front) *cue (body, ball)	RT C >V, W (Back view (body/ball), front view (body/ball)) Accuracy V >W, C (Back view (body/ball), front view (ball)) W >C (Back view (ball))
Vansteenkiste et al. (2014); (9)	Ve: n=10 Age=20.0±1.2 Vi: n=10 Age=20.9±1.8 C: n=17 Age=20.1±1.6	Belgium, Three groups	Ve: Volleyball group (experienced) Vi: Volleyball group (intermediate) C: Control group (no training)	Visual search task	Accuracy Ve >Vi, Vc RT Ve, Vi <Vc approached significance, p: 0.053)
Zach and Shalom (2016); (5)	n=20 Age=27.3±3.2	Israel, one group, supervised	3 session acute exercise V: volleyball A: submaximal aerobic exercise AN: anearobic exercise	Visual memory span Digit span test (before and after the acute exercise)	V >A, AN (WM↑)
Zhang et al. (2009); (6)	V: n=17 Age:=20.2±1.9 C: n=20 Age=19.0±0.8	China, Two groups	V: Volleyball group (8–10 years training) C: Control group	Multiple object tracking task	RT V <C
Zwierko et al. (2014); (7)	V: n=11 Age=15.09±0.53 C: n=7 Age=14.85±0.38	Poland, Two groups	V: Volleyball group (3.37±0.44 years training) C: Control group (no training) Intervention: 2 years of systematic training for V (twice a day)	Visual evoked potentials	P100 latency V↓ N75 latency V↓

Note: ↑= within group improvements or ↓decrements; > or <= group by volleyball interaction effects; F= Female; fNIRS= functional Near-Infrared Spectroscopy; fMRI: Functional magnetic resonance imaging; HbO_2= Oxygenated Hemoglobin; M= Male, n.a= not available; PFC= Prefrontal Cortex; PMC= Premotor Cortex, RT= Reaction Time, WM= Working Memory.

Cognitive and Neurophysiological Outcome Measures

20 studies assessed at least one relevant cognitive function[7,17–35]. Two studies measured both cognitive skills and neurophysiological parameter[19,31]. There was only one study measured only neurophysiological parameter[36]. Executive functions (EFs) refer to a group of cognitive processes that allow humans to concentrate, plan, organize and make complex judgments[37]. We based on the general consensus that defines three core EFs which are inhibition, working memory and cognitive flexibility to assess the studies[38,39]. Given this model, there were fourteen studies that assessed core EFs[7,17,18,20,23–29,33–35].

As displayed in Table 1, eleven studies reported that playing volleyball decreases reaction times in cognitive tasks compared to untrained controls[7,17,21,23–26,30,32,33,35]. Two studies demonstrated that experience had an effect on reaction times;[20,21] experienced volleyball players were faster than novice ones in cognitive tasks. Additionally, two studies pointed out the effect of sport type on reaction times with contradictory findings[21,28].

Fourteen studies reported that playing volleyball increases the accuracy scores in visuopatial attention,[18,20,21,33] prediction,[23,24,26,28,29,32] categorization,[30,31] and working memory[7,34] tasks. See Table 1 for more comprehensive details of the outcome measures.

Discussion

In this review, we present an attentive overview of the effects of playing volleyball on healthy people's cognitive skills and brain functions. We found 21 studies that assessed the effects of playing volleyball on cognitive functions and neurophysiological parameters. Overall, playing volleyball has been shown to improve specific cognitive functions.

The first research that published the data about the association between cognitive functions and physical activity decades ago demonstrated that men regularly participating in sports outperform in reaction time tasks than their sedentary counterparts[40]. Since the first publication, an increasing body of evidence showed that exercise improves cognitive function, particularly EFs[1,41]. Consistent with the literature, most of the research reviewed within this study's scope suggested that volleyball players exhibited superior abilities in EFs as attention management, working memory, inhibition, and tasks of cognitive flexibility.

Effect of Training Characteristics of Volleyball

Motor and cognitive switching tasks are frequent while playing volleyball which is an open-skill exercise. Volleyball players must constantly adapt or switch to more proper actions to respond to the opponent's actions. They have to follow not only the rules of the game but also improve accurate strategies. An exercise that requires substantial cognitive demands such as volleyball may change neurocognitive functioning and affect the brain activation associated with EFs. Previous findings demonstrated that open-skill exercise improves cognitive flexibility at switching tasks[42,43] and led to greater improvement in inhibitory control[44,9], cognitive flexibility,[42,43,45,46] audio-visual perception,[47] problem solving,[48] visuospatial short-term memory[49] and visuospatial attention[50]. In line with the literature, four studies in this review supported that volleyball was more effective to improve cognitive skills than closed-skill sports. Volleyball effects were superior to closed-skill sports such as running, rowing, sprinting, aerobic/anaerobic activity in visuospatial attention processing, inhibition, anticipatory skill, working memory.

In this review, volleyball players were reported to have shown superior cognition scores than karate and badminton athletes[7,20]. Although karate and badminton are open-skill sports, the more significant effect of volleyball may be explained by its being a team sport. The social support that can arise from being a part of a team might positively affect cognitive skills[51].

Motor coordination involves a balanced, fast, and precise motor response that harmonizes the nervous and musculoskeletal systems. Sensory input, perceptual and cognitive processing, action production must occur in the proper sequence. Neuroimaging studies indicate that some brain regions such as the cerebellum and basal ganglia formerly thought to be only related to the motor activity are also activated during specific cognitive activities[52]. The prefrontal cortex, posterior parietal cortex, and cerebellum network are involved in cognitive functions such as working memory, attention, perception[53].

Chasing the ball and response selection in a volleyball match needs attentional control, visual processing (cerebellum, dorsolateral prefrontal cortex, posterior parietal cortex, middle occipital cortices), and planning (anterior cingulate cortex, supplementary motor areas)[53,54]. It was demonstrated that coordinative exercise interventions had shown more positive effects on cognition than standard sport lessons[55]. Studies

showing the linear relationship between motor coordination and academic achievement are also evidence of how motor coordination improves cognition[56,57]. Due to volleyball being a sport involving complex motor tasks such as balance control, quick responses, and task-switches, the network mentioned above may be activated during the game.

A top-down control process is demanded to perform convenient judgment, accurate decision-making, and timely action in a coordinated and flexible way. Decision-making is a part of executive control, and the prefrontal cortex is the main area for this task. It is one of the most effective cognitive processes needed while playing volleyball. That executive function accomplishes identifying and choosing alternatives based on the advantages and preferences[58]. In order to maximize the performance, quick and accurate decision is essential in volleyball. This repetitive cognitive process may explain why the volleyball players are better at decision-making tasks[19,22]. Indeed, the fMRI study included in the review showed that volleyball players' activity in the left primary motor cortex hand area and the left premotor cortex was decreased in impossible actions whereby their accurate decision-making mechanism[31]. One possible explanation is that volleyball experts are able to discriminate possible vs. impossible actions, anticipate the context and use neural resources in this direction. Exposure to regular and repetitive commands and contexts in sport might improve the implicit motor simulation context in expert players so that expert players make more accurate decisions by recruiting fewer neural resources.

Neurobiological Considerations

Greater cardiorespiratory fitness is associated with better cognitive functioning[59]. Some researchers have suggested that exercise-induced increased levels of neurotrophins and increased cerebral blood flow explain the link between cardiorespiratory fitness and cognition. Training methods in volleyball create a higher metabolic profile and involve jumping and plyometric exercises designed to produce quick and explosive movements. Greater explosive strength had been associated with better cognitive function, information processing speed, and inhibitory control[60,61]. One possible explanation for the link between explosive strength and cognitive tasks is that they share similar physiological mechanisms. After the stimulus arrives at the sensory organ, a neural signal is created, and transmission, processing, and muscle activation occur[62]. From this point of view, athletes who can generate faster muscle activation may develop a faster reaction in cognitive tasks. This mechanism might explain the shorter reaction times in volleyball players. EEG study results supported that idea by showing that playing volleyball reduced signal conductivity time through the visual pathway and indicated that playing volleyball can affect very early sensory processing[36].

The location of the mirror neuron system (MNS) in the human and its function in understanding the movement and social cognition was demonstrated by previous work[63–66]. A recent study has shown the positive effect of exercise on the MNS[67]. Because volleyball is a team sport, both the opponent's and the teammates' actions and gestures must be followed during the game.

This recurrent experience may have an effect on the MNS of the volleyball player. It is possible to be activated the MNS to predict the opponent's movement and change or withdraw the planned action during the game so that enhanced MNS activation may contribute to the other cognitive tests that involve these tasks' anticipatory skills and inhibition.

One general hypothesis described as the broad transfer is that skill transfer will occur if the original and transfer tasks include overlapping processing elements and engage, at least in part, the same brain regions[68]. This idea may explain transfer from cognitive skills acquired during sports training and similar processes outside of the domain of sport[69]. Previous research findings into the broad transfer hypothesis have been inconsistent and contradictory. One study supports the broad transfer hypothesis by demonstrating that the expertise of athletes can be transferred to non-sports-specific contexts[70]. On the contrary, one study rejects the idea of the transfer hypothesis[71]. In the majority of research in this review the decrease of reaction times in favor of volleyball players may be the result of a skill acquired by athletes over years' practice and transferred to a non-sporting context[7,17,20,23–26,28,30,33,35]. In line with this opinion, a meta-analysis showed that athletes outperformed non-experts in cognitive skills like processing speed and visual attention[72].

Conclusion

Understanding how the brain differentiates following sports experience is essential to ensure that exercise is part of preventive and remedial interventions. The results presented here demonstrated playing volleyball is an improving way for cognition. Based on these

outcomes, we concluded that the effects of volleyball experience on working memory, inhibition, visuospatial skills, attention shifting, perception, basic processing network are reflected essentially in measures of accuracy and reaction times.

Nevertheless, much remains to be learned about the relationship between sports experience and cognition, particularly influencing factors and underlying mechanisms. Further research on various sports disciplines and cognitive relationships should address different target groups and individual needs.

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Supplementary

Supplementary Table I. Quality assessment of observational studies

1st author, year	Quality scoring	Final score
Alves et al. (2013)	1-1-0-1-0-1-0-1-1-1-1-0	8
Chiu et al. (2017)	1-1-0-1-1-1-0-0-1-1-1-0	8
Costa et al. (2018)	1-1-0-0-1-0-0-0-1-1-1-1	7
Fontani et al. (2006)	1-1-0-1-1-0-0-0-1-1-1-0	7
Giglia et al. (2011)	1-1-0-0-1-1-0-1-1-1-1-1	9
Gil et al. (2012)	1-1-0-1-0-0-0-1-1-1-1-0	7
Kioumourtzoglou et al. (1998)	1-1-0-0-1-0-0-1-1-1-1-0	7
Kioumourtzoglou et al. (2000)	1-1-0-0-0-1-0-1-1-1-1-0	7
Kokubu et al. (2006)	1-1-0-1-1-1-0-1-1-1-1-0	9
Loffing et al. (2015)	1-1-0-1-1-0-0-1-1-1-1-1	9
McAuliffe. (2004)	1-1-0-0-0-0-0-1-1-0-1-0	5
Meng et al. (2019)	1-1-1-0-1-1-0-1-1-1-1-1	10
Nuri et al. (2012)	1-1-0-0-1-0-0-1-1-1-1-0	7
Schorer et al. (2013)	1-1-0-0-1-1-0-1-1-1-1-1	9
Tomasino et al. (2012)	1-1-0-1-1-1-0-1-1-1-1-0	9
Tomasino et al. (2013)	1-1-0-1-1-1-0-1-1-1-1-0	9
Urgesi et al. (2012)	1-1-0-1-1-1-0-1-1-1-1-0	9
Vansteenkiste et al. (2014)	1-1-0-1-1-1-0-1-1-1-1-0	9
Zhang et al. (2009)	1-1-0-1-0-0-0-1-1-0-1-0	6

Supplementary Table II. Quality assessment of intervention study

1st author, year	Quality scoring	Final score
Zach and Shalom (2016)	0-0-0-1-0-0-0-1-1-1-1	5

Supplementary Table III. Quality assessment of cohort study

1st author, year	Quality scoring	Final score
Zwierko et al. (2014)	+ + ? ? - + + + + - ? +	Moderate

A. Items of quality assessment tool for observational studies.

Study purpose

1. Was the study purpose clearly stated?

Study design and methods

2. Were eligibility criteria and the sources and methods of selection of participants clearly defined?
3. Were all outcomes, exposures, predictors, potential confounders, and effect modifiers clearly defined using standardized methods of acceptable quality?
4. Was exposure measurement carried out using standardized methods and measures and with acceptable quality?
5. Were the effects controlled for current (from physical activity assessment to cognitive function assessment) physical activity behavior?
6. Were the results adjusted for sedentary behavior?

Statistical methods

7. Was choice of confounders adjusted for, and in the case of subgroup analysis, was the definition of subgroups appropriate (sex, age, education or IQ, social surroundings, chronic diseases, alcohol, and smoking)?
8. Were all statistical methods, including those used to control for confounding and to examine subgroups and interactions, appropriate (i.e. sample size, statistical power)?
9. Were methods dealing with missing data appropriate?

Results

10. Were descriptive data and results of inductive analysis clearly stated?
11. Were unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval) given?

Discussion

12. Were study limitations clearly stated?

B. Items of quality assessment tool for intervention studies.

1. eligibility criteria
2. randomization
3. concealed allocation
4. similar baseline
5. blinding of all subjects
6. blinding of all therapists
7. blinding of all assessors
8. more than 85% retention
9. intention to treat analysis
10. between-group comparison
11. point measures and measures of variability

C. Critical appraisal skill program (CASP) score criteria of Oxford Center for Evidence-based Medicine

1. Whether the study address a clearly focused issue
2. Whether the cohort were chosen in an acceptable way
3. Whether the exposure precisely measured to reduce bias
4. Whether the outcome precisely measured to reduce bias
5. Whether the authors identified all significant confounding factors
   Whether they considered con-founding factors in the design or analysis
6. Whether the follow up of subjects was complete
   Whether the follow up of subjects was long enough
7. Whether the result of this study in complete
8. Whether the result was accurate
9. Whether the result of the study in believable
10. Whether the result could be applied to local population
11. Whether the result fit with other available evidence
12. Whether this study provided implication for practice

DERLEME / *REVIEW*

Past to Present, Epidemiology of SARS-CoV-2 Infection and Ways of Treatment: A Review

Geçmişten Bugüne, SARS-CoV-2 Enfeksiyonun Epidemiyolojisi ve Tedavi Yolları: Derleme

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ABSTRACT

The rapidly spreading COVID-19 pandemic, which has spread worldwide, emerged at the end of 2019 in Wuhan, Hubei province of China. It has been declared a pandemic by the World Health Organization as of March 12, 2020. The SARS-CoV-2 virus, which caused millions of people to be infected and killed many people worldwide, is a severe public health problem. The average incubation period is 4–5 days (0–14 days). The most common symptoms are fever, dry cough, joint pains, headache, and shortness of breath. The disease can show severe and mortal effects in people with advanced age and different treatments. The disease diagnosis is made by molecular analysis of respiratory samples of suspected cases using special primers. In addition to supportive vitamin and protein support in treatment, specific vaccines are produced, the number of which increases day by day. The absence of close contact, such as handshaking and hugging, and the isolation of possible patients are among the most effective methods to prevent the disease's spread. In addition to personal protective equipment such as masks and gloves, continuous cleaning is essential in the fight against the epidemic.

This study looks at the COVID-19 pandemic from a general perspective, especially with the current advances in vaccines, and evaluates the past pandemic processes worldwide. It is to make inferences by considering the impact of COVID-19 on the world and Turkey.

Key words: *COVID-19; coronavirus; vaccines; pandemics; viruses*

ÖZET

Tüm dünyaya hızla yayılan COVID-19 pandemisi 2019'un sonunda Çin'in Hubei eyaleti Wuhan şehrinde ortaya çıkmıştır. 12 Mart 2020 itibariyle Dünya Sağlık Örgütü tarafından pandemi olarak ilan edilmiştir. Tüm dünyada milyonlarca insanın enfekte olmasına ve çok sayıda kişinin yaşamını kaybetmesine neden olan SARS-CoV-2 virüsü halen daha ciddi bir halk sağlığı problemi olarak etkisini sürdürmektedir. Virüsün yarasa kaynaklı olduğu düşünülmektedir. İnkübasyon süresi ortalaması 4–5 gün (0–14 gün) olarak gösterilmiştir. En yaygın semptomları ateş, kuru öksürük, eklem ağrıları, baş ağrısı ve nefes darlığıdır. Hastalık ileri yaş ve farklı hastalık tedavisi gören kişilerde şiddetli ve mortal etkilerde gösterebilmektedir. Hastalığın teşhisi, şüpheli vakaların solunum yollarından alınan örneklerin özel primerler kullanılarak moleküler analizlerinin yapılmasıyla olur. Temelde tedavide destekleyici vitamin ve protein desteğinin yanında sayıları günden güne artış gösteren spesifik aşılar da üretilmektedir. İnsanların birbirleriyle tokalaşması ve sarılması gibi yakın temasların olmaması ve muhtemel hastaların izolasyonu, hastalığın yayılmasını engellemede en etkin yöntemlerden birisidir. Bulaştan korunmak için maske ve eldiven gibi kişisel koruyucu ekipmanların yanında sürekli bir şekilde temizlik salgın ile mücadelede oldukça büyük öneme sahiptir.

Yapılan bu çalışma; özellikle aşılarda yapılan güncel ilerlemeler ile, COVID-19 pandemisine genel bir perspektifte bakmak ve geçmişte dünyada yaşanan pandemi süreçlerini değerlendirmektir. COVID-19'un dünyada ve Türkiye'de etkisini de dikkate alarak, çıkarımlarda bulunmaktır.

Anahtar kelimeler: *COVID-19; coronavirüs; aşılar; pandemi; virüsler*

Introduction

Although viruses are not cells, they have a nucleic acid genome that encodes functions necessary for the formation and replication of an extracellular form called the virion, which allows the virus to spread from one host cell to another. They are non-living pathogenic agents that live only in the cells of another organism. They are in a structure that is several times smaller than bacteria. Viruses are made up of a protein coat. They contain DNA or RNA as genetic material within this sheath (capsid)[1]. They need a host organism to reproduce. As hosts, they can also infect human cells. The infected adult virus is called a virion and all viruses synthesize their capsid proteins[2]. After viruses infect

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the cell, they inactivate the cell and multiply rapidly by spreading to other cells. Viruses are destroyed in a few hours on non-living surfaces or cells. Antibiotics do not affect viruses. Antiviral drugs are used instead of antibiotics. The most important step in preventing the infection of viruses in the cell is to be vaccinated.

Throughout history, there have been many epidemics caused by viruses. These diseases have affected people negatively. These diseases have been declared a pandemic because they spread in certain geographies. Plague disease, which is called the Black Death, is a disease that started in Central Asia in 1347 and spread to China. It caused the death of 12.5 million people in total[3].

HIV (human immunodeficiency virus), which caused 36 million deaths in 1981, suppresses the immune system of people and reveals the presence of AIDS[4].

The H1N1 virus, which is called the Spanish flu, which surrounds the whole world and causes repercussions with the frequent death of even healthy young people, has caused the death of millions of people[5].

Cholera, the first case of which was detected in 1817 and seen in approximately 2,260,389 patients, is a disease caused by *Vibrio cholerae*, which is transmitted by stale food or water and causes diarrhea in people. These cases resulted in 45,543 human deaths[6].

Typhoid is a disease that causes infection within 7–15 days, transmitted from wastewater caused by *Salmonella typhi*. It is transmitted from person to person with the help of vectors such as lice and fleas. Worldwide, approximately 20 million people suffer from typhoid every year. One out of every 100 people these patients results in death[7].

Ebola, a disease that was first detected in 1976 and lasted until 2014, is the longest-lasting virotic disease. By 2014, more than 1000 people, most of whom were healthcare workers, had died[8]. It was reported that the virus case fatality rate, which increased again on March 21, 2014, was at the level of 59% and in May 2014, the case fatality rate increased to 64%[9]. It has effects such as nausea, vascular rupture, abdominal pain, and bleeding in the gums[10].

Smallpox is the deadliest disease ever. It has continued throughout history. Even the scars on the skin of mummies are reported to be smallpox. It is a disease in which half a million people die today. With the discovery of the vaccine by Dr. Edward Jenner in 1976, humans gained immunity, and deaths decreased[11].

Sars 2003 is a pneumonia-causing disease that originated in southern China. Sars 2003 symptoms are fever and difficulty breathing. In 2003, it spread to other countries and increased its influence[12]. This disease, which can be transmitted to individuals by hanging in the air or by contact, can spread collectively. The World Health Organization announced on March 13, 2003, that it was a global epidemic[12]. In 2003, 8437 cases were recorded and 823 people died.

Influenza A H1N5 is a panzootic disease that can be transmitted from poultry to humans. Bird flu, which poses a great danger to the health of animals and humans, has been accepted as a pandemic[13]. In addition, the infected virus that infects the human respiratory system can cause death[14].

Influenza A H1N1, the causative agent of which is from the Orthomyxoviridae family, emerged in the United States in the spring of 2009 and spread to many regions within a month. It is the most mutated type of influenza among other influenza viruses. This virus, which causes a total of 94,512 cases and 429 deaths in the world, shows the symptoms of headache, sore throat, cough, and runny nose[15].

Mers 2012, known as coronavirus, MERS-CoV is a zoonotic disease that has been proven to exist in the Arabian peninsula[16]. This virus, which caused the death of 858 people in 27 countries in total, can circulate in molecular form in the air and can be transmitted by contact with the surfaces of goods[17].

Influenza A H7N9, which is generally determined to occur after close and long-term contact with bird species, was first detected in March 2013 in China. An (H7N9) virus of avian origin is a serious fatal disease[18].

COVID-19 Epidemiology

In the city of Wuhan, located in the Hubei region of China, cases of pneumonia whose etiology could not be fully determined were recorded by the local representative of the World Health Organization on 31 December 2019. In January 2020, Chinese scientists analyzed the genome of the virus and named it 2019-nCoV. Later, the virus name was changed and given the official name SARS-CoV2. On January 7, 2019, cases were reported to be caused by a new type of coronavirus with no previous evidence that it could infect humans[19]. Research on the disease agent has revealed the similarity of the virus with Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS). The high rate of spread and reproduction of this new virus, called COVID-19, caused

the severity of the virus-borne disease to be described as a global epidemic on March 11, 2020[20].

The first cases in the world were reported in China in December 2019[21]. Between 18 December and 29 December, 5 cases were detected and one of these cases died[22]. By January 2, 41 patients were diagnosed with COVID-19. It has also been reported that many of these patients have sub-diseases such as hypertension and diabetes[23]. The total number of cases reached 571[24]. The number of cases spread very quickly, reaching 5502 cases on January 24[25]. On January 30, the total number of cases in China was 7734, and a total of 54 cases were detected in Singapore, the USA, India, Canada, France, and Germany outside of China. The total case and total mortality rate were calculated as 2.2%[26]. After the case was seen in the United States, the identification of the virus and disease stages were investigated, and mild symptoms turned into pneumonia on the 9th day. The transmission of the virus from person to person was proven for the first time by the USA[27].

On January 10, 2020, before the case was seen in Turkey, the ministry established the scientific committee, which is the COVID-19 research team. At the beginning of the measures taken, thermal cameras were installed at the airports. On March 13, 2020, a total of 5 cases were seen in Turkey, with the first case. Despite many restrictions, on March 19, 2020, the number of cases was 359, while the number of deaths was reported as 4 people[19].

COVID-19 Etiology

Coronaviruses are from the Orthocoronavirinae family, which originates from animals. The coronavirus, which can make humans and animals sick, is positively charged and has a single chain and enveloped spike-shaped cilia. These viruses, which have 4 different genera, are known as α (Alpha), β (Beta), γ (Gamma), and δ (Delta). The genera that can infect humans are α (Alpha) and β (Beta). As a result of the researches, the SARS-CoV-2 Omicron variant, which was first reported to the World Health Organization in South Africa on November 24, 2021, was detected. They can infect not only humans but also all mammals. Coronavirus strains that can infect poultry are known as γ (Gamma), and δ (Delta)[28,29]. As previously known, six types of coronaviruses (HCoV) that can infect humans have been identified[30]. The coronavirus that emerged in Wuhan was determined as the seventh species with three different strains as a result of research, and it was deemed appropriate to be named coronavirus 2 (SARS-CoV-2) by the International Virus Taxonomy Committee[31].

SARS-CoV-2 shows 59% and 79% similarity to MERS and SARS-CoV, respectively. It has been reported that COVID-19 is less associated with MERS-CoV[32]. However, the receptor-binding gene regions are very similar. The receptors use the same intracellular converter, the ACE2 enzyme[33]. Bats are thought to be the host of SARS-CoV-2. Further work is essential to determine the origin of these virüs[34]. In addition, it has been reported in different studies that there are pangolin, snake, and turtle as creatures that infect different animals as intermediate hosts[35]. The source of the virus is not yet clearly known. Knowing and isolating the source is very important in both vaccine and drug studies and reducing the spread of the virüs[36]. Due to the severe Coronavirus-2 (SARS-CoV-2), the COVID-19 pandemic poses an unprecedented challenge to life on Earth. SARS-CoV-2 can result in death by causing diseases such as asymptomatic acute respiratory distress syndrome (ARDS) and multi-organ involvement. Ethnic origins of people also vary according to the people against the COVID-19 pandemic. The genetic susceptibility of people infected with COVID-19 is increasingly known. For this reason, studies on associating COVID-19 with the genome and related genes are continuing.

Ways to Avoid SARS CoV-2 Infection

The coronavirus group viruses that infect humans are transmitted through droplets from the respiratory tract fluids of sick individuals. However, different modes of transmission have been reported during the SARS-CoV-2 pandemic, such as direct contact with contaminated surfaces, inhalation of aerosol containing viral particles, and fecal contamination (Fig. 1)[37].

Learning the transmission routes has paved the way for taking measures to minimize human contact in society to prevent the spread of the disease. The Chinese government imposed a travel ban by closing the city of Wuhan, where the disease was first seen, on January 23, 2020. However, the disease spread to the whole country in a short time like 3 days, and it was made obligatory to wear masks in public spaces to prevent healthy individuals from being infected by carriers of the disease. Therapeutic antibodies that affect the receptor-binding site of the virus, such as REGN-CoV-2, have been noted to provide substantial protection against SARS-CoV-2 infection. In this field, companies such

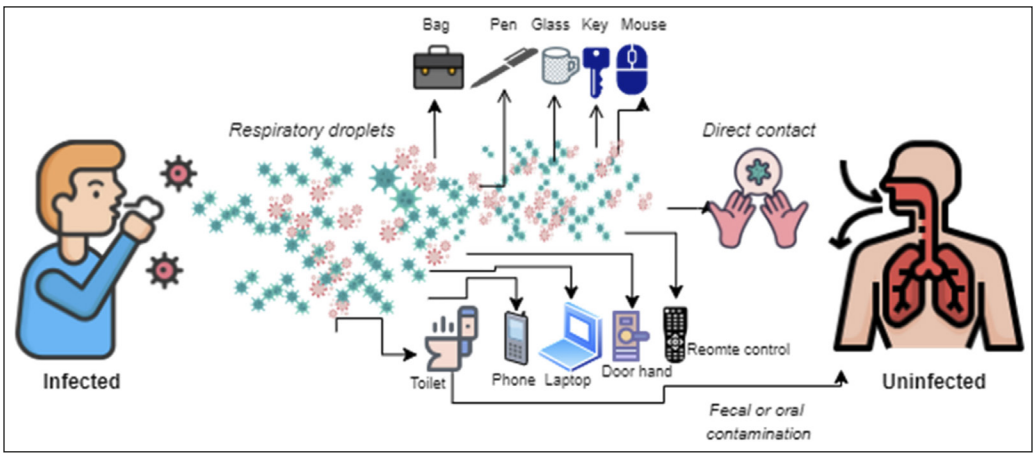

Figure 1. *SARS-CoV-2 transmission routes.*

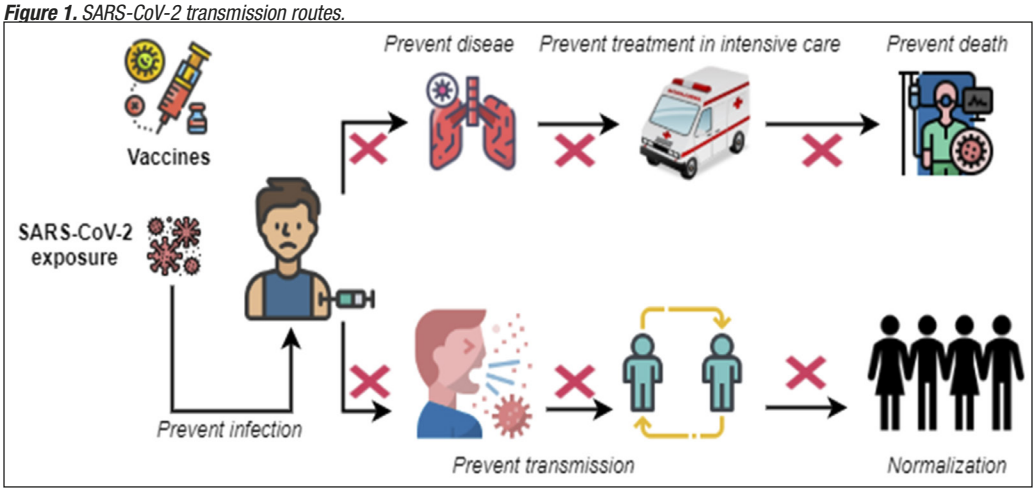

Figure 2. *The possible protection mechanism of Covid-19 vaccines.*

as Regeneron, Eli Lilly, and AbCellera have developed products that have reached the stage of human trials[38].

It is known that vitamin D, an endogenous steroid hormone synthesized in the skin under the influence of UVB rays, plays a role in the prevention of infectious diseases. Since vitamin D deficiency causes immunodeficiency against respiratory diseases, it is thought that supplementing with vitamin D to individuals with vitamin deficiency may be effective in preventing COVID-19[39].

The effect of the herbal mixture called Qingfei Paidu Decoction (QFPD), which is used against pneumonia and viral infections in traditional Chinese medicine, in the prevention and treatment of SARS group viral infections was investigated. Studies have revealed that the product can be effective in the new Coronavirus infection thanks to its anti-viral and anti-inflammatory activity[40,41]

Since the first days of the pandemic, studies have been carried out for the production of vaccines that will be effective on SARS-CoV-2 (Fig. 2). Vaccination aims to prevent the mucosal colonization of the virus and its progression in the lower respiratory tract[42].

Despite many measures taken at the national and international level to prevent the spread of the disease, there are weaknesses in complying with the epidemic measures. In a study conducted with the participation of 4732 people living in Turkey, it was determined that 67% of people with chronic diseases did not comply with epidemic prevention measures[43].

SARS CoV-2 Incubation Time

Angiotensin-converting enzyme 2 (ACE2) is an enzyme that hydrolyzes the hormone angiotensin 2, which causes the narrowing of the vessel walls. This enzyme, which plays a key role in the regulation of the cardiovascular system, is used by viruses of the SARS group as a cell surface receptor that facilitates the entry

of viral RNA into lung cells[44]. Studies have shown that SARS-CoV-2 infection occurs in direct proportion to ACE2 enzyme expression[45]. COVID-19 infection is studied in four stages; The first stage is upper respiratory tract infection. In the second stage, with the progression of the infection, dyspnea (shortness of breath) and pneumonia (pneumonia) are observed. The third stage of infection is characterized by the worsening clinical picture caused by the cytokine storm. In the final stage, death or recovery occurs[46].

In the researche, different results have been reached regarding the incubation period of the virus. In the early stages of the epidemic, it was reported that the number of cases doubled in an average of 7.4 days. In studies on 1099 patients, the estimated incubation period for SARS-CoV-2 was 3.0 days. Symptoms (95%) of the disease began to appear within 10 days of exposure to the virus. In another study conducted on 138 cases, the time elapsed between the onset of the first symptoms and the appearance of the severe acute respiratory syndrome was determined as 5–8 days[47]. Although the incubation period extends to 2–11 days, it is generally accepted as 5–6 days. After the incubation period, the prodormal period begins, in which symptoms begin to appear[37]. Person-to-person transmission of SARS-CoV-2 usually occurs between family members living in the same household, in the hospital, and in public areas. Transmission usually occurs during the incubation period[48].

Symptoms of COVID-19

People infected with SARS-CoV-2 usually develop symptoms related to upper respiratory tract infection in the first stage of the disease. The most common symptoms are high fever, sore throat, cough, and shortness of breath. Studies have shown that most of the patients have complaints such as headache, loss of smell and taste, diarrhea, and abdominal pain in addition to these basic symptoms. Neurological symptoms are quite common in COVID-19 patients. Headache (11–34%) reported complaints of SARS-CoV-2 patients admitted to the hospital. These headaches were defined as abrupt onset, throbbing, or pressure-like moderate to mild pain. Headaches have been noted to have poor sensitivity to common analgesics and have been reported to affect the temporoparietal, forehead, or periorbital region. In addition to headache, changes in mental status, impaired consciousness, paresis, hypo-areflexia, encephalopathy, and cerebrovascular events are other common neurological symptoms. In the analysis of cerebrospinal fluid samples taken from SARS-CoV-2 patients by RT-PCR (reverse transcriptase-polymerase chain reaction), it was noted that viral particles were found in a small number of patients[46,49,50]. In some cases, appetite and weight loss have also been shown as symptoms related to COVID-19 infection[37].

Bilateral pneumonia has been observed in the majority of SARS-CoV-2 patients. In blood tests, findings of leukopenia and lymphopenia were found. It was determined that IL2, IL7, IL10, GSCF, IP10, MCP1, MIP1A, and TNFα values in the blood plasma of patients treated in intensive care units were higher than in other patients (Wang, 2020). Since COVID-19 symptoms vary according to the course of the disease, patients who do not show symptoms are grouped as asymptomatic and other patients according to their symptoms are grouped as moderate and severe patients. High fever was observed in 98% of patients, cough in 76%, and myalgia (muscle pain) or fatigue in 44%. Atypically, 28% of patients reported sputum, 8% headache, 5% bloody cough, and 3% diarrhea. Lymphopenia was found in 63% of the patients and shortness of breath in 50%. The incidence of lymphopenia is increased in patients with hypertension or chronic lung disease[48]. In elderly patients, pneumonia and organ failure may occur in the later stages of the disease[51]. The neuropsychiatric effects of SARS-CoV-2, on the other hand, remain unclear. The data obtained show that psychotic spectrum disorders occur in 0.9–4% of patients. It is thought that neuropsychiatric disorders such as mild cognitive impairment, mood changes, insomnia, psychosis, encephalopathy, and suicide can be seen with the effect of the disease[52].

Effects of COVID-19 Infection by Age

SARS-CoV-2, like all other infectious group diseases, causes different effects to be observed according to age groups in terms of symptoms and severity of the disease[37]. Early research on COVID-19 infection revealed that children are less affected by the infection. In a study conducted on 72,314 patients, only 1% of the patients were under the age of 10, there were no fatal cases in the patient group under 9 years of age, and the mortality rate was 8.0% in cases aged 70–79; In cases over 80 years of age, the mortality rate was recorded as 14.8%. In another study conducted on 2143 pediatric patients, it was stated that children showed mild upper respiratory tract infection symptoms and more severe symptoms occurred in infants under the age of 1, whose immune

system has not yet completed its development[42,48]. It has been determined that morbidity in SARS-CoV-2 infections is associated with advanced age. Scenarios such as the severity of the disease, the presence of comorbidities such as obesity, diabetes, hypertension, increase in D-dimer and C-reactive protein levels, and decrease in lymphocyte count affect the mortality rate[38,46].

In a study in which data from 23 clinical studies were analyzed, it was reported that smell and taste disorders caused by COVID-19 infection mostly affect young female patients, and complaints of loss of smell persist for longer in younger patients[53]. In statistics on COVID-19 patients in the intensive care unit in Italy, it was determined that the average age of the patients was 63 in 1,591 fatal cases, and 82% of them were male. When the data of 1,300 patients who received respiratory support were examined, it was seen that 88% of the patients were treated in the intensive care unit with mechanical and 11% non-invasive respiratory support, and the mortality in the intensive care unit was 26%[38]. When the rates of re-positive PCR tests of the patients were evaluated, it was determined that the patients under the age of 18 had milder symptoms and complaints, but a higher rate of COVID-19 positive results[54].

Studies by the Chinese Center for Disease Control and Prevention have shown that 86.6% of the cases reported to date include patients aged 30–79 years, with patients over 60 years of age at higher risk compared to late patients who experience milder symptoms or are asymptomatic[47]. Age also affects clinical findings. In the studies, it was revealed that the blood urea nitrogen levels, inflammation indicators, and bilateral lesions were higher in COVID-19 patients over the age of 60[48].

COVID-19 Diagnosis and Treatment Ways

Since SARS-CoV-2 infection is a disease that occurs with flu-like symptoms, it is possible with the use of laboratory instruments and radiological methods to make a definitive diagnosis of COVID-19[37]. Laboratory tests are essential to distinguish pneumonia, which is the most serious sign of the disease, from other viral, mycoplasma, and bacterial infections and pneumonia without infection. COVID-19 tests are carried out by laboratory analysis of nasal swab, nasopharynx, and trachea extracts, sputum, lung tissue, blood, and stool samples. To detect viral RNA molecules in early diagnosis, upper and lower respiratory tract samples are analyzed. For this purpose, RT-PCR (Real-Time Polymerase Chain Reaction) method is used[47].

Although RT-PCR is the most actively used analysis method for detecting the SARS-CoV-2 virus, the sensitivity of PCR analysis is affected by variables such as viral load, sample type, and time from sample collection to analysis. Even patients with all symptoms can sometimes be negative due to errors in sampling. In order to minimize these erroneous results, genomic points such as ORF1a and ORF1b nucleocapsid genes and spike protein genes are targeted in RT-PCR[47]. Before the COVID-19 epidemic turned into a pandemic, studies were carried out to develop PCR-based rapid diagnostic kits in January 2020, and these kits began to be used all over the world. In addition to virological tests, it is also diagnosed with serological tests. Pneumonia findings are also evaluated in radiological examinations, and MR and tomography images are used to support positivity in the diagnosis of COVID-19[38].

Favipiravir, Lopinavir, Ritonavir, Hydroxychloroquine sulfate, and Oseltamivir drugs are used in the treatment of COVID-19 in Turkey. Information on the use, dosage, and preparation recommendations of these drugs, as well as possible drug interactions, have been announced by the official authorities and the documents have been uploaded to the relevant websites in a way that can be accessed by everyone.

Hand Hygiene

Hand hygiene is of great importance as the virus is transmitted through contact. Proper cleaning and hygiene of hands are very important. Hands should be washed for at least 20 seconds. When soap and water are not available, hand hygiene can be provided with disinfectants containing at least 60% alcohol. The correct use of disinfectants is very important in terms of hygiene[55].

Mask

It is the barrier that prevents the virus transmitted through airborne droplets from entering the body. Hand hygiene and social distance protection should not be neglected when using masks, gloves and rules should not be forgotten when wearing masks, and hands must be cleaned before wearing a mask. The mask should be worn in such a way that the mouth and nose are completely covered. Moistened masks should be changed while changing the mask, they should be changed by holding the ropes, and then the hands should be cleaned. It is not appropriate to use disposable masks more than once[56]. When medical masks are not available, fabric masks should be used and washed at high temperatures[57].

Social Distancing and Isolation

The virus spreads to a distance of two meters in the air, so it is very important to maintain social distance. We know that people who do not show COVID-19 symptoms are carriers, and they should not go out of the house unless necessary. Public vehicles should not be used unless it is very necessary for transportation. Meetings and events that will require crowded should be postponed or canceled. In this process, the continuity of remote working and distance education systems is also important to prevent the spread of the virüs[58].

Villages, neighborhoods, apartments, etc., where a virus case has been detected. areas should be quarantined immediately. The quarantine application determined by WHO should be followed for people who have contact with the sick person or have symptoms during the trip[59]. Quarantine applications are as follows; stay away from the house for 14 days and at least two meters away from the individuals in the house. Contact should not be made with anyone other than the quarantined individuals in the household. In case of any symptoms that will develop within two days, they should not contact anyone and apply to health institutions.

Protection of Healthcare Workers

High-level measures should be taken to prevent the health workers, who are in the highest risk occupational group in the society, from contracting the epidemic. Isolation rooms should be created for patients with symptoms and the number of personnel working in these rooms should be limited. Masks should be worn everywhere in the hospital. Employees who circulate with patients need to take extra precautions. Ventilation in all waiting areas should work actively, and areas, where verbal conversations with patients are made, should be limited with glass barriers. Cleaning of glass barriers should be done routinely, and employee rotation and working times should be shortened. A new section should be opened for COVID-19 patients and people with symptoms, and they should be prevented from sharing the same environment with other patients. It should be preferred that those who need to go to hospitals for prescriptions and drugs should get help by making phone calls as much as possible. Personnel reinforcement should be provided to intensively working hospital units and departments[60].

Environmental Cleaning

Since the disease is transmitted by contact, routine cleaning of contacted surfaces will greatly reduce the rate of viruses infecting people. Places such as door handles and electrical switches in public areas should be wiped and ventilated at regular intervals. Bleach (1/100 ratio) or alcohol (at least 60%) should be preferred for cleaning. Animal products used in meals should not be preferred raw or undercooked. Vegetables or fruits should be washed with vinegar or plenty of water[61].

Drugs

Favipiravir

Favipiravir, which is effective in reducing mild and moderate complaints of COVID-19 patients in early clinical studies; is a commercial drug that has an inhibitory effect on the RNA polymerase enzyme[62]. Favipiravir is currently used in the inhibition of various influenza group viruses (H5N1, H7N9, H1N1)[63]. There are also studies showing that it is an effective antiviral agent in the Ebola epidemic[64]. In studies on COVID-19 patients who had previously been treated with Lopinavir/Ritonavir, it was determined that the use of Favipiravir was more effective in reducing the symptoms of the disease[62].

According to the Information Guide on Drugs to be Used in the Treatment of COVID-19 (SARS-CoV2 Infection) published by the Ministry of Health of the Republic of Turkey, it is recommended to use Favipiravir 200 mg tablet (2×1600 mg for 5 days). Favipiravir is a drug in tablet form that can be swallowed with water. If the drug cannot be used as recommended for various reasons, it can be prepared in liquid dosage form by hospital pharmacists.

It has been determined that the use of favipiravir during pregnancy may cause fetal anomalies and its interactions with various drugs have been recorded. An increase in exposure to Favipiravir has been observed when Alcuronium from the anesthetic/muscle relaxant group, Moxonidine, and Treprostinil from antihypertensive group drugs, Ipratropium bromide from bronchodilators, and paracetamol derivatives from analgesics are used together with Favipiravir. Similarly; Antibacterials, antidiabetics, contraceptives, and hormone replacement therapy agents can also show overexposure when used with Favipiravir. Although no significant interactions of favipiravir with other antiviral drugs have been reported, concomitant use with Oseltamivir is not recommended.

Hydroxychloroquine Sulfate

Hydroxychloroquine is an immunomodulator used in the treatment of rheumatic diseases and malaria.

Prophylactic use of contacts and healthcare workers in the risk group in the COVID-19 pandemic has been recommended. In a study in which the prophylactic effect of hydroxychloroquine sulfate was observed, 821 asymptomatic volunteers were studied. It was noted that 87.6% of the participants were contacted in the high-risk group. Some of the participants were given hydroxychloroquine and some were given a placebo and were followed for 14 days. No significant prophylaxis was observed in subjects receiving hydroxychloroquine supplementation after 14 days[65].

According to the Information Guide on Drugs to be Used in the Treatment of COVID-19 (SARS-CoV2 Infection) published by the Ministry of Health of the Republic of Turkey, the prescribed use of Hydroxychloroquine is 2 x 200 mg tablets for 5 days in outpatients with mild pneumonia symptoms. Since side effects such as ventricular tachycardia and QT prolongation may occur, it is recommended that patients with cardiovascular disorders should be followed up with ECG. The usual method of use is to swallow the tablet with food or milk, without crushing and chewing. In cases where oral use is not possible, the tablets can be crushed and mixed with liquid/semi-liquid foods and used.

The most common side effect of hydroxychloroquine has been noted as QT prolongation. It should be used with caution because of the possibility of cardiotoxicity and retinotoxicity. Since it is predicted that the use of hydroxychloroquine with analgesics, antiarrhythmics, anticonvulsants, and some antipsychotics may cause serious interactions, it is not recommended to be used together with drugs from this group. However, it is generally in the safe group for patients on hormone replacement therapy, using lipid-lowering, inotropes, or vasopressors.

Lopinavir/Ritonavir

Lopinavir/Ritonavir drug combination is an antiviral used in the treatment of SARS and AIDS prophylaxis[66]. It has common side effects such as gastrointestinal system disorders, headache, fatigue, and QT prolongation. The lopinavir/Ritonavir combination is not recommended for use in neonates. *In vitro* and *in vivo* studies have confirmed the inhibitory effect of Lopinavir and Ritanovir on SARS CoV[66]. In the Information Guide on Drugs to be Used in the Treatment of COVID-19 (SARS-CoV2 Infection) published by the Ministry of Health of the Republic of Turkey, the use of 200 mg Lopinavir and 50 mg Ritonavir combination in the form of 2×2 tablets for 10–14 days is recommended for pregnant women with signs of COVID-19. In infants and pediatric patients older than 14 days, 16 mg of Lopinavir per body mass is recommended depending on the progression of the disease course. QT/PR prolongation has been reported when lopinavir and ritonavir are co-administered with some anesthetics/muscle relaxants, antiarrhythmics, antibacterials, antidepressants, antipsychotics/neuroleptics, antiemetic drugs, beta-blockers, and calcium channel blockers. If these drugs are used together, it is recommended to monitor the patient with ECG.

Oseltamivir

Oseltamivir is an antiviral agent widely used in the treatment and prophylaxis of influenza-related infectious diseases. Its success in shortening the duration of the disease and preventing aggravation of the course has been proven[67]. It is recommended to be used especially in pediatric cases with the severe course and accompanying other diseases[68].

Oseltamivir is used in cases showing signs of viral pneumonia associated with influenza. Its use is not recommended for COVID-19. Since the findings of COVID-19 are very similar to seasonal flu and influenza group diseases, and COVID-19 can be detected in patients who are currently using Oseltamivir, interactions with other drugs used in the treatment have been investigated. It has been noted that exposure to Oseltamivir may be increased by 14% when used with favipiravir.

Vaccines for the COVID-19 Pandemic

To end the pandemic caused by SARS-CoV-2 infection, vaccine, and drug development studies have been carried out since the first days of the epidemic. To create an effective treatment and prevention method, the mechanism of action of the virus has been investigated, and the vaccines and drugs produced have been started to be tested on human and animal models. The effects of the developed vaccines were observed on genetically modified BALB/c mice and human experiments were conducted with products that showed successful results[37].

The main purpose of vaccination; is to protect people from SARS-CoV-2 infection, signs of disease caused by infection, and transmission. For this reason, safety and efficacy principles are observed in the formulation of vaccines. However, the examination of candidate vaccines in different countries within the scope of changing parameters such as duration of protection, immunological response, and cost necessitated the

production of vaccine alternatives that could meet different expectations in vaccine development. It has been suggested by the US Food and Drug Administration (FDA) that vaccines can be considered successful if their effectiveness is at least 50%. In vaccine studies, 3 clinical trials are required to prevent severe illness and death from possible side effects. In these studies, controlled infection studies are carried out on volunteers as well as animal subjects[69].

All known vaccine types have been tested in preclinical studies, including attenuated virus, inactivated virus, DNA, mRNA, virus-like particle, and subunit vaccines against the SARS-CoV-2 virus. In attenuated and inactivated virus vaccines, the genes encoding the replication regions of the virus are removed from the genome or inactivated by physical and chemical processes. As another method, viruses other than SARS-CoV are modified by recombinant methods to synthesize the causative compounds of the SARS-CoV virus. Vaccines derived from virus-like particles are vaccines designed to produce an effect similar to that of the virus in the vaccinated organism[51]. The working mechanism of mRNA vaccines, which stands out as a fast and easily modifiable method during the pandemic process, is based on the principle of transferring the gene fragments encoding the metabolites of the virus to the organism in the form of mRNA by modifying it. In studies conducted on 30,420 volunteers, it was found that the mRNA-based mRNA-1273 vaccine caused regional, transient, or systemic reactions in some participants; however, it was found to be effective at a rate of 94.1%[70].

The biggest concerns in vaccine studies are that the vaccine causes an immune response or that vaccines containing viral particles multiply in the body after vaccination and lead to infection. The fact that the vaccines developed to prevent the H1N1 swine flu epidemic that emerged in 1976 caused autoimmune reactions, especially in children, made it necessary to produce the vaccine meticulously and to launch the products after the clinical trials were completed[71]. Another concern with vaccines is vaccine hypersensitivity. Animal studies have found that vaccines containing viral antigens treated with formalin elicit an immune response that leads to Th2 polarization and deficiency of cytotoxic T cells[72].

When the results are observed, according to covid19. trackvaccines. org data, as of end of August 2022, there are 222 vaccine candidates developed against the SARS-CoV-2 virus worldwide. Trials (774) of these vaccines have been made and 41 of them have been approved. The Pfizer/BioNTech vaccine approved in 149 countries and the Moderna Spikevax vaccines approved in 88 countries are RNA-based; Oxford/AstraZeneca AZD1222 approved in 149 countries, Gamaleya Sputnik V approved in 74 countries, Serum Institute of India Covidshield approved in 49 countries, CanSino Ad5-nCoV approved in 10 countries, Janssen Ad26. COV2. S non-replicating virus-based approved in 113 countries; Sinopharm BBIBP-CorV approved in 93 countries, Sinovac CoronaVac approved in 56 countries, Sinopharm Inactivated approved in 2 countries and Bharat Biotech Covaxin approved in 14 countries are inactive and FBRI EpiVacCorona protein subunit based vaccines approved in 4 countries (https://covid19.trackvaccines.org/). Vaccine studies in Turkey started with the Ministry of Health approved Sinovac and Pfizer/BioNTech BNT162b2. Clinical studies of ERUCOV-VAC vaccines developed by CoronaVac and Erciyes University have been finalized and approved.

Conclusion

The COVID-19 outbreak broke out in Wuhan, China's Hubei province in December 2019 and was recognized as a pandemic by the World Health Organization in March 2020. More than 605 million people around the world have been sick so far due to the disease. In addition, approximately 6.48 million people died from these cases. Along with COVID-19, in addition to epidemics such as Ebola, which are limited and effective only in certain regions, in the developing and constantly circulating the world, a pandemic that has affected the whole world has been encountered for the first time. In the face of this unexpected situation, some circles thought that the disease would only be temporary. Other people, on the other hand, were not given the possibility that such a situation could be real at this time. The realistic view of the citizens on this issue has started to become more believable with the loss of their lives as a result of this disease, such as family members, and friends around them. As a result, when we look at it today, it seems that our most powerful weapon, after vaccinations, is cleanliness and social distance.

Authors' Contribution

Study Conception: OU; Study Design: OU, EU, UA; Supervision: AG; Data Collection and/or Processing: OU, EU, UA; Literature Review: OU, EU, UA; Manuscript Preparation: OU; and Critical Review: OU, AG

Conflict of Interest

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EDİTÖRE MEKTUP / *LETTER TO THE EDITOR*

Melanotic Neuroectodermal Tumor of Infancy

İnfantil Melanotik Nöroektodermal Tümörü

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To the Editor,

I read with interest the article entitled 'Melanotic Neuroectodermal Tumour of Infancy (MNTI)' published in your journal[1]. This article addresses a critical tumor type that should be considered during the differential diagnosis of head and neck tumors (HNT) in infants.

Melanotic neuroectodermal tumor of infancy (MNTI) management is based on reports that indicate complete excision as the best treatment[2]. However, the article states, "During the surgery, a tracheostomy was performed for the oral mass, which comprised a solid tumor that filled the oral cavity and exhibited infiltrative growth into the surrounding maxilla and base of the nose." The standard approach to such a large tumor mass should involve a pediatric oncology consultation to use neoadjuvant chemotherapy to induce tumor shrinkage before surgery. However, this should be mentioned in the article. Pre-operative neoadjuvant chemotherapy may decrease the need for wide-margin resection and, consequently, the disfiguring effect of the surgery[3,4]. Moreover, the article does not mention whether the surgery caused any disfiguration in the patient.

Some necessary conditions for the differential diagnosis of HNT were not sufficiently addressed. Melanotic neuroectodermal tumor of infancy is a neuroectodermal tumor; therefore, it can be easily confused with neural crest origin tumors. Elevated catecholamines and neuron-specific enolase should have triggered an investigation of the rosette pattern in the bone marrow for neuroblastoma. The approach should also include bone marrow aspiration, and the article should include the pre-and post-operative catecholamines and neuron-specific enolase levels.

Melanotic neuroectodermal tumor of infancy differential diagnosis includes other small blue round cell neoplasms of infancy, particularly neuroblastoma, Ewing sarcoma, alveolar rhabdomyosarcoma, desmoplastic small round cell tumors, and lymphoma[1]. Mainly, alveolar rhabdomyosarcoma may occur in the head and neck. However, this needs to be adequately differentiated from MNTI.

During the evaluation of the pigmented mass in the head and neck, the differential diagnosis should have included, but not limited to, lymphoma, malignant melanoma, and clear cell sarcoma of soft tissue[5].

In conclusion, this study addresses a rare head and neck neoplasm in infants. Furthermore, it significantly contributes to raising awareness about the differential diagnosis of HNT.

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