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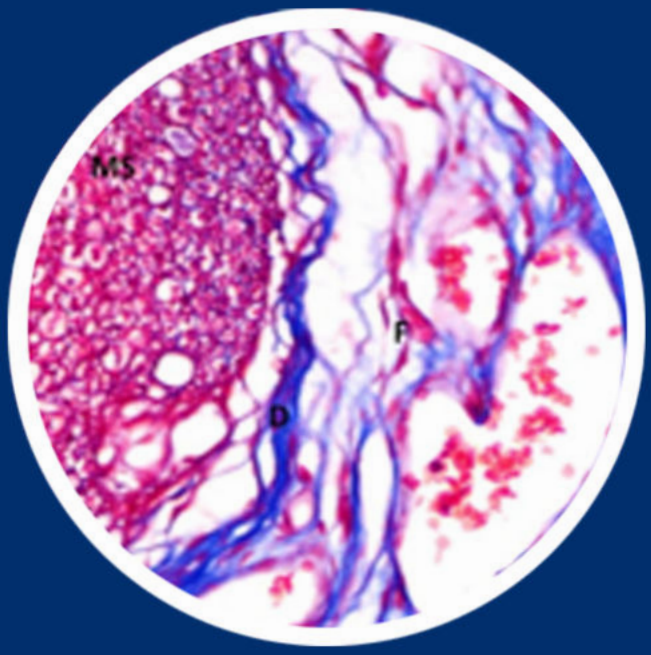

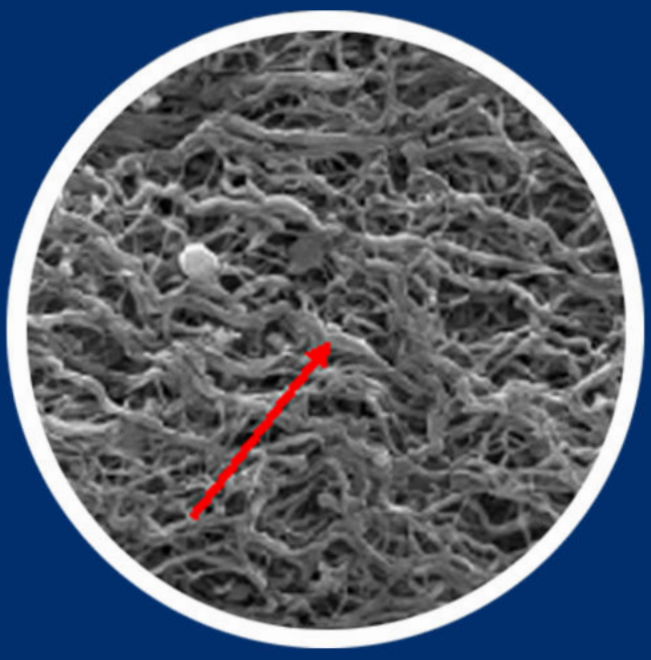

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Original Articles

The European Research Journal 2020;6(6):534-538 Original Article

DOI: 10.18621/eurj.523854 *Neurosurgery*

Dehydrated human amnion/chorion membrane allograft for preventing epidural fibrosis after laminectomy: an experimental rat model

Özhan Merzuk Uçkun[1], Denizhan Divanlıoğlu[1], Rıfat Akdağ[2], Güner Menekşe[3], Ali Dalgıç[1], Ahmet Deniz Belen[1]

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ABSTRACT

Objectives: Post-laminectomy syndrome due to epidural fibrosis (EF) is a common cause of persistent low back pain after lumbar spinal surgery and is challenging for both surgeons and patients. The current experimental study aimed to investigate the effect of dehydrated human amnion/chorion membrane (dHACM) allograft for preventing EF formation following lumbar laminectomy.

Methods: Sixteen Sprague–Dawley rats equally divided into two groups underwent lumbar laminectomy. Group A, the control group, underwent lumbar laminectomy with standard closure. Group B, the experimental group, additionally received epidural dHACM allografts during the procedure. After 45 days of follow-up, the rats were sacrificed, and the harvested tissues were histopathologically evaluated for EF.

Results: Compared with Group A, Group B showed significantly less EF generation ($p < 0.001$), implying that dHACM allografts effectively prevent EF.

Conclusions: This study demonstrated that dHACM can effectively reduce EF formation after spinal laminectomy in rats.

Keywords: Epidural fibrosis, laminectomy, amnion membrane allograft

Failed back surgery syndrome (FBSS) is characterized by refractory back pain with or without lower extremity involvement, occurring after 5%-40% of lumbar disc surgeries [1-3]. Many factors, including inadequate surgical decompression, recurrent disc herniation, epidural fibrosis (EF), and spinal instability, contribute to the risk of FBSS. Of these known factors, EF occurs in 24% of patients with lumbar disc herniation, making it the most common cause of post-laminectomy syndrome [4]. Currently, there exists no known method with proven efficacy for reducing EF after its formation in such cases. Although wide epidural adhesions can be cleared and compressed nerve roots can be relaxed by a secondary surgery, adhesions may recur [5]. As a result, methods involving materials such as free and pedicled oil grafts, synthetic membranes, fibrin foam, gelatin, and dextran sulfate blends (implants containing adcon-l materials) have been explored for preventing EF formation [6-9]. Human amniotic membrane (HAM) is one of promising materials for preventing EF. EF formation after lumbar laminectomy is a multistage process involving

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the accumulation of extracellular matrix components such as collagen, fibronectin, and dermatan sulfate and the reduction of tissue cells [10]. HAM allografts may prevent this process by inhibiting vascularization and apoptosis in epithelial cells along with reducing inflammation [11, 12]. Various methods for processing HAM allografts have been developed, but their usage is associated with challenges such as damaged collagen construction, residual chemical agents, and blood-borne pathogen infections [13]. Recently, an allograft composed of dehydrated human amnion/chorion membrane (dHACM) (AmnioFix, MiMedx, Marietta, GA), which was processed gently for preserving the collagen matrix and its biological activities, was introduced in clinic practice [14]. The current study aimed to investigate if the dHACM is effective for preventing EF formation after experimental lumbar laminectomy.

METHODS

Sixteen adult, male Sprague-Dawley rats weighing between 300 and 350 grams were included in the study. All animals were housed in a suitable animal-care facility under veterinary supervision. During the experiment, until sacrification, all of the subjects were followed in a good state of health without any complications such as wound, infection, hematoma, and cerebrospinal fluid fistula development. All experiments were approved by the Animal Experiments Ethics Committee.Sixteen subjects were equally distributed into two groups: Group A, control group, underwent lumbar laminectomy and Group B, treatment group, received epidural dHACM allografts following lumbar laminectomy. Prior to surgery, general anesthesia with intraperitoneal 60-mg/kg ketamine hydrochloride (Ketalar; Eczacıbaşı, Turkey) and 5-mg/kg xylazine (Rompun; Bayer, Turkey) was administered.

Surgical Technique

Thoracolumbar regions of the subjects were prepared, and the operation site was disinfected using iodine solution. A midline incision between T11 and sacrum was performed. Subsequent paraspinal muscle dissection was followed by T12 to L4 total laminectomies. Adequate care was taken during the entire procedure to protect the spinal cord.

For the subjects in Group A, a standard closure was performed using 0/3 silk sutures for the thoracolumbar fascia and 0/3 prolene for skin. For the subjects in Group B, epidural dHACM allografts were placed before performing the same standard closure.

All animals were housed in a stress-free animal-care facility at room temperature (25 ± 1 °C) with ad libitum access to food and water for 45 days until sacrification using high dose ketamine hydrochloride.

Histopathologic Evaluation

Obtained tissue blocks of muscle, bone, epidural space, and spinal cord structures were sampled and fixed in formalin for histological examination. These samples were then immersed in 10% EDTA for decalcification. To investigate the presence of scar tissue and for microscopic examination, the blocks were stained with hematoxylin-eosin (HE) and Masson's trichrome.

The grade of EF formation was evaluated by a blinded pathologist in accordance with the model recommended by He *et al*. [15] (Table 1). Fig. 1 shows thin fibrous attachments between the dura mater and scar tissue (grade 1). The samples shown in Figs. 2 and 3 demonstrate fibrosis of grade 2 and 3, respectively.

Statistical Analysis

Data were presented as mean ± standard deviation. Differences in histological findings among the two groups were evaluated using the Mann-Whitney U test. The level of statistical significance was set at $p < 0.05$.

RESULTS

The mean histopathological grade of EF was 2.87

Table 1. Grading extent of fibrous tissue

Grade 0	No fibrosis on the dura mater
Grade 1	Thin fibrous bands between the dura mater and scar tissue
Grade 2	Fibrosis forms over less than two thirds of the laminectomy defect
Grade 3	Fibrosis extends over two thirds of the laminectomy defect or to the nerve root

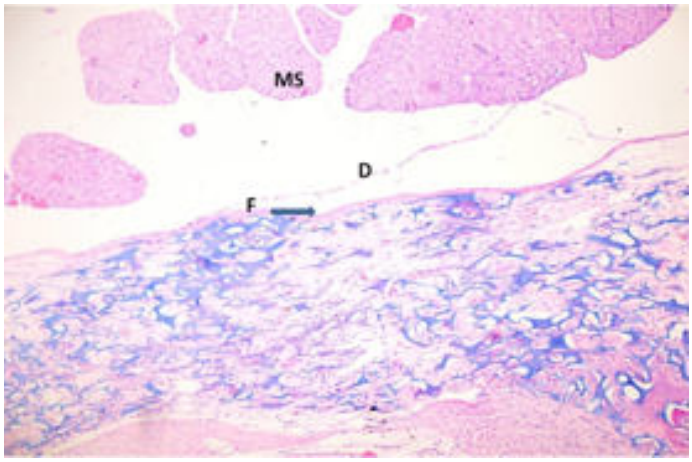

Fig. 1. An example of the thin fibrous attachments between the dura mater and scar tissue (grade 1). [HE ×100] MS: Medulla spinalis, D: Dura mater, F: Fibrosis

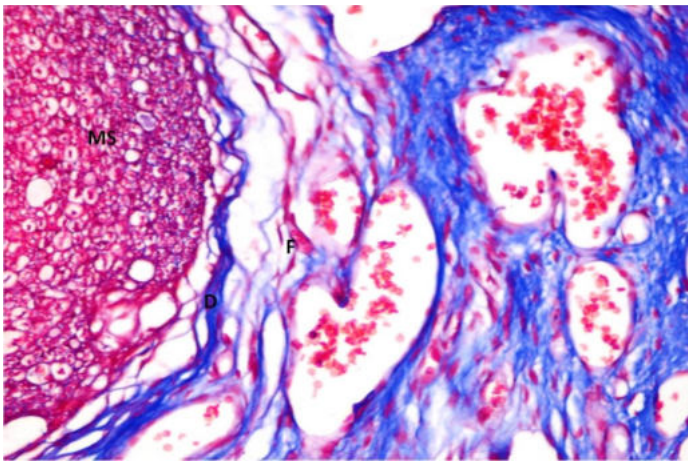

Fig. 2. An example of fibrosis covering less than 2/3 of the laminectomy defect (grade 2). [Masson's trichrome (MT), ×200]

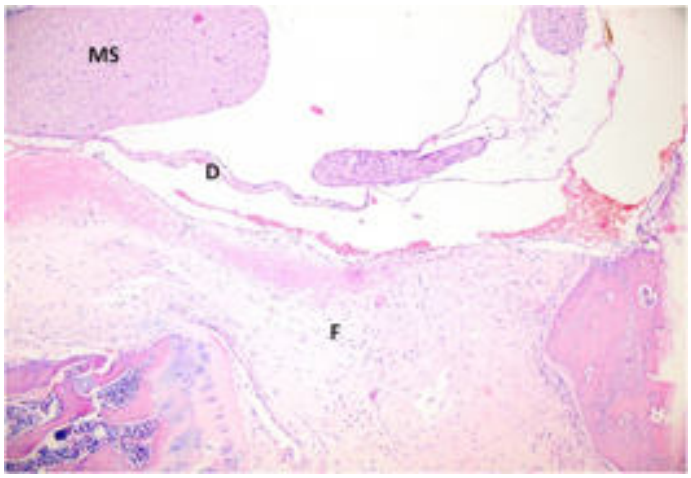

Fig. 3. An example of fibrosis covering more than 2/3 of the laminectomy defect (grade 3). [HE, ×300]

Table 2. Grades of epidural fibrosis among the groups

	Specification	Mean ± SD	Median	Minimum–Maximum	*p* value
Group A	Control (laminectomy)	2.87 ± 0.35	3.00	(2.0-3.0)	**< 0.001****
Group B	dHACM graft (Laminectomy + Graft)	1.62 ± 0.51	2.00	(1.0-2.0)	

SD = Standard deviation, dHACM = Dehydrated human amnion/chorion membrane, The Mann-Whitney U test was used to compare values between groups. ** $p < 0.01$.

± 0.35 in Group A and 1.62 ± 0.34 in Group B (Table 2). The difference between the two groups was statistically significant ($p < 0.001$), indicating that EF formation was greater in the control group than in the dHACM allograft group. The dHACM graft was thus found to decrease EF formation significantly.

DISCUSSION

Epidural fibrosis around nerve roots and the dura mater at the post-laminectomy site is an important cause of FBSS, which causes considerable morbidity in approximately 40% of patients that undergo spinal

surgery and has high health care costs. As EF cannot be effectively treated after its formation, the key is to prevent the migration of fibroblasts to the dura in the early recovery stage after spinal surgery. Thus, the placement of a physical barrier to restrict cell migration may be an effective approach for reducing the incidence of EF. Although various biological and synthetic materials have been tried to prevent EF, this issue has not been fully elucidated [5-9].

HAM dressings are reported to have beneficial effects on tissue reparation and regeneration due to their anti-inflammatory, re-epithelialization, anti-scarring, antibacterial, and analgesic activities. In addition to containing many growth factors, anti-vasculogenic factors, and anti-inflammatory cytokines, HAMs have low immunogenicity. HAMs have been extensively employed in medical fields such as ophthalmology and for treating conditions such as bone defects, skin burns, and bladder or oral cavity reconstruction [16, 17]. However, fresh amniotic membrane carries a risk of disease transmission. Thus, processing methods such as glycerol treatment, irradiation, lyophilization, or cryopreservation are required. However, these methods may damage collagen construction or leave behind residual chemical agents. Therefore, processing methods that protect the biological efficacy of HAMs while providing safety are important [18]. Recently, a method involving mild cleansing and dehydration was used to protect and retain the biological activities of the natural amnion [14]. In this study, the potential of dHACM processed using this method to function as a barrier for preventing EF after laminectomy was assessed. Indeed, the findings of this study support that dHACM can significantly reduce EF formation.

Previous studies, mostly experimental, on the effects of HAM on EF formation following spinal surgery are summarized in Table 3 and favorable outcomes were noted [5, 19-21]. Till date, only one case series by Subach *et al*. [20]has reported the use of dHACM. They reported that dHACM during transforaminal lumbar interbody lumbar fusion (TLIF) can positively prevent EF formation and simplify dissection in secondary surgery.

CONCLUSION

In conclusion, the present study found that

Table 3. Previous studies investigating the effect of HAM on epidural fibrosis formation after spinal surgeries

Authors (Year)	Study Design	Product Type	Study Subjects	Results
Tao *et al.* [5] (2009)	Animal model (dog)	FAM CAM AFF	24 dogs	CAM effectively reduced epidural fibrosis
Choi *et al.* [19] (2011)	Animal model (rat)	Irradiated FAM	20 rats	Irradiated FAM effectively reduced epidural fibrosis
Subach *et al.* [20] (2015)	Human case series	dHACM	5 patients	dHACM implant during TLIF had favorable results on epidural fibrosis formation
Kara *et al.* [21] (2015)	Animal model (rat)	Fresh HAM Human Amniotic fluid	27 rats	Fresh HAM and human amniotic fluid were found to have favorable results for preventing epidural fibrosis but not significantly

FAM = Freeze dried amniotic membrane, CAM = Cross-linked amniotic membrane, AFF = Autologous free fat, TLIF = Transforaminal lumbar interbody lumbar fusion, dHACM = Dehydrated human amnion/chorion membrane, HAM = Human amniotic membrane

dHACM can effectively reduce EF formation after spinal laminectomy. In our knowledge, this is the first study demonstrating reduction epidural fibrosis following laminectomy in a small animal study via dHACM graft. Further studies are needed to verify these findings. Future studies should also compare the efficacy and complications of different HAM types.

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The author disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2020;6(6):539-544 Original Article

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Evaluation of the effects on atherosclerosis and antioxidant and antimicrobial activities of *Agaricus xanthodermus* poisonous mushroom

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ABSTRACT

Objectives: The aim of this study was to determine the total antioxidant capacity, total oxidant capacity, oxidative stress index and antimicrobial activity of a poisonous mushroom *Agaricus xanthodermus.* The effects of mushrooms on atherosclerosis are due to their antioxidant effects.

Methods: Mushroom samples collected from study field were extracted with methanol (MeOH) and dichloromethane (DCM) using soxhlet apparatus. Total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) were measured using Rel Assay trade kits. Antimicrobial activities were tested on 9 microorganisms (*Staphylococcus aureus, S. aureus* MRSA, *Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii, Candida albicans, C.krusei* and *C. glabrata*) using the modified agar dilution method.

Results: In this study *A. xanthodermus* has shown high antioxidant and antimicrobial activities. In addition, the highest activities of MeOH and DCM extracts of the mushrooms were demonstrated against *E. coli, P. aeruginosa,* and *A. baumannii.*

Conclusions: In conclusion, *A. xanthodermus* is considered to be a poisonous mushroom and can be used as a pharmacological natural agent due to its high antioxidant and antimicrobial activities.

Keywords: Medicinal mushroom, poisonous mushrooms, Agaricus xanthodermus, antioxidant, antimicrobial, atherosclerosis

Up to date, approximately 140,000 mushroom species have been identified, and about 2,000 of these species are considered safe for human consumption. It has also been reported that about 700 mushroom species have therapeutic properties [1]. Serious poisoning can occur because of the misidentification natural mushrooms. Some types of mushrooms contain compounds that are toxic to humans [2]. In some countries several mushroom poisoning cases are reported every year. For example; in 1998, 1,675 cases of mushroom poisoning were reported in France and it is estimated that only 8,000-10,000 cases are registered in this country in this year. Most of these cases are due to the misidentification of species because of empirical and traditional knowledge [2-4]. Although the incidence of mushroom poisoning is not known in Turkey, in the United States was reported to be 5 in 100,000 [5].

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Agaricus xanthodermus (Yellow Stainer) is a large white mushroom with a cap resembling the horse or field mushrooms. However, the young caps are often square in shape and older caps usually have a flattened top and straighter, almost vertical sides. The gills are white initially, then pink, and later turn dark purple-brown; the pink shades are deeper than those of field mushrooms. The most distinguishing feature is that the cap and stem base turn bright chrome yellow when scratched. The smell is often unpleasant, especially when cooking [6].

General symptoms of mushroom poisoning are nausea, vomiting, diarrhea, fatigue, abdominal pain and state of consciousness change. The cardiac effects of mushroom poisoning are the most common sinus tachycardia, sinus arrhythmia, ST-T change and hypotension [2]. *A. xanthodermus* can cause serious stomach and intestinal pains, diarrhea, vomiting, hypotension, malaise, sweating, numb and sometimes coma. Symptoms may start after 40 minutes to 4 hours after consumption. Exactly the healing process takes several days. Some people may not develop intoxications in the consumption of mushrooms, in some people poisoning after consumption several times, while in some people shows serious symptoms in the first consumption [6-8].

Mushrooms have been used as food and medicine since ancient times [9]. Mushrooms are well in consumption of food and also in medical features. They produce secondary metabolites which have many different biological effects [10]. In previous studies, it has been reported that different mushroom species have many effects such as antioxidant, anti-lipidemic, antihypertensive, DNA preservative, analgesic, antimicrobial, anticancer, immunosuppressive, anti-allergic, anti-inflammatory and antagonistic activity [11-17]. There are lots of study about edible and toxic mushrooms and their anti-oxidative and anti-lipidemic effects. In fact, we can evaluate the anti-lipidemic and anti-oxidative effects of mushrooms as a group effect. Cardiovascular diseases are one of the most common causes of morbidity and mortality in the world [18]. There are lots of risk factors in cardiovascular disease such as hyperlipidemia, hypertension, cigarette smoking, obesity, diabetes mellitus and a positive family history of cardiovascular disease [19]. Some risk factors can be changed by lifestyle change. We can eliminate most risk factors with a change in diet. Several food ingredients reduce hyperlipidemia, hypertension. On the other hand, the presence of antioxidant [20, 21] and anti-inflammatory [22, 23] compounds in mushrooms might be clinically relevant in the management of heart and circulation health complications. Food constituents with anti-oxidative and anti-inflammatory properties. Anti-oxidative and anti-inflammatory functions and therefore lipid lowering effects correlate with anti-atherogenic effects [24]. And also the consumption of dietary antioxidants could be important in the prevention of cardiovascular diseases [25, 26] and there is evidence that the oxidative modification of LDL (lipids or protein components) play a crucial role in atherogenesis [27].

In this context, it is very important to determine the pharmacological potential of poisonous, edible and non-edible mushroom species. The present study aimed to determine the antimicrobial activity, total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI) of *Agaricus xanthodermus* Genev poisonous mushroom. In addition, the effect of fungus on atherosclerosis depending on antioxidant effect was evaluated.

METHODS

Laboratory Study

A. xanthodermus specimens were collected during routine field studies conducted in Gaziantep in 2017-2018. Mushroom samples was dried at 40 0C. The dried mushroom samples were pulverized by mechanical milling. Powdered mushroom samples were subjected to extraction with methanol (MeOH) and dichloromethane (DCM) at 50 0C for about 6 hours in soxleth apparatus.

TAS, TOS and OSI Tests

Total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) of the mushrooms were determined by Rel Assay brand commercial kits (Assay Kit Rel Diagnostics, Turkey). Trolox was used as calibrator for TAS and the results were expressed as mmoL Trolox equiv./L [28]. Hydrogen peroxide was used as the calibrator for the OS and the results were expressed as μmoL H2O2

equiv./L [29]. When calculating OSI (AU: Arbitrary Unit), which is expressed as the percentage of TOS levels to TAS levels, the mmoL value in the unit of TAS test is converted to µmoL as in the TOS test [29]. Analyses were carried out with 5 replicates.

Antimicrobial Activity Tests

The antimicrobial activity tests of MeOH and DCM extracts of the mushrooms were determined by the agar dilution method recommended by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Minimal inhibitory concentrations (MIC) for each extract were determined against standard bacteria and fungal strains. *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212 were used as gram positive bacteria. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacterbaumannii* ATCC 19606 were used as gram negative bacteria. *Candidaalbicans* ATCC 10231, *Candidakrusei* ATCC 34135 ATCC 13803, *Candidaglabrata* ATCC 90030 were also used as fungi. Bacterial strains were cultured on Mueller Hinton Broth and fungal strains were cultured on RPMI 1640 Broth. To obtain a standard inoculum, the blur of bacteria and fungi was prepared according to McFarland 0.5. All extracts were tested at concentrations of 800, 400, 200, 100, 50, 25 and 12.5 µg/mL and all dilutions were made with distilled water. The solvents used for the extracts were also tested for antimicrobial activity. For fungi Fluconazole, Amphotericin B was used as the reference drug, whereas for bacteria Amikacin, Ampicillin and Ciprofloxacin were used as reference drug. The lowest dilution that inhibits the growth of bacteria and fungi was determined as the minimal inhibitor concentration (MIC) [30-34].

RESULTS

TAS, TOS and OSI Results

According to our studies on *A. xanthodermus* 6 different mushroom samples were taken and these samples were run in 5 replicates. The results are shown in Table 1.

When our studies, it is seen that the endogenous antioxidant compound capacity that the mushroom produces is 4.229 ± 0.153 mmol/L. The oxidant compound level of the mushroom, which was produced as a result of environmental effects and metabolic activities, was determined as 29.065 ± 0.286 µmol/L. It is determined that the oxidative stress level indicating how much the oxidant compounds were tolerated with the endogenous antioxidant compounds in percentage were 0.688 ± 0.021.

Antimicrobial Activity Results

In this study MeOH and DCM extracts of *A. xanthodermus* were used and their activities on test microorganisms were determined. The results of the study were given in the Table 2.

When Table 2 was examined, it was seen that the highest activity was determined as MeOH extract of the mushroom. In this study, the highest activity on *P. aeruginosa* and *A. baumannii* were seen with MeOH extracts in the concentration of 50 µg/mL. The activity on *E. coli* was seen with MeOH and DCM extracts in the concentration of 100 µg/mL. The activity on *C. albicans,C. glabrata* and *C. krusei* were seen with MeOH and DCM extracts in the concentration of 200 µg/mL. The highest activity on *S. aureus* MRSA was seen with MeOH extracts in the concentration of 200 µg/mL. The activity on *S. aureus* and *E. faecalis* were seen with MeOH and DCM extracts in the concentration of 400 µg/mL.In addition, the highest activities of MeOH and DCM extracts of the mushrooms were demonstrated against *E. coli, P. aeruginosa,* and *A. baumannii.*

Table 1. TAS, TOS and OSI values

	TAS (mmol/L)	TOS (µmol/L)	OSI
A. xanthodermus	4.229 ± 0.153	29.065 ± 0.286	0.688 ± 0.021

Values are presented as mean ± SD; number of mushroom samples n = 6, experiments were made in 5 parallels.
TAS = Total antioxidant status, TOS = Total oxidant status, OSI = Oxidative stress index

Table 2. Antibacterial and antifungal activity of *A. xanthodermus*

	A	B	C	D	E	F	G	H	I
DCM	400	400	400	100	100	50	200	200	200
MeOH	400	200	400	100	50	50	200	200	200
Ampicillin	1.56	3.12	1.56	3.12	3.12	-	-	-	-
Amikacin	-	-	-	1.56	3.12	3.12	-	-	-
Ciprofloksasin	1.56	3.12	1.56	1.56	3.12	3.12	-	-	-
Flukanazol	-	-	-	-	-	-	3.12	3.12	-
Amfoterisin B	-	-	-	-	-	-	3.12	3.12	3.12

The MIC values are presented in units of µg/mL. DCM = dichloromethane, MeOH = methanol, A = *S. aureus*, B = *S. aureus* MRSA, C = *E. faecalis*, D = *E. coli*, E = *P. aeruginosa*, F = *A. baumannii*, G = *C. albicans*, H = *C. glabrata*, I = *C. krusei*

DISCUSSION

Most fungi-derived pharmaceuticals have been sourced from Ascomyceteous fungi where most (but not all) species produce microscopic fruiting bodies; for example, those used in pharmaceuticals include *Penicillium*, *Aspergillus*, and so on, whereas perhaps fewer pharmaceuticals, certainly in an industrial context, have been derived from the higher phyla of fungi, the Basidiomycota [35]. In this study, TAS, TOS and OSI values of a basidiomycetes species *A. xanthodermus* were determined using Rel Assay kits.As a result of the studies, the value of TAS was calculated as 4.229 ± 0.153 mmol/L, TOS value was 29.065 ± 0.286 µmol/L and OSI value was 0.688 ± 0.021. No studies were conducted to determine the TAS, TOS and OSI values of A. xanthodermus. In studies conducted on literature on different types of mushrooms in the literature, the values of edible mushroom *Auricularia auricula* and non-edible mushroom *Trametes versicolor* were found to be 1.010 and 0.820, TOS values were 23.910 and 17.760, OSI values were 2.367 and 2.166 [36]. Edible mushroom *Cyclocybe cylindracea* TAS value is 4.325, TOS value is 21.109 and OSI value is reported as 0.488 [37]. In other studies, edible mushroom *Gyrodon lividus* has a TAS value of 2.077, TOS value of 13.465 and OSI of 0.651 [38]. Edible mushroom *Clavariadelphus truncatus* TAS value is 2.415, TOS value is 3.367and OSI value is reported as 0.140 [39]. Poisonous mushroom *Ompholatus olearius* has a TAS value of 2.836, TOS value of 8.262 and OSI of 0.291 [40].

Compared to these studies, *A. xanthodermus* used in our study is lower than *C. cylindracea* and *O. olearius,* C. truncatus, *A. auricula,* T. versicolor and G. lividus have higher TAS values. In value of TOS, *A.xanthodermus* was found to be higher than *C. cylindracea, A. auricula, T. versicolor, O. olearius, C. truncatus*, and *G. lividus.* In addition, *A. xanthodermus* OSI value was lower than *A. auricula* and *T. versicolor* and higher than *C. cylindracea, O. olearius,* C. truncatus, and *G. lividus.* The reason for these differences in TAS, TOS and OSI among mushrooms is due to differences in the capacity of the product oxidant compounds, the capacity of endogenous antioxidant compounds and their ability to tolerate oxidant compounds with endogenous antioxidants with environmental and metabolic factors. As a result, it is thought that *A. xanthodermus* has high antioxidant potential and it can be used as a natural antioxidant agent by detecting the compounds that cause this effect. In addition, the protective effect of atherosclerosis can be utilized due to its antioxidant effects. We all know atherosclerosis risk factors are associated with excess ROS generation and oxidation of LDL. Ox-LDL acting on cell types promotes atherogenesis. Many pharmacologic agents that are currently in use modulate oxidative stress and improve atherogenesis [41]. In some studies traditional antioxidant supplements mostly failed in improving CV event rate some not. There are many factors affecting the cause of these results. One is anti-oxidant therapy was started late or exogenous antioxidants have any effects on this pathway.

Antimicrobial Activity

Today, there are many harmful microorganisms that cause diseases. Unconscious and excessive use of antibiotics in different areas caused these microorganisms to provide resistance to antibiotics. According to clinical epidemiology analysis reports, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* became the most common hospital infection and drug resistant strains with infection rates of up to 50% [42]. Therefore, detection of new antimicrobial agents has become inevitable. Mushrooms are immensely rich in bioactive compounds yet largely untapped resource of useful natural compounds. These bioactive compounds are found in various cellular components and secondary metabolites, which have been isolated and identified from the fruiting bodies [43]. Mushrooms have many biological activities thanks to these bioactive compounds [39]. In the present study, *A. xanthodermus* extracts were tested on 9 different bacteria and fungus strains with antimicrobial activity. As a result of the study, MeOH extracts of mushrooms showed higher activity than DCM extracts. In addition, mushroom extracts were found to be more effective against gram-negative bacteria *E. coli, P. aeruginosa* and *A. baumannii.* It was previously reported that antimicrobial activity on *A. xanthodermus* was effective at different concentrations against *S. aureus, E. coli* and *P. aeruginosa* using different solvents [35, 44]. In our study, MeOH and DCM extracts of *A. xanthodermus* were used and it was found to be effective at 50-400 µg/mL concentrations against test microorganisms.

CONCLUSION

In this study, a poisonous mushroom, *A. xanthodermus's* antioxidant, oxidant and antimicrobial potentials were determined. It was detected that the mushroom has antioxidant, oxidant and antimicrobial potential. Mushroom clearly has a cholesterol-lowering effect or hypocholesterolemic effect by different mechanisms such as decreasing very-low-density lipoproteins, improving lipid metabolism, inhibiting of activity of HMG-CoA reductase, and consequently preventing the development of atherosclerosis. The antioxidant and anti-inflammatory compounds occurring on mushroom also may contribute to reduce the atherosclerosis risk. *A. xanthodermus* should not be consumed through diet. Because *A. xanthodermus* is poisonous. Consumption is extremely dangerous. In our study, antioxidant and antimicrobial activities were determined. It is thought that bioactive compounds that cause this effect can be identified and used in pharmacological designs.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Relationship between ocular and computed tomography findings in patients with spina bifida

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ABSTRACT

Objectives: Spina bifida is one of the most common congenital diseases in Turkey and around the world. Despite the developing diagnostic and therapeutic methods, abnormal ocular characteristicsin spina bifida patients are still quite common. We investigated the ocular characteristics of spina bifida patients with and without hydrocephalus.

Methods: We included 37 patients who were previously referred to the Istanbul Bilim University, Department of Ophthalmology and already had computed tomography (CT) scans. We retrospectively investigated the patients' ophthalmologic findings (refractive errors, strabismus, and optic disc characteristics) and used their recent CT images to measure the Evans ratios (ERs), which indirectly reflect the grade of hydrocephalus. The patients were divided into three groups according to their ERs (ER ≤ 0.3, 0.3-0.5, and ≥ 0.5), and then the ocular characteristics of these groups were compared. In addition, the patients were divided into three groups according to their ages (≤ 1 year, 1-3 years, and ≥ 3 years), and the ERs and rates of refraction defects in these groups were compared.

Results: There was no relationship between specific ocular characteristics and ER or between age and ER. However, refraction errors were observed more frequently as patient age increased.

Conclusions: The degree of hydrocephalus does not affect the ocular characteristics of patients with spina bifida, but emmetropization may be deteriorated in these patients.

Keywords: Spina bifida, ocular findings, computed tomography, emmetropization

Spina bifida is a congenital malformation caused by the embryonic development of a neural tube closure defect, manifesting itself in the form of a split spinal column. It also refers to a neurogenetic disease with a complex aetiology influenced by both genetic and environmental factors [1]. Spina bifida is one of the most common congenital diseases in Turkey and across the world, incidences are 1.04% and 0.31%, respectively [2, 3]. It presents many neurological, urological, and orthopaedic complications, among which ocular manifestations are some of the most common. Patients with spina bifida are at high risk for hydrocephalus and hindbrain herniation, which are characteristic of the Chiari II malformation [4].

Ocular complications induced by hydrocephalus that are frequently seen in patients with spina bifida

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include amblyopia, strabismus, anisometropia, optic atrophy, cortical blindness, and nystagmus [5,6]. According to previous studies, optic atrophy is seen in patients with spina bifida at varying rates [7-10]. Several researchers have investigated refractive errors among patients with optic atrophy, hydrocephalus, and spina bifida, independently. However, the existing literature lacks a dedicated comparison of ocular findings against the severity of hydrocephalus, which constitutes the subject matter of the present study.

It has previously been shown that that spina bifida defect closure surgeries performed within 48 hours of birth ensured a decrease in neurological complications, such as muscle paralysis. Based on these determinations, we first measured the extent of hydrocephalus using the Evans ratio (ER) [11, 12]. We predicted that ocular complications might be less common in patients who underwent early surgery and that they would, therefore, experience less ventricular enlargement. To that end, we aimed to compare ocular complications among participating patients, who were grouped according to their ER scores.

METHODS

This study examined 37 patients with spina bifida aged between 0 and 13 years, including 20 males and 17 females, who applied to our outpatient clinic between February 2015 and August 2016. The mean age of the study patients was three years-two months. This research adhered to the tenets set forth in the Declaration of Helsinki and the approval of the local ethics committee was also obtained. Ophthalmologic examinations were analyzed retrospectively, based on their examination files. All ophthalmologic examinations were performed by the same examiner (OT). Visual acuity examinations were excluded, due to the young ages of patients, as well as a lack of patient cooperation. Patients over 13 years old and those who demonstrated isolated ocular pathology (such as congenital glaucoma, congenital cataracts, etc.) were also excluded from the study.

First, all patients were subjected to refraction measurements by a 2 win hand-held auto refractometer (Adaptica, Padua, Italy) following the application of 1% cyclopentolate eye drops every 15 minutes, i.e. three applications spanning 45 minutes in total. As suggested by the manufacturer, measurements were recorded only when the reliability index was over 5 (maximum 9). Measurement precision was set as 0.25 diopter for power and 5 degrees for the axis. In the case of patients who did not allow for measurement due to cooperation issues, refraction was assessed by manual retinoscopy. In order to rule out normal-ranged refractive errors, which are seen in the first years of life in most patients, cycloplegic refraction measurements for the patients aged 3 years and below were adjusted in accordance with the relevant table in Guidelines for Refractive Correction in Infants and Young Children, published by the American Academy of Ophthalmology in 2012. Patients with eyes that needed refractive correction were considered to have the refractive disorder. In contrast, a refractive defect was not noted down for that did not require refractive correction [13]. For children over three years of age, we followed the methodology adopted by previous studies (Biglan [5], Caines *et al*. [9]). In other words, refractive error was defined as: hypermetropia ≥ 3D, myopia ≥ 1D, and astigmatism ≥ 1D (Fig. 1).

Following refraction measurements, strabismus examinations were conducted. To begin, a Hirschberg test was performed by shining a light in the subject's corneas from fifty centimetres away, at eye level. Any reflex landing on the temporal of the cornea was recorded as esotropia, and any reflex landing on the nasal of the cornea was recorded as exotropia. Then the cover-uncover and alternate cover tests were performed on the patients. During these strabismus examinations, the presence or absence of fixation and nystagmus was also noted. For patients in whom cyclopentolate did not ensure a sufficient level of dilatation, an additional 0.05% tropicamide eye drop was applied, and dilatation was achieved.

Eyelid, conjunctiva, anterior-posterior chamber, and fundus examinations were then conducted using a biomicroscope in the patients who cooperated, whereas those same examinations were completed using a binocular indirect ophthalmoscope (Welch-Allyn, Model: WA 12500) in the patients aged below 3, as well as those who did not cooperate. In the fundus examinations, 90D lenses were employed for the biomicroscope, while 20D lenses were used for thebinocular indirect ophthalmoscope.

Based on the results of our patients' latest follow-

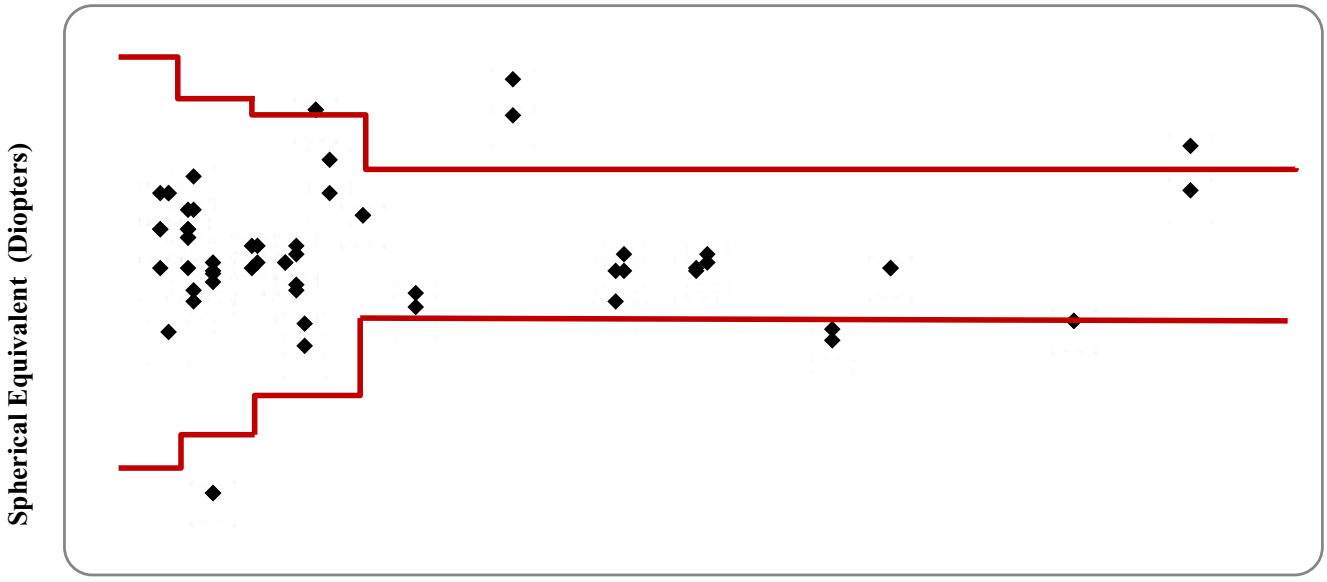

Fig. 1. Spherical equivalent values of patients' eyes refraction measurements. The red line shows the cut-off values which we used for refraction defect. This figure does not include astigmatic refractive errors.

up computed tomography (CT) scans, anterior-posterior optic canal diameters and ERs were calculated. The Somatom Sensation 16 (Siemens, Forchheim, Germany) CT scanner was used to scan all patients. Radiological values used for that purpose were as follows: kilovolt (peak): 100 kV; milliampere-second: 150 mas; section thickness: 0.67 mm; interslice distance: 0.4 mm. In order to determine ERs, a review was held of CT scans after they were retrospectively assessed by a neurosurgery consultant. In the axial plane, the distance between the external walls of both lateral ventricles was measured where anterior horns were found to be the largest in size. Next, that distance was divided by the distance between internal tabula of the cranium, to obtain the ER. An ER $\geq$ 0.3 was considered to indicate the existence of hydrocephalus. The patients were then divided into three groups, based on their ERs: < 0.3, 0.3-0.5, and > 0.5.

Statistical Analysis

In accordance with the purposes of this study, the links between categorical variables were investigated using a Chi-square test (nonparametric test). Cohen's d values were calculated to determine effect size in an independent sample t-test: d values measuring 0-02 indicated a minimal effect, those close to 0.5 represented a moderate effect, and those $\geq$ 0.8 demonstrated a significant effect.

RESULTS

No refraction measurement could be performed on six patients, out of 37 in total, due to a lack of cooperation. According to measurements taken from the 61 eyes of the remaining 31 patients, a refractive defect was found in 19 (31%). Furthermore, 6 (31%) of these eyes featured hypermetropia, 2 (10%) from myopia, 11 (57%) from isolated astigmatism, and 3 (15%) from hypermetropia + astigmatism.

In addition to refraction measurements, CT scans for the 37 patients included in the study were reviewed with the purpose of calculating ERs. However, an ER could not be calculated for one patient, due to an arachnoid cyst. When the remaining 36 patients were divided into groups by calculated ERs, the following results were obtained: ER $\leq$ 0.3 in 8 patients, ER < 0.5 in 20 patients, and ER $\geq$ 0.5 in 8 patients. Upon comparing 59 eyes from 30 patients for whom both refraction measurements and ER calculations were practicable, no statistically significant relationship was found (χ^2=.71, p > 0.05).

Furthermore, all patients included in the study were subjected to fundus examinations, and all patients demonstrated a normal appearance of the retina. Among these findings, only optic disc findings were used for fundus examination. Among the patients, 5 (13%) were found to have bilateral optic atrophy, 1 (2%) to have optic discs with obscure boundaries, and 1 (2%) to show signs of optic disc oedema. For statistical analysis purposes, all patients demonstrating any optic disc symptoms, which are mentioned above, were placed in the "positive optic disc symptom" group. No statistically significant correlation was found between the ER and the presence of optic disc symptoms (χ^2= 3.60, $p > 0.05$). However, a significant relationship was detected between the presence of optic disc symptoms and the presence of refractive error (χ^2= 5.85, $p < 0.05$). This result indicates that patients with optic disc symptoms tended to experience refractive defects less frequently.

For the next series of comparisons, patients were broken into a group according to age: 10 (27%) patients were aged one year or below, 16 (43%) patients were aged between 1 and 3 years, and 11 (29%) patients were above the age of 3 years. As noted previously, the mean age of all subjects was calculated as three years and two months. When examining the distribution of ERs by age groups, it was found that they were distributed normally ($p > 0.05$). To compare ERs by age groups, a one-way analysis of variance (ANOVA) was performed According to the results of this analysis, ERs did not to vary with age groups ($p > 0.05$). In fact, the ERs of patients in different age groups were quite close to one another (Table 1).

Additionally, refractive defect and age were found to be statistically significantly correlated (χ^2=13.25, $p < 0.05$) (Table 2). Specifically, as a patient gets older, the refractive defect is more likely to emerge. Meanwhile, a correlation analysis of spherical equivalents of refraction values of the patients revealed a considerably weak correlation among those values (r = 0.01). This indicates that spherical equivalents of refractions tend to remain unchanged by age.

Lastly, 19 (51%) patients were found to have strabismus. Of these, 16 (84%) cases were esotropia, 2 (10%) were exophoria, and 1 (5%) was esophoria. An examination of the relationship between ERs and strabismus revealed no statistically significant connection (χ2=320, $p > 0.05$) (Table 3).

Table 1. The relationship between Evans ratio and refraction defect

	Refraction Defect		Total	χ^2	*p* value
	Negative	Positive			
Evans Ratio					
≤ 0.30	7	5	12	0.71	0.70
0.31-0.49	25	10	35		
≥ 0.50	8	4	12		
Total	40	19	59		

Table 2. The relationship between refraction defect and age groups

	Refraction Defect		Total	χ^2	*p* value
	Negative	Positive			
Age group					
≤12 months	16	1	17	13.25	**< 0.01**
12-36 months	18	6	24		
≥36 months	8	12	20		
Total	42	19	61		

Table 3. The relationship between strabismus and Evans Ratio groups

	Strabismus		Total	χ^2	*p* value
	Negative	Positive			
Evans Ratio					
≤ 0.30	3	5	8	3.20	0.20
0.31-0.49	12	8	20		
≥ 0.50	2	6	8		
Toplam	17	19	36		

DISCUSSION

Despite technological advances in radiologic imaging systems, the ER, developed in 1942 by William Evans, remains a significant indicator in clinical settings to determine ventricular expansion [14-18]. In their study on patients with meningomyelocele, Stein *et al*. found the prevalence of hydrocephalus to be 80%, which is in keeping with the present study, as we calculated the rate as 77.7% [19].

The existing literature contains many studies on ocular manifestations in patients with hydrocephalus; however, ocular manifestations in spina bifida patients with hydrocephalus have attracted the attention of very few researchers. Refractiveerrors in newborns generally display normal distribution with a wider range, which is also referred to as Gaussian distribution [20]. Since the emmetropization process occurs over the course of years, the average spherical equivalent of this distribution decreases with reduced standard deviation (SD); as such, the distribution tends to be concentrated in a specific interval.

As the present study did not involve a control group consisting of subjects without spina bifida, we were not able to compare the refractive defects of our patients to a normal population. However, other researchers have included such populations in their investigations. For instance, in their study on Swedish children, Caines *et al*. [9] found that 81% of patients with meningomyelocele demonstrated significant refractive error, which is 10.3 times greater than the prevalence of refractive error among normal Swedish children. In a different study on 3,568 primary school children in Turkey, Toygar *et al*. [21] calculated a refraction error prevalence of 10.7%. Based on cycloplegic refraction scores for our subjects, 32.2% of them were found to have refractive error. Although, according to the present study, the prevalence of refractive error among those with spina bifida is 3 times greater, we are of the opinion that extended series are required considering the size of sample and patient age covered by this study. In comparison, Lennerstrand and Gallo [22] found the rate of refractive error to be 54%. We suggest that the difference between this study and ours was caused by the narrow age range and criteria that we adopted for refractive error. The present study could establish no statistically significant difference between the prevalence of refractive error across the patient groups with spina bifida, i.e. those without hydrocephalus, with moderate hydrocephalus (ER: 0.3-0.5), and with severe hydrocephalus (ER ≥ 0.5).

The fact that correlation analysis of spherical equivalents of refraction values against patient age revealed no correlation between age and refractive disorder is indicative of the failed emmetropization process in this group of patients. Similarly, a comparison among age groups, i.e. 0-1, 1-3, and > 3, revealed a statistically significant increase in refractive errors by age, which is also indicative of failed emmetropization. The emmetropization process is affected by many factors, notably genetic and environmental. One of these factors is poor accommodation. For example, Schaeffel *et al*. [23] showed that pupillary response was greater in chicks with damaged accommodation due to defocused lenses.

No study has been conducted thus far to evaluate the emmetropization process in patients with spina

bifida. However, various studies have found the prevalence of refractive error to be as high as 76% in patients with cerebral palsy involving cerebral damage [24]. For instance, McCelland *et al*. [25] showed that these patients suffered from both a higher prevalence of refractive error and a lower accommodation response, as compared to those without cerebral disease. A refractive correction with the help of added plus bifocal lenses at near was shown to be helpful for near reflex [26, 27]. This also highlights the importance of accommodation in emmetropization, because in patients with Down's syndrome, impaired accommodation gave rise to emmetropization failure, even in the absence of any cerebral damage [28, 29].

Additional studies are required to assess accommodation in patients with spina bifida, investigating both the cause of emmetropization disorder and the necessity of employing added plus bifocal lenses for treatment purposes. It is hypothesized that, in addition to impaired accommodation, the transmission of visual signals to the brain would affect emmetropization. Troilo and Wallman [30] showed that chick eyes were made functionally myopic and hypermetropic with lenses adjusted their axial extension, re-approaching emmetropia once lens administration ceased. The same emmetropization process was observed in eyes after the optic nerve was cut [30]. In the present study, patients whose fundus examination revealed atrophy in the optic disc, obscure boundaries, or papilledema did not demonstrate a higher prevalence of refractive error than those in the other patient group; in fact, it was found to be significantly lower. In the same study by Troilo and Wallman [30], refractive error in eyes with cut optic nerves became more prevalent in a reversed manner in the subsequent period. This finding indicates that the emmetropization process involves a feedback mechanism [30]. From this point of view, a prospective investigation on our study group may shed light on the emmetropization process in patients with optic disc symptoms.

In the present study, 51% of patients with spina bifida were found to have strabismus. For context, in a study on 298 patients with spina bifida in 1990, Biglan [5] estimated a strabismus prevalence of 61%. Other studies suggested 42-52% prevalence rates among spina bifida patients [6, 9]. The findings revealed by the current work are in harmony with those of previous studies. For instance, studies on healthy Turkish children indicate that the prevalence of strabismus ranges between 3% and 6.5% [31-33]. It can therefore be argued that strabismus among those with spina bifida has become 7 to 17 times more prevalent in Turkish society. However, no statistically significant relationship could be established between ER and strabismus prevalence. In short, these results show that strabismus is common among patients with spina bifida; however, the prevalence of strabismus remains unchanged concerning the presence of hydrocephalus or its severity.

Finally, in Gaston's 1991 study [6], the prevalence of optic atrophy was found to be 17%. In comparison, the present study found five subjects (13%) with optic atrophy, although no statistically significant relationship was detected between ER and optic disc manifestation. Despite ever-advancing medical technology, this prevalence remains high. Therefore, the present authors suggest that early diagnosis and treatment should more actively be pursued in patients with spina bifida. Limitations of this study include an insufficient number of subjects across groups, as well as a lack of differentiation when interpreting CT results, especially in terms of whether the ER calculations were obtained pre- or post-V-P shunting. In the present study, we sought to investigate the genesis of ocular manifestations in patients with spina bifida in an effort to pave the way for future applications aimed at patients' ocular rehabilitation.

CONCLUSION

According to the results of our study, patients with spina bifida were found to have a higher prevalence of ocular manifestations as compared to the normal population, in keeping with previous studies. However, we found that the severity of hydrocephalus had no impact on the prevalence of ocular manifestations. Furthermore, a comparison among different age groups of patients revealed a higher prevalence of the refractive disorder in older age groups, which might be suggestive of the failed emmetropization process in this group of patients.

Authorship declaration

All authors listed meet the authorship criteria

according to the latest guidelines of the Internaional Committe of Medical Journal Editors, and all authors are in agreement with the manuscript.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The relationship between the attachment styles of the adults with and without depression and the parenting attitudes of their parents

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ABSTRACT

Objectives: In this study, it is aimed to determine the relationship between attachment styles of adults with and without depression and child rearing attitudes of their parents.

Methods: One hundred eighteen patients who are admitted to outpatient clinic of Department of Psychiatry of the Uludağ University School of Medicine, who are diagnosed with depression according to DSMIV-R and DSM V and put on regular medication, and 130 healthy individuals of similar demographic characteristics without any psychopathologic diagnostic, are included in this study. Within the scope of this research "Experiences in Close Relationships Inventory-II", "Child-Rearing Attitudes Scale", "Beck Depression Inventory" and Sociodemographic Information Form were used.

Results: It is found that there is a significant difference between the attachment styles of the individuals with and without depression and their parents' attitudes towards child rearing. Maternal and paternal acceptance / interest scores of the patient group were lower than those of the healthy group, and the parents' supervision / control scores were higher. The anxiety and avoidance attachment scores of the married individuals of the patient group are higher than the scores of married individuals of the healthy group.

Conclusions: High anxiety and avoidance related to attachment and controlling parental attitudes were found to be risk factors for depression.

Keywords: Attachment styles, parent child rearing attitudes, depression

Attachment is an instinctive form of behavior that occurs between the mother (or caregiver) and the child in the early stages of life, and that follows a neurobiopsychological development path with the need of the child to be close to the mother [1].

Attachment behaviors, which are not limited to childhood only, continue in adulthood. The first relationship is established with the mother and this relationship is the basis for the relationships that will occur in other periods of life. In this article, the term "parents" will be used instead of "caregivers".

Attachment theory has been demonstrated by John Bowlby. Bowlby objected to the understanding of Freud that mother's feeding her baby is the reason of the attachment of the baby to the mother. According to Bowlby, attachment is not a process that is only developed by the elimination of basic need for the nutrients. This basic relationship the child has with his mother will affect his personality and his psychological development. In infants aged 7-15 months, baby's

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demonstration of attachment behavior is important for the development of the limbic system and the maturation of cortical attachment regions. Clinging to a baby, singing her a song or a lullaby will increase the bond between a mother and her baby and contribute to the baby's brain development [2].

In 1950, the World Health Organization asked Bowlby, who was working in the field of developmental psychiatry related with the mental health-related İssues of the homeless children, to prepare a report containing recommendations. Bowlby [3], mentioned in the first part of the report, that realization of a baby or a young child's relationship with his mother in a satisfying way giving pleasure is very important for the mental health of the baby or a young child. Bowlby and his colleagues stated that a small child had hunger for the presence and love of his mother at least as much as his hunger for food.

In the early studies on attachment, the relationship of the baby with the mother and the child's attachment behaviors during childhood were discussed. Ainsworth and his friends designed an experimental model, namely Foreign Environment Experiment, in order to observe the reactions of infants to different situations. In this study, mothers, their environment and their interactions with foreigners of the 12-18 months old babies were investigated. Ainsworth and his colleagues concluded after observations that there were three types of attachment style. These attachment styles are: safe, anxious / unstable and avoiding [4]. If the mother is sensitive and can respond to the needs of her child, the child will be securely attached; if the mother is overly controlling, inconsistent or irrelevant, the child will develop an unsafe attachment.

Later, the view of Bowlby [5] that "attachment is a process that continued from the cradle to the grave" was adopted and the boundaries of the attachment studies expanded. In their work, Hazan and Shaver [6] asked adult individuals to evaluate themselves and their partners in romantic relationships. As a result of the study, it was seen that those who established a relationship with their mothers on the basis of trust where their needs are met in childhood, were securely connected to their spouses during adulthood and established a relationship based on reciprocity. It has also been shown that adults who have been subjected to rejection by their parents during childhood have also been insecure (anxious / unstable) in their relations with their spouses [6].

Together with the studies on adult attachment, new classifications for attachment styles have begun to be made. According to Bartholomew and Horowitz [7]; the basic dimensions of attachment styles are composed of positive and negative evaluation of self and others. As indicated in Table 1; while individuals who are securely connected to others have good intentions for others, they find themselves worthy of being loved. Obsessive individuals do not respect themselves, they exalt others over and they are constantly worried about being abandoned. Indifferent individuals only see themselves as valuable, their self-esteem is very high, and they refuse to establish a relationship. Fearfully attaching people find themselves worthless, they think that Noone finds them worthy of loving and find others as unreliable [8].

Development of attachment between mother and baby is very important for the survival of the baby with basic needs such as nutrition, cleansing and temperature. The prerequisite for the development of a secure attachment is that the physical needs of the baby are remedied in a timely manner and that the he feels comfortable near his mother. The lack of a secure relationship between the baby and the mother in the first years of life was thought to be the determinant of psychopathology in the later life period [9].

According to Bowlby, sadness is the usual reaction to losses and adversities in life. Depression occurs when a person reacts differently to negativities of life. Seligman explains the mood of the person who does not take a job he can manage, who does not care about their accomplishments and ignores them as luck coincidences, as "learned helplessness". According to Bowlby, this mood may be a result of some experiences in childhood and makes the person prone to depression. Bowlby is of the opinion that the children who were not appreciated by their parents in childhood and were called to be incompetent even though they did their best to meet the demands of their parents may be inclined to "learned helplessness" in the future, and this feeling of helplessness and hopelessness may lead

Table 1. Attachment styles

	Self (Positive)	Self (Negative)
Others (Positive)	Confident	Obsessive
Others (Negative)	Indifferent	Fearful

to depression [10-15].

According to theory; having inconsistent and insufficient care from the parent in childhood, the individual will experience attachment anxiety in adulthood. Having constantly angry and distant parents in the childhood, the person will avoid expressing his feelings in his relations during his adulthood and will not seek closeness and support. Lack of social support is also a risk factor for depression [11].

The aim of this study is to compare adult attachment styles and parental attitudes of individuals with and without depression, and to investigate the relationship between adult attachment styles and parenting attitudes of their parents.

METHODS

A total of 118 patients (90 women, 28 men) of 18-65 years of age being at least primary school graduates, who were admitted to the Uludağ University Medical Faculty Psychiatry outpatient clinics in the year 2017 and who had been diagnosed with depression according to DSM-IV-IV and DSM-V criteria formerly and who are informed about the study and approved to take part in the study, participated in the data gathering process. The participants completed the questionnaires during the control interviews after diagnosis.

The study was approved by Uludağ University Faculty of Medicine Ethics Committee. The data of the healthy control group were obtained from 130 volunteers consisting of hospital staff and relatives with similar demographic characteristics.

Instruments

Sociodemographic Information Form (SIF)

The socio-demographic data form which is prepared for demographic characteristics such as gender, age, education level, marital status and income level has been applied to all participants. For the patient group, questions about their medical stories were added.

Experiences in Close Relationship Inventory-II (ECR-II)

In 2000, Fraley, Waller and Brennan developed the scale "Experiences in Close Relationship Inventory-II" for a measurement with a higher sensitivity than the previous scales. The reliability and validity study of the scale for the Turkish sample was conducted by Selçuk and colleagues in the year 2005. The items of the scale measure two dimensions of attachment (anxiety and avoidance). The scale, which is evaluated with 7-point Likert-type questions, consists of 36 items. From two sub dimensions scores varies between 18-126 can be obtained [12].

Parenting Styles Questionnaire (PSQ)

Scale is developed by Sümer and Güngör in 1999 based on the studies of Steinberg and colleagues as 30 items and the number of items is reduced to 22 by subsequent analysis. The scale is filled separately for the mother and father. The scale measures two dimensions of parental attitude (acceptance / attention and strict supervision / control). The scores from the scale ranged between 11 and 55 [13].

Beck Depression İnventory (BDI)

The scale developed by Beck and his colleagues in 1961 was adapted into Turkish by Hisli in 1988. On the scale consisting of 21 items of symptoms of depression, each question is evaluated between 0-3 points. The total score from the scale ranged from 0-63 [14].

Statistical Analysis

Statistical analysis of the findings obtained from the study was performed using SPSS (Statistical Package for the Social Sciences) 18 for Windows. Shapiro Wilk test was used to determine the compliance of variables to normal distribution. In the analysis of continuous variables, t test for was used for Independent Samples in normal distributed data, and Mann-Whitney U test was used for data not normally distributed. For the categorical variables the chi-square test was used, and when the expected frequencies were not met the Fisher Exact test was used. Significance was tested at $p < 0.05$ and $p < 0.001$.

RESULTS

Two hundred forty-eight participants, 118 (90 females, 28 males) diagnosed with depression and 130 (100 female, 30 male) healthy volunteers are included in the study. Table 2 shows the demographic

Table 2. Comparison of patient and healthy group in terms of demographic information

	Patient (n = 118)	Healthy (n = 130)	*p value*
Age (years)	45.6 ± 1.1	37.7 ± 1.1	< 0.001[a]
	46 (18-62)	37 (23-67)	
Gender			
Woman	90 (76.3%)	100 (76.9%)	0.904[b]
Man	28 (23.7%)	30 (23.1%)	
Marital status			
Married	79 (67%)	74 (62.7%)	< 0.001[b]
Single	18 (15.3%)	39 (33.1%)	
Divorced or widow	14 (11.9%)	2 (1.7%)	
Has a relationship	7 (5.9%)	15 (12.7%)	
Level of education			
Primary school	47 (39.8%)	29 (22.3%)	< 0.001[b]
Middle school	18 (15.3%)	15 (11.5%)	
High school	19 (16.1%)	29 (22.3%)	
License and above	34 (28.8%)	57 (43.9%)	
Employment status			
Working	42 (35.6%)	104 (80%)	< 0.001[b]
Unemployed	64 (54.2%)	21 (16.2%)	
Retired	12 (10.2%)	5 (3.8%)	
Monthly income			
0-1500	54 (45.8%)	29 (43.5%)	0.001[c]
1500-3000	37 (31.4%)	39 (39.8%)	
3000-4500	20 (16.9%)	61 (42.5%)	
> 4500	7 (5.9%)	1 (4.2%)	

Data are expressed in terms of the mean ± standard deviation, median (minimum: maximum) and n (%).
[a]Mann-Whitney U test, [b]Chi-Square test, [c]Fisher Exact test

characteristics of the patient and the healthy group. There is a significant difference between the patient and the healthy group in terms of marital status, education level, working status and income level ($p <$ 0.001). The education and income level of the patient group are significantly lower. In terms of the scores obtained in the study; Beck depression scores, maternal and paternal acceptance / interest scores, strict control / inspection points of the parents are significant in terms of patient and healthy group ($p <$ 0.001). The results obtained from the comparison are shown in Table 3. The Beck depression scores and parental control / inspection scores of the patient group are significantly higher than healthy group; while maternal and paternal acceptance / interest scores are lower. When the literature is examined, there is no findings that parental attitudes and attachment styles are affected by age, education level and working status variables. In order to eliminate the intermixing effect of the marital status variable, only married individuals were included in the comparison to be conducted in terms of the attachment styles between the patient and the healty gruop, considering that marital status may have an impact on the attachment style of the individual. This comparison is shown in Table 4. Different groups of participants who are single, have

Table 3. Comparison of patient and healthy group in terms of scores obtained from the scales

	Patient (n = 118)	Healthy (n = 130)	*p value*
Beck depression points	18.8 ± 0.8	7.1 ± 0.6	< 0.001[a]
	18 (1-38)	5 (0-32)	
Mother acceptance / interest points	36.4 ± 0.9	43.5 ± 0.7	< 0.001[a]
	38 (11-55)	44 (11-55)	
Father acceptance / interest points	32.5 ± 0.9	40.8 ± 0.7	< 0.001[a]
	32 (11-52)	44 (19-55)	
Mother's strict supervision / control	34.5 ± 0.9	29.2 ± 0.7	< 0.001[a]
	35 (17-55)	41(11:52)	
Father's strict supervision / control	34.5 ± 0.8	28.2 ± 0.7	< 0.001[b]
	35.5 (11-55)	28 (11-48)	

Data are expressed as mean ± standard deviation and median (minimum: maximum).
[a]Mann-Whitney U test, [b]Independent Samples t test

Table 4. Comparison of married individuals in terms of attachment scores

	Patient (n = 78)	Healthy (n = 74)	*p value*
Anxious attachment points	66.6 ± 2.1	54.1 ± 1.9	**< 0.001[b]**
	64 (30-111)	54 (25-107)	
Avoiding attachment points	56.4 ± 2.6	45.3 ± 2.1	**< 0.001[a]**
	50 (21-112)	43 (18-90)	

Data are expressed as mean ± standard deviation and median (minimum: maximum).
[a]Mann-Whitney U test, [b]Independent Samples t test

a relationship, are divorced and widowed were excluded from the comparison analysis because the number of participants within the each group were insufficient. With the aim of qualifying the patient profile, the clinical characteristics of the patients are presented in Table 5. The correlation between anxious and avoidance attachment scores, parental acceptance/interest and strict supervision/control scores, and depression scores of the patient group were shown in Table 6

DISCUSSION

In terms of sociodemographic characteristics evaluated in the study, the result that patients with depression are more disadvantaged than healthy individuals, is compatible with the literature. It has been seen that this difference is tried to be explained with the approaches of social selection and social causation in the literature [15].

In this study, firstly the patient group data, then the healthy group data with similar characteristics were collected. Although there were some matched groups in terms of living place and gender, it was observed that there was a difference between the patient and healthy group in terms of age, marital status, income level, education level, and working status. There were no evidence in the literature that age, income, education, and working status had an

Table 5. Clinical presentation of the disease in the patient group

Treatment time (years)	
0 – 5 years	83 (70.3%)
6 – 10 years	20 (16.9%)
> 10 years	15 (12.7%)
Hospitalization	
Yes	14 (11.9%)
No	104 (88.1%)
Number of hospitalization	
0 – 5	13 (92.9%)
6 – 10	1 (7.1%)
Hospitalization Time (for a single time)	
0-30 days	6 (42.9%)
30 – 61 days	6 (42.9%)
61 – 120 days	2 (14.3%)
How many years ago has the disease started?	
0 – 5	60 (50.8%)
6 – 10	26 (22%)
> 10	32 (27.2%)
Is the period of depression repeated?	
Yes	47 (39.8%)
No	45 (38.1%)
Continued	26 (22.1%)
Number of Attacks	
0 – 5	9 (41.60%)
6 – 10	3 (6.30%)
> 10	25 (52.10%)
Medication time (year)	
0 – 5	94 (79.6%)
6 – 10	19 (16.1%)
> 10	5 (4.24%)
Suicide	
Yes	17 (14.4%)
No	101 (85.6%)

The data are expressed as n (%).

impact on attachment styles. However, since it was thought that marital status could affect the attachment styles, only the attachment scores of married individuals were compared. It was found that patient and married individuals were more anxious and avoidant in their emotional relations than healthy and married individuals. Since there was no evidence in the literature on the fact that the variables of age, income, marital status, education, and working status had an impact on the individual's assessment of the parental attitudes, all participants were included in the comparison between the Parenting Styles Questionnaire scores in which the adult individuals evaluated their parents retrospectively. According to the Parenting Styles Questionnaire, where parental attitudes of adults are assessed, the patient group perceives more control of their parents; while it was found that the healthy group perceived their parents as more caring and accepting. According to Bowlby, the failure to safely develop the attachment or interruption of the attachment in childhood triggers the formation of a neurotic personality in advanced ages [16]. It has been shown in a prospective study that insecure attachment is a feature of personality structure predisposed to depression [17].

Correlation analysis was performed to see the relationship between anxiety and avoidance attachment scores of the patient group, parental acceptance/interest and strict supervision/control scores, and depression scores. A significant, positive and weak correlation was found between anxiety and avoidance scores ($r(118) = 0.36$, $p = 0.001$). A significant, positive and weak correlation was found between anxiety and depression scores ($r(118) = 0.36$, $p = 0.001$). A significant, positive and weak correlation was found between avoidance and depression scores ($r(118) = 0.23$, $p = 0.001$). No relation was found between the depression scores and the scores regarding the parental child-rearing attitudes. A significant, negative and weak correlation was found between maternal acceptance/interest and strict supervision/control scores ($r(118) = -0.20$, $p = 0.001$). A significant, positive and moderate correlation was found between maternal acceptance/interest and paternal acceptance/interest scores ($r(118) = 0.48$, $p = 0.001$). A significant, positive and high correlation was found between maternal strict supervision/control scores and paternal strict supervision/control scores ($r(118) = 0.66$, $p = 0.001$). A significant, negative and weak relationship was found between avoidance and maternal acceptance / interest level ($r = -0.34$, $p < 0.001$). As the perceived acceptance and caring from

Table 6. Correlation analysis between subgroups of the patient group's attachment styles and parental attitudes

	ANP	AVP	MCP	MAP	FCP	FAP	DP
ANP	-	0.36**	0.08	-0.06	-0.07	0.05	0.36**
AVP	0.36**	-	0.09	-0.34**	0.04	-0.14	0.23*
MCP	0.08	0.09	-	-0.20*	0.66**	-0.14	-0.07
MAP	-0.06	-0.34**	-0.20*	-	-0.09	0.48**	-0.06
FCP	-0.07	0.04	0.66**	-0.09	-	-0.20*	0.05
FAP	0.05	-0.14	-0.14	0.48**	-0.20*	-	-0.01
DP	0.36**	0.23*	-0.07	-0.06	0.05	-0.01	-

ANP = Anxious Attachment Points, AVP = Avoiding Attachment Points, MCP = Mother's Strict Supervision/Control Points, MAP = Mother Acceptance/Interest Points, FCP = Father's Strict Supervision/Control Points, FAP =: Father Acceptance/Interest Points, DP = Depression Points.
**$p < 0.001$, *$p < 0.05$

mother decreases, avoidance is increased. In our study, no relation was found between anxiety level and parental child rearing attitudes.

CONCLUSION

According to the data obtained from this study, it is observed that anxious and avoidant attachment is a depressing factor. The lack of acceptance / interest of the parent is seen to be predictive of the avoidance of the close relations of adulthood, and it is thought that the avoidant attachment is susceptible to depression, because it deprived the individual from social support. Parental control is also found to be a risk factor for depression. Parent's insufficient and inconsistent care may lead the child's mental schemes to be built on anxiety, hopelessness and despair, and these schemes make the individual prone to depression. It is considered that the studies that will be carried out with the healthy control group matching one-to-one with a large scale of the patient and the healthy group will be important in terms of investigating the causality of the findings.

Authorship declaration

All authors listed meet the authorship criteria according to the latest guidelines of the Internaional Committe of Medical Journal Editors, and all authors are in agreement with the manuscript.

Conflict of interest

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Prophylactic and therapeutic parotidectomy for secondary neoplasms of the parotid gland

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ABSTRACT

Objectives: The aim of this study was to review the demographic and clinicopathological findings of the patients who underwent prophylactic or therapeutic parotidectomy due to the secondary neoplasm of the parotid gland (SNP), the outcomes of the patients with SNP and to provide a perspective to the parotid and neck approach in head and neck skin cancer cases.

Methods: The clinical data of 178 patients who underwent parotidectomydue to neoplasm in the Department of Otorhinolaryngology, Head and Neck Surgery of a tertiary referral center from 2012 to 2017 were reviewed.

Results: Among 178 patients, twenty-two (12.4%) patients were found to underwentprophylactic or therapeutic parotidectomy due to SNP. Six of these patients had pathologically positive parotid metastases (therapeutic parotidectomy). The median age of six patients was 67 years (ranging from 2 to 83 years). Five patients were male and one was female. The most common indication of parotidectomy (prophylactic and therapeutic) and pathology of parotid mass due to SNP and Cutaneous Squamous Cell Carcinoma (SCC) (36.3% and 50%, respectively).

Conclusions: Owing to rapidly progressive feature and high morbidity, close observation of parotid in case of metastasis is crucial for recurrent SCC of the face and scalp.

Keywords: Head and neck neoplasms, neoplasm metastasis, parotid neoplasms

Secondary neoplasm of the parotid gland (SNP) is a rare condition. Metastasis can both occur via lymphatic and hematogenous systems. The lymphatic system is the main route of head and neck tumors' metastases. The majority of these tumors are cutaneous malignancies of the scalp and face region [1]. The lymphatic system of the parotid space can be classified into three groups which are determined embryologically: The first group is intraparenchymal, the second group is intrafascial and extraparenchymal, and the last and third one is extrafacial and extraglandular such as the preauricular lymph nodes. The auricule, cheek, parietal scalp and ipsilateral side of the forehead are the primary sites that drain into the lymph nodes of the parotid space and through the deep cervical [1].

The thoracic duct and the Batson's paraspinal venous plexus were also reported to be the route of the distant metastases of breast, prostate, kidney and gastrointestinal carcinomas to the parotid gland [1]. However, the hematogenous system is the main route for the distant metastases of tumors such as sarcomas.

This study aimed to review the demographic and clinicopathological findings of the patients who under-

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went prophylactic or therapeutic parotidectomy due to SNP, the outcomes of the patients with SNP, and to provide a perspective to the parotid and neck approach in head and neck skin cancer cases.

METHODS

The clinical data of 178 patients who underwent prophylactic or therapeutic parotidectomy due to neoplasm in the Department of Otorhinolaryngology, Head and Neck Surgery (ORL-HNS) of a tertiary referral center from 2012 to 2017 were reviewed. The demographic and clinicopathological features of the patients with SNP were recorded.

All patients underwent superficial, total or radical parotidectomy as a part of the prophylactic, therapeutic and/or diagnostic purposeof a primary neoplasmother than the parotid gland. The finding asto the malignant mass or lymph node of the parotid gland with imaging studies such as US, CT, MRI and/or PET-CT and/or confirming fine needle aspiration cytologyor thru-cut aspiration biopsy (FNA, TCB); parotidectomy was added to the treatment protocol. En-block resection of the primary tumor with or without neck dissection was also performed for the tumors of head and neck other than nasopharynx carcinoma.

The indications of prophylactic or therapeutic parotidectomy due to SNP with subheadings can be assessed as:

Squamous Cell Carcinoma (SCC) of the Skin

In case of the parotid malignant mass finding with imaging studies and confirming FNA or TCB, the therapeutic parotidectomy was performed. In case of the local recurrence of the cutaneous SCC and Basosquamous Cell Carcinoma (BSCC)with one or more risk factors and/orsuspicious findings, lymph node of the parotid gland with imaging studies, the prophylactic parotidectomy was performed. Risk factors include tumor size more than 2 cm, invasion into subcutaneous fat, poorly differentiated or metatypical or morpheophorm phenotype histopathology, high grade or desmoplasia, perineural invasion, lymphovascular invasion, location near parotid or lip, local recurrence, SCC in a preexisting scar, and immunosuppression [2].

Malignant Melanoma (MM)

The prophylactic, superficial parotidectomy was performed as a part of the sentinel lymph node biopsy (SLNB) with suspicious imaging findings. The therapeutic, radical parotidectomy was performed in one patient with intraoperative positive frozen biopsy for MM.

Buccal and Lip (Oral Commissure) Carcinoma

Buccal and Lip (oral commissure) Carcinoma that invade the skin (T4a) with suspicious imaging findings of the parotid; the prophylactic, superficial parotidectomy was performed with neck dissection and en-block tumor excision.

Lymphoma (in the follow-up period, after the previous treatment of lymphoma)

In case of the suspicious findings of FNA and PET-CT, the prophylactic (diagnostic), superficial parotidectomy was performed for the possibility of relapse.

Nasopharynx Carcinoma (NPC)

In case of the metastasis of NPC to the parotid gland that does not respond to the CRT treatment, the therapeutic, and total parotidectomy was performed.

Rhabdomyosarcoma

In case of the metastasis to the parotid space that does not respond to the CRT treatment, the therapeutic, total parotidectomy and tumor excision was performed.

According to the American Joint Committee on Cancer (AJCC) 2017, the malign neoplasms of the head and neck region were staged retrospectively. Neoadjuvant or adjuvant radiotherapy (RT) or chemoradiotherapy (CRT) was carried out, depending on the pathological evaluation of the surgical specimen includingthe primary tumor.

The approval was taken from the local institutional research committee (5417/4). The research was conducted in compliance with the Ethical Principles of the Declaration of Helsinki.

Statistical Analysis

In descriptive statistics related to continuous data; mean, standard deviation, median, minimum, maximum values; number and percentage values were

given. The Kaplan-Meier method was used for survivor analysis of six patients with pathologically positive parotid metastasis.

RESULTS

Among 178 patients, 156 patients had primary tumors (134 benign, 22 malign tumors) of the parotid gland. Twenty-two (12.4%) patients were found to underwentprophylactic or therapeutic parotidectomydue toSNP. Six of these patients had pathologically positive parotid metastases (therapeutic parotidectomy) and 18 underwent prophylactic parotidectomy.The SNP constituted 3.7% (6/160) of all parotid neoplasms. The median age of six patientswas 67 years (ranging from 2 to 83 years). Five patients were male and one was female.

Among 22 patients underwent prophylactic or therapeutic parotidectomy; eight patients had SCC of the skin, two BSCC of the skin, four MM of the skin, two Buccal Carcinoma, one Lip (oral commissure) Carcinoma, two Lymphoma, one NPC, one Rhabdomyosarcoma.

Squamous Cell Carcinoma of the Skin

Eight (36.3%) patients with SCC of the skin underwent parotidectomy: Five patients hadprophylactic, three patients had therapeutic parotidectomy. Six of the patients were male and two were female. The averageage of the patients was 70.6 years (range 21 to 87 years). The youngest one of these patients (21 years old) had Xeroderma Pigmentosum. Superficial, prophylactic parotidectomy was added to the treatment protocol of five patientswho had all of three risk factors liketumor size more than 2 cm, recurrence, and location near parotid; as a result of the recurrence of the primary skin lesion. No pathological risk factor was reported as insufficient surgical margin, perineural or lymphovascular invasion in the first pathology reports of these patients. Parotid metastasis was not found in the surgical specimen of these five patients, pathologically.

In the remaining three patients with parotid mass and metastasis confirmed with the imaging studies and FNA, the parotid metastasis of SCC was found in the surgical specimen. One of these patients had a recurrenceone year after the surgery of the primarycutaneous zygoma lesion (pT2N0M0), as a parotid mass that invaded the facial nerve in the foramen stylomastoideum and caused total facial paralysis. Radical parotidectomy with radical neck dissection was performed. This patient presented intracranial metastasis in the follow-up period (six months later) and had died of thedisease. Another patient had parotid metastasis six months after the surgery of the primary cutaneous neck lesion (pT2N1M0). Total parotidectomy with radical neck dissection was performed. One year after the surgery, the patient presented iliac bone metastasis and

Table 1. The patients with pathologically positive parotid metastasis

No.	Age (years)	Gender	Location	Histopathology (TNM stage)
1	83	Male	The superficial and deep lobe of the parotid	SCC of zygoma skin ($pT_2N_1M_0$)
2	81	Male	The superficial and deep lobe of the parotid	SCC of neck skin ($pT_2N_1M_0$)
3	67	Female	The superficial and deep lobe of the parotid	SCC of lateral canthal skin ($pT_1N_1M_0$)
4	67	Male	The deep lobe of the parotid	SCC of nasopharynx ($pT_2N_3M_0$)
5	65	Male	The superficial and deep lobe of the parotid	Malignant melanoma (unknown primary tumor)
6	2	Male	The extraglandular region of the parotid	Rhabdomyosarcoma of the lower limb

palliative CRT treatment was carried out. One year after the treatment, he died of thedisease. The third patient admitted to our clinic with grade 4 peripheral facial paralysis as a result of 2×3 cm parotid mass, two years after the surgery of the primary cutaneous lateral canthal lesion (pT1N0M0). The infratemporal fossa surgery with radical parotidectomy was performed. Two years after the surgery, the patient presented intracranial metastasis and palliative CRT treatment was carried out. The common features of these three patients were the surgeries of the primary skin lesion were performed in another center and all of them had died of the disease (Table 1).

Adjuvant RT or CRT was carried out in all of the patients with skin SCC and parotid metastases.

Basosquamous Cell Carcinoma of the Skin

Two (9.1%) patients with basosquamous cell carcinoma of the skin underwent parotidectomy with en-block tumor resection. Superficial, prophylactic parotidectomy was added to the treatment protocol of these two patients with risk factors like tumor size more than 2 cm, recurrence and location near parotid; as a result of the localrecurrence of the primary skin lesion. No pathological risk factor was reported as insufficient surgical margin, perineural or lymphovascular invasion in the first pathology reports of these patients. No parotid metastasis was found in the surgical specimen of these patients.

Malignant Melanoma

Four (18.2%) patients with MM underwent parotidectomy: One patient with evident parotid mass was misdiagnosed as a warthin tumor with FNA, preoperatively. As a result of the detection of the invaded facial nerve with parotid mass, frozen section biopsy was studied intraoperatively. After the diagnosis of MM with frozen section biopsy, radical parotidectomy with radical neck dissection was performed. The primary skin lesion was not found in this patient. Adjuvant CRT was carried out. No recurrence was detected in the second year of follow-up (Table 1).

In the remaining three patients, superficial, prophylactic parotidectomy was performed as a part of the sentinel lymph node biopsy (SLNB) with suspicious imaging findings. However, parotid metastasis was negative in the surgical specimen of the parotid. Two of these patients presented distant metastasis in the follow-up period. Palliative CRT was carried out and they died of thedisease.

Buccal and Lip Carcinoma

Superficial, prophylactic parotidectomy was performed in three (13.6%) patients with two buccal and one lip (oral commissure) carcinoma with suspicious imaging findings. The stages of the tumor of these patients were pT4a, as a result of the invaded skin by the primary tumor. No parotid metastasis was found in the surgical specimen of these three patients. Selective or radical neck dissection with en-block tumor excision was also performed in these patients. In the follow-up period, one female patient with buccal carcinoma presented second primary neoplasm in the oral cavity as SCC of the tongue, four years later. After the treatment, this patient presented recurrence as tongue lesion and neck metastasis, she died of disease as the result of the neck metastasis that invaded the internal carotid artery. Adjuvant RT or CRT was carried out in all of these patients.

Lymphoma

Two (9.1%) patients who were in the follow-up period after the treatment of lymphoma (one Hodgkin and one Marginal), presented with parotid mass. As the suspicious finding of FNA and PET-CT, superficial, prophylactic (diagnostic) parotidectomy was performed. No malignancy was found in the surgical specimen.

Nasopharynx Carcinoma (NPC)

Three years after the treatment of NPC with CRT, one (4.5%) patient presented with a parotid mass in the deep lobe of the parotid gland, confirmed as NPC metastases with FNA preoperatively that do not respond to the CRT treatment. Total, therapeuticparotidectomy was performed. After the detection of multiple distant metastases in the follow-up period of two years, palliative CRT was carried out but the patient died of the disease (Table 1).

Rhabdomyosarcoma

One (4.5%) patient who is two years old, presented with parotid space mass, six months after the treatment of the rhabdomyosarcoma of the lower limb. Total, therapeutic parotidectomy, and tumor

excision were performed for the metastasis which was extrafacial and extraglandular that do not respond to the chemotherapy treatment. CRT was carried out after the surgery. No recurrence was detected in the follow-up period of two years (Table 1).

The Kaplan-Meier method showed that median survival time of six patients with pathologically positive parotid metastasis was 24 months (6-48). The cumulative survival rate in the first year was 66.7% (95% confidence interval: 29-100%).

DISCUSSION

Parotid gland tumors constitute 4-8% of the head and neck tumors. Malignancy rate for the parotid gland tumor is 0.2 [3, 4]. SNPs havean average incidence of 6-8% of all parotid gland tumors, metastases originate from head and neck malignancies commonly and distant metastases rarely [5, 6]. As a rare entity, there are few series of SNP and the literature mostly arises from the case reports. In our series, SNPs constituted 3.7% of all of the parotid gland tumors, the metastasis of head and neck malignancies were the most common pathology, compatible with the literature.

Rouviere [7] was first to declare the association of head and neck lymphatic drainage with the lymph nodes of parotid space in 1938. The parotid space takes the lymphatic drainage of the auricule, cheek, ipsilateral side of the forehead, parietal scalp and neck that are anterior and lateral to the parotid and drains into the internal jugular lymph nodes. The Cutaneous SCC of these anatomical regions is the most common pathology resulting in SNP. In our series, the most common indication of (prophylactic and therapeutic) parotidectomy and pathology of parotid mass due to SNP was Cutaneous SCC (36.3%, 50% respectively), consistent with the literature. As in our series, it characteristically involves the patients older than 65 years that have the primary tumor in the head and neck region [8]. In the presence of the parotid mass, preoperative FNA may affect the treatment decision with the assessment of malignancy and the distinction of primary and secondary neoplasms with high sensitivity and specificity [9]. It prepares the patient and the surgeon for a more extensive surgery such as radical parotidectomy and neck dissection, if needed [1]. In this series, there was only one false-negative case with a rare parotid metastasis of MM from an unknown primary.

The treatment of the Cutaneous SCC of the head and neck region is wide local excision of the lesion. The TNM classification based on AJCC 2017 has included prognostic risk factors that also give direction to the treatment approach (Tables 2 and 3) [10]. The recommended surgical margin of the lesion is 5 mm for minimal risk group that has 2 mm or lower vertical tumor thickness (VTT), 5-10 mm for the low-risk group (2.01-6 mm VTT), and 10 mm for the high-risk group (VTT > 6 mm). As a footnote; the low-risk group with one or more risk factors should be managed as the high-risk group [11]. Insufficient surgical margin increases morbidity with the risk of local and regional recurrence. The small subset of Cutaneous SCC metastasizesto regional lymph nodes. In a systemic analysis, Cutaneous SCC of head and neck was reported to have 5% risk of regional metastasis [12]. In this context, the neck treatment is still a controversial issue. Elective neck dissection with parotidectomy for SCC with cN0 neck was recommended only for the risk of occult metastases

Table 2. The pathological tumor (pT) classification of Invasive Cutaneous Squamous Cell Carcinoma based on AJCC 2017 [10]

pT classification	Definition
pT_1	Tumor ≤ 2 cm at largest horizontal width +0-1 high-risk feature
pT_2	Tumor ≤ 2 cm at largest horizontal width +2-5 high-risk features or tumor >2 cm at largest horizontal width
pT_3	Infiltration of facial and cranial bones
pT_4	Infiltration of skeletal bone or skull base

pT = pathological tumor

Table 3. Prognostic risk factors of Invasive Cutaneous Squamous Cell Carcinoma based on AJCC 2017 [10]

Prognostic Risk Factors	Low Risk Factors	High Risk Factors
Tumor diameter	Less than 2 cm	More than 2 cm
Location	Sun exposed sites (except ear/lip)	Ear/lip Non sunexposed sites SCC arising in radiation sites, scars, burns or chronic inflammatory conditions Recurrent SCC
Depth/level of invasion	Less than 6 mm/ invasion above subcutaneous fat	More than 6 mm/ invasion beyond subcutaneous fat
Histologic features	Well differentiated grade Common variant or verrucous	Moderately, or poorly differentiated grade Acantholytic, spindle, or desmoplastic subtype Perineural invasion
Surgical margins	Clear	Incomplete excision
Immune status	Immunocompetent	Immunosuppressed (organ transplant recipients, chronic immunosuppressive disease or treatment)

more than 19% as the classical knowledge [13]. D'Souza and Clark [2] defined clinical and pathological risk factors (noted in the methods section) to determine the patients who have a higher risk of regional metastases. The temporal region, cheek, auricle, and forehead are the most common sites of Cutaneous SCC that metastasize to the parotid gland [14]. As a multicenter study, Andruchow *et al*. [15] reported the rate of parotid involvement as high as 80% for Cutaneous SCC. Kadakia *et al*. [16] reported 25% of occult parotid metastases rate in the patients with Cutaneous SCC of Temporal Region of at least 2 cm and recommended prophylactic parotidectomy especially for tumors with the perineural or vascular invasion at the primary site.

Considering the low cure rate of salvage surgery, increasing distant metastasis risk of loco-regional involvement, the high morbidity of N+ neck dissection; the morbidity of parotidectomy and neck dissection (if necessary) may be thought to be negligible [14, 17]. However, Hoch *et al*. [18] and Osborne *et al*. [19] recommended against prophylactic parotidectomy, who studied Cutaneous SCC of head and auricle, respectively. These patients are also recommended to be screened with parotid MRI for infraclinical metastases in the Guidelines of the French Society of ORL [20].

In this study, prophylactic parotidectomy was added to the treatment protocol of five patients with recurrent SCC and two patients with recurrent BSCC that had two or more risk factors (commonly tumor size more than 2 cm, recurrence and location near parotid), as the clinical preference. No parotid metastasis was found in the surgical specimen of these patients. There was no local or regional recurrence in these cases in the follow-up period.

Tumors located on the scalp and face anterior to the vertical line drawn through the external auditory canal commonly spread to the parotid and through upper cervical nodes; parotidectomy should be considered as a part of the neck dissection. The incidence of occult neck metastasis in the presence of parotid metastasis was reported as between 29-35% in the literature [1, 21]. For the management of cutaneous SCC with parotid metastasis, a combined modality treatment including neck dissection with parotidectomy and RT is recommended as in our series [22]. The survival rates are low in our series, as all of the patients had died of the disease in 6-24 months after the treatment, compared to other series [23, 24]. In this context, loco-regional treatment and close follow-up are crucial for these patients.

SCC of the oral cavity and oropharynx were also reported as the primary sites of the parotid metastases [4, 5, 25, 26]. Increased number of cancer-positive lymph nodes were reported to increase the risk of parotid involvement by Harada *et al*. [27]. The skin involvement of SCC of the oral cavity and oropharynx were also considered to increase the possibility of metastasis to the parotid gland. In the present series, no pathologically positive metastases were assessed in the parotid specimens. In the presence of advanced imaging methods, parotidectomy with suspicious findings is questionable. However, the facial nerve involvement and facial paralysis occurrence are seen to be rapidly progressive in these patients with parotid metastases. Also, these cases require extensive treatment or become unsalvageable compared with low morbidity of parotidectomy.

The parotid gland contains an average of 5-10 lymph nodes as distinct from other salivary glands. Lymphoproliferative diseases such as lymphoma may involve lymph nodes in the parotid gland and also glandular parenchyma [28]. As the low diagnostic value of FNA for lymphoproliferative diseases, excisional lymph node biopsy as superficial partial parotidectomy was performed to the patients who were cured and in the follow-up period of lymphoma. These were pathologically negative for lymphoma. In this regard, TCB might be an alternative method to the excisional lymph node biopsy for the diagnosis.

In NPC, parotid metastasis may occur via retropharyngeal lymph nodes, indirectly through periparotid extension, retrograde extension from massive neck metastasis and rarely skip metastasis [29, 30]. Parotid lymph node (PLN) sparing intensity-modulated radiotherapy (IMRT) to reduce the side effect of xerostomia was reported to cause 2.8% of PLN metastasis after IMRT. PLN metastasis of NPC was recommended to assess as N3 and treat with aggressive concurrent chemoradiotherapy to decrease the possibility of distant metastasis. Parotidectomy is a choice for treatment-resistant (CRT) cases, as the cases with NPC and Rhabdomyosarcoma in the present series [31-33].

, the distant metastases of the upper aerodigestive tract, lung, breast, pancreas, and colon cancers to the parotid gland have been reported in the literature. Hematogenous dissemination is the main route for these cancer types [4, 5, 10, 34]. Rhabdomyosarcoma is extremely rare as metastatic disease to the parotid space. There was only one case with parotid, auricle and lung metastases in the literature that had died of disease 14 months after parotidectomy [1]. In the present series, two years old patient had no distant metastases other than parotid space and he has been in the follow-up period of 24 months after parotidectomywithout recurrence.

CONCLUSION

Although secondary neoplasms of the parotid gland are uncommon, it should be taken into consideration for the differential diagnosis of the mass of the parotid gland.Owing to rapidly progressive feature and high morbidity, close observation of parotid in case of metastasis is also crucial for recurrent SCC of the face and scalp.The larger number of patients is needed to recommend a therapeutic approach.

Conflict of interest

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The relationship between smartphone using style and sleep quality and psychiatric symptoms among a foundation university students

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ABSTRACT

Objectives: We aimed to measure problematic smartphone use frequency and it's association with sleep problems and psychiatric symptoms in a sample of university students.

Methods: The data in the study was collected by using a sociodemographic data form, the Smartphone Addiction Scale (SAS), the Pittsburgh Sleep Quality Index (PSQI), the Epworth Sleepiness Scale (ESS) and the Brief Symptom Inventory (BSI).

Results: Based on the SAS median score, the participants were divided into two groups as the low-level smartphone use (SAS score < 79) and the high-level smartphone use (SAS score > 79) groups. In the high-level smartphone use group, general sleep quality was lower, sleep latency was longer, scores of sleep disorder and daytime dysfunction were higher as measured by PSQI and the BSI scores were higher ($p < 0.001$). As a result of the study, it was observed that, as smartphone use increased and became problematic, sleep quality decreased, and daytime dysfunction and psychiatric symptoms increased. SAS scores were positively correlated with both PSQI and ESS scores. SAS score was found as a predictor of PSQI scores in the regression analysis.

Conclusions: This study shows that the relationship between problematic smartphone use and decreased sleep quality and increased psychiatric symptomatology in university students is still an important public health issue. Responsible and mindfull use of smartphones by the students throughout the day may provide a positive effect on their sleep.

Keywords: Smartphone addiction, behavioral addiction, sleep

In recent years smartphones are being used for various purposes such as contacting people every time and everywhere, connecting to the internet, conducting banking operations, shopping, taking care of state-related processes, playing games and listening to music. As the usage of smartphones has become widespread, it is observed that some people have started to show a problematic form of using smartphones that may result in psychological, physiological and social problems. While the term "smartphone addiction" was proposed as a type of behavioral addiction [1], more recent articles suggested that, instead of the term addiction, naming this issue as 'problematic smartphone use' would be more appropriate [2, 3]. Whether it is called 'smartphone addiction' or 'problematic smartphone use', it may be argued that it has become a significant

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public health problem that is increasingly prevalent all over the world [3].

Smartphones may be functional or dysfunctional tools of coping with stress depending on the form of usage. While the stress-reducing applications on the phone may be functional [4, 5], playing games or gambling for a long time, or, staying on social media without a purpose may not only increase the stress load on the person but also turn into a problematic behavior due to temporary feelings of relaxation provided by these practices. For example, a meta-analysis that discussed 39 independent studies reported that stress and anxiety increased alongside increase usage of smartphones [6].

Sleep is a vital and physiological state that makes our entire body ready again for the active daytime [7]. If the person feels vigilant, fit and ready for a new day after they wake up, it may be argued that they have good sleep quality. In addition to the quantitative aspects of sleep such as sleep latency and number of times of waking up in one night, sleep quality is also related to the subjective aspects of sleep such as its depth and restfulness [8]. People who do not sleep enough may experience problems such as fatigue, weariness, reduced attention, increased sensitivity that leads to pain, irritability, illogical thoughts, chronic headache, loss of motivation, hallucinatory perceptions, loss of appetite, immune dysfunctions, increased cardiovascular risk, weight gain and metabolic problems [9, 10].

The factors that disrupt sleep among students include increased number of academic responsibilities, consumption of caffeine and alcohol, insufficient or incorrect physical activity, dietary habits and smartphone use [11-14]. A study that was carried out in Switzerland with 362 high-school students reported that the teenagers who had smartphones had delayed times of going to bed, they experienced more sleep problems in comparison to those that did not have smartphones, and they slept less in the weekends, which was related to depressive symptoms [15]. Demirci *et al*. [12] found that 39.8% of Turkish university students had high rates of smartphone use, 37.9% had low rates of smartphone use. They observed that depression and anxiety levels increased while sleep quality decreased in problematic smartphone users. Mohammadbeigi *et al*. [14] reported that 10.7% of 380 university students had problematic smartphone use, and problematic smartphone use disrupted sleep quality significantly [14]. Matar Boumosleh and Jaalouk's study [16] in Lebanon with 688 university students determined that 35% of the sample felt tired in daytime in relation to using smartphones until late hours at night, 38.1% had reduced sleep quality, and 35.8% slept less than four hours on multiple occasions due to smartphone use. Tao *et al*. [17] conducted a study with 4747 college students and found problematic smartphone use in 28.2% of the sample, as well as sleep problems in 9.8% by using PSQI. Accordingly, they did not find a significant relationship between problematic smartphone use and psychiatric diseases in those with good sleep quality. As a result, it was proposed that a good quality of sleep may be preventive against psychopathology development even in cases of problematic smartphone use [17]. It was reported that using smartphones at night was among the reasons of sleep problems also in 906 athlete students around the age of 19 who could be expected to have high quality of sleep as they are involved in regular physical activity [18]. A study that was carried out with 82 mid-level and high-level managers showed that using smartphones at night for job-related purposes affected their ability to focus on their job in daytime as a result of interruption of sleep at night [19]. A 1-year-long prospective study that was conducted with 4156 young adults revealed that excessive smartphone use resulted in higher stress loads, depressive symptoms and sleep disorders in comparison to the initial statuses [20]. A study which monitored 383 teenagers for 2 years reported that those who owned a smartphone experienced reductions in sleeping times [21]. All these studies suggest that there is an association between problematic smartphone use and sleep disorders. Given the high number of studies conducted on the subject, we thought people and especially university students must have become more aware of the consequences of smartphone use and that they might have started developing healthier ways of using their phones. Therefore, we wanted to measure problematic smartphone use frequency and it's association with sleep problems and psychiatric symptoms with a new sample of university students. We carried out a questionnaire study that investigated the relationship between the smartphone use characteristics of a group of university students and their sleep quality and psychiatric symptoms.

METHODS

Participants

The sample of the study consisted of a total of 362 volunteer students including 242 women and 120 men in a foundation university.

Measures

The data in the study was collected by using a sociodemographic data form, the Smartphone Addiction Scale (SAS), the Pittsburgh Sleep Quality Index (PSQI), the Epworth Sleepiness Scale (ESS) and the Brief Symptom Inventory (BSI). Sociodemographic data form was developed by the researcher to collect information from the participants regarding their age, gender, marital status, class year, department and smartphone checking activity.

The Smartphone Addiction Scale (SAS) is a self-report scale that measures smartphone addiction under six dimensions (daily-life-disturbance, positive anticipation, withdrawal, cyberspace-oriented relationship, overuse and tolerance) consisting of 33 6-point Likert-type items that was developed by Kwon *et al*. [1]. The test is valid and reliable in Turkish [22]. No cut-off point was determined for the scale. Kwon *et al*. [1], found the mean score of those who accepted that they had problematic smartphone use as 120.15 ± 31.55. In the Turkish reliability and validity study, based on the median value of the sample, those with 72 points or above were considered to be high-level users, while those with 71 points or below were considered to be low-level users. Higher scores indicate more serious smartphone addiction.

Pittsburgh Sleep Quality Index (PSQI) is a self-report scale developed by Buysse *et al*. [23] which measures the quality of life and provides information on the type and severity of sleep disorders for the previous month. The test is valid and reliable in Turkish [24]. The total index scores of 6-10 indicate poor sleep, while over 10 points indicate long-term sleep disorders.

Epworth Sleepiness Scale (ESS) is a self-report scale developed by Johns [25]. It questions the general sleepiness level of the individual. It is an 8-item scale and is valid and reliable in Turkish [26]. If the total score is higher than 5, it suggests daytime sleepiness. Scores of 10 or higher may be considered as severe sleepiness.

Brief Symptom Inventory (BSI) is a scale composed of 53 items developed by Derogatis to catch psychiatric problems in various medical cases [27]. The inventory is valid and reliable for Turkish youth [28]. The scale has a 5-factor structure. These factors are: 'Anxiety', 'Depression', 'Negative Self', 'Somatization' and 'Hostility'.

Procedures

The questionnaire forms were distributed to the students of associate's, bachelor's and master's degrees in person in closed envelopes between the dates of 1 and 30 November 2017 by the first author.

Statistical Analysis

All statistical analyses were performed using SPSS v.18.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics are presented as mean ± standard deviation, median (25th–75th percentile), frequencies and percentages. Kolmogorov-Smirnov test was used to assess whether the continuous data fit normal distribution. Independent-samples t-test or Mann Whitney U test was used to compare continuous variables when appropriate. The categorical variables were compared with Pearson chi-squared test. Pearson's and Spearman's correlation tests were used to determine the strength of the linear relationships between variables. Multiple linear regression analysis was conducted to identify the predictors of smartphone addiction and PSQI score. A p value of < 0.05 was accepted as statistically significant.

RESULTS

Our study included a total of 362 participants including 242 (66.9%) women and 120 (33.1%) men. The mean age of the participants was 24.9 ± 7.1 years. 239 of the participants (66.0%) were 24 years old or younger, while 123 (34.0%) were 25 years old or older. Among the 362 individuals, 47 (13.0%) were married, while 315 (87.0%) were not.

The median value of the scores that was obtained from SAS was 79. Based on the SAS median score, the participants were divided into two groups as the low-level smartphone use (SAS score < 79) and the high-level smartphone use (SAS score > 79) groups. The descriptive characteristics of the

Table 1. Descriptive characteristics of the sociodemographic variables in low-level and high-level smartphone users

	Low-level smartphone user group n = 181	High-level smartphone user group n = 181	*p value*
Age (years) (mean ± SD)	27.0 ± 8.2	22.7 ± 5.1	**< 0.001**
Gender, n (%)			
Male	54 (29.8)	66 (36.5)	0.180
Female	127 (70.2)	115 (63.5)	
Education level, n (%)			
Associate's	21 (11.6)	82 (45.3)	**< 0.001**
Bachelor's	36 (19.9)	33 (18.2)	
Master's	124 (68.5)	66 (36.5)	
Purpose of smartphone use, n (%)			
Talking	146 (80.7)	139 (76.8)	0.369
Message texting	140 (77.3)	144 (79.6)	0.609
Social media	130 (71.8)	167 (92.3)	**< 0.001**
Mobile applications	75 (41.4)	101 (55.8)	**0.006**
Online gaming	40 (22.1)	65 (35.9)	**0.004**
Frequency of checking smartphone, n (%)			
10-19	66 (36.6)	20 (11.0)	**< 0.001**
20-29	46 (25.6)	38 (21.0)	
30-39	34 (18.9)	43 (23.8)	
40 and higher	34 (18.9)	80 (44.2)	
Keeping the smartphone in bed			
Yes	142 (78.5)	165 (91.2)	**0.001**
No	39 (21.5)	16 (8.8)	

sociodemographic variables of the two groups are given in Table 1. The mean age of the high-level smartphone use group (22.7 ± 5.1) was significantly younger than that of the low-level smartphone use group (27.0 ± 8.2) ($p < 0.001$). There was no significant difference between two groups regarding distributions of gender ($p = 0.180$). With regard to the education levels, the ratio of the associate's degree students among the high-level smartphone use group was significantly high ($p < 0.001$). The participants in the high-level smartphone use group stated that they used their smartphones significantly more frequently for social media ($p < 0.001$) mobile applications ($p = 0.006$) and playing games ($p = 0.004$) in comparison to the low-level smartphone use group. When the participants were compared based on their frequencies of checking their smartphones, the ratio of those who checked their smartphones 40 or more times a day was significantly higher in the high-level use group ($p < 0.001$). In the same group, the share of those who kept their smartphones with them before going to be was also significantly high ($p = 0.001$).

In the high-level smartphone use group, general sleep quality was lower, sleep latency was longer, scores of sleep disorder and daytime dysfunction were higher as measured by PSQI and the BSI scores were higher. It was found that the high-level smartphone use group had significantly higher values in comparison to the low-level smartphone use group regarding their ESS scores, PSQI total scores, PSQI subscale scores

Table 2. Comparison of the scale scores in low-level nd high-level smartphone user groups

	Low-level smartphone user group n = 181	High-level smartphone user group n = 181	*p value*
Epworth sleepiness scale	14 (11-17)	17 (13-20)	**< 0.001**
Pittsburgh sleep quality index	11 (7.5-16)	15 (12-21)	**< 0.001**
Subjective sleep quality subscale	1 (1-1)	1 (1-2)	**< 0.001**
Sleep latency subscale	1 (0-2)	1 (1-2)	**< 0.001**
Sleep duration subscale	0 (0-1)	0 (0-1)	0.802
Habitual sleep efficiency subscale	3 (2-3)	3 (3-3)	0.254
Sleep disorder subscale	5 (2-8)	8 (5-11)	**< 0.001**
Sleeping pill use subscale	0 (0-0)	0 (0-0)	0.071
Daytime dysfunction subscale	1 (0-2)	2 (1-3)	**< 0.001**
Brief Symptom Inventory	67 (58.5-91)	94 (70.5-120)	**< 0.001**
Anxiety subscale	16 (13-21)	21 (17-28)	**< 0.001**
Depression subscale	16 (13-23)	23 (17-32)	**< 0.001**
Negative self subscale	15 (12-20)	20 (16-26)	**< 0.001**
Somatization subscale	11 (9-15)	16 (12-21)	**< 0.001**
Hostility subscale	9 (7-14)	14 (9.5-19)	**< 0.001**

Table 3. Correlations between SAS score and the variables

	SAS	
	r_s	*p value*
Age	- 0.342	**< 0.001**
Epworth sleepiness scale	0.314	**< 0.001**
Pittsburgh sleep quality index	0.385	**< 0.001**
Subjective sleep quality subscale	0.245	**< 0.001**
Sleep latency subscale	0.246	**< 0.001**
Sleep duration subscale	0.011	**0.841**
Habitual sleep efficiency subscale	0.134	**0.011**
Sleep disorder subscale	0.362	**< 0.001**
Sleeping pill use subscale	0.091	0.084
Daytime dysfunction subscale	0.306	**< 0.001**
Brief Symptom Inventory	0.445	**< 0.001**
Anxiety subscale	0.413	**< 0.001**
Depression subscale	0.405	**< 0.001**
Negative self subscale	0.411	**< 0.001**
Somatization subscale	0.432	**< 0.001**
Hostility subscale	0.377	**< 0.001**

SAS = Smartphone Addiction Scale

Table 4. Linear Regression Analysis Results for SAS

Model	Variables	Standardized Coefficients (Beta)	t	p	R^2	Model *p* value
1	Somatization subscale	0.433	9.094	< 0.001	0.185	**< 0.001**
2	Somatization subscale	0.385	8.215	< 0.001	0.239	**< 0.001**
	Age	-0.242	-5.158	< 0.001		

SAS = Smartphone Addiction Scale

of subjective sleep quality, sleep latency, sleep disorder, daytime dysfunction, BSI total scores and scores of all subscales of BSI ($p < 0.001$, Table 2).

SAS score was significantly and positively correlated with age and negatively correlated with the scores of ESS, PSQI total, PSQI subscales of subjective sleep quality, sleep latency, habitual sleep efficiency, sleep disorder and daytime dysfunction, BSI total, BSI subscales of anxiety, depression, negative self, somatization and hostility. Table 3 shows the correlations between SAS scores and the scores of other scales.

A linear regression analysis was carried out to find the variables that predicted SAS scores. It was found that the BSI subscale of somatization and age predicted SAS scores to a significant extent (Table 4). According to the linear regression analysis, the significant predictors of PSQI scores were the BSI subscales of somatization and depression, SAS scores and age (Table 5).

DISCUSSION

This study investigated the smartphone use behaviors of 362 students who were enrolled at a foundation university and the effects of these on different aspects of sleep and psychiatric symptoms. As a result of the study, it was observed that, as smartphone use increased and became problematic, sleep quality decreased, and daytime dysfunction and psychiatric symptoms increased. SAS scores were positively correlated with both PSQI and ESS scores. SAS score was found as a predictor of PSQI scores in the regression analysis. The findings that were obtained here were in agreement with the literature which suggested that problematic smartphone use behaviors have negative effects on the quality of sleep.

Table 5. Linear Regression Analyses Results for PSQI

Model	Variables	Standardized Coefficients (Beta)	t	*p*	R2	Model *p* value
1	Somatization subscale	0.659	16.587	< 0.001	0.432	< 0.001
2	Somatization subscale	0.453	7.418	< 0.001	0.459	< 0.001
	Depression subscale	0.266	4.346	< 0.001		
3	Somatization subscale	0.414	6.669	< 0.001	0.470	< 0.001
	Depression subscale	0.248	4.079	0.001		
	SAS	0.123	2.867	0.004		
4	Somatization subscale	0.405	6.591	< 0.001	0.480	< 0.001
	Depression subscale	0.271	4.460	< 0.001		
	SAS	0.154	3.526	< 0.001		
	Age	0.116	2.871	0.004		

PSQI = Pittsburgh Sleep Quality Index

Development of decision-making skills is dependent on the changes the brain experiences throughout life. Insufficiency of decision-making skills increases the tendency towards abuse of any substance. The changes that the brain experiences in adolescence increase impulsivity, and therefore, sensitivity about substance abuse [29]. With the transition to adulthood, de- creases in neuroticism and impulsive behaviors reduces substance abuse problems [30]. In parallel to this information, studies that have been conducted so far found that problematic smartphone use is inversely proportionate to age. We also observed that, as age decreased, smartphone usage became significantly more problematic, and age predicted SAS scores. Individual and social responsibilities and liabilities that increase with age may also contribute to the reduction of time that is spent on smartphone. We found that the associate's degree students have significantly higher problematic smartphone use in comparison to the bachelor's and master's degree students. The younger age of associate's students might explain this finding. The course loads and academic responsibilities of associate's degree students are lighter, and they may have a tendency to spend more time using smartphones.

In previous research, some researchers suggested that, the addiction develops to the social media applications and games provided by smartphones, instead of the phone itself [2]. Likewise, in our study, the high-level smartphone use group used their smartphones significantly more for social media, gaming and other mobile applications in comparison to the low-level smartphone use group. Engaging with social media is one of the significant factors that disrupt sleep. An epidemiological study that was conducted in the USA used online questionnaires to investigate the relationship between social media usage and sleep disorders among 1788 individuals with the age range of 19-32 [31]. The median social media use duration of the sample was found as 61 minutes, and more than half of the sample had moderate or severe sleep disorders. A directly proportional relationship was found between social media use and sleep disorders. The reason for the relationship between problematic smartphone use and sleep disorders in our study could be the need for connecting to social media. Future studies should particularly and distinctively investigate the relationship between using social media via smartphones and sleep disorders.

Previous studies suggested that compulsively looking at one's smartphone may be a part or promoter of problematic behavior. The results of our study supported this issue. SAS scores increased as the frequencies of checking smartphones increased. The behavior of checking increases the mental attachment with the smartphone. Levenson *et al*. [31] found a relationship between sleep disorders and frequency of visiting social media, rather than the time spent on social media. They argued that this may be an obsessive checking behavior [31].

The dimensions of smartphones make them easier to use practically in every form of posture including lying down and almost everywhere, especially in bed, and development of sleep disorders may be easier. Using smartphones before sleep may also make it difficult to fall asleep by causing cognitive, emotional and physiological arousal [32]. Sleep quality may be disrupted due to the electromagnetic field created by smartphones and reduced secretion of melatonin [33-35]. The blue-purple light emitted by the backlight of smartphone screens may delay the time of falling asleep by disrupting the circadian rhythm [36-39]. Telzer *et al*. [35] reported that, as a result of insufficient sleep in smartphone users, processing decreased in the dorsolateral prefrontal cortex, and the connection between the ventral striatum and insula was reduced during reward processing. In other words, lack of sufficient sleep may be leading to increased usage of smartphones by disrupting impulse control skills [35].

A study that was conducted with 844 Flemish adults at the ages of 18 to 94 revealed that six in every 10 people took their phone with them to the bedroom [40]. As the rate of messaging and making phone calls in bed increased, sleep quality had a tendency to decrease. It was reported that spending time with smartphones in bed increased sleep latency, worsened sleep efficiency, delayed the time of waking up in the morning, increased insomnia scores and daytime fatigue. Our study also showed that those who kept their smartphones with them before sleep had lower sleep quality and higher daytime dysfunction levels. A study by Gradisar *et al*. [41] in the USA reported that more than half of people who use their phones

before falling asleep left their phone on, and because of this, they woke up with the incoming notification and found it difficult to fall asleep again [41]. Adams and Kisler [42] stated that 47% of university students woke up at night to answer messages, while 40% woke up at night to respond to calls [42]. Rosen's study [43] reported that most students woke up to check their messages, and a third of the sample had short sleep durations.

Presence of psychiatric symptoms may lead to problematic smartphone use or continuation of existing problematic use. In some cases, problematic smartphone use may lead to development of psychiatric symptoms. Additionally, both the presence of psychiatric symptoms and problematic smartphone use may increase tendencies towards each other. Consequently, an underlying genetic or environmental risk may lead to the development of both psychiatric symptoms and problematic smartphone use. In our study, there was a positive correlation with SAS scores and all subscales of BSI, and the BSI subscale of somatization predicted SAS scores. Yen *et al*. [44] found the scores of the BSI subscales of hostility, depression and somatization higher among teenagers with substance abuse problems. The same study observed that teenagers who had internet addiction had higher BSI hostility, depression and phobic anxiety scores. In a logistic regression analysis, internet addiction was found to be related to hostility, depression and substance abuse disorders [44].

Somatization represents the form of expression of psychological problems especially in underdeveloped societies, and it frequently accompanies depression and anxiety disorders [45]. Somatization is used to express problems of psychological origin more in people with insufficient problem-solving skills and lack of psychological mindedness [46]. If the smartphone is used for coping with psychiatric problems, presence of somatization in these individuals may be understandable.

Excessive use of smartphones may lead to serious deformations in the anatomic stance of the body and various musculoskeletal system symptoms such as pain in the wrists or the neck may develop [1]. The constant position of the head in an angle and fixed position of the arms may lead to contraction of muscles and show an effect that reduces relaxation by lack of antagonistic motion. Smartphone use may lead to damage in the cervical vertebrae [47], a sedentary lifestyle, and as a result of this, feelings of low energy and physiological problems may ensue [48]. In our study, the orthopedic problems caused by smartphones may have increased the BSI somatization scores by increasing somatic complaints. However, we have not addressed this question in the study. Xie *et al*. [49] showed a relationship between excessive smartphone use and physical symptoms in teenagers. This relationship was mediated by sleep quality [49]. Future studies may investigate the effects of musculoskeletal symptoms that develop as a result of smartphone usage on sleep quality.

While sleep disorders are among the symptoms of depression, depression may also develop in relation to sleep disorders. Smartphone use may have an effect on both sleep and mood. The person may be spending time with their smartphone because they have trouble sleeping, which in turn increases sleep latency and develops smartphone addiction. Having to sleep late and wake up early may increase somatic and depressive complaints such as concentration problems, irritability, fatigue, reluctance towards physical or social activities, headache or diffuse bodily pain due to sleep deprivation. Similarly, if the person spends time with their smartphone to improve the depressive mood, this may not only turn smartphone use problematic and develop sleep disorder. Therefore, there is not a one-way, but a two-way relationship here. A study in Nepal that was carried out with 937 university students at 27 different campuses stated that the state of being addicted to the internet mediated 16.5% of the indirect effects of sleep quality on depressive symptoms, while sleep quality mediated 30.9% of the indirect effects of internet addiction on depressive symptoms [50]. Some people use social media to fall asleep, but as a result of this strategy, sleep durations get shorter and daytime dysfunction increases [51]. A longitudinal study that was carried out with university students for 3 years found that presence of sleep disorders predicted usage of online social media and other media tools such as television. It was stated that spending time on social media was a result of sleep disorders [52].

The fact that all the scales that were used in the study were self-report increased the probability of bias. Future studies may obtain more qualified data regarding the characteristics of sleep, based on long-

term data records by using actigraphy and sleep diaries. Selection of the sample from only amongst foundation university students prevents us from generalization of the findings to all university students or the entire society. The period when the questionnaire forms were distributed to the students corresponded to the period of their mid-term exams. Mid-term periods are when students stay awake for long times to study, their hours of falling asleep become irregular, and therefore, sleep quality decreases, and daytime dysfunction decreases. Collection of the data in the mid-term period may have been the reason for the finding of dis- rupted sleep quality. Repeating the study at the beginning of the term when students have more regular sleep may increase the quality of data.

BSI, which is used to measure psychiatric symptoms, is not sufficient to make a clinical diagnosis, and it uses a limited number of psychiatric symptoms in screening. Psychiatric syndromes should be diagnosed by standardized and structured clinical interviews, and accordingly, it may be recommended to differentiate whether sleep disorders are caused by problematic smartphone use or a psychiatric syndrome.

CONCLUSION

This study shows that the relationship between problematic smartphone use and decreased sleep quality and increased psychiatric symptomatology in university students is still an important public health issue. Responsible and mindfull use of smartphones by the students throughout the day may provide a positive effect on their sleep. Prohibiting students from using phones may increase their anxiety due to nomophobia, and because of this, they may be encouraged to turn off their phones during classes as a form of a technology break instead of prohibition. Collaboration of physicians that provide public health services, educators and institutions that provide preventive mental health services will be beneficial for informing students about the relationship between sleep disorders and smartphone using styles and developing healthy smartphone usage skills.

Ethics

The study was approved by the Ethics Committee of the university and complies with the Declaration of Helsinki. All subjects were informed about the study and all provided informed consent.

Authors' contribution

EEY planned the study and collected the data. BÖÜ did the statistical analysis and wrote the manuscript.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Comparison between periarticular injection and intraarticular infusion for pain control following total knee arthroplasty

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ABSTRACT

Objectives: To compare periarticular infiltration and intraarticular continuous infusion methods in pain management following total knee arthroplasty (TKA).

Methods: Patients who underwent TKA from May 2015 through September 2015 according to their postoperative pain protocol were compared. The patients who received bupivacaine by periarticular infiltration (PAI group) and intraarticular infusion (IAI group) were included in the study. Patients also received a treatment through intravenous patient-controlled analgesia (PCA) device. The frequency of patients' bolus need and the tramadol dose used via PCA device, Visual Analogue Scale (VAS) scores and clinical evaluation as active knee flexion at the postoperative 3th day were obtained from the patient follow - up forms and records. Side effects related to narcotic analgesic medications were also obtained.

Results: The study included 90 patients, of whom 46 were in the PAI group (median age, 65.5 years; females, 82.6%) and 44 were in the IAI group (median age: 65.5 years; females, 81.8%). The VAS pain scores assessed at various postoperative time points and tramadol consumption were usually lower in the IAI group than in the PAI group. No difference was determined between the groups regarding the 3th-day VAS scores in flexion and in terms of analgesia-associated side effects.

Conclusions: Bupivacaine administration by IAI for postoperative pain management following TKA is associated with lower pain and lower tramadol consumption as compared with bupivacaine administration by PAI. The groups were comparable in terms of side effects. Accordingly, IAI seems to be an effective and safe analgesic technique for patients undergoing TKA.

Keywords: Arthroplasty, replacement, knee, pain, postoperative, infusion pumps, analgesia, patient-controlled

Postoperative pain is one of the important problems in modern medicine. Severity of pain may sometimes outweigh the success of surgery; in fact, surgery becomes questionable from the patient's point of view. Therefore, postoperative pain management has currently become one of the critical components of the patient care [1]. Effective pain management not only provides patients with comfort and satisfaction but also enables early mobilization, lower pulmonary and cardiac complications, reduced risk of deep vein thrombosis, and faster recovery and thereby results in decreased cost of care [2].

Total knee arthroplasty (TKA) is one of the most frequently performed surgical procedure in orthopedic

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surgery and annual number of this procedure is increasing gradually worldwide due to prolonged life expectancy [3]. Complaint of pain is frequent after TKA and causes significant problems concerning both postoperative rehabilitation and patient comfort [4]. New methods such as peripheral nerve blocks, periarticular injections, intravenous or epidural patient-controlled analgesia (PCA), and intraarticular continuous infusion pumps have been implemented to provide an effective analgesia after TKA [4, 5].

Periarticular infiltration and intraarticular continuous infusion pumps are the locally effective methods that are frequently preferred for pain management following TKA [6-9]. The present study aimed to compare these two local effective methods 'periarticular infiltration and intraarticular continuous infusion methods' in pain management following TKA.

METHODS

A prospective controlled study was conducted in patients with severe knee osteoarthritis scheduled for TKA surgery. Two groups were constituted regarding the two different post-operative pain management preference of the two senior surgeons as; periarticular infiltration (PAI) and intraarticular continuous infusion (IAI). After the approval of the local ethics committee, consecutive patients who had gonarthrosis were enrolled in the study. Informed constents of the patients was obtained from all patients. Patients were excluded if; the ASA (American Society of Anesthesiologists) score was IV or more, patients had previously diagnosed inflammatory arthritis, patients had severe knee deformity, there was a previous knee surgery, patients had an advanced liver or kidney disorders, patients had neuropathic pain, psychiatric disorders and documented allergy against local anesthetics.

Spinal anesthesia through administration of 15 mg hyperbaric bupivacaine (0.5%) at the L2-3 or L3-4 level was performed in all patients routinely by the same anesthesia team. The patients were placed in the supine position after anesthesia. The surgical procedure was started when the spinal block reached to the level of T8-10. Thigh tourniquet was applied for all patients; however, it was inflated only during bone cement application. Fixed-bearing, cruciate-retaining cemented total knee prostheses (Zimmer Warsaw, IL, USA) were implanted for all patients included in the study. The native patella was retained in all patients.

The patients who had periarticular infiltration (PAI) as postoperative analgesia assigned as group PAI and those who had intraarticular infusion (IAI) for postoperative analgesia assigned as group IAI. In the PAI group, a 60-mL solution composed of 40 mL of 0.5% bupivacaine and 20 mL of saline was infiltrated into the posterior capsule and medial and lateral collateral ligaments prior to the implantation and into the patellar tendon, fascia and subcutaneous tissue along the incision after the implantation. In the IAI group, ON-Q elastomeric infusion pumps (I-Flow LLC/Kimberly Clark) Hopkins, Flrd, USA) catheter was placed into the joint along the lateral margin. A solution composed of 200 mL 0.5% bupivacaine and 100 mL saline was prepared; 40 mL of this solution was infused as bolus into the joint without deflating the tourniquet. Infusion pump clamp was opened and infusion continued for 48 hours at a rate of 5 mL/h. A hemovac drain was not placed in any of the patients. The tourniquets were deflated in all patients after applying a compressive Jones bandage. The bandages were removed after 24 hours of surgery and the patients were allowed assisted walking and to perform active and passive movements.

In addition to the above-mentioned pain management methods, the study groups also received a treatment through intravenous (IV) PCA device; the treatment was the same for both groups. PCA device is a patient-controlled device which allows the patient to receive additional analgesic at a time determined by him/her and with the maximum dose limited by the device. Using a PCA device (Abbott Laboratories, Chicago, IL, USA), a 48-hour IV tramadol infusion was performed at a rate of 5 mg/h with a bolus dose of 10 mg and with a lockout time of 20 minutes. The frequency of patients' bolus need and the total amount of tramadol used via PCA device were recorded. For nausea and vomiting, IV metoclopramide (20 mg) was administered maximum three times a day. The patient records were assessed for any sign of side effects related to narcotic analgesic medications and the findings were recorded.

The cost of the pain management methods was measured as the cost of infusion pump and total used medications for IAI group and the cost of medications

used for PAI group.

All the patients who underwent surgery were informed about the horizontal Visual Analogue Scale (VAS; 0: No pain, 100: Unbearable pain) that was used for pain assessment. Resting VAS scores were recorded at 4-hour intervals in the first 24 h after the surgery and then on the 2nd and 3th days. The patients were allowed to exercise after the removal of Jones bandage at the 24th hour; VAS scores during exercise were recorded at on the 2nd and 3th days.. The patients were clinically evaluated based on their degree of active knee flexion on the postoperative 3th day.

Statistical Analysis

Data analysis was performed by the Predictive Analytics Software (PASW) 18.0 (SPSS Inc., Chicago, IL, USA) for Windows program. Descriptive statistics were expressed as number and percentage for categorical variables and as mean, standard deviation, median, and quartiles Q1 (25th percentile) and Q3 (75th percentile) for numerical variables. Comparison of two groups for categorical variables was performed using chi-square analysis, and when chi-square condition was not met, Fischer's exact test was performed. Comparison of two groups for non-normally distributed numerical variables was performed by Mann-Whitney U test. The level of statistical significance was accepted as $p < 0.05$.

RESULTS

A total of 112 consecutive patients, operated on from May 2015 through September 2015 by two experienced surgeons were included in the study. 22 patients were excluded from the study for several reasons (6 for having previous knee surgery, 6 for having ASA score of 4 or more, 1 for having neuropathic pain, 1 for not co-operating regarding the use of PCA device, 1 for having chronic liver-kidney disorder, 2 for having early postoperative infection, 4 for technique problems about the infusion device and 1 for having psychiatric disorder). Finally there were 90 patients (46 in PAI group and 44 in IAI group) in the study. The study groups were comparable in terms of age and gender (Table 1).

The VAS scores of the patients are summarized in Table 2. While the median resting VAS scores at the 4th hour and on the 3th day were significantly lower in the PAI group ($p = 0.001$ and $p < 0.001$, respectively), the median resting VAS scores were significantly lower at the 8th, 12th, 16th, 20th and 24th hours in the IAI group ($p < 0.001$ at all). The median VAS scores during exercise at the 24th hour and on the 3th day were significantly lower in the IAI group ($p < 0.001$ and p < 0.001, respectively). No difference was determined between the groups in terms of the 3th-day VAS scores in flexion ($p = 0.102$).

Comparison of the PCA use between the groups revealed that the median tramadol dose used within 0-6 hours was lower in the PAI group, whereas the median total tramadol dose was lower in the IAI group (Table 3). The cost of pain management protocols were approximately 265€ for patients in IAI group and 10€ for patients in PAI group.

The most common analgesia-associated side effect was nausea followed by headache and constipation. The distribution of side effects in the PAI and IAI groups is demonstrated in Table 4. No significant difference was observed between the two groups in terms of the distribution of side effects.

Table 1. Demographic characteristics of the patients

	Group PAI (n = 46)	Group IAI (n = 44)	*p* value
Age, Median (Q1-Q3)	65.5 (62-69)	65.5 (62-69)	0.903[a]
Gender, n (%)			
Female	38 (82.6)	36 (81.8)	0.922[b]
Male	8 (17.4)	8 (18.2)	

PAI = periarticular infiltration, IAI = intraarticular infusion, Q1 = the 1st quartile, Q3 = the 3rd quartile
[a]Mann-Whitney U test, [b]Chi-square test

Table 2. Visual analog scale pain scores

	Group PAI (n = 46)	Group IAI (n = 44)	*p* value*
Resting VAS scores	Median (Q1-Q3)	Median (Q1-Q3)	
4th hour	10 (10-10)	10 (10-20)	**0.001**
8th hour	30 (30-40)	20 (20-20)	**< 0.001**
12th hour	40 (40-40)	30 (30-30)	**< 0.001**
16th hour	40 (40-40)	20 (20-30)	**< 0.001**
20th hour	40 (40-40)	30 (30-30)	**< 0.001**
24th hour	40 (40-40)	30 (30-35)	**< 0.001**
2nd day	30 (30-30)	30 (30-30)	0.068
3th day	30 (30-40)	40 (40-40)	**< 0.001**
VAS scores during exercise			
24th hour	55 (50-60)	50 (40-50)	**< 0.001**
2nd day	55 (50-60)	50 (40-50)	**< 0.001**
3th day	40 (40-50)	40 (40-40)	0.055
3th-day VAS score in flexion	85 (85-90)	90 (85-95)	0.102

PAI = periarticular infiltration, IAI = intraarticular infusion, VAS = Visual analog scale, Q1 = the 1st quartile, Q3 = the 3rd quartile

*Mann-Whitney U test

Table 3. Amount of tramadol used via patient-controlled analgesia device

	Group PAI (n = 46)	Group IAI (n = 44)	*p* value*
Tramadol use	Median (Q1-Q3)	Median (Q1-Q3)	
0-6 hours, mg	30 (30-35)	35 (35-40)	**< 0.001**
Total, mg	400 (380-420)	360 (350-370)	**< 0.001**

PAI = periarticular infiltration, IAI = intraarticular infusion, VAS = Visual analog scale, Q1 = the 1st quartile, Q3 = the 3rd quartile

*Mann-Whitney U test

DISCUSSION

Perioperative pain management in patients undergoing TKA remains to be one of the most challenging issues for both the surgeons and the anesthesiologists. Reducing pain is the essential component of patient satisfaction, functional outcomes, and duration of hospital stay [10]. In addition to the changes and evolutions in the surgical techniques, anesthesia and analgesia techniques have also evolved over time. Regional anesthesia techniques have replaced the general anesthesia [11]. This also applies to the postoperative pain management; local implementations are gradually becoming more popular as the traditional opioid-based methods are associated with numerous side effects [12]. Kerr and Kohan [13] defined local infiltration analgesia (LIA) as a simple, practical, safe and effective method and stated that it targets to achieve satisfactory pain management with little physiological disturbance. Gibbs *et al*. [14] conducted a review including 29 randomized trials and concluded that LIA following TKA was successful in postoperative pain management. It has been reported that continuous LIA is superior to placebo in relieving pain but that it might be associated with increased risk of infection. Nevertheless, continuous LIA has not

Table 4. Analgesia-associated side effects

	Group PAI (n = 46)	Group IAI (n = 44)	*p* value
Nausea, n (%)	11 (23.9)	9 (20.5)	0.693[a]
Headache, n (%)	8 (17.4)	6 (13.6)	0.623[a]
Constipation, n (%)	8 (17.4)	5 (11.4)	0.416[a]
Pruritus, n (%)	7 (15.2)	5 (11.4)	0.591[a]
Vomiting, n (%)	6 (13.0)	5 (11.4)	0.808[a]
Dry mouth, n (%)	6 (13.0)	5 (11.4)	0.808[a]
Urine retention, n (%)	6 (13.0)	4 (9.1)	0.740[b]
Dizziness, n (%)	6 (13.0)	4 (9.1)	0.551[b]
Sleepiness, n (%)	6 (13.0)	3 (6.8)	0.486[b]

PAI = periarticular infiltration, IAI = intraarticular infusion, VAS = Visual analog scale
[a]Chi-square, [b]Fisher's exact test

been found to be associated with prolonged surgical duration or prolonged length of hospital stay [15]. Moreover, LIA results in low hospital cost as compared with the standard analgesia [16].

During local interventions, various agents in different combinations and at different doses are infiltrated into the periarticular tissue or injected or infused into the intraarticular space via catheters during surgery and/or after surgery [14]. It is not apparent from which tissue the pain following TKA primarily arise; additionally, which one of the methods used for pain management is the most optimal is also controversial. Karlsen *et al*. [17] conducted a systematic review to identify the most effective and safe method for postoperative pain management and included 113 eligible study identified by literature search; they concluded that it was difficult to determine the optimal therapeutic regimen because of small sample sizes, heterogeneous study designs, and low quality of evidence.

The PAI and IAI have been evaluated in clinical studies. The main finding of this study was the better pain management of IAI when compared to PAI with comparable side-effects of the drugs. Although these protocols are widely questioned in literature individually, there is not enough knowledge comparing these techniques in terms of pain management and side effects. PAI is widely studies by many authors and its favorable outcomes were reported. Chaumeron *et al*. [18] reported the PAI as effective as femoral nerve block and Kerr and Kohan [13] reported the reduced need for morphine with PAI. Mullaji *et al*. [19] studied the effectiveness of PAI in patients with bilateral TKA and demonstrated that patient felt less pain in their PAI side. Yuenyongviwat *et al*. [20] conducted a placebo-controlled study and administered bupivacaine by the PAI for postoperative pain control in patients undergoing TKA and demonstrated the decrease in morphine consumption in PAI group. The use of intraarticular agents for postoperative pain management has also been evaluated before. Browne *et al*. [21] reported lower pain and narcotic consumption in the patients receiving intraarticular bupivacaine injection than in those receiving placebo (saline), following capsule closure during TKA; however, they reported that the difference did not reach a statistical significance. Kazak *et al*. [22] reported that intraarticular bupivacaine administration was associated with better postoperative analgesia, lower tramadol consumption, and shorter hospital stay as compared with placebo administration. However there are limited studies aimed to compare these two protocols in patients undergoing TKA surgery. Perret *et al*. [23] compared the outcomes of PAI and IAI methods in patients undergoing TKA and reported no difference between groups regardin postoperative opioid consumption. As compared with the intraarticular group, the VAS scores on the postoperative 1st day and during hospital discharge were reported to be lower and the duration of hospital stay was reported to be longer in the peri-

articular group. Based on these results, Perret *et al*. [23] concluded that none of the methods was superior to another. But contrary to Perret *et al*. [23], this study demonstrated the better pain control of IAI day after surgery. Although PAI patients had better pain control at first hours of surgery which was probably caused from the higher amount of bupivacaine in the bolus doses, the IAI was superior for pain control when the postoperative days were considered. In addition, although patients in IAI groups received more bupivacaine through 3 days, the side effects of the groups were comparable.

Bupivacaine, with its proven efficacy, is one of the agents used frequently as a part of multimodal pain management in TKA [24]. In the present study, we also used bupivacaine for pain management following TKA and compared PAI and IAI methods. In the present study, the VAS pain scores assessed at various postoperative time points were usually lower in the IAI group than in the PAI group. The total amount of tramadol consumption was also lower in the IAI group. No difference was determined between the two groups in terms of analgesia-associated side effects. It has been reported that demographic characteristics such as age and gender are effective in postoperative pain following knee surgeries [25]. In the present study, the groups were comparable in terms of age and gender. This eliminated the effects of demographic characteristics on study outcomes. In the present study, surgical procedures were performed by two surgical teams which graduated from the same institution and performs the TKA in a very similar way on the other hand the same anesthesia team was on duty in all patients, which was also an advantage for making accurate evaluations. It has been reported that perioperative anesthesia and analgesia are effective also on the outcomes after one month of surgery [26].

Limitations

Lack of assessment of the long-term outcomes can be considered as a limitation of the present study.

CONCLUSION

In conclusion, bupivacaine administration by IAI for postoperative pain management following TKA is associated with lower pain and lower tramadol consumption as compared with bupivacaine administration by PAI. The groups were comparable in terms of side effects. Accordingly, IAI seems to be an effective and safe technique.

Authors' Contribution

MA = Study design; İBA = Performing anesthetical procedures and technical review; HAA = Patient admission, surgical team; AÖ = Critical review, technical revisions; YGB = Data collection, statistical work; and ÖE = Critical scientifically revision

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

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Micro- and nanoscale characterization of different natural biomaterials for ocular surface regeneration

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ABSTRACT

Objectives: This study aims to characterize the widely used biological derived membranes in clinics in terms of micro-nano scale mechanical and morphological properties. Within this scope, advanced platelet-rich fibrin (A-PRF), leucocyte-and platelet-rich fibrin (L-PRF) and human amniotic membrane were studied in this research study.

Methods: Nano-indentation, optical coherence tomography (OCT), scanning electron microscopy (SEM), and in vitro degradation test were performed for material characterization.

Results: The nano-indentation test revealed significantly higher modulus of elasticity and hardness values in A-PRF group, while OCT presented significantly higher thickness measurements when compared L-PRF. A loose 3D architecture formation due to the large pores formed by means of large fiber diameter were observed in A-PRF group. Besides, platelets were observed among the large fibers in A-PRF membranes on the contrary of L-PRF membranes. Low fiber diameter and high cellular separation were recorded in L-PRF group due to the high centrifugal force application. Therefore, it was observed that the platelets were located mostly on the surface of the membranes in L-PRF. The loose 3D architecture of A-PRF membranes is thought to release growth factors for a longer period of time, ensuring cellular integrity. On the other hand, degradation test results indicated that amniotic membranes degrade to about 85% in one week, while L-PRF and A-PRF were lost their initial weights approximately 31% and 40%, respectively.

Conclusions: This comparative characterization study of three different natural biomaterials used in a wide range of clinical applications, from dentistry to ophthalmology, was thought to guide surgeons on the selection of site-specific material.

Keywords: Leucocyte- and platelet-rich fibrin, advanced platelet-rich fibrin, amniotic membrane, nano-indentation, optical coherence tomography

Tissue engineering is an interdisciplinary field, which combines life sciences, engineering techniques, cell biology, and biomaterials science. It targets maintenance, improvement or regeneration of tissue function with modernized approaches including cellular and/or scaffold-based applications [1]. The

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major concern in this field is to obtain the most safe, simple and clinically applicable and also the least harmful and expensive product. Unfortunately, the compatibility, degradability, and versatility of many biomaterials are not satisfactory for desired regeneration of soft and hard tissue defects [1, 2]. The researchers have therefore focused on blood-derived autologous biomaterials which are important source of essential cellular and protein-based products that cannot be obtained from other synthetic biomaterials [2-5].

Platelet concentrate trapped in 3 dimensional (3D) fibrin architecture is one of the blood derivative products and has gained popularity in the last two decades with major innovations of different preparation protocols [4-8]. Leucocyte- and platelet-rich fibrin (L-PRF), derived from individual's own blood sample by Choukroun's technique, is a member of platelet-rich product family and consists of leukocytes, cytokines, circulating stem cells, matrix proteins andgrowth factors (GFs) within the platelet granules which induce and accelerate tissue regeneration [6, 7]. Unlike the previously proposed platelet-rich preparation method in the literature, Choukroun's recommendation does not use techniques that prolong the duration of the procedure and have side effects, such as anticoagulants, bovine serum or multi-step centrifugation [4, 6, 7]. When preparing the standard L-PRF, firstly venous blood sample of a patient is obtained by syringe and transferred to anticoagulant-free glass tube in which the blood sample starts to coagulate rapidly when contacted with the tube wall. Following, the tubes provide L-PRF elution when centrifuged at 2,700 rpm for 12 minutes due to its clot activator feature. Primarily, fibrinogen is formed at the top of the tube. The circulating thrombin then transforms the fibrinogen into the fibrin, and this fibrin clot accumulates in the middle of the tube like a buffy coat between the red corpuscles at the bottom and the acellular plasma at the top [6, 7]. Although the proposed standard L-PRF matrices seem to be a good source of biomaterials for the healing and regeneration process, it is not clear that which kind of size, shape, density of fibrin matrices together with cell distribution in the fibrin architecture will provide the optimized conditions for regeneration. Studies in recent years mostly seeks to determine the optimum centrifugation process to obtain more cellular and GF-enriched fibrin matrix that supports GF release to the regeneration side and therefore cell migration [9, 10]. Ghanaati *et al*. [9] demonstrated that decrease in centrifugation speed (1,500 rpm) together with increase in centrifugation time (14 minutes) result in higher platelet concentrations and enhanced presence of neutrophilic granulocytes in the distal part of the clot away from the buffy coat. In addition to high platelet and GF concentrations in platelet granules, the presence of neutrophilic granulocytes in the microenvironment contributes to monocyte-macrophage differentiation that resulting in accelerated tissue regeneration due to additional GF release from macrophages. Because of this improved cell and GF composition, the advanced PRF (A-PRF) acronym was assigned for the description of this matrix [9]. Like the Ghanaati's investigation, Kobayashi *et al*. [10] showed that A-PRF releases significantly higher total quantities of GF throughout a 10-day period and is suitable for conditions that require long-term release. However, in another study, biological features such as GF release and degradation time of standard L-PRF was compared with A-PRF and the released GF from standard L-PRF was found two times higher than A-PRF. In addition, it was observed that A-PRF membranes lost their initial shape by degrading earlier than the L-PRF membranes [11]. Due to the increasing accumulation of confounding results of A-PRF, the idea of evaluating the morphological and mechanical properties of this new matrix has emerged.

Both forms of PRFs have been widely used in clinical approaches by surgeonsdue to their reconstructive properties. Further, recent human and animal studies reported that the PRF enhances surface regeneration by giving rise to epithelial cells to migrate and grow because of its biologically derived scaffold function [12, 13]. Although the L-PRF's and amniotic membrane's biological, mechanical and morphological features [5, 14-16], and A-PRF's cellular composition and release dynamics [9-11] have been investigated, there have been no studies to investigate the A-PRF's mechanical and morphological properties in the literature.In addition, the lack of comparison between these biomaterials can lead to confusion in pre-determining the appropriate material for the relevant surgical procedure.

The objective of this in vitro study is to elucidate the structure of A-PRF by nanomechanical, morphological and degradation test methods and to compare

it with autogenous L-PRF and allogenic human amniotic membrane, which is frequently preferred in clinical practice for ocular surface reconstruction.

METHODS

This experimental study was conducted at Ophthalmology Departments, Yıldırım Beyazıt University School of Medicine and Keçioren Training and Research Hospital; and Institute of Science and Engineering, Bioengineering Division, Hacettepe University, Ankara, Turkey, according to the tenets of the declaration of Helsinki. Approval was obtained from the Institutional Review Board of Kecioren Training and Research Hospital (2012-KAEK-15/1066) and written informed consent was obtained from all participants after the explanation of the research design.

Test Subjects

We enrolled a total of 10 healthy volunteers for the obtainment of PRF membranes (PRF group) and 10 healthy, volunteer pregnant femalesunderwent caesarean section for the obtainment of 10 pieces of amniotic membrane (amniotic membrane group). Patients with a history of smoking, anticoagulant medication, systemic disease, contagious diseases including Acquired Immune Deficiency Syndrome, hepatitis and syphilis were excluded. For each individual in the PRF group, 20 ml peripheral venous blood sample was obtained and immediately transferred to centrifugation process. 10 ml of the blood substitutes were applied standard L-PRF preparation protocol (L-PRF group) while the other 10 ml underwent A-PRF preparation protocol (A-PRF group).

Preparation of L-PRF and A-PRF

Twenty-ml blood sample wasobtained by a vacutainer needle from the patient's antecubitalvein under sterile conditions. Samples were divided into 2 separate 10 ml glass tubes without anticoagulant and immediately transferred to centrifugation process. In L-PRF group, the blood sample was centrifuged at 2,700 rpm for 12 minutes (standard protocol) using a table centrifuge system (Hettich EBA-20; Hettich Holding GmbH & Co. oHG, Germany). In A-PRF group, the blood sample was centrifuged at 1,500 rpm for 14 minutes with the same device. The fibrin clots formed between the red blood corpuscles at the bottom and platelet poor plasma at the top were removed (Fig. 1a), and remnants of red blood cells were scraped off and discarded with gauze (Fig. 1b). The clotswere then transferred to a PRF box (Medisoft Medical, Turkey) and compressed to obtain L-PRF and A-PRF membranes (Fig. 1c). All of the membranes were transferred into pH 7.4 phosphate buffer solution (PBS) in sterile transportation tubes.

Preparation of Human Amniotic Membrane

The placentas, obtained from Caesarean section of

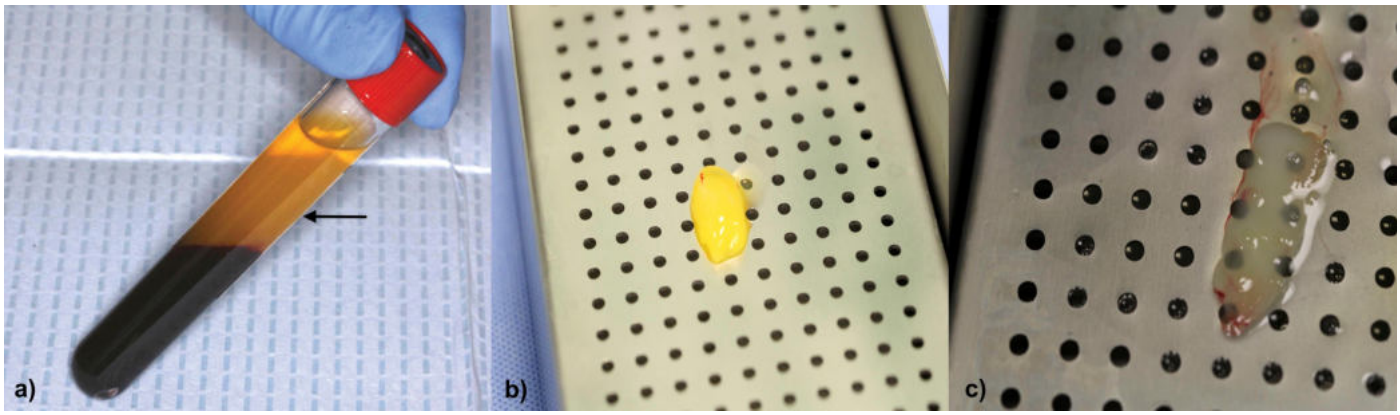

Fig. 1. The preparation of PRF after one-step centrifugation process: a) Fibrin clot was concentrated (arrow) between the red blood cell corpuscule at the bottom and the acellular plasma at the top of the tube, b) Separation of the PRF clot from red blood cell layer, c) Suturable membrane prepared from the fibrin clot by pressing in the PRF membrane box. Note the semi-transparency and smooth surface of the membrane.

10 females, were cleaned with pH 7.4 PBS containing 50 mg/ml penicillin (Sigma-Aldrich, Germany), 50 µg/ml streptomycin (Sigma-Aldrich, Germany), 100 mg/ml neomycin (Biowest, USA) and 2.5 mg/ml amphotericin B (Hyclone, England) under sterile conditions. The amnion was separated from the chorion by blunt dissection and remnants of chorion were removed by extra-washing with pH 7.4 PBS.The proximal part of the amniotic membranes were placed on nitrocellulose paper strips within pH 7.4 PBS in sterile transportation tubes.

Evaluation of Thickness by Spectral Domain Optical Coherence Tomography (SD-OCT)

The membranes were placed between 2 glass slides without any pressure and these slides were positioned in front of the fixation point of the SD-OCT (Optovue Inc., USA) (regularly calibrated). Images were taken using the anterior segment option (CAM-L) that provided a radial scan with 12 spaced lines around the buffy coat areain L-PRF and A-PRF groups,and central region in amniotic membrane group by same examiner (GDC).A total of 3 measurements for each membrane were recorded, and the mean thickness values were calculated.

Evaluation of Mechanical Properties by Nano-Indentation

Nano-indentation studies were conducted to investigate the mechanical properties of the membranes. Membranes were cut into strips of 10 mm × 5 mm in size including the central buffy coat area in L-PRF and A-PRF groups and central region in amniotic membrane group, and kept in the pH 7.4 PBS before being subjected to mechanical testing. The modulus of elasticity and hardness values of the membranes were recorded by using a nano-indentation tester (CSM Instrument Nano-indentation Tester, Switzerland) (regularly calibrated) with Olivier Pharr method. It was excavated to a depth of 6 µm from the membrane surface and 10 mN load was applied for all samples. Measurements were taken from 5 different points of the membrane surface and mean values were calculated together with standard deviation.

Evaluation of Morphology by Scanning Electron Microscope (SEM)

Structure and surface morphology of the membranes were examined using SEM (Supra 50VP, Germany) (regularly calibrated). Samples were first cut into strips of 10 mm × 5 mm in size including the central buffy coat area and distal area separately in L-PRF and A-PRF groupsand central region in amniotic membrane group, then fixed with 2.5% glutaraldehyde (Sigma Aldrich, Germany) and subjected to series of increasing concentrations of alcohol. In order to remove the water inside the samples completely, hexamethyldisilazane (Sigma Aldrich, Germany) was applied. Finally, the materials were sputter-coated with gold-palladium alloy before SEM imaging. Operated voltage was set at 20 kV and images were obtained from the samples at different magnifications.Fiber diameters were calculated by an image analysis program (Image J; National Institutes of Health, USA) through the obtained photographs.

In Vitro Degradation

Samples from each experimental group were cut into strips of 10 mm x5 mm in size. In order to obtain the dry weight of the membranes, samples were placed in an incubator set to 37°C for overnight. Initially, the membranes were weighed on an electronic micro weighing scale (Denver Instrument, Germany) (regularly calibrated). When they reached at constant weight, each sample was immersed inglass vials containing 10 ml pH 7.4 PBS supplemented with 1% v/v penicillin-amphotericin B.The glass vials containing samples were fixed on a shaker set (Gerhardt, Germany) set to 60 rpm, 37°C and the degradation medium was changed daily. At the end of 1-week, the membranes were taken out of the incubation medium, washed with distilled water, dried, and weighed. The degradation results were given as a graph in comparison with the initial dry weight.

Statistical Analysis

Statistical analysis was performed by using the Statistical Package for Social Sciences software version 20.0 (SPSS Inc, Chicago, IL, USA).All quantitative data was presented as mean ± standard deviation for all groups. Significance of difference in the variables was assessed by independent samples t-test and one-way analysis of variance (ANOVA), and correlation analysis was performed by Pearson correlation analysis. Differences were considered statistically significant at $p \leq 0.05$. Correlation

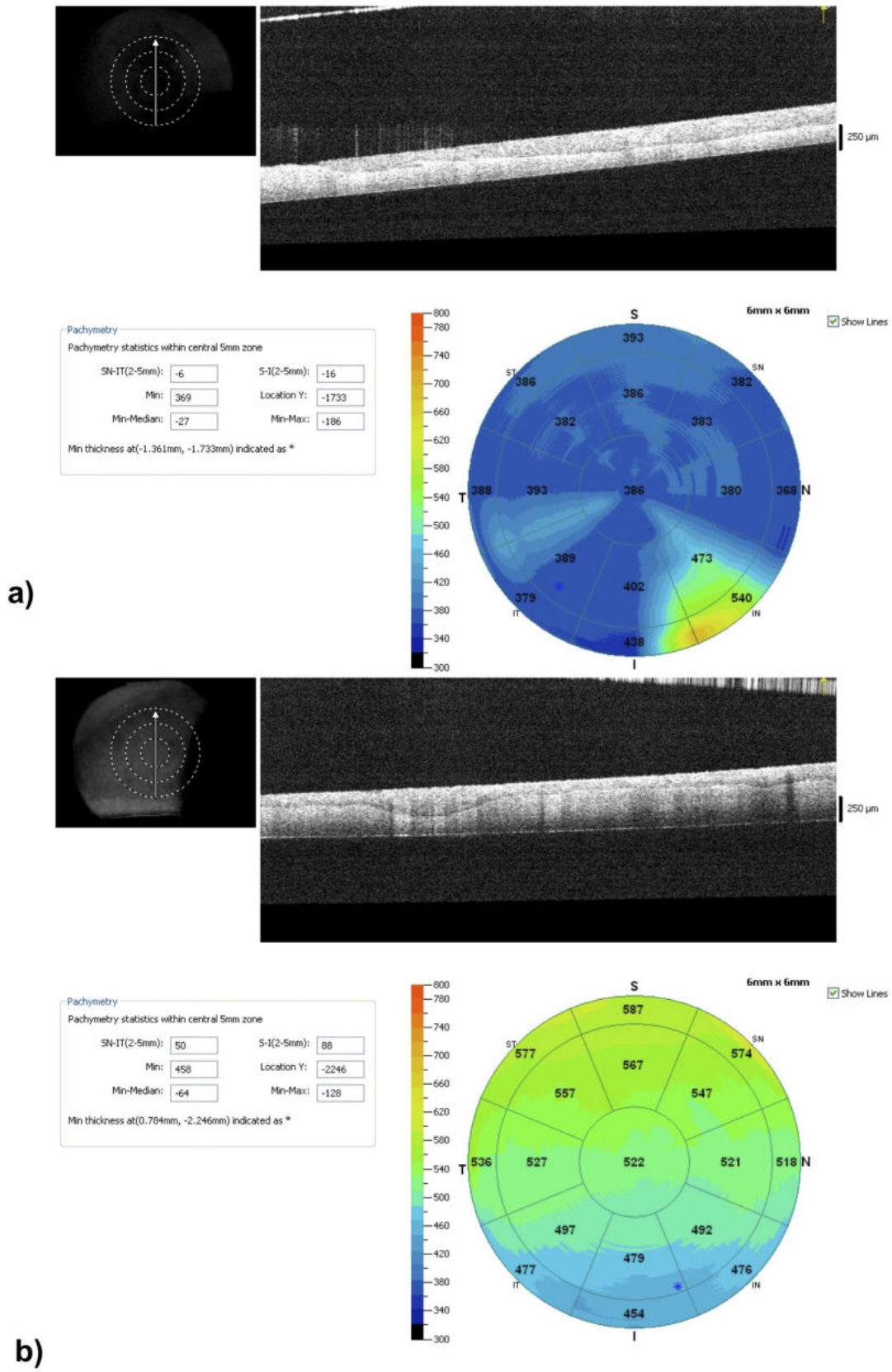

Fig. 2. Optical Coherence Tomography (OCT) images of L-PRF and A-PRF membranes: a) Thickness measurement of a L-PRF membrane. Note irregular demarcation line at middle part of the membrane, b) Thickness measurement of an A-PRF membrane. Note irregular demarcation line at middle part of the membrane.

coefficient (r) showed a strong association between variables if it was higher than 0.5.

RESULTS

Demographics

There were 5 (50%) female and 5 (50%) male subjects in PRF group, 10 (100%) pregnant female subjects in amniotic membrane group. The mean ages of the subjects in PRF group and amniotic membrane group were 31 years (range between 20-40 years) and 28 years (range between 22-36 years), respectively (p = 0.548).

Modulus of Elasticity and Hardness

In L-PRF group, mean modulus of elasticity value was calculated as 0.2 ± 0.13 GPa (range between 0.04-0.5 GPa) while mean hardness value was computed 11.44 ± 6.98 MPa (range between 2.69-26.97 MPa). On the other hand,mean modulus of elasticity and hardness values in A-PRF group were calculated as 0.66 ± 0.63 GPa (range between 0.09-2.15 GPa) and 36.92 ± 34.73 MPa (range between 7.09-125.7 MPa), respectively. Differences for modulus of elasticity and hardness values between L-PRF and A-PRF groups were found statistically significant (p = 0.03, for both). In amniotic membrane group, mean modulus of elasticity value was calculated as 0.14 ± 0.12 GPa (range between 0.01-0.4 GPa) and found significantly lower than the L-PRF and A-PRF groups (p = 0.02). Moreover, mean hardness value in amniotic membrane group was calculated as 18.64 ± 11.96 MPa (range between 2.26-41.34 MPa) and it was found higher than L-PRF group and lower than A-PRF group (p = 0.06). The results of mechanical evaluation were summarized in Table. Statistically significant positive correlation between modulus of elasticity and hardness values for all groups were revealed. (r = 0.95, p < 0.001).

SD-OCT

Mean thicknesses of the PRF membranes were obtained as 429.3 ± 77.04 µm (range between 345-543 µm) and 566.1 ± 146.71 µm (range between 389-866 µm) in L-PRF group and A-PRF group, respectively (p = 0.018) while the mean thickness of the amniotic membranes was calculated as 132.11 ± 29.86 µm (range between 92-189 µm). Thickness measurements revealed that significantly lower values were recorded in L-PRF group when compared to A-PRF group (p = 0.018). Likewise, in amniotic membrane group mean thickness measurement was found significantly lower when compared to L-PRF and A-PRF groups (p < 0.001). Interestingly, an irregular demarcation line was observed approximately at middle part of the membranes probably due to the difference of light reflectivity in both L-PRF and A-PRF groups (Fig. 2).

SEM

Buffy coat sections of fibrin matrices were monitored by SEM analysis and cell behavior and cell contents were tried to be illuminated in the distal region of prepared samples. Scanning electron microscopyimages were obtained under the same magnifications and were shown in Fig. 3 comparatively. The fibrin structure and cellular locations of the L-PRF membranes were depicted in Fig. 3a and Fig. 3c, respectively while the A-PRF membranes were depicted in Fig. 3b and Fig. 3d, respectively.

Fiber diameter was found in the range of 50 nm to 480 nm in L-PRF group, while it was in the range of 140 nm to 740 nm in A-PRF group. The mean value was calculated as 210 ± 80 nm in L-PRF group, while it was computed as 390 ± 160 nm in A-PRF group. It was observed that the diameters of the fibers formed in the structure of L-PRF membranes were smaller than the A-PRF membranes.

In the L-PRF group, cells were observed to be located on the surface of the formed fibrin structure. Contrary to this, cells were observed among the fibrin structure in the A-PRF group. These observations were shown in Fig. 4. The conducted Image J analysis revealed that the fiber size of the amniotic membrane was in the range of 30 nm to 140 nm and the mean value was calculated as 80 ± 30 nm.

Macroscopic Observation

Although same amount of blood sample was utilized for the obtaining of both membranes, mean length of L-PRF membranes was measured higher that of A-PRF membranes (Fig. 5). The mean length of the L-PRF membranes was measured as 3.32 ± 0.13 cm (range between 3.2-3.5 cm), while the mean length of the A-PRF membranes was computed as 2.52 ± 0.14

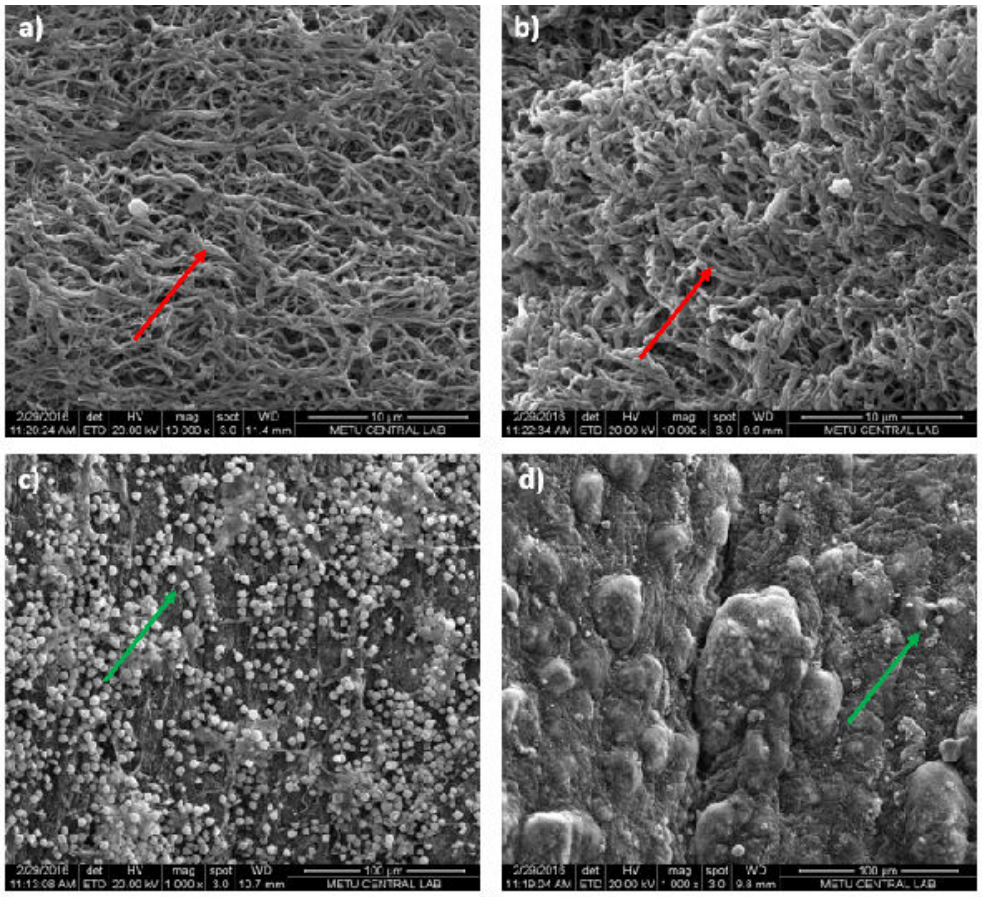

Fig. 3. Scanning Electron Microscopy (SEM) images of L-PRF and A-PRF membranes: a) Small-diameter fibrin fibers formed a dense 3D architecture with lower porosity and smaller pore diameters in L-PRF group, b) Larger diameter fibrin fibers formed a loose 3D architecture with higher porosity and larger pore diameters in A-PRF group, c) Small, round shape cells were observed to be located on the surface of the formed fibrin structure in L-PRF group, d) Small, round shape cells were observed among the fibrin structure in A-PRF group (Red arrows: fibers, green arrows: cells).

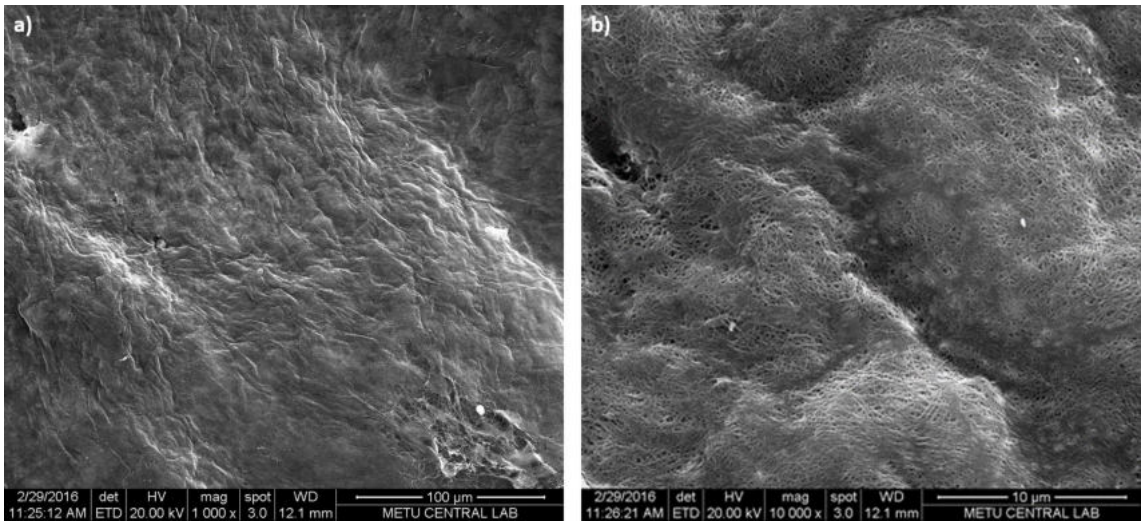

Fig. 4. Scanning Electron Microscopy (SEM) images of amniotic membranes: a) Surface topography of amniotic membrane, b) Thinner fibers firmly cross-matched and formed a stiff 3D architecture with lower porosity and smaller pore diameters in amniotic membrane group.

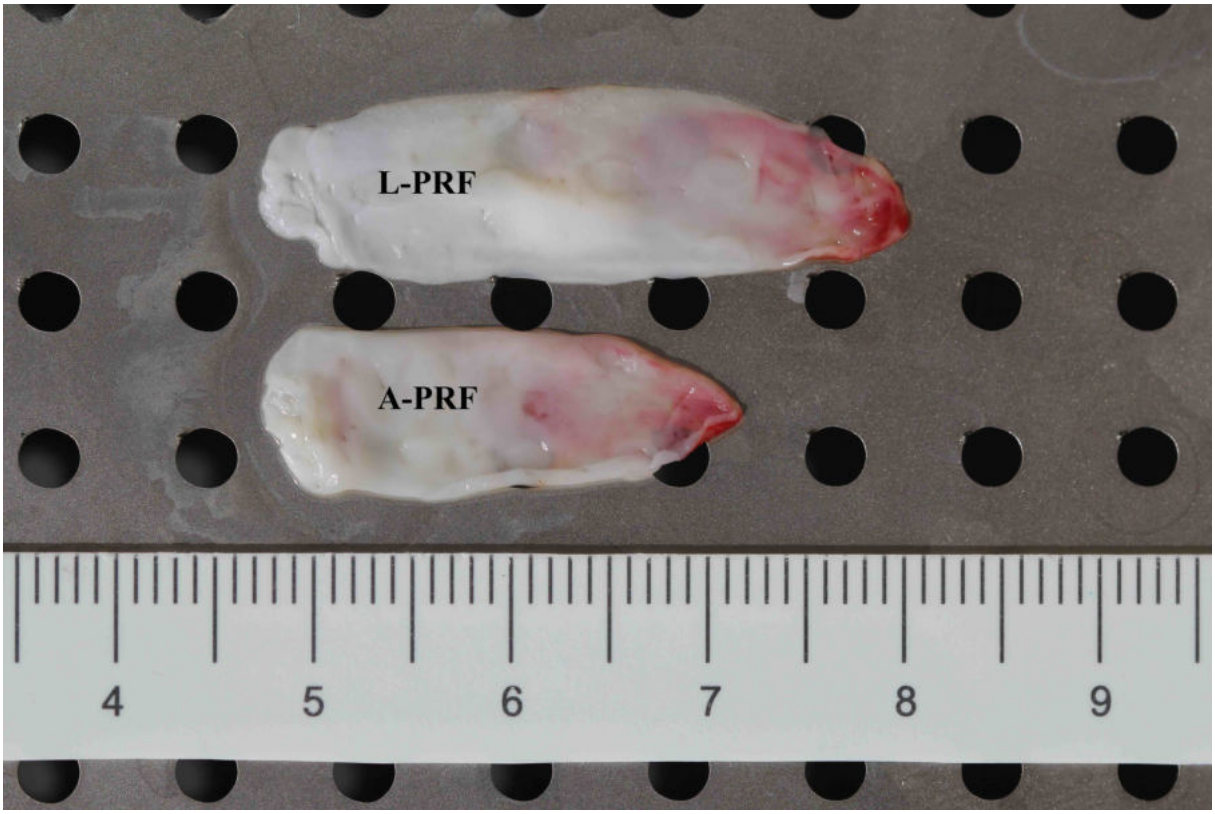

Fig. 5. **Macroscopic appearance of L-PRF and A-PRF membranes after pressing.**

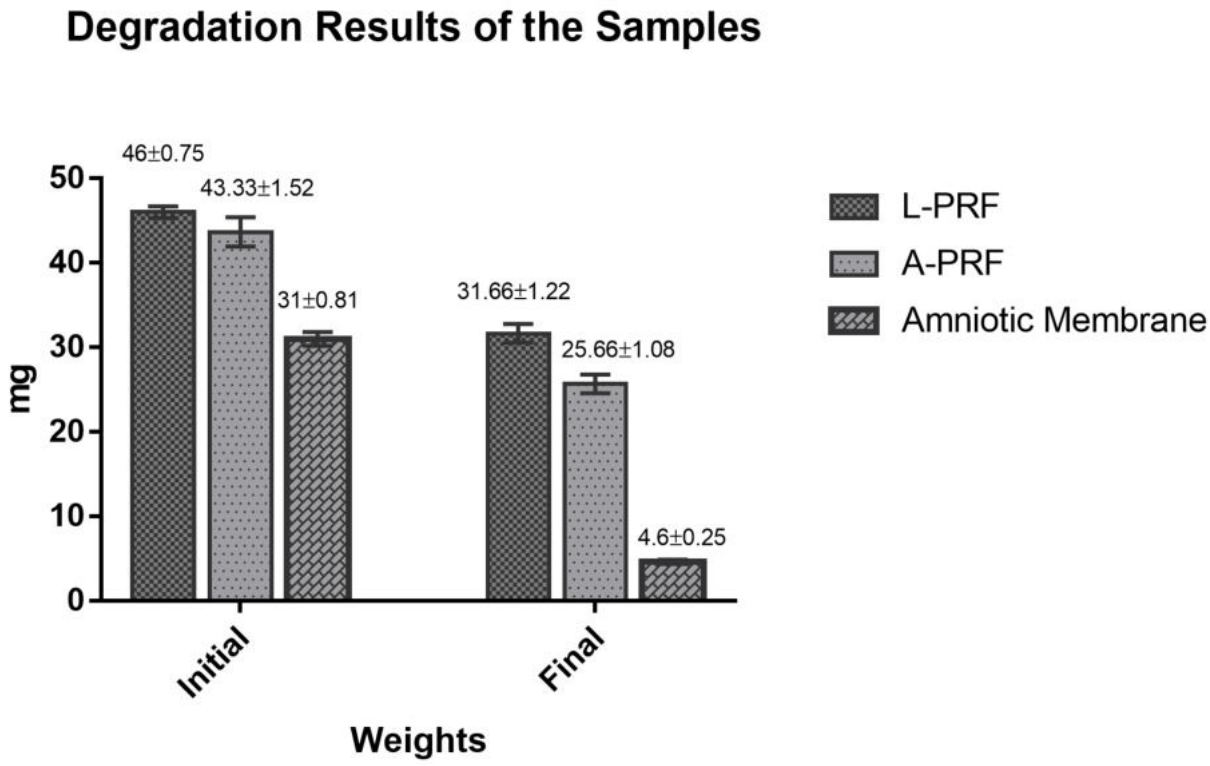

Fig. 6. **Results of in vitro degradation test.**

cm (range between 2.3-2.7 cm). The L-PRF membranes were found significantly longer than the A-PRF membranes ($p < 0.001$).

In Vitro Degradation

Degradation profiles of the membranes were depicted in Fig. 6. Advanced PRF membranes lost its 40.77% ± 0.73 of initial weight at the end of the in vitro shaking test, while L-PRF membranes lost 31.15% ± 0.85 of its initial weight. On the other hand, amniotic membranes degraded by 85.16% ± 1.27 at the end of the 1 week. These results showed thatamniotic membranes degraded remarkably faster than L-PRF and A-PRF membranes.

DISCUSSION

In recent years, studies have demonstrated that the use of platelet enriched fibrin products which are known to be a rich source of autologous chemokines and GFs, provide faster healing rates and enhanced recovery for tissue regeneration [5, 17-20]. Choukroun's L-PRF is a second generation platelet concentrate and can be convertible into a well-organized fibrin scaffold (L-PRF membrane) consisting of endogenous bio-signal molecules such as platelet-derived GF (PDGF), insulin-like GF (IGF), vascular endothelial GF (VEGF), and transforming growth factor-Beta (TGF-β), circulating stem cells, and proteins such as vitronectin, fibronectin, and Bone Morphogenetic Protein (BMP) [4-7]. These leucocyte- and platelet-rich fibrin membranes are one of the convenient graft material among other members of fibrin family due to its facile production process and self-regeneration capacity [21]. Because of L-PRF's natural fibrin framework properties, GFs can keep their activity in fibrin mesh that allows for their progressive release over time (7-11 days) [22, 23]. The accumulation of these autogenous sources in an excellent 3D architecture therefore provides it desirable applications in clinical fields such as oral, ear–nose–throat, orthopedic, plastic, and ophthalmologic surgeries [13, 24-27]. However, different preparation protocols have been trending upward in order to obtain more vigorous bio-functional material. To this end, a new concept "A-PRF", has increasingly become popular since 2014 [9-11]. However, the aforementioned studies mostly focused on this novel architecture in terms of cellularity by histological examinations up to now [9-11], and there have been no studies showing mechanical and morphological properties of A-PRF that are important for surgeons during the operations.

Human amniotic membrane, the innermost layer of the placenta, is a multi-layered fetal membrane that is composed of a single epithelial layer, an avascular stroma and a thick basement membrane [28]. The epithelial cells are considered as an important source of anti-inflammatory, anti-scarring and anti-microbial bio-signals, and show advantages like stem cells for tissue regeneration [28-30]. It has been used for the reconstruction of skin, oral cavity, head and neck, bladder and ocular surface as a biostatic allograft [29, 31-36], and for the stem cell cultivation as afeeder layer [37]. However, some drawbacks such as complicated preparation process, transmission of infection, and requirement of special storage conditions set to -80 0C may restrict its clinical use. In addition, variable biomechanical properties of amniotic membranes due to the variation of the components such as collagen, proteoglycan and elastin should be considered in the reconstruction of large and deep tissue defects in the clinical field [38].

In the present study, three different natural biomaterials were compared in terms of mechanical strength by nano-indentation, morphological characteristics by OCT and SEM, and degradation profile by in vitro degradation test. The nano-indentation test revealed significantly higher modulus of elasticity and hardness values in A-PRF group when compared to L-PRF and amniotic membrane groups. A study of Sam *et al*. [16] investigated mechanical properties of L-PRF membrane by a triboindenter (T1 950, Hysitron Nanotechnology, US) revealed that the modulus of elasticity and hardness values of L-PRF membrane were 0.35 GPa and 10.67 MPa, respectively. In consistence with the findings of this study[16] we demonstrated that the modulus of elasticity and hardness values of L-PRF membrane were 0.2 GPa and 11.44 MPa, respectively. Moreover, a recent study by Khorshidi *et al*. [15] evaluated the tensile strength, stiffness, and toughness of the early L-PRF membranes and compared it with PRGF/Endoret membranes which can be obtained by a multi-step process. They demonstrated that early L-

PRFmembranes had stronger mechanical properties and might have easier clinical handling as a scaffold in periodontal regenerative procedures.

One of the most importantfindings of the present work was that changing the centrifugation force leads to significantly different fiber diameter and configuration in micro-architecture of the PRF membranes. In SEM analysis, the major finding was that the fiber diameters of A-PRF membranes measured from the buffy coat area by Image-J analysis were remarkably higher than those of the L-PRF membranes. Additionally, larger diameter fibers formeda loose 3D architecture with higher and larger diameter pores. These findings could be related to the lower centrifugal speed (1,500 rpm) in A-PRF group. Because of the high centrifugal speed (2,700 rpm) in L-PRF group, more intense fibrin fragmentation occurs and small-diameter fibers are formed firmly which results in dense and thin structure with lower porosity. Furthermore, the OCT analysis revealed significantly higher thickness measurements for A-PRF membranes when compared to L-PRF membranes. It was thought that loosely cross-matched thicker fibers in A-PRF membranes configure thicker membranes. Additionally, the macroscopic sizes of A-PRF membranes were remarkably smaller than the L-PRF membranes. It can be speculated that higher centrifugal speed for L-PRF procedure followed by pressing in order to obtain L-PRF membranes results in thinner fibrin fibers condensed into a more stiff and dense structure with small pore sizes. In contrast, lower centrifugal speed for A-PRF procedure protects fibrin fibers from disruption and induces the formation of thicker membranes, which cannot be easily pressed. Consequently, we obtained small and thick A-PRF membranes and large and thin L-PRF membranes by altering the centrifugal force. The aforementioned study by Sam *et al*. [16] investigated the surface morphology and cell types of L-PRF membranes by SEM and revealed that dense clusters of platelets which are resulted from extensive aggregation and clotting were resting on a mature fibrin structure. In a previous study by Hatakeyama *et al* .[39], investigators evaluated the ultra-structural morphology of the L-PRF membranes and demonstrated a more highly condensed fibrin fiber network that was regularly arranged. In conclusion, the analysis of previous literature studies clearly shows that the buffy coat area of the L-PRF membrane is made up of thick fibers which make it a dense material.

To the best of our knowledge, no research to date has focused on the mechanical properties of PRF despite its clinical use for over a decade [40], and this is the first experimental study investigating the mechanical and morphological properties of A-PRF membrane. The results of the present work demonstrated that the A-PRF membranes exhibit higher modulus of elasticity and hardness values. It is our opinion that higher fibrin diameter within the A-PRF structure might provide enhanced mechanical strength to this material. As far as we know, this is the first attempt also to evaluate the thickness of the PRF membranes with OCT. We demonstrated that A-PRF membranes exhibit higher thickness measurements that can be linked with the increased fiber diameter within the structure. As a result, it can be interpreted that sparsely located thick fibrin strands within the structure of A-PRF lead to thick membrane formation and provide higher mechanical strength. Interestingly, an irregular demarcation line was observed approximately in the middle part of the all membranes in both L-PRF and A-PRF groups. We speculated that, it is due to the difference of light reflectivity originated from variation of cellular density in upper and lower part of the PRF membranes.

As an evaluation of cellular locations in L-PRF and A-PRF membranes, we visualized the distal part of both membranes (neighboring red blood cells) under the same magnifications. In contrast with the findings of the study reported by Hatakeyama *et al*. [39] platelets seemed to be located on the surface of the formed fibrin structure in the L-PRF group. Contrary to this, cells are observed among the fibrin structure in the A-PRF group. These findings were exhibited correlation with our SEM results mentioned above. Here, it can be speculated that the high centrifugal speed might cause dense and firmly adhered fibrin architecture that might force the cells to migrate to the surface of the membrane. On the other hand, cells were thought to be hold into the sparse fibrin structure with more inter-fibrous space in A-PRF group. Although the study of Hatakeyama *et al*. [39] showed that the platelet concentrates were embedded within the highly condensed fibrin fibers in L-PRF group, our results were found distinct from

their analysis by means of applied centrifugal force. Yet, it needs to be thoroughly determined whether the centrifugal force is the only important indicative for cell distribution within the fibrin architecture. It was suggested that further studies need to be performed to address this issue.

The present study basically demonstrates that the lower fiber disintegration together with lower cellular scattering were succeeded by means of lower centrifugal force in A-PRF group. Therefore, it was also refrained from cell death due to the cell scattering. Additionally, thicker fibers together with more inter-fibrous space in A-PRF group might provide the cellular integrity within the 3D structure. On the other hand, this study did not evaluate the cellular types of PRF membranes with histological analysis. However, the morphological results might be related to previous studies in terms of protected cellular sources within the fibrin meshesthat provide higher and longer GF release [9, 10]. Cellular distribution, penetration and allocation were compared between the L-PRF and A-PRF in a study presented by Ghanaati *et al*. [9] and it was shown that the penetration of T-lymphocytes, CD34+ stem cells, macrophages and neutrophilic granulocytes in A-PRF group was deeper than the L-PRF group resulting the higher GF intensity within the structure.

Another important finding of our study was that the change in degradation profile of the PRF membranes depending on the centrifugal force. The degradation of L-PRF membranes was approximately 10% lower than the A-PRF membranes at the end of 1-week period. These results could be explained by the formation of more close and robust fibrin strands consisting of small inter-fibrous space in L-PRF group that restrict the structure against PBS infiltration which led to fiber disintegration. However, the degradation rate of both membranes was comparable in terms of maintaining its initial physical features up to 5 days. Moreover, remaining membranes after 1 week in the surgical side might continue to provide mechanical and chemical support to the wound gap.

CONCLUSION

In conclusion, it would be more advantageous to apply more practical graft materials to achieve faster, innocuous and cost-effective treatments in regenerative medicine. A-PRF, a novel tissue engineered product, is a temporary matrix protein consisting GFs and stem cells that have a pivotal role in wound healing. A-PRF might be able to promote cell growth and migration in order to achieve acceleration of wound healing while promoting vascularization of tissue gap. Although this study mostly focused on the advantages of the A-PRF membranes, the applicability of this new concept has to be proven through clinical studies such as pterygium surgery, scleral regeneration, corneal repair and orbital socket reconstruction. On the other hand, this study was intended to guide surgeons in the selection of PRF membranes, since morphological and mechanical properties of the graft materials are discussed in detail here.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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A comparison of clinical, hepatic and immunological effects of three different parenteral lipid emulsions in children

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ABSTRACT

Objectives: Lipid emulsions are not only considered as a source of energy, but also as a regulatory substance for key metabolic functions in different diseases. The aim of the present study was to compare clinical, biochemical and immunological effects of olive oil, soybean oil and omega-3 fatty acid which are administrated to the children in parenteral nutrition products.

Methods: Patients who were admitted to pediatric intensive care unit and had parenteral nutrition for 7 days are included in the study. The patients were randomised into 3 groups based on the admission order, including 10 patients in each group. The children in Group O were fed by a lipid emulsion based on olive oil; the children in Group S were fed by a soybean based lipid emulsion and the children in group F were fed by soybean based lipid emulsion with omega-3 based solution. Analysis were made on the first and the seventh day. Laboratory parameters as well as hemogram, biochemical analysis, immunoglobulins, lymphocyte subgroup panel and cytokine levels were evaluated just before parenteral nutrition (day 0, basal value) and at day 7 of parenteral nutrition. Clinical monitoring parameters included body weight, and circumference of the mid-upper arm.

Results: Thirty children were enrolled into the study. Total cholesterol and triglyceride levels significantly increased in Group O whereas triglyceride level significantly increased in Group S. Hemogram, liver function tests, cytokine levels, lymphocyte sub-group distribution, immunoglobulin levels and total antioxidant capacity measurements were not different.

Conclusions: Three lipid emulsions which are used for parenteral nutrition treatment in intensive care unit patients are clinically and biochemically useful.

Keywords: intravenous lipid emulsions, parenteral nutrition, olive oil, fish oil, total antioxidant capacity, soybean oil

Lipid emulsions are safely used in parenteral nutrition (PN) products for pediatric intensive care unit (PICU) patients who do not have sufficient enteral nutrition [1]. Lipid emulsions are one of the main components of parenteral nutrition to give sufficient energy, essential fatty acids and fat soluble vitamins [1]. The issue that use of which lipid emulsion obtained from which herbal source for PN is still conflicting. Since the soybean oil (SO) based intravenous lipid emulsions (ILE) have excess linoleic acid (n-6 polyunsaturated fatty acid; PUFA), they thought to be responsible for increase in proinflammatory cytokines

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levels, decrease in immunological response, increase in free radicals and lipid peroxidation [2]. It was reported that olive oil (OO) based lipid emulsions would be more advantageous due to antioxidant protection with natural vitamin E and lower PUFA ingredient and the effect on immunological and inflammatory processes would be neutral due to dominant monounsaturated fatty acid (MUFA) content [2]. Use of fish oil (FO) was reported to provide antiinflammatory effects of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), omega-3 fatty acids [1]. As a result of these, lipid emulsions are not only considered as a source of energy, but also has a roleas a regulatory substance for key metabolic functions in different chronic and acute diseases. Possible effects of the lipids on such inflammatory processes are very important for the patients with sepsis whose excessive production of pro-inflammatory mediators contribute to mortality [3]. Furthermore, the risk of hypertriglyceridemia increases in intensive care unit patients under metabolic stress and this persists during intravenous lipid administration [4].

The aim of this study was to review and compare the effects of lipid emulsions based on soybean, olive oil and soybean with omega-3 fatty acid on hepatic, immunological and clinical processes.

METHODS

Study Design

This interventional study was admitted to the ethical board of Uludağ University School of Medicine. The ethics committee approval date and number is 2005-3. After approval of the ethics committee, 30 patients who were admitted to PICU within a year and had PN for 7 days were included in the study. Verbal and written consent was obtained from the patients. The patients were randomised into 3 groups based on the admission order, including 10 patients in each group. The children with type 1 diabetes, acid-base imbalance, metabolic disease, kidney failure and those who use anticoagulant, immunosuppressive agent, steroids and carnitin were excluded. The children in Group O were fed by a ILE based on OO (20%, ClinOleic; Baxter, Maurepas, France); the children in Group S were fed by a SO based ILE (20%, Ivelip; Clintec, Amilly, France) and the children in Group F were fed by SO based ILE (Ivelip) with omega-3 based 10% FO solution (Omegaven; Fresenius Kabi, Bad Hamburg, Germany). Characteristics and content of the lipid emulsions used for the patients are shown in Table 1.

Fluid, electrolytes, trace elements, protein and calorie support were provided with PN to all patients. Lipid emulsions were administrated in a dose of 1 to 3 g/kg/day to provide the protein-free energy between 20% to 50% in the nutrition fluids. Omega-3 fatty acid solution was administrated as 1 ml/kg/day.

Laboratory Tests

Laboratory parameters as well as hemogram, biochemical analysis, immunoglobulins, lymphocyte subgroup panel and cytokine levels were evaluated just before PN (day 0, basal value) and at day 7 of PN. Clinical monitoring parameters included body weight, and circumference of the mid-upper arm. Measurements were done by the same person with the same device. Biochemical analysis included blood glucose, total and direct biluribin, total protein, albumin, prealbumin, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), total cholesterol, low-density lipoprotein cholesterol (LDL), triglyceride (TG), high-density lipoprotein cholesterol (HDL), serum biliary acid level and they were detected through an Abbott Aeroset fully-automated biochemistry device. Total antioxidant capacity (TAC) levels were measured by calorimetric method (Aeroset, Abbott Diagnostics, IL, USA).

Hemogram, prothrombin time (PT), activated partial thromboplastin time (APTT) and international normalized ratio (INR) were analyzed. Immunoglobulins and C-reactive protein (CRP) were analyzed in Dade Behring BN II device through nephelometric method. Lymphocyte subgroups were detected in Beckman Coulter device through flow-cytometry method. Cytokines were analyzed through ELISA (Enzyme Linked Immuno Sorbent Assay) method. BioSource International Elisa kit was used for cytokine analysis. The values obtained in a Tecan brand optic density device at 450 nm wavelength were reevaluated on a graphic drawn according to optic density levels of standard solutions and cytokine levels (pg/ml) were found.

Statistical Analysis

The statistical analysis were run through SPSS-23 (Statistical Package for Social Science) computer software.Kruskal Wallis test was used to compare variables in more than two groups without normal distribution, and the groups with significant differences in median values were exposed to binary comparison through Mann Whitney U test. Chi-Square test was used to evaluate differences between categorical variables. Median values of the parameters measured before and after PN were compared with Wilcoxon test. Percentage change of the patients in all groups at days 0 and 7 was calculated by the formula [(value at day 7 - value at day 0) / value at day 0]. The comparisons with a p value <0.05 were accepted as statistically significant. Bonferroni's correction was done for p value in multiple comparisons.

RESULTS

There was no statistically significant difference

Table 1. Composition of fat emulsions used in the study [19, 21, 24]

Contents of products	Intravenous fat emulsion products		
	Fish oil-based	Soyabean oil-based	Olive oil-based
Composition (g/100 mL)			
Soyabean oil	-	20	4
Olive oil	-	-	16
EYPL	1.2	1.2	1.2
Glycerol	2.5	2.5	2.25
Sodium oleate	-	0.03	0.03
Fish oil	10	-	-
Fatty acids content (mol %)			
Palmitic acid	2.5-10	11	14.5
Stearic acid	0.5-2	4	3
Oleic acid	6-13	20	60
Linoleic acid	4.4	52	18.5
α-Linolenic acid	1.2	8.5	2
Arachidonic acid	1-4	0.1	0.5
EPA	12.5-28.2	-	-
DHA	14.4-30.9	0.34	0.23
PUFA	57.5	60.5	20.5
MUFA	23	22.3	65
ω-6 PUFA	3.4	56	18
ω-3 PUFA	54	5.6	2
ω-6 FA / ω-3 FA ratio	1:8	7:1	9:1
Vitamin E			
α-tocopherol µg/mL	230	12.8	32
γ-tocopherol µg/mL	0.15	77	13.9
δ-tocopherol µg/mL	0.01	51	10.5
α-tocopherol/ PUFA mg/g	2	0.2	0.75

EYPL = egg yolk phospholipids, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, PUFA = polyunsaturated fatty acid, MUFA = monounsaturated fatty acid

detected in age, gender, weight and diagnosis distributions of 30 patients enrolled into the study (Table 2).

When measurements of all groups at day 0 were compared, no statistically significant difference was detected except APTT. Initial APTT levels of all groups were within normal limits. The APTT value in Group S at day 0 was found significantly lower than Group F [median = 22.5 sn(min = 18, max = 51) vs median = 38 (min = 22, max = 60), $p = 0.023$, respectively].

Within groups O, S and F, levels of the parameters measured before and after PN were compared statistically. Body weight was detected statistically and significantly higher in day 7 when compared with day 0 ($p = 0.026$, $p = 0.018$, $p = 0.012$, respectively).
Within the groups O, S, and F, prealbumin levels at day 7 were found to be significantly higher in comparison to the levels of day 0 ($p = 0.005$, $p = 0.008$, $p = 0.005$, respectively). Levels of ALP, TG and total cholesterol was detected significantly higher in Group O at day 7 when compared with the initial level ($p = 0.005$, $p = 0.037$, $p = 0.025$, respectively). In Group S, a statistically significant increase was detected in TG and APTT levels at day 7 when compared with the levels at day 0 ($p = 0.005$, $p = 0.018$, respectively). There was no significant difference in glucose, AST, ALT, serum biliary acids, PT, INR, HDL, LDL, total bilirubin, direct bilirubin, total protein, albumin, TAC between days 0 and 7. The results were shown in Table 3.

There was no significant difference in CRP levels between the groups at days 0 and 7 ($p = 0.477$, $p = 0.176$, respectively). None of the patients had positive blood culture during PN period. The CRP levels of day 7 were detected significantly lower than day 0 in all groups ($p = 0.046$ in Group O, $p = 0.036$ in Group S, $p = 0.033$ in Group F) (Table 3).

There was no significant difference in hemogram levels, immunoglobulin levels (IgG, IgM, IgA), lymphocyte subgroup distribution (CD4, CD8, CD19), cytokine levels (IL-1 beta, IL-12, IL-2, TNF-alpha) between day 0 and day 7. Hemogram, immunological values and statistical evaluation are presented in Table 4.

Day 7 measurements showed significant increase in APTT levels ($p = 0.013$, $p = 0.006$, respectively) and decrease in total bilirubin levels ($p = 0.029$, $p = 0.01$, respectively) in Group S compared to Group O and Group F. There was no significantly changes between the groups in clinical presentation and laboratory analysis between day 0 and day 7.

DISCUSSION

PN contributes positively to the growth and causes some positive or negative changes in biochemical, immunological, hematological and inflammatory parameters depending on lipid solutions. The present study reviewed effects of 3 different lipid solutions on clinical findings and laboratory tests prospectively.

In the literature review, it is reported that body weight monitoring in critically ill children is important to evaluate nutritional status [5]. Furthermore, similar to SO and OO based ILEs, short term PN procedure

Table 2. Demographic features of patients

Gender, age and diagnosis	Group O (n =10)	Group S (n = 10)	Group F (n = 10)	*p* value
Male/female, n	6/4	6/4	5/5	0.87
Age (months), median (minimum-maximum)	6 (2.5-132)	38.5 (2-144)	8.5 (2.5-96)	0.492
Weight (kg), median (minimum-maximum)	4.75 (2.5-43.5)	13.5 (2.3-38)	5.78 (3.3-19)	0.356
Sepsis, n (%)	8 (80)	8 (80)	8 (80)	0.99
Coma, n (%)	1 (10)	1 (10)	1 (10)	NA
Malabsorbtion, n (%)	1 (10)	1 (10)	1 (10)	NA

Table 3. Clinic and hepatic measurements on the day 0 and 7

	Group O			Group S			Group F		
	Day 0	Day 7	*p* value	Day 0	Day 7	*p* value	Day 0	Day 7	*p* value
Weight (kg)	4.75 (2.5-43.5)	4.82 (2.6-43.5)	**0.026**	13.5 (2.3-38)	13.6 (2.7-38)	**0.018**	5.78 (3.3-19)	5.95 (3.5-19)	**0.012**
MUAC (cm)	12.75 (10.1-25)	12.85 (10.1-25)	0.083	18.4 (10.8-28)	18.4 (11-28)	0.059	14.1 (11.8-23.4)	14.2 (11.8-23.4)	0.052
Glucose (mg/dl)	85.5 (64-110)	84.5 (61-108)	0.959	95 (64-122)	88 (66-105)	0.721	87 (60-110)	93.5 (72-120)	0.153
AST (UI/L)	41 (15-232)	40 (12-276)	0.386	54 (11-279)	56 (17-372)	0.26	68 (14-571)	53.5 (16-224)	0.646
ALT (UI/L)	23.5 (10-141)	20.5 (9-354)	0.953	31.5 (10-255)	29 (10-124)	0.169	19.5 (10-378)	23.5 (9-241)	0.202
ALP (UI/L)	135.5 (57-954)	142 (83-1051)	**0.005**	161.5 (97-431)	176.5 (103-372)	0.799	239 (51-783)	257 (72-652)	0.475
GGT (UI/L)	55 (24-200)	58 (24-170)	0.959	51.5 (15-545)	93 (12-345)	0.508	54 (14-240)	39 (14-238)	0.859
T. bilirubin (mg/dl)	0.5 (0.2-16)	0.6 (0.2-18)	0.440	0.5 (0.2-25)	0.3 (0.2-20)	0.06	0.3 (0.2-18.6)	0.4 (0.2-18.8)	0.301
U. bilirubin (mg/dl)	0.3 (0.1-7.6)	0.35 (0.1-7)	0.752	0.25 (0.1-10)	0.15 (0.1-10.8)	0.063	0.2 (0.1-11.8)	0.2 (0.1-11.6)	0.197
Bile acid (umol/L)	9.8 (0.1-109)	6.6 (0.2-31)	0.575	5.8 (0.1-120)	7.6 (0.7-195)	0.878	4.3 (0.1-18.6)	4.1 (0.1-6)	0.878
PT	14.1 (11.5-92.8)	14.5 (12-45.6)	0.813	13.2 (11-76)	13.5 (12-71)	0.610	15 (12-43)	14.2 (12-39)	0.074
APTT (s)	30.2 (21-103)	31 (21-59)	0.359	22.5 (18-51)	27 (20-48)	**0.018**	38 (22-60)	32 (25-50)	0.114
INR	1.2 (0.9-10.1)	1.25 (1-4.3)	0.602	1.1 (0.9-6.5)	1.1 (1-6.1)	0.957	1.3 (1.1-3.5)	1.2 (1-3.1)	0.071
Total protein (g/dl)	5.4 (4.2-6.3)	5.8 (4.4-7.3)	0.066	6.1 (3.4-8.2)	6.5 (3.6-7.4)	0.359	5.8 (4-7.4)	6.3 (4.5-7.6)	0.413
Albumin (g/dl)	3.3 (2.8-3.9)	3.6 (3-4.1)	0.073	3.9 (2.5-4.9)	3.9 (2.9-4.7)	0.765	3.7 (2.2-4.5)	3.8 (2.8-4.7)	0.258
Prealbumin (g/dl)	0.14 (0.07-0.4)	0.18 (0.08-0.5)	**0.005**	0.13 (0.04-0.32)	0.18 (0.08-0.38)	**0.008**	0.13 (0.02-0.22)	0.21 (0.1-0.26)	**0.005**
Cholesterol (mg/dl)	136 (71-172)	152 (86-198)	**0.025**	150 (116-307)	143 (110-304)	0.386	136 (46-217)	133 (82-187)	0.445
Triglyceride (mg/dl)	124 (52-346)	153 (103-305)	**0.037**	116 (29-143)	154 (98-209)	**0.005**	118 (47-205)	125 (73-355)	0.575
HDL (mg/dl)	30 (2-57)	36 (8-42)	0.281	29 (10-49)	35 (10-41)	0.444	28 (10-48)	31 (15-43)	0.259
LDL (mg/dl)	74 (12-113)	82 (18-156)	0.286	85 (73-231)	81 (44-239)	0.059	83 (26-133)	78 (25-136)	0.878
TAC (mmol/L)	1.15 (0.92-1.67)	1.12 (0.87-1.63)	0.721	1.08 (0.86-1.55)	1 (0.52-2.4)	0.262	1.1 (0.74-1.29)	1 (0.92-1.52)	0.203
CRP (mg/dl)	3.7 (0.6-12)	1.5 (0.2-6.2)	**0.046**	7.2 (0.9-14)	4.6 (0.37-6.6)	**0.036**	3.77 (0.5-12)	2.8 (0.2-5.6)	**0.033**

Data are shown as median (minimum-maximum). MUAC = mid-upper-arm-circumference, AST = aspartate transaminase, ALT = alanine transaminase, ALP = alkaline phosphatase, GGT = gamma glutamyl transferase (GGT), T. Bilirubin = total bilirubin, U. Bilirubin = unconjugated bilirubin, PT= prothrombin time, APTT = activated partial thromboplastin time, INR =international normalized ratio, HDL = high-density lipoprotein cholesterol, LDL = low-density lipoprotein cholesterol, TAC = Total antioxidant capacity, CRP = C-reactive protein

Table 4. **Hemogram, immunologic and cytokine measurements on the day 0 and 7**

	Group O			Group S			Group F		
	Day 0	**Day 7**	***p* value**	**Day 0**	**Day 7**	***p* value**	**Day 0**	**Day 7**	***p* value**
Hb (g/dl)	10.6 (8.9-12)	10.4 (7-11.8)	0.138	11.1 (8.4-14)	10.5 (8-13.8)	0.386	10.4 (8.1-12)	10.1 (7.1-13.6)	0.594
Platelet (K/uL)	219500 (22000-559000)	274000 (130000-495000)	0.799	236500 (22000-450000)	220000 (40000-615000)	0.721	354000 (83000-658000)	344000 (122000-670000)	0.721
WBC (K/uL)	10350 (5700-20700)	13700 (4900-26000)	0575	9700 (4920-14500)	10600 (5030-25100)	0.760	11750 (5100-19000)	12560 (4000-19800)	0.575
IgG (mg/dl)	880 (263-1420)	911 (302-1750)	0.333	926 (424-2520)	944 (432-2310)	0.508	813 (346-1550)	840 (396-1470)	0.721
IgA (mg/dl)	62 (14-365)	74 (16-214)	0.799	59 (24-443)	73 (25-341)	0.333	64 (12-134)	60 (16-112)	0.285
IgM (mg/dl)	120 (36-236)	105 (33-263)	0.76	128 (37-266)	124 (39-322)	0.767	101 (41-192)	99 (46-201)	0.721
CD4	39 (29-57)	37 (20-52)	0.203	34 (29-51)	38 (30-48)	0.959	41.6 (33-49)	39 (33-52)	0.505
CD8	24 (19-36)	22 (19-31)	0.074	21 (13-28)	20.5 (12-29)	0.721	26.7 (19-32)	26 (17.6-34)	0.504
CD19	21 (7.6-34)	21 (8.7-39)	0.241	26 (9-46)	25 (11-39)	0.093	22 (16-34)	22 (14-29)	0.609
IL-1beta (pg/ml)	0 (0-14.4)	0 (0-0.53)	0.18	0 (0-1.2)	0 (0-5.73)	0.317	0 (0-1.23)	0 (0-107)	0.414
IL-2 (pg/ml) (pg/ml)	0 (0-15.8)	0 (0-0)	0.18	0 (0-2.3)	0 (0-0)	0.317	0 (0-227)	0 (0-16.3)	0.50
IL-12 (pg/ml) (pg/ml)	51.5 (0-442)	54 (0-293)	0.721	44 (0-344)	67 (0-258)	0.678	135 (0-384)	94 (17-348)	0.508
TNF-α (pg/ml) (pg/ml)	0 (0-193)	3.8 (0-150)	0.735	0 (0-186)	7.5 (0-192)	0.249	14.5 (0-220)	1.4 (0-67)	0.093

Data are shown as median (minimum-maximum). P values were obtained by Wilcoxon, p values < 0.05 were accepted as statistically significant. Hb = hemoglobin, WBC = white blood cell, Ig = immunoglobulin, CD = cluster of differentiation, IL = interleukin, TNF = tumor necrosis factor

was reported to have no significant effect on upper arm circumference and body weight [6]. Three lipid emulsions were found effective in terms of weight gain and no significant difference was found between those emulsions in the present study.

Goulet *et al*. [7] reported that there was not any significant change in leukocyte, platelet and hemoglobin levels in some children who had long term soybean oil and olive oil based lipid emlusions. In this study, as the other studies, no effect of OO, SO and omega-3 fatty acid was observed on hemogram analysis.

Hyperglycemia or hipoglycemia was not detected in any of the patient. This may indicatethe protective effect of lipid emulsion for hyperglycemia [8]. Such effect was observed in all ILEs used in three groups in the present study. Furthermore, addition of lipid to PN protects from hyperinsulinism and hepatic steatosis due to excessive glucose intake [9]. There was no significant change in AST, ALT, GGT, direct bilirubin levels in any of the groups.The studies showed positive effect of FO procedure to reduce direct bilirubin level [1]. There are studies reporting PT and APTT prolongation by use of SO ILE [10]. In the present study, a significant increase of APTT levels within normal limits was observed in the group where SO based lipid emulsion was used at day 7. The patients receiving SO based ILE should be closely monitored for APTT levels. An increase in ALP level within normal limits was observed in those receiving OO based lipid emulsion. For this increase, there was no difference between the other groups. Significant disruption was not detected in hepatic functions during short term use of SO based ILE, OO based ILE and omega-3 fatty acid solutions.

There is a study reporting that excess use of omega-6 PUFA caused a decrease in bile flow [11]. In the present study, an increase was detected in serum bile acids in the group using soybean based ILE with PUFA content whereas a decrease was observed in the group where OO based lipid emulsion was used. However, such changes were not statistically significant.

Despite the increase in hepatic cholesterol production under stress, plasma cholesterol concentration decreased with both HDL and LDL [12]. A significant increase in triglyceride levels within normal limits was detected in those who receive OO and SO in the present study. We also detected a significant increase in total cholesterol levelsin the group receiving OO. These increased cholesterol levels were in normal limits.The condition that TG level exceeds 400 mg/dl requiring restriction of ILE administration was not detected in the patients of our study [13]. The HDL levels in our study increased in those receiving olive oil and omega-3 fatty acid and tended to decrease less in those receiving soybean oil. Goren *et al*. [14] reported a decrease in TG and total cholesterol levels by use of omega-3 fatty acid in the children and an increase in HDL cholesterol. Calkins *et al*. [15] detected an increase in TG level in the patients receiving SO and a significant decrease in TG level in those who receive FO in addition to their PN. Park *et al*. [16] reported an increase in lipoprotein lipase and a decrease in TG levels by use of omega-3 fatty acid. Similar to the aforesaid studies, we did not detect any significant increase in TG and total cholesterol levels in the group where omega-3 fat acid was added; HDL cholesterol level increase was not significant.

Leite *et al*. [17] and Delgado Artur Figueiredo *et al*. [18] reported an increase in prealbumin level by short term parenteral nutrition in the children with critical disease. Prealbumin levels significantly increased in all groups at day 7. Such increase in prealbumin level was not different between the groups.

In a previous study analysing antioxidant alpha tocopherol content in ILE detected the highest level by FO and then OO; the lowest level was detected in SO based lipid emulsions [19]. Pironi *et al*. [20] reported that lipid peroxidation increases in PN by use of ILE with high PUFA content. Goulet *et al*. [7] detected higher lipid peroxidation product in SO based lipid emulsions than OO based ILE in their study. Antebi *et al*. [21] reported that addition of omega-3 into the PN create higher antioxidant level when compared with use of soybean oil only. In the present study, antioxidant capacity levels were preserved in OO receivers, decreased in SO receivers and increased in the group where omega-3 was added. However, such changes were not significant. This may depend on natural antioxidant characteristics of OO and omega 3.

Dahlstrom *et al*. [10] reported no change in immunoglobulin levels in their study on the children who received SO based lipid emulsion. In our study, there was not any significant change in IgG, Ig A, Ig M levels in any of the groups before PN and no change was detected at day 7.

In previous study where SO rich in omega-6 PUFA and effects of omega-3 were compared, n-3 PUFA products were reported to suppress production of proinflammatory cytokines and use of SO based ILE increase production of such cytokines [3]. OO based ILE is reported to be immunologically neutral [22]. Furthermore, TNF-alpha and IL-1 beta production are reported to decrease by both soybean oil and olive oil [23, 24]. Granato *et al*. [24]suggested that OO and SO based lipid emulsions cause a tendency of decrease in IL-1 beta and TNF-alpha levels. The previous studies reported that blood level of IL-12 is significantly lower by use of omega-3 fatty acid when compared with OO and n6-PUFA receivers [25]. We observed that blood levels of cytokines did not change by short term use of intravenous lipid emulsions.

It was reported that all PUFAs may suppress lymphocyte proliferation [23, 26, 27]. Among these, DHA and EPA were detected as a strong inhibitor of lymphocyte proliferation [26, 27]. It is also reported that lymphocyte proliferation suppressed with SO is not affected by OO based lipid emulsion [23, 24, 27]. It was reported in a study associated with use of fish oil that fish oil does not affect lymphocyte subgroup distribution and lymphocyte proliferation [28]. It is also reported that omega-3 PUFAs may reduce T cell ratio [29]. Such different results reported for omega-3 fatty acids may depend on the dosage. The patient groups of the present study did not present a significant change in lymphocyte subgroup distribution by PN. OO and SO based lipid emulsion and omega-3 fat acid solution did not affect lymphocyte subgroup distribution in short term use.

The balance between omega-6 and omega-3 fatty acids seem to be important for cytokine production. Sole use of ILE rich in omega-6 PUFA should be avoided to prevent immune suppression. Different possible effects of lipid emulsions on immunomodulatory and inflammatory cytokines may be used to treat the underlying disease. Until such effects are clearly understood, administration of SO, OO and FO based lipid emulsions may be more physiological rather than providing ILE from a single source in PICU patients.There are no definite suggestions on the best form or start time of parenteral nutrition in critically ill children [30].

Limitations

The limitations of this study are that the number of patients is small and the follow-up period is short. The lack of this study is due to many factors that affect biochemical test results and cytokine levels in patients.

CONCLUSION

The use of OO, SO and FO based lipid emulsions in pediatric intensive care unit patients with short term parenteral nutrition is clinically and biochemically useful. FO and OO based lipid emulsions may provide more contribution to protection of antioxidant capacity in critically ill children. These three lipid emulsions have no effect on immune system in pediatric intensive care unit patients during short term use. OO, SO and FO based lipid emulsions may be used safely in critically ill patients. More randomized controlled studies with larger patient series are needed to prevent deficiency of essential fatty acid and to clarify immunological effects of ILEs as a main energy source.

Conflict of interest

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Evaluation of tear function tests and corneal thickness in patients with meibomian gland dysfunction

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ABSTRACT

Objectives: To investigate whether central corneal thickness (CCT) and tear function test differ from healthy controls in the ones suffering meibomian gland dysfunction (MGD).

Methods: This prospective study was carried out with 99 individuals in total (50 patients MGD, 49 healthy individuals). Schirmer-1, tear break-up time (TBUT), the Ocular Surface Disease Index (OSDI) questionnaire and CCT measurmentwas administered to all patients.

Results: The average Schirmer 1 measurements were 16.6 ± 3.3 mm for right eyes, 16.4 ± 3.6 mm for left eyes in the MGD group and 17.1 ± 3.7 mm for right eyes, 17.0 ± 4.4 mm for left eyes in the control group. The mean TBUT values were 10.1 ± 3.6 seconds for right eyes, 10.2 ± 3.4 seconds for left eyes in the MGD group and 14.7±3.7 seconds for right eyes, 15.8 ± 4.1 seconds for left eyes in the control group (p = 0.001). The mean OSDI score values were 40.3 ± 23.7 in the MGD group and 19.4 ± 8.7 in the control group (p = 0.001). The average CCT in the MGD group was 539.4 ± 30.0 µm and 539.7 ± 33.0 µm (right and left, respectively). The average CCT in the control group was 551.6 ± 32.8 µm and 550.7 ± 32.2 µm (right and left, respectively). The mean CCT measurements in the MGD group were not statistically significant compared to the healthy control group (p = 0.059, and p = 0.097, right and left, respectively).

Conclusions: The Schirmer test and CCT measurements are not significantly different in patients with MGD compared to healthy control subjects.

Keywords: Meibomian gland dysfunction, Schirmer-1 test, tear break-up time, ocular surface disease index, central corneal thickness

Meibomian glands are known as a kind of modified sebaceous glands which are positioned in parallel rows inside the tarsi of the eyelids, amounting 30-40 nodes in the upper lid and 20-30 nodes in the lower lid [1], and help forming the lipid layer of the tear film to decrease aqueous tear evaporation by discharging lipids to the surface of the eye [2]. Meibomian gland dysfunction (MGD) is a chronic, common anomaly of the meibomian glands, described by terminal duct obstruction and/or qualitative/quantitative alterations in the glandular secretion. It may result in change of the tear film, indications of eye irritation, clinically significant inflammation, and ocular surface disease. The role of inflammation in the etiology of MGD is controversial and uncertain [3].

This leads to a decreased distribution of meibomian lipid to the lid margin and is connected with a changing level of lid inflammation [4]. MGD is a widespread eyelid disorder with a broad frequency of 39-50% in the US population, incidentally increasing

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with age [5].

MGD is usually clinically categorized with posterior Blepharitis; however, the terms posterior blepharitis and MGD can not be used interchangeably [3], since posterior blepharitis, as a definiton, involves significant inflammation and no significant inflammation is seen in all variations of obstructive MGD [6].

Meibomian glands, through holes located just in front of the mucocutaneous junction as a clear liquid, terminologically meibomian oil or meibum [7], synthesize and secrete a mixture of lipid. This makes the release and spread of meibum to the tear film lipid layer easier while the blink strokes upwards over the aqueous subphase. The role of tear film lipid layer is crucial in retarding the evaporation of tear fluid from the preocular tear film, so as to preventing dryness. At the same time, it helps tear film stability to improve by lowering outer tension and avoiding tear film from being contaminated by sebum [8]. Tear film instability and evaporative dry eye problems may occur due to the disorders in meibomian gland function which affect meibomian quality and quantity [9].

Chronic irritation of the ocular surface as a result of dryness is followed by immune activation and results in a decrease in film thickness, which is normally ranging from 3 to 40 μm [10, 11].

Assessment of central corneal thickness (CCT) informs about corneal health. CCT assessment has a clinical and practical precaution in practices such as refractive surgery, contact lens use and ocular diseases such as glaucoma and keratoconus [12, 13]. Karadayi *et al*. [14] suggested using CCT assessment as a diagnosis and follow-up criteria in dry eye patients. The Meibomian gland dysfunction is one of the most common factor that cause increase of tear film evaporation. Mathers [15] demonstrated that the loss of meibomian glands decreases production of tear, and increases tear evaporation rate and tear osmolarity. It has been reported that removal of lipid layer of the tear film increases 4-fold of the evaporation of the aqueous tear [16]. In normal conditions, the aqueous tear layer film is isotonic or mildly hypotonic. Increase of tear film osmolarity and inflammation of the ocular surface are likely to be the cause of thinning of the corneal thickness [17]. Hence, in all types of dry eye, including tear deficiency and incresed evaporation, may be associated with a decreased corneal thickness.

Given the incidence of dry eye together with MGD and the possible effect of dry eye on corneal thickness measurements, we conducted a study to determine if CCT and tear function tests differed in the ones suffering from MGD compared to the ones in healthy controls.

METHODS

The prospective study groups consisted of 198 eyes:100 eyes of 50 patients diagnosed with MGD aged between 18 and 65 years and 98 eyes of 49 healthy individuals (control group) aged between 18 and 60 years without any ophthalmic or systemic pathology following their application to our outpatient for routine examination. Written consent was obtained from all patients before the study, conducted according to ethical committee guidelines and accordingly to the principals of the Helsinki Declaration.

Patients with a history of ophthalmological or systemic pathology or who used topical medication during the study were excluded from the study. For each participant, a full ophthalmological examination including refraction, external eye examination, slitlamp examination and evaluation of posterior segments was performed.

In addition to subjective complaints in the diagnosis of MGD, biomicroscopic examination depended on the existence of all of the following: frothy tear accumulation on lower eyelid edge; shortening of tear break up time; redness in tarsal and bulbar conjunctiva; papillary reaction on the tarsus; irregularity at the eyelid margin; telangiectatic vessel formation; and inclusion in the mouth of the meibomian glands (plugging) [9].

Along with the common check-up, first tear break-up time, then Schirmer-1 was measured. The tear break-up time (TBUT) was evaluated by applying one drop of 2% sodium fluorescein dye into the eye without anesthesia. After the patient had been blinded three to four times, the tear film was observed using a biomicroscope with a broad light blue cobalt filter. The time to the first break in the tear film complex after the last blink was measured. The Schirmer 1 test was detected by putting aSchirmer strip into the temporal inferior fornices. Topical anesthesia was not preferred. The outcomes were obtained as the number of millimeters of wetting at five minutes.

Table 1. Comparison of CCT and dry eye tests between groups

Parameters	MGD group		Control group		*p value*	
	R	L	R	L	R	L
CCT (μm)	539.4 ± 30.0	539.7 ± 33.0	551.6 ± 32.8	550.7 ± 32.2	0.059	0.097
Schirmer 1 (mm)	16.6 ± 3.3	16.4 ± 3.6	17.1 ± 3.7	17.0 ± 4.4	**0.001**	**0.001**
BUT (sec)	10.1 ± 3.6	10.2 ± 3.4	14.7 ± 3.7	15.8 ± 4.1	**0.001**	**0.001**
OSDI	40.3 ± 23.7		19.4 ± 8.7		**0.001**	

Data are shown as mean ± standard deviation. CCT = central corneal thickness, TBUT = the tear break up time, OSDI = Ocular Surface Disease Index, R = right eye, L = left eye

The Ocular Surface Disease Index (OSDI) questionnaire was administered to all patients. The OSDI questionnaire, consisting of 12 questions, aims to question the patient's complaints about the dry eye in the last two weeks, and scales the severity of the disease by scoring the effects of these complaints on daily activities, environmental causes, duration of the complaints and the severity, along with the complaints of the patient [18].

Finally, the CCT was measured using an ultrasonic pachymetry (Echoscan US 500 Nidek Co. Ltd, Aichi, Japan). Topical proparaine hydrochloride was used as anesthesia in the cornea. Measurements were taken as the point of the probe was targeted to the center of the pupil and was vertical to the cornea while the subject was looking at a planned spot. Three consecutive measurements were taken at the center of the cornea of each eye and the average value was measured. A 20-minute period was applied between all measurements.

Statistical Analysis

The measurements were described as the mean and standard deviation. The normality of the data was assessed using the Shapiro-Wilk test and the difference between the two groups using Student t-tests. Pearson correlation test was performed for the correlation between variables. A value of $p < 0.05$ was considered statistically significant.

RESULTS

While mean age was 42.0 ± 13.8 years in the MGD group, mean age level was found as 41.2 ± 11.2 years among the controls (p = 0.642). The best corrected visual acuity of all patients in both groups was 1.00 according to LogMAR. While the female to male ratio was 1.7/1 in the MGD group, the ratio was found as 2.5/1 the controls. There was no statistically significant difference between groups with respect to sex comparison (p = 0.429). The mean Schirmer 1 values were 16.6 ± 3.3 mm for right eyes, 16.4 ± 3.6 mm for left eyes in the MGD group and 17.1 ± 3.7 mm for right eyes, 17.0 ± 4.4 mm for left eyes in the control group (p = 0.894, and p = 0.365, right and left, respectively). The mean TBUT values were 10.1 ± 3.6 seconds for right eyes, 10.2 ± 3.4 second for left eyes in the MGD group and 14.7 ± 3.7 seconds for right eyes, 15.8 ± 4.1 seconds for left eyes in the control

Table 2. Correlation coefficients between CCT, Schirmer 1, TBUT and OSDI

		Schirmer 1		TBUT		OSDI	
		R	L	R	L	R	L
CCT	***p value***	0.411	0.273	0.515	0.692	0.314	0.223
	r	-0.084	-0.111	-0.066	-0.040	0.102	0.121

Data are shown as mean ± standard deviation. CCT = central corneal thickness, TBUT = the tear break up time, OSDI = Ocular Surface Disease Index, R = right eye, L = left eye

group (p = 0.001). The mean OSDI score values were 40.3 ± 23.7 in the MGD group and 19.4 ± 8.7 in the control group (p = 0.001).

The average CCT in the MGD group was 539.4 ± 30.0 µm and 539.7 ± 33.0 µm (right and left, respectively). The average CCT in the control group was 551.6 ± 32.8 µm and 550.7 ± 32.2 µm (right and left, respectively). The mean CCT measurements in the MGD group were not statistically significant compared to the healthy control group (p = 0.059, and p = 0.097, right and left, respectively) (Table 1).
There was no statistically significant correlation between CCT, Schirmer 1, TBUT and OSDI tests in the eyes MGD (p > 0.05) (Table 2).

DISCUSSION

MGD is one of the most frequently encountered disorders in ophthalmic practice. Bron and Tiffany [4] reported that 38.9% of the patients admitted to the routine ophthalmic examination suffer from MGD. Meibomian gland dysfunction is a chronic disease that results in decreased lipid secretion to the ocular surface due to occlusion of the meibomius gland. People with MGD usually suffer from notable obstacles, such as burning, itching, irritation, and photophobia. They might have a cloudy vision, contact lens intolerance, ocular surface damage and ocular infection. The use of contact lenses can aggravate symptoms of MGD even if the contact lens intolerance does not ocur [19, 20].

This study shows that the mean CCT and Schirmer 1 test was not significantly different from normal eyes in patients with MGD with dry eyes. However, according to the control group, it was found that there was thinning of the central corneal thickness more than 10 microns. There was a statistically significant difference between the control group and the MGD group in terms of the mean values of TBUT and OSDI scores.

Akyol-Salman *et al*. [21] assessed CCT values in patients with MGD and in healthy individuals. They also did not find significant differences between the groups [21]. Ultrasonic pachymeter (UP) devices are still accepted as the gold standard technique and commonly used because of the easiness of its handling, portability and rapid measurement time [22]. In our study, an ultrasonic pachymeter was used for CCT measurement like Akyol-Salman *et al*. [21]. Yuksel *et al*. [23] found a statistically significant decline in CCT as a result of MGD in the 6-month treatment of patients treated with systemic isotretinoin for acne vulgaris. Meibomian glands discharge lipids into the tear film and create a superficial lipid layer stabilizing it. MGD is identified by terminal duct blockage or glandular secretion changes and immediately afterwards, tear evaporates, tear osmolarity increases, aqueous tear volume declines, and inflammation cycle begins [4, 24]. Additionally, apoptosis in the ocular surface tissues, including the central and peripheral corneal epithelial, was shown in an experimental dry-eye study in mice[10]. Sanchis-Gimeno *et al*. [25] and Liu and Pflugfelder [27] found a statistically significant decrease in corneal thickness values in dry eyes. Pole and Batzer [11] observed in their studies that there was a reduced corneal thickness in dry eyes, yet dry and normal eyes did not significantly differ from each other. Likewise, Van Bijsterveld and Baardman [27] obtained compared mean CCT values in patients with keratoconjunctivitis sicca and healthy control subjects. We can say that the differences between the CCT measurement values in the studies in which the dry eye and the CCT relationship in the literature are studied are derived from the measurement methods. However, we believe that these measurements should be repeated in a wider population with newdevices developed to measure CCT.

In our study, all patients with MGD had various symptoms and specific lid changes. Despite TBUT shortening due to dry eye as a result of excessive evaporation, the Schirmer test results were normal due to the absence of aqueous insufficiency. Demircan *et al*. [28] found that Schirmer test results were similar to healthy subjects and that TBUT was significantly shorter in the study of patients with chronic blepharitis. Sayman Muslubaş *et al*. [29] showed increased score in OSDI questionnaire and shortening in TBUT without any decrease in the Schirmer test measurements in their studies comparing healthy individuals with MGD patients. Taşkıran Cömez *et al*. [18] found, in a study comparing 40 patients with dry eye syndrome and 39 healthy subjects, that the OSDI score average was statistically significantly higher in the patients with dry eye syndrome. Similarly, in our

study, OSDI score was found to be higher in the MGD group which caused dry eyes due to evaporation increase. We also examined whether there was a correlation between CCT and Schirmer 1, TBUT, OSDI score in our study, but no significant correlation was found between these parameters.

The weakness of our study is not being able to measure tear osmolarities and apply non-contact meibography. The strength of our study is that more patients are included in the study than the number of patients in the literature. In addition, the correlation between CCT and Schirmer 1, TBUT and OSDI score, which had not been observed before, has been examined in our study.

CONCLUSION

As a result, dry eye syndrome may occur depending on the MGD, as is the cause of the MGD. High score in OSDI questionnaire, the fact that Schirmer test measurements are not different from healthy individuals and shortening of TBUT in MGD support the diagnosis. For this reason, using TBUT and OSDI questionnaires will be appropriate to determine the presence of a dry eye in such cases. In addition, the CCT analyzes are not significantly different between patients with MGD and healthy control subjects.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Evaluation of myocardial functions in pregnant women with speckle-tracking echocardiography

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ABSTRACT

Objectives: The speckle-tracking technique calculates the regional rate, the strain and the strain rate from two-dimensional gray-scale visualizations. The aim of this study was to evaluate with speckle-tracking echocardiography the effects of the first, second and third trimesters of pregnancy on cardiac functions.

Methods: One hundred five voluntary pregnant and 35 healthy women of reproductive age were included in the study. For echocardiographic evaluations, the 111 highest-quality visualizations were chosen: 24 cases in the first trimester, 31 cases in the second trimester, 32 cases in the third trimester and 24 healthy women as a control group. Global longitudinal, radial, and circumferential strain, and left ventricular (LV) rotation and twist were evaluated by two-dimensional speckle tracking echocardiography.

Results: During pregnancy, the diameter, and volume of the left atrium, LV stroke volume, and the heart rate significantly increased beginning in the first trimester ($p < 0.0125$). The parameters of pulse-Doppler E velocity and tissue Doppler Em velocity significantly increased in the first trimester ($p < 0.0125$), whereas in the second and third trimester they decreased to control levels. Global longitudinal strain was significantly decreased in the third trimester of the pregnancy ($p < 0.0125$). Basal and apical LV rotation and twist were significantly increased in the third trimester of pregnancy ($p < 0.0125$). LV apical and basal reverse rotation rate were significantly increased in the first trimester of pregnancy ($p < 0.0125$).

Conclusions: In the third trimester global longitudinal strain decreased whereas LV rotation and twist increased. Speckle-tracking echocardiography may be used to evaluate the effects of pregnancy and that provide further data on cardiac functions.

Keywords: Strain, echocardiography, tissue Doppler imaging, pregnancy

During the process that includes pregnancy, birth and the period after birth, important cardiovascular changes occur [1]. The increase of the volume burden that happens during pregnancy, the increase of heart rate and the decrease of the vascular resistance constitute the principle changes that occur in the cardiovascular system. These changes happen gradually and have different effects on the heart at each trimester.

The echocardiographic examination is the most important technique used to evaluate the effects of pregnancy on the heart. The speckle tracking echocardiography (STE) technique calculates the regional rate, strain and strain rate from two-dimensional gray-scale visualizations and is an angle-independent technique [2, 3]. With the speckle-tracking technique,

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it is possible to measure the transverse displacement as well as the left ventricle longitudinal strain parameters in apical visualizations. This technique also allows us to evaluate the radial and circumferential displacements. The evaluation of radial and circumferential displacements enables the calculation of the rotation and twist of myocardium [4].

The goal of this study is to use STE to evaluate the effects of pregnancy on cardiac functions in the first, second and third trimesters.

METHODS

Selection of Cases

This study included 105 volunteers pregnant and 35 volunteer age-matched healthy women of reproductive age who applied to the Gynecology and Cardiology clinics of Bursa Yüksek İhtisas Traning and Research Hospital. The study was approved by the Bursa Yüksek İhtisas Traning and Research Hospital Ethics Committee.

A detailed history of all the cases was taken. After written informed consent was obtained the demographic details (such as the week of pregnancy and age) of the cases were registered, physical examinations were conducted, and heart rate and blood pressure were measured. After taking ECG, standard echocardiographic analysis was performed. Based on the standard echocardiography analysis, the 111 highest-quality images were used.

Exclusion criteria were hypertension, diabetes, coronary artery disease, valvular heart disease, pulmonary hypertension, heart failure, atrial fibrillation, thyroid function disorder, and multiple pregnancies.

The week of pregnancy was calculated by taking into consideration the last day of menstruation. The cases were separated into three trimesters according to their week of pregnancy. Echocardiography records from 24 women in the first trimester, 31 women in the second trimester and 32 women in the third trimester and, as the control group, 24 healthy women are taken. The echocardiographic records for the cases in the first trimester were taken between weeks 7 and 11 of pregnancy, for cases in the second trimester between weeks 20 and 24 and for cases in the third trimester between weeks 32 and 36.

Echocardiographic Evaluation

Standard Echocardiography

Transthoracic echocardiography was performed using an echocardiography machine (Vivid 7, GE Medical System, Horten, Norway) with subjects in the left lateral decubitus position. A variable-frequency phased-array transducer (2.5-3.5 MHz) was used for thoracic echocardiographic imaging. A routine standard echocardiographic examination was performed, which included measurements of LV systolic and diastolic dimensions, LV ejection fraction (EF), mitral inflow velocities and mitral annular velocities. LVEF was calculated using Teicholz's method [5]. Left atrium (LA) volume was estimated using biplane area length method and indexed to body-surface area. Stroke volume was calculated using '' Left ventricular outflow tract (LVOT) VTI x Cross Sectional Area of the LVOT'' formula. Peak early (E) and late (A) diastolic velocities of mitral inflow were measured using pulsed-wave Doppler imaging at the tip of the mitral valve leaflets. Systolic (Sm) as well as early (Em) and late (Am) diastolic mitral annular velocities were acquired at the septal and lateral annulus in the apical four-chamber view.

2D Strain Echocardiography

Parasternal short-axis [apical, mid (level of the papillary muscle), and basal segments] and apical four-chamber, two-chamber, long-axis views of the LV were recorded at end-expiration (70 to 140 frames/s) and probe frequency (range 1.6 to 3.2 MHz). All measurement performed with simultaneous electrocardiography at a sweep speed of 50–100 mm/sec. Timing of aortic valve closure was assessed looking at the aortic valve motion in the long-axis apical view. To standardize short-axis image planes among the individuals, we identified the basal LV segment at the level of the mitral valve leaflet tips and the apical segment at the level just proximal to LV luminal obliteration at the end-systolic period. To obtain reliable LV 2D strain and rotation values, three consecutive heart beats were digitally saved in cine loop format for offline analysis with EchoPac BT 12 software (GE-Vingmed; Norway). LV endocardial borders were manually traced at the end-systolic phase, a second, larger, concentric circle was then automatically generated near the epicardium in order to include all the LV myocardium for STE analysis.

The three apical views were used for global longitudinal strain measurements. Short-axis views were used for measurement of global radial strain, global circular strain, and rotation. Radial strain and circular strain were measured on the short-axis view obtained at the level of the mid-ventricle (papillary muscle). LV rotation was measured on the short-axis views obtained at basal and apical levels.

LV rotations, at the basal or apical short-axis planes were determined as the average angular displacement of six myocardial segments. Counterclockwise LV rotation as viewed from the apex is expressed as a positive value and clockwise LV rotation as a negative one. The LV twist was calculated as the net difference between apical and basal rotation. We also measured the peak rotation rates during ejection and reverse rotation rates during diastole. Peak positive rotation rate was measured as time to peak positive rotation velocity and peak reverse rotation rate was measured as time to peak reverse rotation velocity.

Image analysis was performed by two independent cardiologist who was not involved in the image acquisition and had no knowledge of other characteristics of cases.

Inter and intraobserver agreement of strain measurements were assessed in 20 randomly selected patients (five patients for each group) by two independent observers who analyzed the data blind to the other observer results. Intraobserver agreement was assessed by one observer who analyzed the 20 randomly selected patients twice, more than 15 days apart.

Statistical Analysis

SPSS (Statistical Package for the Social Sciences ver. 10.0, SPSS Inc, Chicago, Illinois, USA) software was used for all statistical analyses. The numerical variants are expressed as the average ± standard deviation and the categorical variants as percentage. The normality of distribution of the averages in each group was evaluated with the Shapiro-Wilk test. Logarithmic conversion was applied for variants that did not show normal distribution, and all averages were compared by one-way ANOVA. In one-way ANOVA, the p values of parameters in each group were compared with the control group by using Dunnett's post-test. The categorical variants were compared with the chi-square test or with Fisher's exact test. Inter and intraobserver agreements were assessed with Pearson's correlation coefficients. Bonferroni correction was applied for p-value when more than one group was compared, for four group comparison $p < 0.0125$ value was accepted as significant. In standard statistical analysis, $p < 0.05$ value was accepted as significant.

RESULTS

Among the cases whose visualization were recorded, the visualization of 111 cases were approved for evaluation (24 cases in the first trimester, 31 cases in the second trimester, 32 cases in the third trimester, 24 cases in the control group). The demographic characteristics and physical examination findings were compared between the pregnants and the control group. Heart rate gradually increased beginning in the first trimester, whereas systolic blood pressure (SBP) and diastolic blood pressure (DBP) gradually decreased ($p < 0.001$, $p < 0.001$, $p = 0.004$, respectively) (Tables 1 and 2).

Conventional and tissue Doppler echocardiography

The comparing of the conventional echocardiography parameters, the diameter, and volume of the LA increased beginning in the first trimester of pregnancy, whereas the velocity of the mitral E wave and the E/A ratio gradually decreased during the advancement of pregnancy despite their increase in the first trimester ($p < 0.001$, $p < 0.001$, $p = 0.004$, respectively) (Tables 1 and 2). LV stroke volume increased beginning in the first trimester of pregnancy (Tables 1 and 2).

The comparing the tissue Doppler parameters, the velocities of the mitral lateral and medial Em velocity increased in the first trimester, but afterward, they decreased to the level of the control group ($p < 0.001$, $p < 0.001$, respectively) (Tables 1 and 2). Tricuspid lateral Sm velocities increased in the first and second trimester, but it decreased to control levels in the third trimester ($p = 0.001$) (Tables 1 and 2).

Left Ventricular Strain and Rotation

Global longitudinal strain significantly decreased in the third trimester according to control ($p < 0.001$) (Tables 3 and 4). Global circular strain and global

Table 1. Demographic and echocardiography characteristics (conventional and tissue Doppler parameters)

Variant	1st Trimester	2st Trimester	3st Trimester	Control	F value	*p* value
Age (year)	26 ± 3.5	26 ± 3.8	27 ± 4.5	27 ± 3.7	1.30	0.27
Heart rate (pulse/min)	81.0 ± 7.6	81.0 ± 5.1	86.0 ± 5.8	78.0 ± 7.1	8.09	**< 0.001**
SBP (mmHg)	122.5 ± 9.6	111.7 ± 10.2	104.3 ± 9.3	118.1 ± 14.4	15.42	**< 0.001**
DBP (mmHg)	72.9 ± 6.3	67.9 ± 5.8	68.8 ± 5.6	72.5 ± 7.9	4.74	**0.004**
LVEDD (mm)	46.21 ± 3.72	46.19 ± 3.92	46.61 ± 2.94	44.72 ± 3.26	1.44	0.23
LVESD (mm)	28.64 ± 4.53	28.83 ± 3.24	29.93 ± 3.21	27.56 ± 3.17	2.19	0.09
Septum (mm)	8.19 ± 1.44	8.23 ± 1.36	8.79 ± 1.38	8.21 ± 1.05	1.34	0.26
Posterior (mm)	8.21 ± 1.42	8.35 ± 1.08	8.67 ± 1.09	0.82 ± 0.89	0.96	0.41
EF (%)	66.50 ± 8.85	66.00 ± 6.58	64.37 ± 6.24	67.58 ± 7.87	0.93	0.42
Stroke volume (ml)	54.87 ± 4.65	56.14 ± 8.78	58.75 ± 5.39	48.65 ± 4.77	5.79	**0.009**
Left atrium volume index (ml/m^2)	25.81 ± 3.12	30.71 ± 2.15	32.19 ± 2.43	23.69 ± 3.43	6.78	**< 0.001**
Left atrium (cm)	3.43 ± 0.34	3.58 ± 0.32	3.62 ± 0.30	3.26 ± 0.35	6.44	**< 0.001**
PASP (mmHg)	25.69 ± 7.76	26.78 ± 6.59	27.42 ± 9.38	25.55 ± 4.73	1.21	0.87
E velocity (cm/s)	109.14 ± 35.27	98.32 ± 20.15	82.65 ± 21.37	87.66 ± 14.42	6.81	**< 0.001**
A velocity (cm/s)	72.23 ± 31.45	67.56 ± 20.54	68.42 ± 17.27	59.31 ± 12.18	1.37	0.25
E/A ratio	1.59 ± 0.35	1.46 ± 0.40	1.24 ± 0.35	1.51 ± 0.34	4.74	**0.004**
Mitral lateral Sm (cm/s)	10.24 ± 2.32	9.34 ± 2.42	9.65 ± 1.74	10.43 ± 2.27	0.69	0.55
Mitral Lateral Em (cm/s)	20.13 ± 3.15	17.21 ± 3.43	15.27 ± 3.22	17.36 ± 3.31	7.49	**< 0.001**
Mitral lateral Am (cm/s)	8.78 ± 2.85	9.87 ± 1.65	10.15 ± 1.27	9.32 ± 2.29	1.48	0.22
Mitral medial Sm (cm/s)	10.56 ± 1.42	9.75 ± 1.68	9.72 ± 1.76	9.45 ± 1.27	2.71	**0.04**
Mitral medial Em (cm/s)	15.98 ± 3.42	14.34 ± 3.67	11.76 ± 2.32	12.34 ± 2.27	11.56	**< 0.001**
Mitral medial Am (cm/s)	9.75 ± 1.27	10.42 ± 2.25	10.76 ± 2.45	10.69 ± 3.38	2.25	0.08
Tricuspid lateral Sm (cm/s)	16.47 ± 3.78	16.85 ± 2.36	14.65 ± 2.53	14.17 ± 2.76	6.19	**0.001**
Tricuspid lateral Em (cm/s)	18.78 ± 4.47	19.85 ± 3.47	17.93 ± 4.54	16.43 ± 3.67	3.34	**0.02**
Tricuspid lateral Am (cm/s)	14.89 ± 4.68	16.68 ± 5.87	19.45 ± 1.47	14.38 ± 4.21	1.74	0.16

BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, LVEDD = left ventricle end-diastolic diameter, LVESD = left ventricle end-systolic diameter, EF = ejection fraction, PASP = Pulmonary artery systolic pressure

Table 2. Comparison with Dunnett's test of pregnant and control groups (physical examination findings, conventional and tissue Doppler echocardiography parameters)

Variant	(I)	(J)	Average difference (I - J)	*p* value
Heart rate	1st Trimester	Control	2.41 ± 1.84	0.41
	2st Trimester	Control	3.06 ± 1.74	0.48
	3st Trimester	Control	7.87 ± 1.72	**< 0.001**
SBP	1st Trimester	Control	4.37 ± 2.82	0.27
	2st Trimester	Control	-6.35 ± 2.84	0.07
	3st Trimester	Control	-13.76 ± 3.01	**< 0.001**
DBP	1st Trimester	Control	0.46 ± 1.74	0.98
	2st Trimester	Control	-3.95 ± 1.86	0.08
	3st Trimester	Control	-4.59 ± 1.75	**0.02**
Left atrium	1st Trimester	Control	0.17 ± 0.09	0.18
	2st Trimester	Control	0.31 ± 0.09	**0.002**
	3st Trimester	Control	0.35 ± 0.08	**< 0.001**
Left atrium volume index	1st Trimester	Control	2.12 ± 0.03	0.08
	2st Trimester	Control	6.98 ± 1.02	**0.001**
	3st Trimester	Control	7.95 ± 1.15	**< 0.001**
LV stroke volume	1st Trimester	Control	6.20 ± 1.05	**0.04**
	2st Trimester	Control	7.11 ± 2.19	**0.002**
	3st Trimester	Control	8.32 ± 2.43	**< 0.001**
E velocity	1st Trimester	Control	0.21 ± 0.06	**0.005**
	2st Trimester	Control	0.10 ± 0.06	0.26
	3st Trimester	Control	-0.05 ± 0.06	0.73
E/A ratio	1st Trimester	Control	0.08 ± 0.10	0.77
	2st Trimester	Control	-0.04 ± 0.10	0.93
	3st Trimester	Control	-0.26 ± 0.09	0.23
Mitral lateral Em	1st Trimester	Control	0.03 ± 0.01	**0.01**
	2st Trimester	Control	0.006 ± 0.01	0.83
	3st Trimester	Control	-0.001 ± 0.009	0.25
Mitral medial Em	1st Trimester	Control	0.02 ± 0.008	**0.004**
	2st Trimester	Control	0.01 ± 0.007	0.12
	3st Trimester	Control	0.01 ± 0.007	0.12
Tricuspid lateral Sm	1st Trimester	Control	0.01 ± 0.007	**0.03**
	2st Trimester	Control	0.02 ± 0.007	**0.003**
	3st Trimester	Control	0.001 ± 0.007	0.99

BMI = body mass index, SBP = systolic blood pressure, DBP = Diastolic blood pressure

Table 3. Evaluation of the left ventricular global strain and rotation with speckle-tracking

Variant	1st Trimester	2st Trimester	3st Trimester	Control	F value	*p* value
Global longitudinal strain (%)	-23.76 ± 2.67	-23.40 ± 3.03	-20.85 ± 2.95	-23,95 ± 1.50	7.53	**< 0.001**
Global circumferential strain (%)	-22.99 ± 2.75	-23.50 ± 3.35	-23.09 ± 4.65	-26.13 ± 4.53	3.12	**0.03**
Global radial strain (%)	37.72 ± 13.78	39.27 ± 10.23	41.50 ± 12.57	40.19 ± 6.21	0.44	0.72
Basal maximum rotation (degree)	-8.71 ± 1.73	-8.20 ± 2.06	-10.00 ± 2.77	-8.12 ± 2.10	3.91	**0.01**
Apical maximum rotation (degree)	11.76 ± 2.40	12.33 ± 4.20	15.94 ± 2.94	14.00 ± 3.98	7.46	**< 0.001**
Twist (degree)	20.47 ± 3.85	20.54 ± 5.60	26.16 ± 4.75	22.19 ± 5.58	7.37	**< 0.001**
Basal rotation rate (degree/sec)	31.55 ± 6.28	30.97 ± 8.29	36.57 ± 10.29	30.09 ± 11.11	2.62	0.05
Apical rotation rate (degree/sec)	42.55 ± 9.05	47.13 ± 16.47	56.31 ± 12.03	49.79 ± 18.12	3.11	**0.03**
Basal reversed rotation rate (degree/sec)	42.86 ± 13.01	33.71 ± 8.47	35.29 ± 7.34	34.07 ± 12.68	4.16	**0.008**
Apical reversed rotation rate (degree/sec)	65.47 ± 15.44	51.78 ± 16.91	49.06 ± 12.86	56.39 ± 22.35	3.54	**0.01**

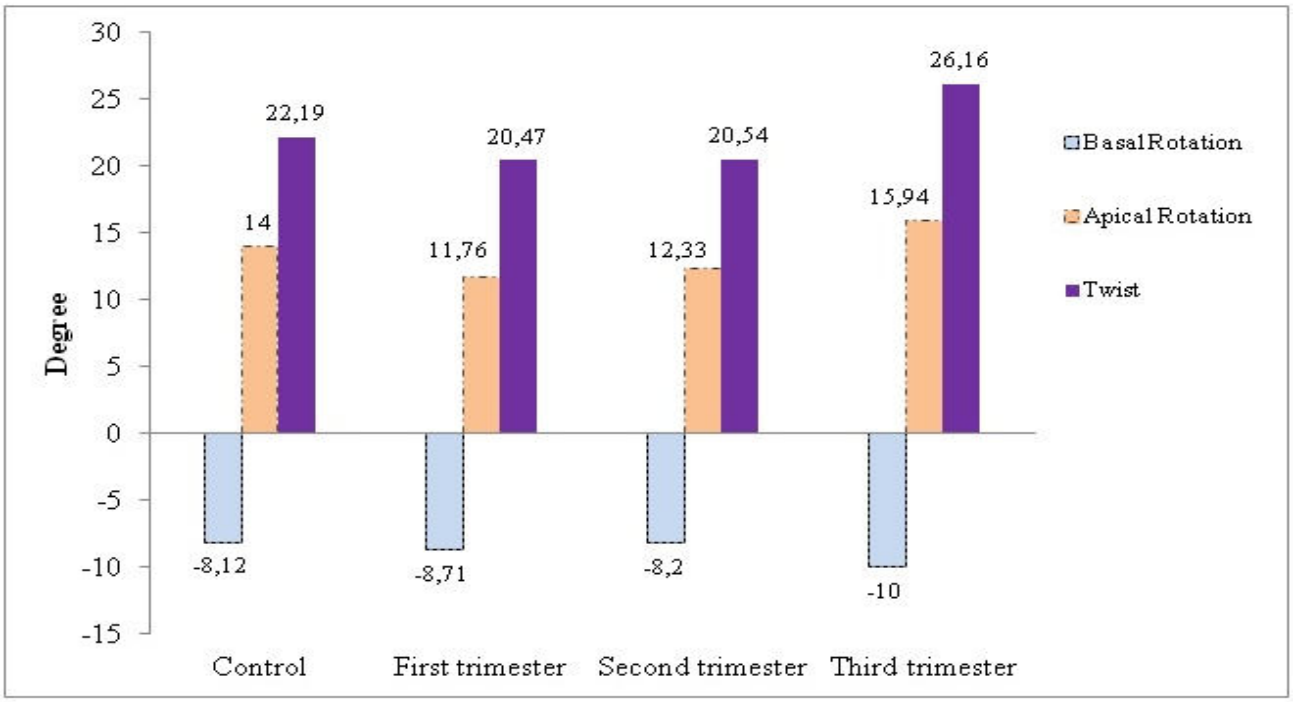

Fig. 1. Left ventricular rotation and twist.

Table 4. Comparison of the cases and the control groups with the Dunnett's test (Speckle tracking parameters)

Variant	(I)	(J)	Average Difference (I - J)	*P* Value
Global longitudinal strain (%)				
	1st Trimester	Control	-0.19 ± 0.82	0.99
	2st Trimester	Control	-0.55 ± 0.81	0.82
	3st Trimester	Control	-3.09 ± 0.78	**< 0.001**
Basal maximum rotation (degree)				
	1st Trimester	Control	0.53 ± 0.65	0.75
	2st Trimester	Control	0.02 ± 0.64	0.99
	3st Trimester	Control	1.82 ± 0.62	**0.01**
Apical maximum rotation (degree)				
	1st Trimester	Control	-2.24 ± 1.02	0.07
	2st Trimester	Control	-1.67 ± 1.00	0.23
	3st Trimester	Control	1.93 ± 0.98	0.12
Twist (degree)				
	1st Trimester	Control	-1.71 ± 1.47	0.51
	2st Trimester	Control	-1.65 ± 1.44	0.52
	3st Trimester	Control	3.97 ± 1.42	**0.01**
Basal reversed rotation rate				
	1st Trimester	Control	8.78 ± 3.08	**0.01**
	2st Trimester	Control	-0.35 ± 3.19	0.99
	3st Trimester	Control	1.22 ± 3.30	0.96
Apical reversed rotation rate				
	1st Trimester	Control	9.07 ± 5.17	0.20
	2st Trimester	Control	-4.61 ± 5.22	0.71
	3st Trimester	Control	-7.33 ± 5.60	0.42

radial strain did not differ between pregnant and control (Table 3).

Basal maximal rotation significantly increased in the third trimester according to control (p = 0.01) (Tables 3 and 4) (Fig. 1). Apical maximal rotation significantly decreased at first (11.76 ± 2.40 vs 15.94 ± 2.94; $p < 0.001$) and second trimester (12.33 ± 4.20 vs 15.94 ± 2.94; p = 0.002) according to third trimester and control (Fig. 1). The twist degree significantly increased at the third trimester according to the first (20.47 ± 3.85 vs 26.16 ± 4.75; p = 0.001), second (20.54 ± 5.60 vs 26.16 ± 4.75; p = 0.001) trimester and control group (22.19 ± 5.58 vs 26.16 ± 4.75; p = 0.01) (Tables 3 and 4) (Fig. 1). The rates of basal and apical reversed rotation rate increased in the early period of pregnancy (p = 0.008, p = 0.01, respectively) (Table 3). Compared to the control group at each trimester, the rate of basal reversed rotation rate was significantly increased in the first trimester (42.86 ± 13.01 vs 34.07 ± 12.68, p = 0.01) (Table 4).

High interobserver correlation was found between the two observer measurements; global longitudinal strain (r = 0.91; p = 0.001), global radial strain (r = 0.90; p = 0.001) and global circular strain (r = 0.89; p = 0.002). Also high intraobserver agreement was found between the two measurement of the same operator, global longitudinal strain (r = 0.93; p = 0.001), global radial strain (r = 0.92; p = 0.001) and

global circular strain (r = 0.92; p = 0.001).

DISCUSSION

In this study, the diameter of the left atrium increased during pregnancy. The diastolic parameters of the pulse-Doppler and tissue Doppler increased in the first trimester, whereas they decreased to the control level during the second and third trimester. Global longitudinal strain decreased in the third trimester according to the first, second trimesters and control group. Left ventricular rotation and twist increased in the third trimester. Left ventricular reversed rotation rate increased in the first trimester.

When the parameters of conventional echocardiography were examined, the dimensions of the left atrium started to expand from the first trimester, reaching a maximum in the third trimester. Because left atrium volume increases during pregnancy, the venous return of the left atrium increases [6]. Because of the increased venous return, the dimension of the left atrium expands. Recently Tasar *et al*. [7] showed that left atrial area and volume increase whereas left atrial strain decrease from the first trimester to the third trimester during pregnancy. LV diameters increased during pregnancy but did not reach significance. However, the stroke volume increases significantly since the beginning of pregnancy. Increased stroke volume and heart rate provide the cardiac output increase that requires during pregnancy.

The pulse-Doppler images showed that the E wave velocity and E/A ratio increased in the first trimester and decreased afterward. Mitral lateral and medial tissue Doppler Em increased in the first trimester, in the second and third trimesters they gradually decreased. The compliance of the left ventricle, which decreases with the myocardial hypertrophy, is thought to be the most important factor responsible for these changes [8-11]. The increasing tricuspid Sm values reflect the contractility of the right ventricle, which increased in early pregnancy. These echocardiographic changes may explain with pregnancy-related preload increasing and left ventricular physiological myocardial hypertrophy. Previous studies have shown similar results [10, 12].

The quantitative assessment of global longitudinal is an important part of STE analysis. The longitudinal movement of the ventricular wall is one of the principal deformations of the heart, which represents as shortening and lengthening of myocardial fibers from the base to the apex. In this study, we found the ventricular global longitudinal strain decreased significantly in the third trimester of pregnancy. This is similar to the previous studies, [13, 14]. Although there are no significant changes in global radial and circular strain parameters during pregnancy, left ventricular rotation and twist values increase significantly in the 3rd trimester [13, 14]. In the evaluation made in terms of the rotation rate, reversed rotation rate increased in the early period of pregnancy and decreased to control values in the last trimester of pregnancy.

Along with changes in systemic vascular resistance (afterload) and volume load (preload) during pregnancy, there are also changes in left ventricular dimensions and wall thicknesses. Together with these changes, the combination of changes in left ventricular deformation provides the necessary cardiac functions in pregnant women.

Despite the decrease in diastolic functions because of increasing volume burden and left ventricular mass during pregnancy, the systolic functions were protected. In spite of the decrease in longitudinal deformation, the necessary increase of stroke volume during pregnancy was provided with the increasing rotation and twist of the left ventricle. The increase in the left ventricle twist may be a systolic compensation mechanism. The increase in a myocardial twist has also been shown in patient groups who have concentric left ventricle hypertrophy and diastolic dysfunction related to age [15, 16]. The findings of these studies support our results.

In our study, an increase of left ventricle early diastolic velocities and reversed rotation rate during the early period of pregnancy while in rotation degrees during the third trimester was observed. From these findings, we can conclude that the hemodynamic changes that appeared during pregnancy were compensated for with the augmentation of diastolic functions in early pregnancy and with the augmentation of systolic functions in the later period.

Clinical recommendations

The STE technique, which enables the evaluation of strain and rotational mechanics quantitatively will

contribute to the understanding of what changing of myocardial function during pregnancy.

Limitations

There were some main limitations to this study: (1) small number of cases however the number of cases for each trimester is enough to prevent evaluation errors, (2) the fact that for each trimester group different patients were evaluated however evaluation of different pregnancies for each trimester can provide an overall average and better reflect the population, and (3) the fact that endocardial and epicardial rotations were not evaluated separately. These factors may affect myocardial functions.

CONCLUSION

In healthy pregnant, global longitudinal strain decreased whereas left ventricular rotation and twist increased in the third trimester. These additional quantitative data to standard echocardiographic methods provides us better understanding of the functional changes of myocardium during pregnancy.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The floppy infants and metabolic causes

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ABSTRACT

Objectives: To describe the clinical, metabolic and genetic characteristics of the floppy infants diagnosed at a tertiary care center.

Methods: A retrospective analysis was performed on the medical files of 90 floppy infants diagnosed in the pediatric metabolism department of our tertiary care center. Baseline descriptives, prenatal and perinatal data, results of genetic and metabolic tests as well as neuroradiological imaging findings were overviewed.

Results: Our series was comprised of 42 (46.7%) females and 48 (53.3%) males. Consanguineous marriages were detected in 60 (66.7%) cases. There was no history of prenatal comorbidity or birth-related trauma or infections. Gestational age was ≥ 37 weeks in 78 (87%) infants. The average body weight was 2521.2 ± 839.4 grams (range: 1510-4300). The average height and head circumference were 48.9 ± 0.9 cm and 33.9 ± 0.7 cm, respectively. The etiology of hypotonia was found to be central in 89 (98.8%) infants. The most frequent diseases diagnosed were vitamin B12 deficiency (14.4%), dystrophinopathy (7.7%), spinal muscular atrophy (5.5%), gangliosidosis (3.3%), peroxisomal disease (3.3%), Pompe disease (3.3%), and Zellweger disease (3.3%).

Conclusions: The floppy infant still constitutes a diagnostic challenge in spite of the technological advances in genetic, molecular and metabolic test methods. The priority and selection of diagnostic measures need to be determined on an individualized basis, and multidisciplinary and collaborative work is mandatory to set the diagnosis cost-effectively without delay. An algorithm based and individualized approach may provide a high diagnostic yield for the clinician.

Keywords: Floppy infant, hypotonia, newborn

The floppy infant is characterized by congenital hypotonia, and it is a common disorder in the newborn period. It occurs as the subjective decrease of resistance to a passive range of motion in a newborn [1]. Some publications imply that the majority of hypotonic newborns are floppy due to central nervous system disorders; some hypotonic newborns have genetic diseases and metabolic disturbances, whereas the minority of hypotonic infants has neuromuscular and connective tissue disorders [2].

The clinical approach and investigative methods to be employed for the floppy infant had been well described in the medical literature [3-6]. Even though the identification of a neonate or infant lying in the supine position with a lack of power for spontaneous movements is easy, the appropriate route of diagnostic and investigative steps to be followed may pose a challenge. Before the popularization of DNA-based diagnostic studies, the diagnosis could be made using the identification of the specific syndromes that have cer-

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tain dysmorphic characteristics and clinical presentation. In some circumstances, invasive procedures such as electrophysiological studies or muscle biopsies may be necessary to rule in the diagnosis accurately. Moreover, molecular diagnostics and recognition of certain mutations linked with neuromuscular disorders facilitated the diagnostic processes for the floppy infant [3-5]. An appropriate diagnostic approach for the floppy infant involves selective use of available measures and integrative evaluation of data harvested from history, physical examination as well as the results of metabolic and genetic tests and radiologic imaging studies [4, 5]. To effectively employ the advances in laboratory diagnosis and imaging, multidisciplinary evaluation by departments of genetics, pediatric neurology, pediatric metabolism, and pediatric neuroradiology is mandatory [6]. Careful clinical observation is vital to the proper evaluation of these critically ill children [7].

A better knowledge of the relative frequency of disorders that present with hypotonia may be helpful to select the appropriate investigations. Herein, we describe the perinatal and clinical features and to present the results of laboratory tests and imaging studies in the floppy infants. Therefore, we aimed to achieve an insight into the diagnostic profile of the floppy infants. We hope that our data may be beneficial to determine a stepwise algorithm in the diagnostic process for these patients.

METHODS

Study Design

This retrospective study was performed on data extracted from the medical files of 82 floppy infants diagnosed in the metabolism department of our tertiary care center between May 2017 and May 2018. Ethical approval for this study was obtained from Bursa Yüksek İhtisas Training and Research Hospital Clinical Research Ethics Committee (No: 2011-KAEK-25/2018/11-04, Date: 07/11/2018) in accordance with the declaration of Helsinki-Ethical principles. All cases were infants younger than 12 months who underwent examination for generalized hypotonia, and neurologic, metabolic and genetic evaluation were carried out. Data were derived from the records of floppy infants and baseline descriptives, physical examination findings, prenatal and perinatal factors, diagnostic methods and results of imaging studies (computed tomography and magnetic resonance imaging [MRI]) were noted. The collection of data was performed after the written permission of hospital administration and the local ethics committee. Strict adherence to patient confidentiality was provided, and patients were randomly ordered by a computer program without the announcement of identity information.

Our Clinical Approach

In the first presentation of patients presenting with hypotonia, detailed history (prenatal, natal and postnatal history, neuromotor development steps, family history, similar story history, etc.) were taken. Height, body weight and head circumference were taken and a detailed physical examination was performed.

Complete blood count, biochemical tests (including glucose, alanine aminotransferase, aspartate aminotransferase, uric acid, creatine kinase, cholesterol etc.), thyroid function tests (free thyroxine and thyroid stimulating hormone), vitamin B12, folate, homocysteine, TANDEM-MS, blood gas analysis, ammonia and lactate tests were analyzed from all patients.

Blood amino acid chromatography, urine organic acids, very-long-chain fatty acids, and transferrin electrophoresis tests were performed as the second step in patients who could not be diagnosed by first step tests. Cranial MRI performed in patients who were considered necessary.

Patients who were required by the guidance of clinical and laboratory findings were consulted to the departments of pediatric neurology, ophthalmology, and pediatric cardiology for differential diagnosis.
The diagnosis that reached with these investigations, confirmed genetically. For the patients who could not be diagnosed, a clinical exome sequencing which is scanning 4900 genetic diseases, was performed.

Statistical Analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA). Descriptive statistical methods were used and expressed as means, proportions, percentages and 95% confidence intervals.

Table 1. Descriptive baseline variables

Variables	Data (n = 90)
Gender	
Female	42 (46.7%)
Male	48 (53.3%)
Consanguineous marriage	60 (66.7%)
Prenatal morbidity	0 (0%)
Birth trauma	0 (0%)
Gestational age (weeks)	
33-36	12 (13%)
Term	78 (87%)
Birth weight (grams)	2521.2 ± 839.4
Height (cm)	48.9 ± 0.9
Head circumference (cm)	33.9 ± 0.7
Admission age (min-max)	7 days-12 months
Hypotonia	
Peripheral	1 (1.2%)
Central	89 (98.8%)
Convulsion story	19 (21%)

Data was expressed as the mean ± standard deviation or n (%).

RESULTS

An overview of baseline descriptives is presented in Table 1. Of 90 patients, 42 (46.7%) were females and 48 (53.3%) were males. The admission ages of babies were between seven days and 12 months. Consanguineous marriages were detected in 60 (66.7%) cases. There was no history of prenatal comorbidity or birth-related trauma or infections. Gestational age was ≥ 37 weeks in 78 (87%) infants. The average body weight was 2521.2 ± 839.4 grams (range: 700-4300). The average height and head circumference were 48.9 ± 0.9 cm and 33.9 ± 0.7 cm, respectively. The etiology of hypotonia was found to be central in 89 (98.8%) infants, whereas peripheral cause was determined in only 1 (1.2%) case. There was a history of convulsion in 19 (23.2%) of floppy infants in our series (Table 1).

Table 2 demonstrates the results of biochemical tests carried out in our patient population. Thyroid function tests, serum levels of vitamin B12, homocysteine, ammonium, CK, lactate, pyruvate, and uric acid were within normal range in the majority of our patients.

In Table 3, the results of metabolic tests carried out in selected patients were presented. Transferrin electrophoresis was performed to identify congenital disorders of glycosylation. One out of 16 patients revealed pathological results in transferrin electrophoresis. Very long chain fatty acid (VLCFA) levels were assessed to rule out peroxisomal diseases in 17 patients, and 2 of these infants yielded high levels of VLCFA. On electroencephalogram (EEG), 21 infants exhibited normal findings; whereas 19 infants had dysrhythmia. Magnetic resonance images were normal in 18 out of 43 patients. In contrary, in 25 patients had pathological changes such as thinning of the corpus callosum, cortical atrophy, cortical dysplasia, cerebral atrophy and glial changes in the cerebellum, pons and mesencephalon.

The most frequent diseases diagnosed were vitamin B12 deficiency (14.4%), dystrophinopathy (7.7%), spinal muscular atrophy (5.5%),

Table 2. The results of biochemical tests

Variables	Data
Thyroid function tests (n = 90)	
Normal	90 (100)
Vitamin B_{12} & homocysteine (n = 90)	
Normal	77 (85.6)
Low Vitamin B_{12} level	4 (4.4)
Low vitamin B_{12} level, high homocysteine level	9 (10)
Ammonium (n = 90)	
Normal	87 (96.7)
High	3 (3.3)
Creatine kinase (n = 90)	
Normal	82 (91.1)
High	8 (8.9)
Uric acid (n = 90)	
Normal	88 (97.8)
Low	2 (2.2)
Lactate (n = 90)	
Normal	88 (97.8)
High	2 (2.2)
Pyruvate (n = 22)	
Normal	22 (100)

Table 3. The results of magnetic resonance imaging, electroencephalogram, metabolic tests, and clinical exome sequencing

Variables	n (%)
Electroencephalogram (n = 40)	
Normal	21 (52.5)
Dysrhythmic	19 (47.5)
Magnetic resonance imaging (n = 43)	
Normal	18 (41.8)
Pathological finding	25 (48.2)
Transferrin electrophoresis (n = 16)	
Normal	15 (94)
Congenital Defect of Glycosylation 2 (pattern 1)	1 (6)
Very Long Chain Fatty Acids (n = 17)	
Normal	15 (88)
High levels of c26 and c26:c22	2 (12)
Tandem (n = 90)	
Normal	88 (87.8)
C3 carnitine levels ↑	1 (1.1)
C5DC carnitine levels ↑	1 (1.1)
Urine organic acids (n = 70)	
Normal	60 (85.7)
Urinary methylmalonic acid levels ↑	3 (4)
Urinary glutaric acid levels ↑	1 (1.4)
Oxoglutaric acid ↑	1 (1.4)
N-acetylaspartate levels ↑	1 (1.4)
Oxoproline levels ↑	1 (1.4)
3-methylglutaconic acid, fumaric acid, 3-OH isovaleric acid ↑	2 (3.3)
Methylsitric acid ↑	1 (1.4)
Blood amino acid levels (n = 71)	
Normal	66 (93)
Alanine levels ↑	2 (2.8)
Cystine levels ↓	2 (2.8)
Glycine ↑	1 (1.4)
Clinical exome sequencing (n = 69)	
Normal	5 (7.2)
Diagnostic finding	64 (92.8)

Table 4. The final diagnosis of floppy infants

Diagnosis	n (%)
Vitamin B12 deficiency	13 (14.4)
Dystrophinopathy	7 (7.7)
Spinal muscular atrophy	5 (5.5)
Early infantile epileptic encephalopathy	5 (5.5)
Pompe disease	3 (3.3)
Gangliosidosis (GM1: 2 and GM2: 1)	3 (3.3)
Peroxisomal disease	3 (3.3)
Canavan disease	2 (2.2)
Molybdenum cofactor deficiency A	2 (2.2)
Nieman Pick disease (Type A: 1 and type C: 1)	2 (2.2)
Leigh Syndrome	2 (2.2)
Creatine transporter defect	2 (2.2)
Glutaricaciduria (Type 2:1 and type 3:1)	2 (2.2)
Congenital disorders of glycosylation	2 (2.2)
Angelman syndrome	2 (2.2)
Sandhoff disease	1 (1.1)
EhlerDanlos syndrome	1 (1.1)
Trichohepatoenteric syndrome	1 (1.1)
Kabuki syndrome	1 (1.1)
Tay Sachs disease	1 (1.1)
Leukoencephalopathy with vanishing white matter	1 (1.1)
Lissencephaly	1 (1.1)
Combined oxidative phosphorylation deficiency type 3	1 (1.1)
Metachromatic leukodystrophy	1 (1.1)
Cat Cry syndrome	1 (1.1)
Kleefstra syndrome	1 (1.1)
I-cell disease	1 (1.1)
Methylmalonicacidemia	1 (1.1)
Walker Warburg syndrome	1 (1.1)
Bohring Opitz syndrome	1 (1.1)
Cornelia de Lange syndrome	1 (1.1)
Mucopolysaccharidosis type 3d	1 (1.1)
Sepiapterinreductase deficiency	1 (1.1)
Pyroglutamicacidemia	1 (1.1)
Pyridoxine-dependent epilepsy	1 (1.1)
Biotin-thiamine-responsive basal ganglia disease	1 (1.1)
Undiagnosed	14 (15.5)

gangliosidosis (3.3%), peroxisomal disease (3.3%),Pompe disease (3.3%), and Zellweger disease (3.3%). Despite all these investigations, approximately 15% of the patients could not be diagnosed (Table 4).

DISCUSSION

The objective of the present study was to share the experience with the evaluation and diagnostic investigation of the floppy infant in our tertiary care center. Our retrospective series had a relatively large sample size; however, the applicability of our results to the general population necessitates implementation of further prospective, multi-centric trials on hypotonic infants. The main restrictions of the current study were retrospective design, missing information and lack of a control group for comparison. Furthermore, a standard set of tests cannot be administered to all infants routinely attributed to the unavailability of some molecular diagnostic tests within certain periods during this study.

We observed that the vast majority of the floppy infants revealed generalized hypotonia, which may be accompanied by additional symptoms such as convulsions, mental motor retardation. Congenital anomalies may be detected in a considerable proportion of infants. The gender distribution was fair. The lack of a control group limits the interpretation of the impacts of prenatal and perinatal factors on hypotonia. Genetic factors, family history for neuromuscular disorders as well as maternal diseases may affect the development of hypotonia [6]. Further trials are required for a better understanding of the combined effects of maternal, environmental and genetic factors in the pathogenesis of the floppy infants.

In previous reports, the advanced paternal age was supposed to be a confounding factor since it might increase the possibility of new dominantly inherited conditions [7]. However, we did not come across such a finding in our series. We noted that the majority of floppy infants were either term or near-term infants, who were appropriate for gestational age. A high rate of consanguineous marriages (66.7%) was detected in our patient population, and this was one of our most remarkable outcomes. We suggest that discouragement of consanguineous marriages can be an important precaution to diminish the incidence of floppy infants. Tandem mass spectroscopy did not yield any abnormal results in the majority (98.8%) of floppy infants. Similarly, blood amino acid levels were mostly within normal limits. On MRI views, findings such as thinning of the corpus callosum, cerebral or cortical atrophy and cortical dysplasia were detected. Electroencephalogram demonstrated multifocal epileptiform activity and cerebral dysfunction in some of our series. Clinical implications and the prognostic value of radiological, metabolic and genetic tests need to be elucidated in further trials. Serum levels of thyroid hormones, vitamin B12, homocysteine, ammonium, creatinine kinase, lactate, and pyruvate were generally within normal range. In conjunction with a report by Birdi *et al*. [3], central etiology was responsible for hypotonia in 98.8% of the floppy infants. Transferrin electrophoresis, which was used to identify the congenital disorders of glycosylation and evaluation for VLCFA, may be useful to identify the underlying condition in selected cases. Their priority and timing in the diagnostic study algorithm need to be well established since they are expensive and may not always be readily available.

The neuromuscular diseases were also diagnosed in our patient population. These disorders may be underdiagnosed, or they may be identified later following the onset of other specific symptoms else than hypotonia [8]. A proper sub-classification for floppy infants is essential to determine the risk and prognosis groups and employ the selective diagnostic modes. A small number of our patient population underwent MRI, and this was because CT scans were usually sufficient to identify the cranial abnormalities; thereby reducing the need for further MRI assessment. Integrative and combined use of radiological imaging studies in conjunction with genetic and metabolic tests may provide a highly sensitive and specific diagnostic tool for floppy infants. Advances in neuroimaging studies, as well as deoxyribonucleic acid (DNA)-based diagnostic tests, can improve the diagnostic accuracy and save time during diagnostic work. It has been recommended that considering these 2 options priorlycan improve cost-effectivity [9]. The indication and prognostic value for performance of other tests can be considered on an individualized basis.3 Inborn

metabolic errors and genetic diseases constitute a very wide spectrum, and clues such as multisystem involvement as well as typical dysmorphic features must be carefully overviewed before ruling in the diagnosis in floppy infants. Electrophysiological studies and muscle biopsy must be evaluated together for lower motor neuron disorders. It must be remembered that a good correlation between the experience and expertise of the clinician and laboratory is crucial [3]. David *et al*. [10] reported that there was a poor correlation between muscle biopsy results and electromyography findings. To overcome this diagnostic challenge, the combined use of muscle ultrasonography and electrophysiological studies can be useful [11].

The stepwise approach to the evaluation of a floppy newborn has already been proposed by Richer *et al*. [4]. Paro-Panjan *et al*. [1] have further divided this approach into six steps and proved that evaluation by the first five steps revealed the etiology in the majority of central hypotonia cases and 94% of all classifiable cases during the neonatal period. The diagnostic profile of the floppy infant is quite diverse. A good medical history, proper clinical observation, including neurologic examination, and the use of dysmorphology databases may aid in recognition of the majority of cases during the newborn period. The selective use of neuroimaging and genetic and biochemical methods contributed to the diagnosis in remaining cases. On the other hand, neurophysiologic investigations, specific molecular tests, and muscle biopsy may contribute to the diagnosis in minority of hypotonic newborns. Nevertheless, some cases including congenital myasthenic syndromes, some severe hypotonic forms of cerebral palsy, and syndromes of joint hyperlaxity are diagnosed only after close follow-up and frequent repetition of some neurophysiologic investigations [1].

To sum up, we determined that pathology was mainly central in the floppy infants and patients were usually appropriate for gestational age. In addition to metabolic and genetic diseases, connective tissue disorders must also be included in the differential diagnosis. Our results support the results of Birdi *et al*. [3], who suggested that clinical or whole exomesequence analysis, and neuroimaging studies should be considered first in infants with generalized hypotonia. If there is further need, more invasive test methods should be taken into account.

Limitations

The limitations of our study are that it is a retrospective study and that only hypotonic patients referred to the metabolism outpatient clinic were examined.

CONCLUSION

In conclusion, inborn errors of metabolism must be kept in mind in the differential diagnosis of the floppy infants. The priority and selection of diagnostic measures need to be determined on an individualized basis, and multidisciplinary and collaborative work is mandatory to set the diagnosis cost-effectively without delay. An algorithm based and individualized approach may provide a high diagnostic yield for the clinician.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Is fibromyalgia accompanying Behçet's disease more severe than primary fibromyalgia?

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ABSTRACT

Objectives: Although there are studies in the literature about how Behçet's disease is affected in the presence of fibromyalgia, there is no information about how the severity of fibromyalgia is in the presence of Behçet's disease. This study aims to evaluate the severity of fibromyalgia by comparing the impact of fibromyalgia and pain thresholds between fibromyalgia patients with and without Behçet's disease.

Methods: Twenty-five fibromyalgia patients with Behçet's disease and 34 primary fibromyalgia patients were included in this cross-sectional study. All participants completed the fibromyalgia impact questionnaire, and pain thresholds were measured at 18 tender points using algometry.

Results: The mean fibromyalgia impact questionnaire scores in fibromyalgia patients with and without Behçet's disease were 66.6 ± 18.4 and 60.4 ± 14.5, respectively ($p = 0.157$). Pain thresholds were not different between the groups in 15 of the 18 tender points (TP). In fibromyalgia patients with Behçet's disease, pain thresholds in TP-15, TP-16 and TP-17 were 11.44 ± 4.84, 11.92 ± 5.00 and 15.16 ± 4.89 pounds, respectively, while in primary fibromyalgia patients those were 8.41 ± 2.68, 8.14 ± 2.76 and 12.14 ± 4.06 pounds respectively. Pain thresholds in TP-15, TP-16 and TP-17 were significantly different between the groups ($p = 0.003$, $p = 0.003$, $p = 0.014$, respectively).

Conclusions: According to the literature data, although fibromyalgia has an effect on Behçet's disease, especially in cases associated with central sensitization syndromes, the severity of fibromyalgia was not found to be different in fibromyalgia patients with and without Behçet's disease in this study. There may be a relationship between Behçet's disease and fibromyalgia due to non-inflammatory causes such as central sensitization.

Keywords: Behçet's disease, fibromyalgia, pain thresholds

Fibromyalgia (FM) is a common cause of chronic widespread musculoskeletal pain, often accompanied by fatigue, cognitive disturbance, psychiatric symptoms, and multiple somatic symptoms. There are many factors affecting the severity of FM. These include; working status, coping ability, mood disorders, eveningness, inflammation, arterial stiffness, education, and exercise status [1-8]. The majority of the factors affecting the severity of FM are factors that are not based on inflammation. However, in recent studies, it has been found that inflammation-related conditions such as obesity, arterial stiffness, IL-6, and

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IL-8 levels affect the severity of FM [4, 6, 9]. There are also studies showing that FM is associated with inflammation markers, systemic inflammation, and neuroinflammation [10, 11]. Moreover, the frequency of FM in many diseases associated with inflammation is higher than the frequency of FM in the healthy population [12-18]. Although the relationship between FM and inflammation is increasing, and FM is more common in chronic inflammatory diseases, there is not much known about how inflammatory diseases have effects on FM.

Behçet's disease (BD) is an inflammatory disease characterized by recurrent oral aphthous ulcers and numerous potential systemic manifestations. The incidence of FM in BD is higher than in the healthy population, and its frequency ranges from 5.7% to 37.1% [19]. The presence of FM has a negative effect on depression, anxiety, sleep quality, and quality of life in patients with BD [19-21]. However, there is no literature information about how FM is effected in the presence of BD. The aim of this study is to evaluate the severity of FM by comparing pain thresholds and FIQ scores between FM patients with and without BD.

METHODS

Study design

This cross-sectional study was conducted between March 2018 and February 2019. The study protocol was approved by the local Ethics Committee (2011-KAEK-25 2018/03-14), and written informed consents were obtained from each participant. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Selection of the participants

Behçet patients included in this study were selected among patients who met the international classification criteria [22] and were being followed up from the rheumatology outpatient clinic of an education and research hospital, and primary FM patients were selected among the patients followed from the physical therapy and rehabilitation outpatient clinic of the same hospital. Volunteers aged 18-75 were included in this study. A detailed physical examination of all volunteers was performed, and previously performed laboratory tests were examined from electronic medical records, and the medications they used were questioned.

Inclusion and exclusion criteria

Those with a previously known systemic rheumatic or comorbid disease, pregnancy, and depression were excluded from this study. In addition, other than those used in the treatment of BD, those with chronic drug use were not included in the study.

Screening of fibromyalgia

After the first evaluation, the presence of FM in all participants were screened according to the 2013 American College of Rheumatology (ACR) alternative criteria [23]. Alternative criteria were derived from the Symptom Impact Questionnaire (SIQR) symptoms and the 28-area pain location inventory (PLI). In SIQR symptoms, each of the intensities of the typical ten symptoms of fibromyalgia over the last seven days was graded over 10 points by the participants. The symptom impact questionnaire symptoms score was obtained as half of the total score obtained from the SIQR. In PLI, the participants were asked about the 28 specific localizations of the body in which they defined persistent pain in the last seven days. The total number of painful localizations is the pain location score of the patient. A participant fulfilling the following was defined as having FM: SIQR symptom score $\geq$ 21 and pain location score $\geq$ 17. Those with FM according to alternative criteria were included in the study.

Assessment of the participants included in the study

In order to determine the severity of FM in the participants, all participants included in the study filled the FM impact questionnaire (FIQ), and the pain thresholds of all participants were measured.

Fibromyalgia Impact Questionnaire (FIQ)

FIQ is a self-filled test that quantitatively evaluates the functional status, stiffness and pain level, anxiety, and depression status of patients with FM, which was first described in 1991 by Burckhardt *et al.* [24]. FIQ is the most commonly used test to detect the severity of FM. All participants completed the validated Turkish version of the FIQ under the supervision of a researcher [25]. FIQ is composed of 10 items; the

first item consists of sub-items that question how easily some activities in normal daily life can be performed and the responses given to each sub-item were evaluated between 0 (always able to do) and 3 points (never able to do) according to the Likert scale. The second item questions how many days the participant feels good in the last one week, and scoring is based on the number of days he feels bad. The third item questions how many days of the week, the participant is unable to work due to FM. The scores obtained from each of the first three items were standardized according to the 0-10 scale and were then included in the scoring. In the other seven items that were evaluated for scoring, participants evaluated the severity of work status, pain, fatigue, well being, morning stiffness, anxiety, and depression between 0-10 on the Likert scale. FIQ score is the sum of the scores obtained from the ten items (0-100).

Assessment of pain thresholds

In this study, the pain thresholds were determined using dolorimeter at the tender points (TPs) defined in the ACR 1990 classification criteria defined for FM [26]. These TPs consist of 18 points, in which nine sensitive point regions are evaluated on both sides (Fig. 1). These nine sensitive point regions are as follows; suboccipital muscle insertions (TP-1, TP2), anterior aspects of the intertransverse spaces at C5-C7 (TP-11, TP-12), the midpoint of the upper border of the trapezius muscle (TP-5, TP-6), origins of supraspinatus muscle (TP-3, TP-4), second costochondral junctions (TP-13, TP-14), lateral epicondyles (TP-15, TP-16), upper outer quadrants of buttocks in anterior fold of muscle (TP-7, TP-8), posterior to the trochanteric prominence (TP-9, TP-10), medial fat pad proximal to the knee joint line (TP-17, TP-18). Dolorimeter is an instrument that objectively measures pain threshold and pain tolerance. The dolorimeter used in this study (Baseline® Dolorimeters, New York/USA) consists of a metal piston with a round rubber disc about 1 cm in diameter attached to a dial measuring pressures in pounds (Lbs). The user can apply the desired case pressure by holding the handset. Investigators advance the instrument at a rate of approximately 6-8 Lbs per second and instruct the patient to "tell me when this becomes painful." The initial pressure at which the patient felt pain was recorded in pounds. The procedure was repeated three times at intervals of 60 seconds to evaluate the mean pound pressure pain threshold.

Comparisons between groups

Demographic characteristics, FIQ total scores, and pain thresholds measured at the tender points with the dolorimeter were compared between the groups.

Statistical Analysis

SPSS Version 16.0 for Windows (SPSS Inc,

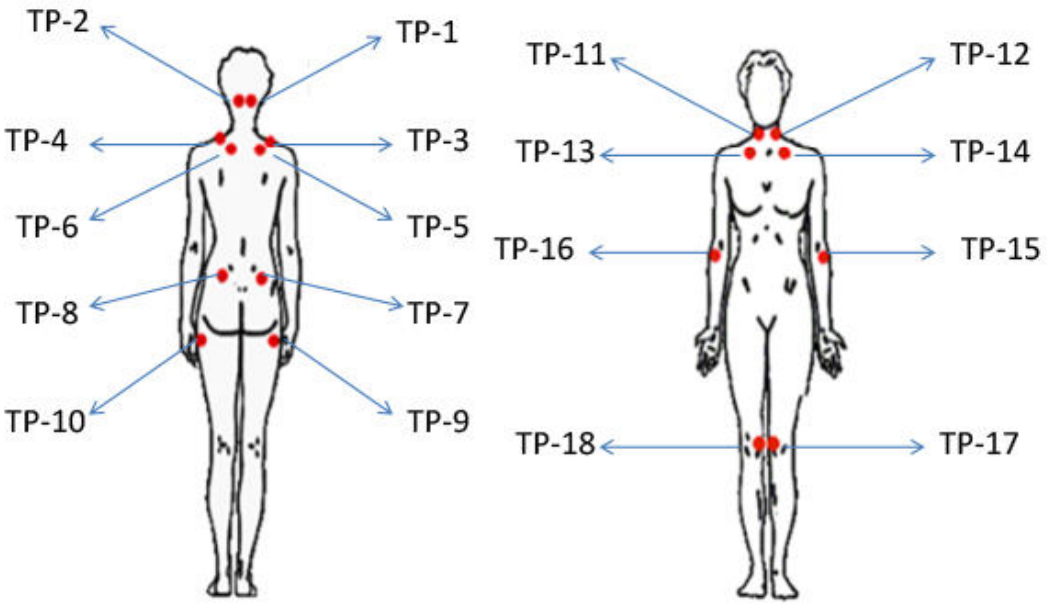

Fig. 1. The tender points where pain threshold levels were measured with a dolorimeter.

Chicago, IL, USA) was used to analyze all data statistically. The data had previously been subjected to a normal distribution test (Kolmogorov-Smirnov). In comparing the quantitative variables, student's t-test was used for the normally distributed variables, and Mann–Whitney U-test was used for the variables that were not normally distributed. For the comparison of qualitative data, Fisher's χ^2 or χ^2 test was used. All tests were two-tailed, and p values < 0.05 were considered to indicate statistical significance.

RESULTS

Ninety-nine participants from the study group, 34 participants from the control group were screened for alternative criteria with fibromyalgia, and 25 participants (19 females, six male) from the study group, and 34 participants (28 females, six male) from the control group were included in the study. Disease-specific features and drugs of patients with BD with FM are shown in Table1. Human leukocyte antigen (HLA)-B51 positive in 14 (56.0%) of Behçet's patients. The comparison of demographic data, PLI, SIQR symptoms, and FIQ total scores of the study and control groups are shown in Table 2. The mean age of patients with BD with FM was 40.0 ± 10.6 years, and the mean age of patients with primary FM was 44.3 ± 8.9 years. Age and gender were not different between the groups (p = 0.095, p = 0.549, respectively). Mean PLI and median SIQ scores were not different between groups (p = 0.636, p = 0.589, respectively). The mean FIQ scores were 66.6 ± 18.4 in the study group, 60.4 ± 14.5 in the control group, and the difference between the groups was not statistically significant (p = 0.157). A comparison of pain thresholds in patients with BD with FM and primary FM patients are shown in Table 3. When compared for each TP individually, the pain thresholds between the groups were not different, except for three of the TPs. The median pain thresholds in TP-15 and TP-16 were 10.0 (minimum-maximum: 3-20 Lbs) and 12.0 Lbs (minimum-maximum: 4-20 Lbs), respectively in patients with BD with FM, and 8.0 (minimum-maximum: 4-14 Lbs) and 8.0 Lbs (minimum-maximum: 4-14 Lbs), respectively in primary FM patients. Pain thresholds in TP-15 and TP-16 were significantly different between the groups (p = 0.003, p = 0.003). The median pain thresholds in TP-17 were 14.0 (minimum-maximum: 7-24 Lbs) in patients with BD with FM, and 11.0 (minimum-maximum: 7-22 Lbs) in primary FM patients. Pain thresholds in TP-17 were significantly different between the groups (p = 0.014).

Table 1. Disease-specific features and drugs of patients with Behcet's disease with fibromyalgia

	Patients with BD with FM (n = 25)
Disease specific features, n (%)	
Oral ulcerations	25 (100)
Genital ulcerations	19 (76)
Erythema nodosum	17 (68)
Acneiform lesions	19 (76)
Pathergy	12 (48)
Eye lesions	4 (16)
Venous disease	3 (12)
Arterial disease	1 (4)
Neurologic disease (Parenchymal)	3 (12)
Drugs, n (%)	
Colchicine	15 (60)
Corticosteroid	10 (40)
Azathioprine	3 (12)
Infliximab	1 (4)
No medication (newly diagnosed)	8 (32)

BD = Behçet's disease, FM = fibromyalgia

DISCUSSION

In this study, we compared the severity of FM in terms of FIQ scores and pain thresholds in FM patients with and without BD, and we did not find the severity of FM generally different between the groups. To the best of our knowledge, there is no study in the literature investigating the effect of BD on the severity of FM, and this is the first study to demonstrate that disease severity is not different in FM patients with and without BD. Very limited data are available on the effects of rheumatic diseases on FM. In the study of

Tablo 2. Comparison of demographic data, fibromyalgia impact questionnaire scores and total number of tender points between patients with primary fibromyalgia and patients with Behçet's disease with fibromyalgia

	Patients with BD with FM (n = 25)	Primer FM (n = 34)	*p* value
Female, n (%)	19 (76.0)	28 (82.4)	0.549
Age, years	40.0 ± 10.6	44.3 ± 8.9	0.095
PLI	32.7 ± 5.9	33.6 ± 7.3	0.636
SIQ	21.0 (16-28)	19.5 (17-28)	0.589
FIQ	66.6 ± 18.4	60.4 ± 14.5	0.157

BD = Behçet's disease, FMS = fibromyalgia syndrome, PLI = pain location inventory, SIQ = symptom impact questionnaire, FIQ = fibromyalgia impact questionnaire, TPs = tender points

Tablo 3. Comparison of pain thresholds in patients with Behçet's disease with fibromyalgia and primary fibromyalgia patients

	Patients with BD with FM (n = 25)			Primer FM (n = 34)			*p* value
pounds	Mean ± SD	median	min-max	Mean ± SD	median	min-max	
TP-1	9.64 ± 4.27	8.0	5-20	10.00 ± 3.01	10.0	6-16	0.275
TP-2	9.28 ± 3.93	8.0	4-18	9.70 ± 3.12	10.0	5-19	0.426
TP-3	12.72 ± 5.2	14.0	4-28	11.67 ± 3.55	11.0	6-20	0.392
TP-4	12.84 ± 5.47	13.0	5-26	10.91 ± 3,26	10.0	4-18	0.097
TP-5	12.80 ± 4.89	13.0	4-22	11.97 ± 3.28	12.0	7-21	0.440
TP-6	13.08 ± 5.52	13.0	4-24	11.79 ± 3.35	12.0	5-18	0.272
TP-7	14.68 ± 5.20	14.0	6-26	14.38 ± 4.87	14.0	5-29	0.823
TP-8	16.00 ± 5.25	16.0	7-26	14.32 ± 4.39	13.5	8-26	0.188
TP-9	15.20 ± 4.98	15.0	5-24	14.14 ± 3.97	13.5	7-24	0.371
TP-10	14.36 ± 4.60	15.0	4-24	13.05 ± 4.45	12.5	6-26	0.279
TP-11	4.96 ± 1.48	6.0	3-8	5.35 ± 1.63	6.0	3-11	0.432
TP-12	5.44 ± 1.98	6.0	3-8	5.55 ± 1.95	5.0	3-11	0.882
TP-13	7.12 ± 3.01	7.0	2-14	8.02 ± 2.43	8.0	3-13	0.203
TP-14	7,44 ± 3.24	7.0	3-14	8.29 ± 2.38	8.0	3-15	0.114
TP-15	11.44 ± 4.84	10.0	3-20	8.41 ± 2.68	8.0	4-14	**0.003***
TP-16	11,92 ± 5.00	12.0	4-20	8.14 ± 2.76	8.0	4-14	**0.003***
TP-17	15.16 ± 4.89	14.0	7-24	12.14 ± 4.06	11.0	7-22	**0.014***
TP-18	13.00 ± 3.92	12.0	6-22	12.55 ± 4.13	12.0	6-21	0.579

BD = Behçet's disease, FM = fibromyalgia, TP = tender point, min = minimum, max = maximum, SD = standard deviation, *$p < 0.05$

De Araújo *et al*., the FIQ scores of FM patients with and without systemic lupus erythematosus (SLE) were compared, and no significant difference was found between the groups [27]. In a prospective study conducted by Jiao *et al*. [28], FM patients with and without rheumatic diseases (undifferentiated inflam-

matory arthritis, rheumatoid arthritis, undifferentiated connective tissue disease, Sjögren's syndrome, SLE, polymyalgia rheumatic, psoriatic arthritis, reactive arthritis, antiphospholipid antibody syndrome) were evaluated prospectively before the fibromyalgia treatment program (FTP) began, and 6 and 12 months after the FTP. As a result, they found that after the FTP, FM patients with rheumatic diseases improved FIQ subscales less than those without rheumatic diseases [28]. In this study, similar to the study conducted by De Araújo *et al*. [27], the FIQ scores were not different in FM patients with and without BD. However, since the treatment response was not evaluated in this study, we do not know how the presence of BD affects the treatment response in FM.

FIQ is the most commonly used test to measure the severity of disease in FM patients, but whether it is indicative of inflammation is not clear. In some studies, it was found to be associated with indicators of inflammation in FM, while in some studies, it was not correlated with the markers of inflammation [9, 29, 30]. When Gunturk *et al*. [29] evaluated the parameters of arterial stiffness in FM patients, they found that the parameters of arterial stiffness were highly correlated with the FIQ score. However, in the study of Ranzolin *et al*. [30], there was no correlation between biomarkers such as IL-6, IL-8, IL-10, TNF-alpha, and FIQ scores in FM patients. Since BD is a systemic inflammatory disease and FM has also found to be associated with inflammation in recent years, we assumed that BD might have an impact on the severity of FM. However, we found that the disease severity of FM patients evaluated by FIQ did not differ according to the presence of BD. Since we do not know to what extent FIQ scores are associated with inflammation in FM in this study, whether there is an association on the basis of inflammation between these two diseases can only be determined by studies that quantitatively investigate inflammatory markers when both diseases are together and not together.

Measurement of pain threshold is another method used in the quantitative evaluation of disease severity in FM. In general, the pain threshold in fibromyalgia is lower than the healthy population, and the main reason for this is exaggerated modulation of the pain stimulus delivered to the brain in FM patients, which is described as central sensitization [31]. Of course, this is true for patients who assume no pathology in the pathways, such as peripheral nerves, where the pain stimulus is delivered to the brain. There is very limited information in the literature about how pain thresholds are affected in FM patients, depending on whether they are accompanied by a rheumatic disease. In a single study from Ostuni *et al*. [32], algometry values were compared between FM patients with and without Sjögren syndrome, and algometry values were found to be lower in primary FM patients. In this study, although the pain thresholds were found to be low in primary FM patients in a few regions as in the study of Ostuni *et al*. [32], in contrast, pain thresholds were not different in the majority of the regions where the assessment was performed. Painful stimulus arising in the periphery is received by specialized nociceptors, and peripheral nociceptive stimulus is transmitted to the central nervous system through the dorsal horn of the spinal column. Finally, after modulating the painful stimulus in the central nervous system, the painful stimulus is perceived by the person. Disorders in the modulation of the stimulus transmitted to the central nervous system, as well as the pathologies that may affect the transmission of the impulse in the peripheral nerves can affect the perceived severity of the painful stimulus. For example, in cases such as central sensitization that causes the amplification of the painful stimulus in the central nervous system, the painful stimulus can be exaggerated, and the pain threshold may decrease as in FM [31]. In cases where peripheral nerves are affected, such as polyneuropathy due to any disease, the pain threshold may increase as the transmission rate will be affected [33]. Central nervous system involvement can be seen in both BD and Sjögren's syndrome [34, 35]. We do not know whether there is peripheral nerve involvement in BD with the literature information so far; however, in Sjögren's syndrome, peripheral nerves are frequently affected [36]. In the study of Ostuni *et al*. [32], higher pain thresholds in the presence of Sjögren's syndrome in FM patients may be related to the effect of Sjogren's syndrome on the peripheral nerves. In this study, the fact that pain thresholds were not different from primary FM patients in FM patients with BD may be due to the fact that peripheral nerves were not affected much in BD. However, in order to test the accuracy of this hypothesis, there is a need for controlled studies in Behçet patients, in which pain thresholds are measured simultaneously with elec-

tromyography tests.

Although the effect of BD on FM has not been studied, there are some studies evaluating the effect of FM on BD. The effects of FM on disease activity in BD are contradictory, and there are studies showing that disease activity in BD did not change in the presence of FM, as well as studies showing that disease activity was increased [19, 20]. In these studies, when the activity of BD was evaluated using a measurement method such as BD current activity form, which includes parameters such as fatigue, joint pain, and headache, which can be seen frequently in FM, disease activity in BD was found to be high in the presence of FM [37]. However, when disease activity was evaluated according to more systemic inflammation-related parameters such as erythrocyte sedimentation rate, C-reactive protein, and clinical activity index, the presence of FM was not associated with disease activity in patients with BD [20]. The effect of FM on BD may be related to the effects of central sensitization related conditions such as pain, fatigue, depression, and anxiety on BD rather than affecting systemic inflammation. As a matter of fact, in the study of Lee *et al*. [20], anxiety and depression scores were higher in Behçet's patients who were accompanied by FM than those who did not. Since the disease most closely associated with central sensitization is FM, the effect of FM on BD can be expected to be higher than the effect of BD on FM. The results of this study support this hypothesis. However, more comprehensive and prospective studies are needed to clarify this issue.

Limitations

This study was conducted in a limited number of patient populations, and further studies are needed to clarify this issue. The disease activity of fibromyalgia was assessed through self-assessed questionnaires and pain thresholds. Perhaps, evaluating arterial stiffness or studying some inflammation markers like IL-6 or IL-8 could provide more objective information about the effect of BD on FM. The fact that we did not investigate disease activity in FM by using objective markers is one of the shortcomings of the study. One investigator made the measurements of pain thresholds. In this study, conditions that may have the potential to affect the severity of FM, including; coping, depression, socioeconomic level, and exercise status, were not compared between the groups. The most important limitation of this study is that the participants included in the study were not evaluated for other conditions that might affect the severity of FM.

CONCLUSION

The results of this study show that the presence of BD does not affect the severity of FM. However, according to literature data, the clinical conditions associated with central sensitization in patients with BD were more severe in the presence of FM. There may be a relationship between BD and FM due to non-inflammatory causes such as central sensitization.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Frequency and predictors of access site complications of the transfemoral diagnostic coronary procedures

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ABSTRACT

Objectives: Access site complications (ASC) remain important and common adverse events after coronary angiography. The aim of this study was to evaluate the frequency and predictors of ASC in patients undergoing diagnostic coronary angiography (DCA) via the femoral artery.

Methods: In this prospective analytical cross sectional study, DCA was performed in 3972 patients by transfemoral approach. The femoral access site hemostasis was obtained by manual compression in all patients. Demographic characteristics of the patients, size of the arterial sheaths, duration of compression time, patients blood pressure, medications, hemoglobin and platelet levels were recorded. All patients were evaluated for ASC (pseudoaneurysm, bleeding, dissection, hematoma). The ASC rate was calculated and ASC predictors were determined by multivariate analysis.

Results: The ASC rate was 1.3% (53 of 3972 patients). The complications were more frequent in female (female: 29 of 53 [54.7%] vs male: 24 of 53 [45.3%]; $p = 0.007$) and chronic renal failure (CRF) patients (103 of 3919 [2.6%] vs 4 of 53 [7.5%]; $p = 0.02$). Hemoglobin level was lower (13.40 ± 1.73 vs 12.86 ± 1.75; $p = 0.02$) and manual compression time was longer (9.19 ± 3.28 min vs 14.53 ± 6.47 min; $p < 0.001$) in the ASC group. Clopidogrel (188 of 3919 [4.8%] vs 7 of 53 [13.2%]; $p = 0.005$) and low molecular weight heparin (LMWH) using were more frequent (37 of 3919 [0.9%] vs 7 of 53 [13.2%]; $p < 0.001$) in the ASC group. According to multivariate analysis; female gender; OR: 2.13, 95% CI: 1.14-3.99 ($p = 0.017$), presence of CRF; OR: 3.15, 95% CI: 1.06-9.29 ($p = 0.038$), manual compression time; OR: 1.23, 95% CI: 1.17-1.30 ($p < 0.001$) and LMWH using; OR: 15.68, 95% CI: 6.05-40.61 ($p < 0.001$) were predictors of ASC.

Conclusions: The incidence of femoral ASC rate was 1.3% in patients with DCA. Female gender, presence of CRF, using LMWH and long manual compression time were predictors of ASC.

Keywords: Vascular access devices, coronary angiography, femoral artery, complication

Over decades, percutaneous coronary angiography has been standard diagnostic strategy for coronary artery disease [1]. Access site complications (ACSs) after diagnostic coronary angiography (DCA) include local site and retroperitoneal hematomas, significant bleeding, peripheral embolization, dissection, aneurysm, pseudoaneurysms, infection, injury to other local structures and arteriovenous fistulas [2, 3]. The common femoral artery has long been the access site for performing coronary angiography and angioplasty. The vascular complication or bleeding risk associated with the transfemoral approach (TFA) is reported up

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to 10% [4, 5]. The use of the transradial approach (TRA) is nowadays more widespread and the international guidelines have recommended radial access as a preferred approach in patients with high bleeding risk. Nevertheless, the radial access takes longer procedural time, with higher radiation exposure and cost than TFA. And also, the use of vascular closure device (VCD) for TFA has increased dramatically. The efficacy of achieving hemostasis with VCD seems similar to traditional manual compression [6]. The use of VCD for TFA, results with procedural cost increases. Additionally, the use of these devices is not without complications [7, 8].

The aim of this study was to identify incidence and predictors of ASCs with TFA and to determine the patients group who will have ASCs with TFA, during DCA.

METHODS

Study Design and Patients

A total of 3972 consecutive patients who underwent elective DCA by TFA over a one−year period (2015 to 2017) were enrolled in to this prospective analytical cross sectional study. The study protocol was approved by the local ethics committee. All the patients provided written informed consent. Exclusion criteria included severe sepsis, local site infection (femoral site), previous contrast allergy, severe intrinsic/iatrogenic coagulopathy (INR > 2), patients DCA performed by TFA in last four week and peripheral vascular disease (iliofemoral disease). Patients who were treated with low molecular weight heparin (LMWH) within the last 12 hours were excluded from the study. Acute coronary syndrome patients, the patients who had planned to undergo ad-hoc percutaneous coronary intervention (PCI) were also excluded. No patient was excluded due to possible femoral complications (obecity, etc).

Each patient was interviewed and examined by a physician before the DCA. The clinical variables included patients' age, sex, body mass index (BMI), presence or absence of hypertension, diabetes mellitus and chronic renal failure (CRF), medications, number of intervention, blood pressure level, and hemoglobin and platelet levels were recorded. The procedural parameters included the size of the arterial sheaths, the peri- or post-procedural use of antiplatelet and anticoagulant, and the duration of compression time after sheath removal were also recorded.

Patients whose systolic blood pressure (SBP) ≥ 140 mmHg, or whose diastolic blood pressure (DBP) ≥ 90 mmHg and patients who use anti-hypertensive medication were accepted as hypertension. Patients were defined as diabetic if fasting blood glucose level was ≥ 126 mg/dL blood on two consecutive measurements or if they used oral antidiabetics/insülin. Patients with an estimated glomerular filtration rate (eGFR) of < 15 ml/min/1.73 m2, or who require hemodialysis or peritoneal dialysis treatment were defined as in CRF. eGFR was calculated with Cockcroft-Gault formula; [(140-age) × body weight (kg)] / [72 × serum creatinine] (if women × 0.85) [9].

Coronary Angiography Procedure

All procedures have been performed by 6 experienced (operator with annual volumes of > 75 PCI procedures) cardiologist at our institution. Either 6F or 7F sheaths were introduced into the femoral artery by using standard modified Seldinger technique. After the procedure, the femoral sheath was removed in the catheter lab. Access site hemostasis was achieved by manual compression in all patients by experienced cath lab staff. Manual compression interval was defined as the time between sheath removal to hemostasis. A sand bag has been applied for 6 hours over access site after hemostasis and patients were followed for occurrence any acute complications at the Cardiology ward. VCD was not used in any patient. No patient was required to switch from TFA to TRA. Access sites was also checked before discharge by performing physician. Vascular Doppler evaluation was performed for patients with suspected ASCs. Both index in-hospital data and post-procedural 2nd week outpatient visit data regarding ASCs were recorded in all patients.

Study Outcomes

The primary endpoint was the occurring of major ASCs. The major ASCs were defined as presence of groin hematoma > 10 cm in diameter, aneurysm or pseudoaneurysm, arteriovenous fistula, significant bleeding, dissection and retroperitoneal hemorrhage. Significant bleeding was defined as blood loss with a

>3 g/dl drop in hemoglobin or a 10% decrease in hematocrit [10]. Aneurysm, pseudoaneurysm and arteriovenous fistula were diagnosed by vascular Doppler ultrasound evaluation. Dissection was diagnosed by conventional angiographic evaluation. Retroperitoneal hemorrhage was diagnosed by computed tomography.

Statistical Analysis

The data were analyzed using the SPSS 10.0 statistics package (SPSS Inc., Chicago, Ill, USA). Continuous variables are reported as means ± standard deviation and categorical variables are reported as percentages. Student's t test was used for comparison of normal distributed variables and Mann-Whitney U test was used for non-normally distributed variables. Categorical variables were compared by the Chi-Square test or Fisher's exact test as appropriate. Univariate and multivariate logistic regression analyses were used to determine significant predictors of ASCs. The sensitivity and specificity of compression time of after sheath removal to predict ASCs was analyzed by receiver operating characteristic (ROC) analysis. *P* values less than 0.05 were considered significant.

RESULTS

Access site complications were observed in a total of 53 (1.3%) patients. The baseline demographic, hematologic, access site characteristics and anticoagulant and antithrombotic medications of the patients with and without ASC were represented in table 1.

The female gender, presence of CRF, clopidogrel use and low molecular weight heparin (LMWH) use

Table 1. Baseline characteristics of the study patients

	Group 1 (n = 3919)	Group 2 (n = 53)	*p* value
Age (years)	59.57 ± 11.90	57.47 ± 11.34	0.20
Gender, n (%)			**0.007**
Male	2482 (63.3)	24 (45.3)	
Female	1437 (36.7)	29 (54.7)	
BMI (kg/m^2)	28.44 ± 4.84	28.10 ± 4.89	0.61
Hypertension, n (%)	1982 (50.6)	28 (52.8)	0.74
Diabetes Mellitus, n (%)	1172 (29.9)	17 (32.1)	0.73
CRF, n (%)	103 (2.6)	4 (7.5)	**0.02**
Systolic blood pressure (mmHg)	141.78 ± 22.55	143.07 ± 23.13	0.68
Diastolic blood pressure (mmHg)	80.28 ± 11.43	79.61 ± 14.13	0.67
Hemoglobin (gr/dl)	13.40 ± 1.73	12.86 ± 1.75	**0.02**
Platelet (× 10^3/ul)	248.04 ± 75.17	236.67 ± 53.46	0.27
Manual compression time (min)	9.19 ± 3.28	14.53 ± 6.47	**< 0.001**
Sheath Size, n (%)			0.36
6F	3858 (98.4)	(100)	
7F	61 (1.6)	-	
Medication, n (%)			
Aspirin	1881 (48)	28 (52.8)	0.48
Clopidogrel	188 (4.8)	7 (13.2)	**0.005**
LMWH	37 (0.9)	7 (13.2)	**< 0.001**

BMI = Body mass index, CRF = Chronic renal failure, LMWH = Low molecular weight heparin. Analyses were performed using Student's t-test or Mann–Whitney U test for between-group comparisons. Categorical variables were compared with Chi-Square or Fisher's exact test.

Table 2. Univariate and multivariate predictors of the access site complication

	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Age	0.98 (0.96-1.008)	0.19		
Gender, female	2.08 (1.21-3.59)	**0.008**	2.13 (1.14-3.99)	**0.017**
BMI	0.98 (0.92-1.04)	0.61		
Hypertension	0.91 (0.53-1.57)	0.74		
Diabetes mellitus	0.90 (0.50-1.61)	0.73		
CRF	3.02 (1.07-8.53)	**0.03**	3.15 (1.06-9.29)	**0.038**
Systolic blood pressure	1.003 (0.99-1.01)	0.68		
Diastolic blood pressure	0.99 (0.97-1.01)	0.67		
Hemoglobin	0.85 (0.74-0.98)	**0.02**	0.94 (0.79-1.11)	0.47
Platelet	0.99 (0.99-1.002)	0.25		
Manual compression time	1.23 (1.17-1.29)	**< 0.001**	1.23 (1.17-1.30)	**< 0.001**
Aspirin	1.21 (0.70 – 2.08)	0.48		
Clopidogrel	3.02 (1.34-6.77)	**0.007**	2.00 (0.79-5.08)	0.14
LMWH	15.96 (6.76-37.67)	**< 0.001**	15.68 (6.05-40.61)	**< 0.001**
Number of intervention 1 versus ≥ c2	1.03 (0.59-1.81)	0.90		

BMI = Body mass index, CRF = Chronic renal failure, LMWH = Low molecular weight heparin. The access site complication predictors were determined with univariate and multivariate logistic regression analysis.

were found more frequent in ASC group. The hemoglobin level was lower and manual compression time was longer in the ASC group (Table 1). The ASCs rate were more frequent in female patients (ASC rate; male: 1% [24 / 2507] vs female: 1.7% [25 / 1465], $p = 0.03$). Sheath sizes were similar in male and female patients (female; 7F / 6F: 19 [1.3%] / 1446 [98.7%] vs male; 7F / 6F: 42 [1.7%] / 2465 [98.3%], $p = 0.34$).

In univariate logistic regression analysis female gender, presence of CRF, low hemoglobin level, use of clopidogrel and LMWH, longer manual compression time were found as predictors of ASCs (Table 2). According to multivariate logistic regression analysis female gender; OR: 2.13, 95% CI: 1.14-3.99 ($p = 0.017$), presence of CRF; OR: 3.15, 95% CI: 1.06-9.29 ($p = 0.038$), use of LMWH; OR: 15.68, 95% CI: 6.05-40.61 ($p < 0.001$) and manual compression time; OR: 1.23, 95% CI: 1.17-1.30 ($p < 0.001$) were predictors of ASC (Table 2).

A ROC analysis was performed to assess the predictive power of the manual compression time for ASCs. The area under the curve was calculated 0.76 and longer manual compression time was related with ASC ($p < 0.001$) (Fig. 1). According to ROC analysis, longer than 10.5 minutes manual compression time had 62% sensitivity and 82% specificity for ASCs.

The distribution of 53 ASCs were; 6 pseudoaneurysm, 2 bleeding (> 3 g/dl drop in hemoglobin), 2 dissection and 43 hematoma (hematoma > 10 cm in diameter). Three pseudo-aneurysm and 3 big hematomas were treated by surgery, the remaining 3 pseudoaneurysm and 40 hematomas were treated by manual compression. Two dissection were treated conservatively, the 2 bleeding (one of them was retroperitoneal bleeding, the other patient bleed out of the groin because sand bag was not correct position) were treated with blood transfusion.

DISCUSSION

The principal findings of the present study are as follows: 1) the femoral ASC rate was 1.3%, 2) female gender, presence of CRF, LMWH using and longer

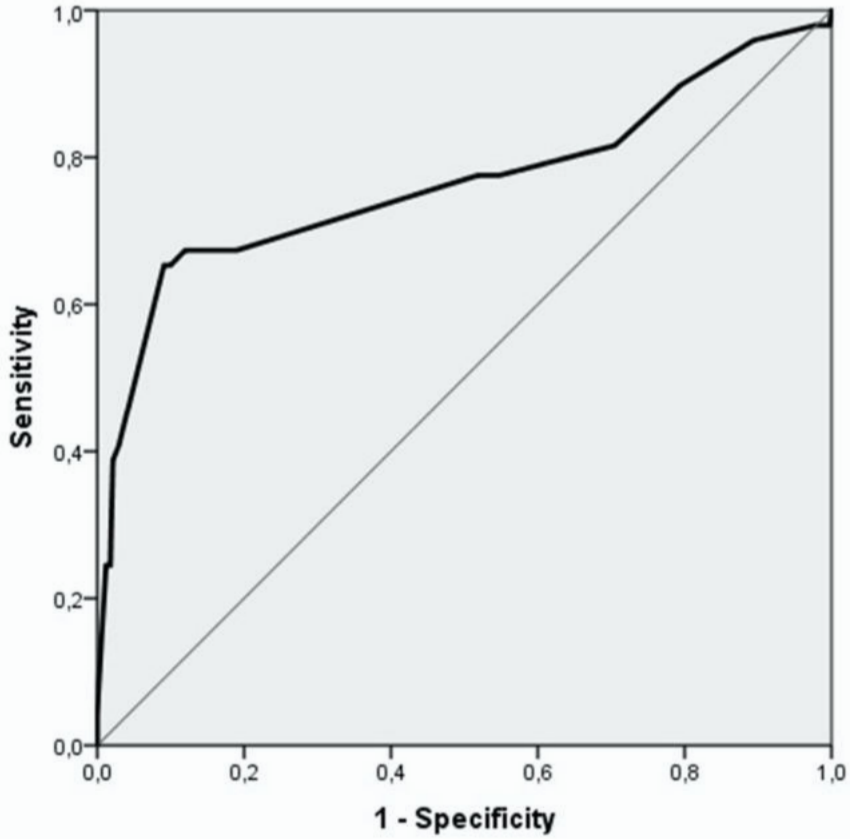

Fig. 1. ROC analysis between the manual compression time and access site complication.

manual compression time were predictors of femoral ASC.

ASC remains an important and common adverse events after DCA and could be concluded with increased risk of morbidity, prolonged duration of hospitalization and procedural costs. In recent years, catheter technologies have improved. So that ASC rate may become lower. According to these knowledge, we decided to assess femoral ASC rate and determine the predictors of ASC after DCA in our experience. In our population (3972 consecutive patients) we performed DCA via TFA, the incidence of ASC rate was 1.3 %. The incidence of ASC rate was consistent with recent studies. While 7-8 F catheters were used in the 1980s, DCA can now be performed using 5-6 F catheters [11]. Over the years, the operator experience has increased, and we enrolled the study only DCA patients. Because of these factors the ASC rate was as low as 1.3%.

In the RIVAL trial, major vascular access complication rate was 3.7 % in the femoral group [12]. However, the RIVAL study population consisted of patients with acute coronary syndrome. The study population was treated with anticoagulant and antiagregan agent. In an other study of acute coronary syndrome, the incidence of entry site complications were found 7.5% [13]. The ASC rate was nearly 5%, pseudoaneurysm and hematoma >5 cm were seen in 1% and 1.5% rate respectively [13]. Low rates of ASC in our study compared to the acute coronary syndrome trials could be explained with study population and medication differences (antiagregan and anticoagulant treatment). Our study population consisted with only DCA patients.

The previous studies demonstrated that risk factors for femoral access complications include female gender, over- and underweight, older age, uncontrolled hypertension, previous catheterization at the same site, high level of anticoagulation, larger arterial sheaths, renal failure, concomitant venous sheath, prolonged sheath duration time [14, 15]. Yatskar *et al*. [16] also showed similar results in their study female sex, concomitant renal disease were significantly associated with access site hematoma requiring transfusion. The incidence of hematoma requiring transfusion was 1.8% and also they found that glyco-

protein IIb/IIIa inhibitors, thrombolytic therapy, and post procedure heparin were more commonly used in hematoma requiring transfusion patients [17]. And also, in our study we demonstrated that the incidence of ASC was related with female gender, CRF, LMWH using and long manual compression time.

In this study we showed that patients who have impaired renal function could tend to be at increased risk of ASC and most of study results were consistent with our study [17, 18]. In renal insufficiency patients, the complications such as hematomas and pseudo aneurysms could be seen more frequently because the hemostatic functions are impaired. The cause of the hemorrhage is platelet dysfunction due to guanidine succinic acid accumulation. This substance causes platelet factor 3 release, platelet adherence and aggregation disorders, and thereby prolonging bleeding time. Likewise the ASC rate was higher with LMWH using, because of the anticoagulant effect.

Female gender was related with higher incidence of ASC, previous studies showed that female gender was an independent predictor of ASC [19, 20]. The differences with gender could be explained with the ratio of sheath size to access artery. The female patients femoral artery are smaller than male patients [21]. The sheath size is an independent predictor of ASC. We use the same size sheath in female and male patients but the ratio of sheath size to access artery is should be higher in female group than male group. The next studies could evaluate the access site femoral artery size to sheath ratio.

Manual compression time is related with the vascular hemostasis of the access site. Anatomical features of access site and coagulation features of the patients are directly affect the vascular hemostasis of the access site. Longer hemostasis time need longer manual compression. The ASC rate is increase with delayed hemostasis. Repeated interventions in the same region may alter the anatomical characteristics of that region, resulting in delayed vascular hemostasis and increased risk of complications. As a result, our study is the first study, showing that manual compression time is a predictor of ASC. Patients who need long compression times to achieve hemostasis can be followed up more carefully and complications could be prevent.

There are different methods to achieve vascular hemostasis after DCA. These methods have not superiority to each other. Previous studies showed that manual compression as effective as mechanical compression for achieving hemostasis and there is no real improvement in clinical outcomes with vascular closure devices when compared with manual compression [22, 23]. The latest trial (The SAFARI-STEMI trial) failed to show that radial access was superior to femoral access in primary PCI [24]. However this is a randomized trial in selected patients trial (female gender, CRF, anticoagulant using) may found superiority of the radial access.

ASC is also related to anatomic risk factors include blind puncture of femoral artery, vessel calcification, placement of venous sheath [22]. Anatomic risk factors could be modified to reduce the risk of complications by using ultrasound scan or fluoroscopy to aid in the optimal puncture site.

Limitations

First; our study population include only DCA patients so that we cannot have data about the percutaneous coronary intervention patients. Second; this study is a single center study and include only one operator team. Third we have not got femoral artery anatomical features, like as femoral artery size and calcification in our study population. Fourth, we used 6F sheath for DCA nearly all patients, therefore we do not have data about smaller and larger size sheath.

CONCLUSION

Incidence of ASC related to DCA in our study was found to be similar to data throughout the world and as low as 1,3%. Female gender, presence of CRF, LMWH using and long manual compression time were independent predictors of ASC. Operators should perform TRA or use vascular closure devices, in patients who had these risk factors to reduce ASC rate.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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A perspective of biometric, visual and refractive outcomes of cataract surgery: a report of an ophthalmologist in compulsory governmental service

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ABSTRACT

Objectives: Knowledge of normal values of biometric parameters in cohort and relationships of them is helpful for ophthalmologists who want to achieve successful results. The aim of this study is to define these parameters in the light of experiences of an individual cataract surgeon after her residency program.

Methods: This is a retrospective, register-based study. Preoperative biometric and postoperative refractive data including axial length (AL), mean keratometry (K), anterior chamber depth (ACD), astigmatism, lens thickness (LT) of 310 patients who had cataract surgery were reviewed. Ultrasound and optical biometry were used to evaluate the biometric parameters of the eyes.

Results: 0.5 D of refractive target (RT) was obtained in 52.1% (186 eyes) of eyes, 1.0 D of RT in 82.20% (293 eyes) and 2.0 D of RT in 98.3% (351 eyes). Analyses of visual acuity show that 43.7% and 86.3% of eyes reached 0.00 and 0.30 log MAR or better. Age, ACD, LT, AL, preoperative corrected distance visual acuity, preoperative astigmatism and preoperative K were correlated between each other significantly ($p < 0.001$). The longer AL was found associated with older age ($\beta = .745$, $p = 0.003$), and lower K ($\beta = -0.327$, $p < 0.001$). A significant association between K value and older age ($\beta = .680$, $p = .029$) and shorter AL ($\beta = -.660$, $p < 0.001$) was seen.

Conclusions: These data show the normative parameters of biometry values for the Turkish population. These results may be helpful in calculating intraocular lens power and evaluating refractive error for young ophthalmologists who have to work with ultrasound biometry in compulsory service.

Keywords: Cataract, biometric parameters, Turkish population, young ophthalmologists

Nowadays, the cataract surgery has taken the first line among the most frequently performed surgeries by ophthalmologists [1]. With the development of biometric devices and surgical techniques, cataract surgery is also considered a refractive form of surgery.

Therefore, it is an essential component of ophthalmology education for residents. The graduates of the ophthalmology residency program complete a number of phacoemulsification cataract surgeries, which vary depending on the clinic they are trained during their ophthalmology residency.

The parameters of ocular biometry including axial length (AL), keratometry (K), corneal and intraocular lens power calculation are known to be essential for

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achieving satisfactory refractive results in cataract surgery [2, 3].

Biometry measurements made with optical methods have higher accuracy than ultrasonic methods. Operator-dependent factors are more prominent in ultrasound biometry (UB) measurements, but not in optical biometry (OB) [4, 5]. Unfortunately, OB devices are still unavailable in manycenter where compulsory service is provided in Turkey.

Although there are many studies that define these main parameters by experienced surgeons, little attention has been paid to studies with new graduates from residency programs.

This study mainly has two purposes. Firstly, this study aims to present over 2-year retrospective review of consecutive series of a young ophthalmologist who has completed her residency program. The other purpose is evaluating the parameters of ocular biometry and relationships of these parameters in the cohort of cataract surgery and contributing to national survey in Turkey.

METHODS

Study Design

All patient charts undergoing corneal phacoemulsification at AdıyamanGölbaşı State Hospital (Turkey) between August 2017 and January 2020 were retrospectively reviewed. All patients were informed about the details of the study in brief and obtained with consent.The study was approved by the local ethics committee.

Study Population

A total of 377 eyes of 324 who were operated within 2 years and had no exclusion criteria were included in the study. The records of patients were obtained for clinical, biometric and demographic data. The criteria for exclusion from the study were determined as < 18 years, >90 years, < 2 0 mm AL, > 30 mm AL, < 35 D preoperative mean K, > 47 D preoperative mean K, corneal astigmatism was > 3 D and aphakia.

All eligible patients underwent a complete ophthalmologic examination with a standard protocol preoperatively and postoperatively including fundus examination, slit-lamp biomicroscopy, corrected distance visual acuity (CDVA) and intraocular pressure measurement preoperatively and postoperative first day, first week and first month.

Surgical Procedure

Surgery was conducted by 1 skilled surgeon who used the same method with Ozil torsional handpiece (Infiniti; Alcon Laboratories, Inc.) for all surgeries. All eyes had phacoemulsification cataract surgery using a standard method consisting of a 2.8 mm clear corneal incision with a 45-degree bevel Kelman con-figuration tip with an ultra-sleeve. Continuous curvilinear capsulorhexis was made using cystotome needle. Hydrodelineation and hydrodissection were performed using a 27-gauge hydrodissectioncannula. The cataract was extracted with a similar prechop and vertical chop technique. Bimanual irrigation/aspiration (I/A) was performed for cortex and viscoelastic removal. The IOL was implanted in the remaining lens capsule and side ports were hydrated. Acrylic aspheric mono focal IOL was used for all patients [Eyecryl Plus HSAS600, Biotech Vision Care] except IOLs implanted in sulcus [which was used 3 pieces IOL Model MN60AC (AlconLaboratories, Inc.).

Data Collection

Collected data included patient clinical history (age-related macular degeneration and diabetes), demographics (gender, age), preoperative measurements like uncorrected visual acuity [UCVA], CDVA, manifest refraction [sphere and cylinder] and K [measurements were obtained with auto refractor keratometer (Topcon KR 8900)], AL [measured by ultrasound with contact method (Nidek US-3300) and by optical method (IOL Master)], and cataract grade [non-dense (grade1-2) or dense (grade 3-4)], procedural details (operated eye, procedure date, IOL power, refractive target), and postoperative outcomes at 1 day and 1 month (manifest spherical equivalent (SE), UCVA, CDVA). The same physician assessed all biometric values.

Visual acuity evaluated with Snellen chart and Snellen UCVA and CDVA were converted to log MAR for analyses. For patients with an AL of below 22 mm the Hoffer Q formula was used and for those 22 mm and over the SRK/T and SRK II formula was used.

Statistical Analysis

TheSPSS Software (Version 22.0, SPSS Inc.)was used for data analysis. The evaluations of visual acuity were based on log MAR units. Continuous variables were presented as mean ± standard deviation (SD). Categorical variables were presented as numbers (n) and percentages (%). The distribution was reached with the Kolmogorov–Smirnov test. Continuous variables were evaluated by Student t-test or Mann-Whitney U test, while categorical variables among the groups were tested by Chi- Square or Fisher's exact test when appropriate. Wilcoxon test was used for comparison of preoperative and postoperative values. The Spearman test was performed to analyze the correlation between parameters. Regression models considering age, gender, K, anterior chamber depth (ACD), cornealastigmatismand lens thickness (LT) were performed to analyze relationships with associated biometric parameters (K, AL and ACD). A P <0.05 value was acceptedsignificant.

RESULTS

Fourteen patients (20 eyes) were excluded from the study because of the missing data, age, biometric and refractive measurements. Patient demographic characteristics, preoperative ocular and biometric features and operative targets are shown in Table 1. At surgery the mean age was 67.5 ± 10.5 years, and 39.4% of patients were female.

Surgical details and complications

Local anesthesia (34.7% subtenon anesthesia and 65.3% topical anesthesia) was used for all operations, except one which general anesthesia was needed. The IOL was implanted in the capsular bag in 96.6% eyes, in the sulcus for 2.5% eyes, and in 0.8% eyes no IOL was implanted (aphakic). Overall 3.3% (95% CI: 3.1-3.5%) cases had a peri-operative complication, the most common being rupture of posterior capsular (PCR) or loss of vitreous or both (1.68%, 95% CI: 1.59-1.92%). PCR was occurred in 6 patients. Peri-operative complication rates were lower for the last six months than first six months but this is not significant statistically ($p > 0.05$). No retinaldetachment and endophthalmitis were seen within 6 months of surgery.

Visual and refractive outcomes

Visual and refractive outcomes of the study patients during the preoperative and postoperative follow up were summarized in Table 1. Preoperative CDVA was 1.40 ± 0.64 log MAR and improved to 0.05 ± 0.06 log MAR at postoperative 1 month follow up. When we look the SE and astigmatism results, preoperative SE was -1.25 ± 5.8 D and changed significantly to -0.04 ± 1.2 D at the postoperative 1 month ($p < 0.05$). Similarly, preoperative astigmatism was -1.8 ± 2.06 D and postoperative astigmatism was -0.73 ± 0.82 D and this change was statistically significant ($p < 0.05$). 0.5 D of refractive target (RT) was obtained in 52.1% (186 eyes) of eyes, 1.0 D of RT in 82.20% (293 eyes) and 2.0 D of RT in 98.3% (351 eyes) postoperatively.

Biometricparameters

Histograms showing the distribution of measured K, AL, ACD and corneal astigmatism values are given in Figu 1. The mean AL found as 23.32 ± 0.87 mm. 15 (4.2%) eyes had an AL < 22.0 mm, 319 (89.3%) between 22.0 and 24.5 mm, 20 (5.6%) between 24.5 and 26.0 mm and 3 (0.8%) > 26.0 mm. Male eyes had more non-dense cataract grade than female eyes ($p < 0.001$); Nevertheless, there was no statistically significant difference when looking at other parameters. Subgroup analysis was also performed according to UB and OB of IOL. The achieved refraction results were not found statistically significant in UB or OB ($p > 0.05$), and when the two different of IOL measurement methods were compared, no statistically significant differences was seen.

Correlations

Correlations were constructed for age, visual outcomes and ocular biometric parameters. Age, ACD, LT, AL, preoperative CDVA, preoperative astigmatism and preoperative K were correlated between each other significantly ($p < 0.001$). No significant correlation was found when looking at the other parameters ($p > 0.05$). Correlations are summarized in Table 2.

Regression models

The models of regressions were assessed for K, AL and ACD in regarding to gender, age, LT, ACD, K, and Corneal astigmatism. A longer AL was associated

Table 1. Baseline characteristics of the study patients

Demographics	Total	Male	Female
Age	67.5 ± 10.5	66.13 ± 10.7	69.46 ± 9.6
Gender		216 (60.6%)	141 (39.4%)
Side			
Right	177 (49.7%)	109 (50.4%)	68 (48.5%)
Medical and Ocular History			
Diabetes	32 (8.9%)	19 (8.7%)	13 (9.2%)
ARMD	7 (1.9%)	5 (2.3%)	2 (1.4%)
Preoperative Ocular Characteristics			
Cataract Grade			
Dense Cataract	147 (41.1%)	69 (31.9%)	78 (55.3%)
Non-dense Cataract	210 (58.8%)	147 (68.1%)	63 (44.7%)
CDVA (Log MAR, median)	1.40 ± 0.64	1.27 ± 0.64	1.60 ± 0.57
Intraocular Tonus	16.92 ± 2.5	17.10 ± 2.7	16.63 ± 2.3
Preop SEQ (D)	-1.25 ± 5.8	-0.67 ± 6.02	-2.20 ± 5.3
Preop Astigmatism (D)	-1.8 ± 2.06	-1.65 ± 2.11	-2.14 ± 1.94
Preoperative Biometry Measurements			
Biometry Type			
Manuel Biometry	299 (83.8)	174 (80.6%)	125 (88.7%)
Optic Biometry	58 (16.2%)	42 (19.4%)	16 (11.3%)
AL (mm)	23.32 ± 0.87	23.38 ± 0.85	22.93 ± 0.84
K (D)	43.60 ± 1.79	43.20 ± 1.69	44.22 ± 1.78
ACD (mm)	3.41 ± 0.21	3.55 ± 0.24	3.21 ± 0.19
Corneal Astigmatism (D)	0.87 ± 0.51	0.83 ± 0.49	1.15 ± 0.60
Lens Thickness (mm)	4.14 ± 0.4	4.18 ± 0.44	4.09 ± 0.39
Operative Details			
Intraoperative Complications	15 (4.2%)	9 (4.1%)	6 (4.2%)
Postoperative Complications	1 (0.02)	1 (0.46%)	0 (0%)
IOL Power	21.81 ± 1.82	21.65 ± 1.86	22.06 ± 1.75
Postoperative Measurements			
Postop SEQ (D)	-0.04 ± 1.2	-0.20 ± 1.32	0.30 ± 1.05
Postop Astigmatism (D)	-0.73 ± 0.82	-0.67 ± 0.83	-0.88 ± 0.80
CDVA (Log MAR, median)	0.05 ± 0.06	0.03 ± 0.5	0.9 ± 0.7

SD = standard deviation, N = number, ARMD = Age related macular degeneration, CDVA = Corrected distance visual acuity, log MAR = log of the minimum angle of resolution, Preop = preoperative, SEQ = spherical equivalent refraction, AL = Axial length, K = keratometry, ACD = Anterior chamber depth, IOL = Intraocular lens. Values are presented as Median or Mean ± SD / N (%)

Table 2. Correlations of age, visual outcomes and ocular biometric parameters of study population

		R	*p* value
Age			
	ACD	-.675	< 0.01
	LT	.859	< 0.01
LT			
	ACD	-.532	0.019
	AL	-.570	0.011
Preoperative CDVA		.459	< 0.01
	Preoperative Astigmatism		
AL		-.518	< 0.01
	Preoperative K		

R = Correlation coefficient, *p* = Significant correlation (0.05), ACD = Anterior chamber depth, LT = Lens thickness, AL = Axial length, CDVA = Corrected distance visual acuity, K (D) = mean Keratometry.

with older age (β = .745, *p* = 0.003), and lower K (β = -0.327, *p* < 0.001). When we look at K, we found a significant association with older age (β = .680, *p* = 0.029) and shorter AL (β = -.660, *p* < 0.001). No significant relationship was found with the other parameters analyzed.

DISCUSSION

The present study has been conducted for a few purposes. The primary aim in carrying out is to reflect the experience of a young ophthalmologist in cases of cataracts encountered in compulsory service after residency training. In addition to, It is aimed to reach the desired refractive error target after cataract surgery. For this purpose, investigations considering current status of using different ocular biometry methods on refractive results of phacoemulsification surgery are insufficient in the literature. So, this study also aimed

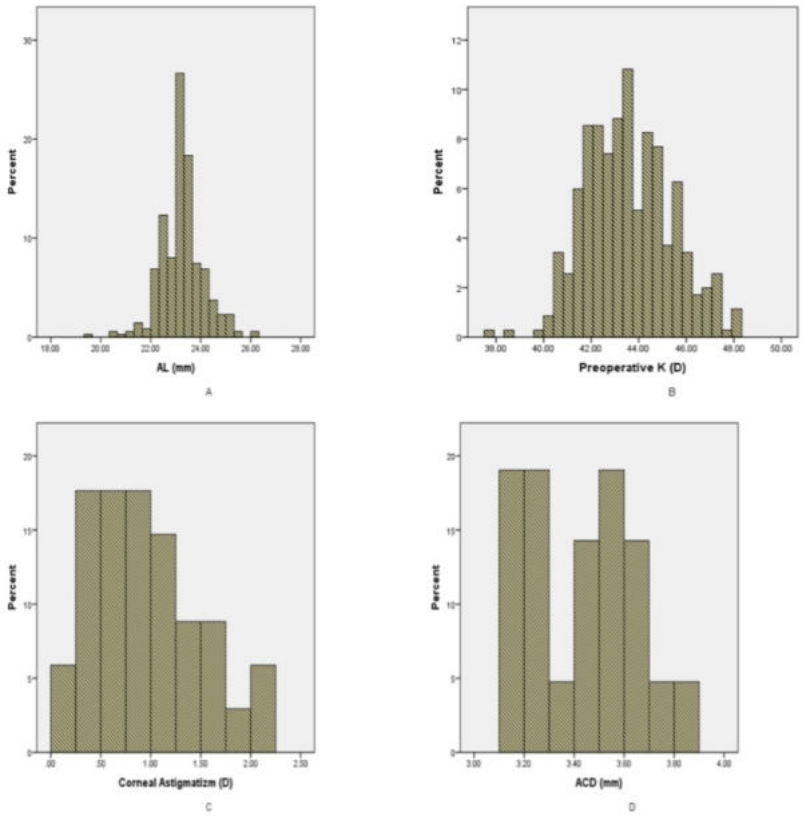

Fig. 1. A. Histogram of axial length (AL) of the study population, B. Histogram of mean keratometry (K) of the study population, C. Histogram of corneal astigmatism of the study population and D. Histogram of anterior chamber depth (ACD) of the study population.

to presents mainly the average values of the biometric parameters, which assessed using OB and UB and secondly refractive and visual outcomes of candidates for phacoemulsification surgery in Turkish population.

In terms of visual and refractive outcomes, it was seen that a statically significant improving of all parameters that we evaluated had stabilized at 1-month postoperatively. The analyses of postoperative visual acuity demonstrated that 43.7 and 86.3% of all eyes achieved 0.00 and 0.30 log MAR or better. And, the analyses of postoperative refractive target showed 52.1%, 82.20% and 98.3% all eyes were within 0.5 D, 1.0 D and 2.0 D respectively. Taken together, these results are comparable to those reported in The Royal College of Ophthalmologists' National Ophthalmology Database study of cataract surgery and Cataract National Dataset [6, 7]. Cataract surgery is not free of risk. In our study procedure-related complication rate is 3.3%. These results appear to be compatible with the literature [8]. Over the study period, the rate of complications appears stable. Because of lower number of complication, it is not possible to make a meaningful comparison to detect predictors.

In the AL analysis, the mean AL was 23.32 ± 0.87 and also, AL had a non-normal distribution with high kurtosis. Longer AL was associated with lower corneal refractive power and thinner LT. These findings are in agreement with the data obtained in The Ural Eye and Medical Study (23.30 ± 1.1 mm) [9], in the Los Angeles Latino Eye Study (23.4 ± 1.1 mm) [10], and in the Beijing Eye Study (23.25 ± 1.14 mm) [11]. Interestingly, there was no significant difference between the genders in terms of AL.

The mean K in our study was 43.60 ± 1.79D as shown in Table 1, mean K was not significantly different in terms of gender. Mean K increased linearly to older age. However, this association was not statistically significant. The distribution of the K has been reported in different age groups and populations. When comparing K values with other populations, our value is lower than that reported in the Portugal population [12], mean K was 43.91 ± 1.71 D but higher than that reported in the Iranian population, was 43.48 D (95% CI: 43.41-43.56) [13]. Our findings are similar to those reported in the Canadian population, mean-K was 43.85 D and USA, mean K was 43.41 ± 1.60 [14].

In our study population, the mean corneal astigmatism value was 0.87 ± 0.51 D, with 58.8% of the eyes showing astigmatism < 1 D, in other words, almost half of eyes has corneal astigmatism value which is enough to prevent optimal visual acuity without optical correction. The current results did not identify significant differences between female and male patients. Our result is not surprising and is supported by previous studies, which conducted by Hoffmann and Hütz [15] in Germany (64%), by Ferrer-Blasco *et al*. [16] in Spain (65.2%) and Duman *et al*. [17] in Turkey (70.1%). And also, this current report did not detect any associations between corneal astigmatism and other biometric variables and age. This may be the result of the limited sample size.

The mean ACD in our population was 3.41 ± 0.21mm and ACD were negatively correlated with age and LT ($p < 0.05$). These findings described also in a study that conducted by Zong *et al*. [18] in China. On the other hand, because of its relationship to primary angle-closure glaucoma (PACG), correlation between ACD and age is not surprising. It is known that age is one of major predisposing factors for the development of PACG [19]. The current results also agreed with study, which conducted by Ferreira *et al*. [12] in Portugal. Our result was in contrast to the cross-sectional analysis of the Blue Mountains Eye Study in which ACD was not related with age [20].

Although LT was not previously used as a variable in calculating IOL power, fourth-generation formulas like Olsen began computing taking this into account [21]. For this reason there may be noticeable trend on impact of LT in future studies. The mean LT in our study was 4.14 ± 0.4 mm and agreed with the literature. Another finding of this study was the positive relation between age and LT and in contrast, negative correlation between ACD, AL and LT. More extensive studies may be needed on these findings.

Limitations

The current study had some strengths and weaknesses. One of the strength is that the data were aggregated and non-selective. Another strength is that all operations were made by the same surgeon and, so less publication bias than multiple-surgeon case series. A weakness of our study is that this is a retrospective, register-based study. Another weakness is that the

study sample size had limited number and did not include very long eyes (AL, >3 0.0 mm) or short eyes (AL, < 20.0 mm). Therefore, the number of peripheral groups for subgroup analysis was inevitably small.

CONCLUSION

In this study, data regarding preoperative biometric and postoperative refractive were reviewed in 310 patients undergoing cataract surgery. The results of our analysis showed that (1) There was no statistically significant difference on achieved refractions prediction when comparing the UB and optical methods of IOL measurement, (2) Male eyes had more non-dense cataract grade than female eyes, (3) Because of associations between each other pre-operative ocular biometryparameters played an important role in IOL power selection and patient assessment. The present study is the first report which providing valuable information about operation volumes of an individual cataract surgeon after her residency program. It also should be remembered that this study is one of few to present normative parameters of biometry and their relationships in Turkish population. The author expects this study will be helpful for future works.

Conflict of interest

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The European Research Journal 2020;6(6):655-660 Original Article

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An approach to diagnosing brain death in patients undergoing extracorporeal membrane oxygenation

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ABSTRACT

Objectives: This study was intended to discuss the process of brain death diagnosis of patients undergoing extracorporeal membrane oxygenation (ECMO) and our approach regarding the existing literature.

Methods: Demographics data (age, gender, admission diagnosis) were noted. ECMO type applied (veno-venous or veno-arterial), time of diagnosing brain death (the time from admission time till diagnosis), being a donor or not, apnea testing application, supplementary tests applied at diagnosis stage (cerebral angiography, computerized tomography angiography, electroencephalography, transcranial doppler ultrasonography), and time of cardiac death (the time elapsing from the moment of brain death diagnosis till cardiac arrest) were noted.

Results: Forty-two patients data were exaimed and ECMO was applied to 8 patients, 4 of whom are female and the others are male. The average age of the patients is 61.8 ± 9.8 years. The average time elapsing from the hospitalization till brain death diagnosis was 2.5 ± 0.5 days. Apart from that, only 2 (25%) of the patients were donors.

Conclusions: The use of modified apnea testing on patients undergoing ECMO could be proliferated if such tests are standardized and reliable guidelines are set. For this reason, we think that cerebral angiography should be kept in mind in addition to apnea test especially on ECMO-treated patients.

Keywords: Brain death, apnea test, extracorporeal membrane oxygenation, cerebral angiography

Brain death is a condition that involves the complete absence of voluntary movements, response to stimuli, consciousness, lower brain stem functions and spontaneous breathing accompanied by prolonged and permanent loss of all brain activity without reversible conditions. Apnea testing is a key component for diagnosing brain death. Apnea testing is regarded to yield a positive result if there is lack of spontaneous respiratory effort and increasing PaCO2 pressure above 60 mmHg or an increase equal to or above 20 mmHg from the initial value [1]. Although apnea testing application is determined with a standard protocol, there are many modified tests which is used different methods in the literature. During apnea testing, many unwanted complications might occur, at varying levels, such as hypoxia, hypotension, barotrauma, pneumothorax, and cardiac arrest [2, 3]. For all these reasons, apnea testing should be performed after optimum conditions are met and the patient should be closely monitored.

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Extracorporeal membrane oxygenation (ECMO) is a procedure used to provide oxygenation in the case of cardiogenic shock and respiratory failure. It has two types as veno-venous and veno-arterial ECMO. Veno-venous ECMO is often applied to patients who suffer from an acute respiratory failure such as refractory hypoxia (acute respiratory distress syndrome [ARDS], etc). On the other hand, veno-arterial ECMO is most suitable for sudden cardiac failure cases (after cardiac arrest, acute heart failure, post-cardiac surgery, etc). Nowadays ECMO is used with increasing frequency especially in medical centers where cardiac surgery is performed and, positive reports are shared about on its effectiveness [4]. Although it is not prevalent in the literature, there is no consensus on the protocol of apnea testing to be applied in patients who are undergoing resuscitative ECMO in intensive care units.

This study was intended to discuss the process of brain death diagnosis of patients undergoing ECMO and our approach regarding the existing literature.

METHODS

In the study, once consent was obtained from the local ethics committee (ref: 66/04, 28/06/2019), 42 patients data who were diagnosed with brain death from 2017 to 2019 were retrospectively reviewed. Eight of the patients, were included in the study as they were administered ECMO (Fig. 1). However, the patients for whom a written consent could not be obtained from their family and those who did not treat with ECMO were excluded from the study. A set of basic information was noted about the patients including demographics such as age and gender as well as admission diagnosis, ECMO type (veno-venous or veno-arterial), time of diagnosing brain death (the time from admission time till diagnosis), being a donor or not, apnea testing application, supplementary tests applied at diagnosis stage (cerebral angiography, computerized tomographic angiography, electroencephalography, transcranial doppler ultrasonography), and time of cardiac death (the time elapsing from the moment of brain death diagnosis till cardiac arrest).

RESULTS

The patients' main data such as demographics (age and gender), admission diagnosis, type of ECMO, being a donor or non-donor, supplementary testings,

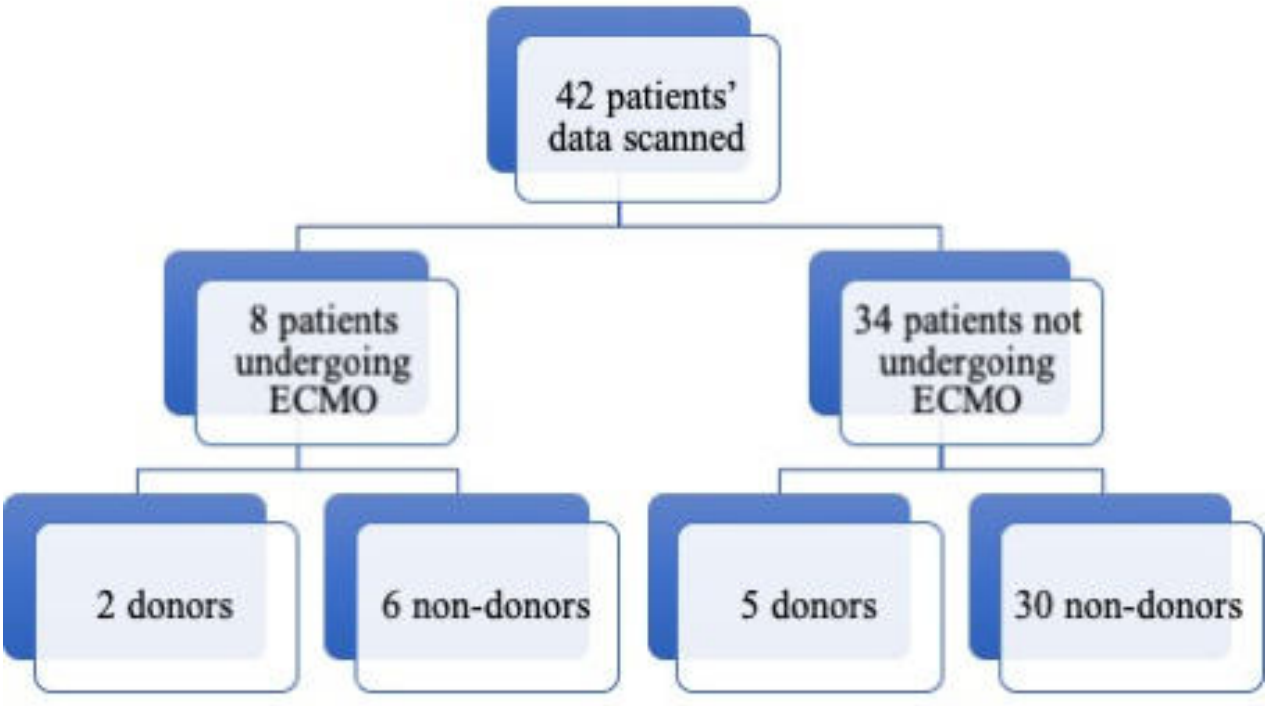

Fig. 1. Flow chart diagram about distribution of the cases.

Table 1. Patients' characteristic data

Age (years)	Gender	Admission diagnosis	ECMO indication	ECMO type	BD diagnosis time (days)	Supplementary testings	Time of arrest (days)	Donor
67	Female	Dissection	Cardiovascular failure	V-A	3	EEG, CA	2	No
58	Female	CHD	Cardiovascular failure	V-A	2	EEG, CA	1	No
42	Male	Cardiac trauma	Cardiovascular failure	V-A	3	CA	1	Yes
69	Male	Dissection	Cardiovascular failure	V-A	2	CA	1	No
74	Male	Aorta Aneurysm	Cardiovascular failure	V-A	2	EEG, CA	2	No
67	Female	Mitral and aortic valve replacement	Cardiovascular failure	V-A	3	CA	1	No
58	Female	Dissection	Cardiovascular failure	V-A	2	CA	1	Yes
60	Male	Acute MI	Cardiovascular failure	V-A	3	EEG, CA	2	No

ECMO = Extracorporeal membrane oxygenation, BD = Brain death, CHD = Coronary heart disease, MI = Myocard infarctus, V-A = Veno-arterial, EEG = Electroencephalogram, CA = Cerebral angiography

and time of cardiac arrest are given in Table 1. ECMO was applied to 8 patients, 4 of whom are female and the others are male. The average age of this patients is 61.8 ± 9.8 years. In addition, V-A ECMO was applied to all this patients because of cardiovascular failure after cardiac surgery. With regards to diagnostic supplementary tests, four of the patients underwent electroencephalography as well as cerebral angiography, and the other four underwent only cerebral angiography. The average time from admission to the diagnosis of brain death was 2.5 ± 0.5 days. Only 2 (25%) of this eight patients were donors.

DISCUSSION

The process of brain death diagnosis is important due to several reasons. In a study in Europe suggested that 2-3% of all patients dying in a hospital and 14% of those dying in intensive care units result in brain death [5]. Firstly, the diagnosis should be completed in the most appropriate time and as soon as possible. All kinds of delays that may occur during the diagnosis and the decision stage affect organ donation adversely. Diagnosis of brain death in some specific cases is known to be difficult and time-consuming. Particular caution is required in cases where the gas exchange is carried out externally and stopping or interrupting the Exchange endangers the patient's life (ECMO, carbon dioxide absorbers, etc.). ECMO is an advanced technique which is often applied as the last chance in cardiac and respiratory failure. However, many neurological complications, especially brain death, occur during the performance of it. The diagnosis of brain death does not come into mind so fast in ECMO patients. Therefore, in such cases, the main goal of the diagnosis should be to shorten the time and keep the patient more stable. One component of brain death diagnosis, apnea testing, is an indispensable part of the diagnostic process. Despite its crucial value, apnea testing can cause many complications including cardiac arrest [2, 3]. Besides, the diagnosis of brain death by apnea testing in ECMO patients involves a difficult and complex process. Especially in veno-arterial ECMO, application and interpretation of the apnea testing become even more difficult due to both gas exchange and circulatory support [6]. Since there is no valid guideline published on this subject, practices are limited to clinical

experience. For all these reasons, apnea testing was not administered no patients who was treated with ECMO in this study.

Apnea testing is a recommended and obligatory test at the diagnosis stage of brain death. Although modified methods have recently been used to prevent complications related to apnea testing, those testings that was applied especially in ECMO patients are mostly at the level of case presentation [7]. So far, apnea testing has been tried by adding exogenous CO_2 to ECMO [8], monitoring the respiratory effort by applying continuous positive pressure [9], and reducing the sweep gas flow [10, 11]; nonetheless, no successful apnea test has been found in all cases presented. For example, Giani *et al*. [12] found that the rate of severe hypoxia during modified apnea testing was 8% in the ECMO-treated group, it was 2.4% in the control group. Furthermore, those in the ECMO group required higher FiO_2, higher PEEP, higher tidal volume, and higher airway pressures than patients in the non-ECMO group. In a study where modified apnea testing was applied successfully; the patient was taking triple inotrope and although the apnea testing was completed, hemodynamic changes or arrhythmias were not noted during or after the testing [10]. Like the apnea testing, reliability of biomarkers such as BIS, transcranial Doppler, or glial fibrillary acidic protein (GFAP) was not fully established [13-15]. Absence of cerebral circulation is important for the diagnosis of brain death. Cerebral angiography is a procedure that directly demonstrates the absence of cerebral blood flow. Duly performed cerebral angiography and carotid bifurcation or absence of flow above the Willis polygon is particularly important for the diagnosis [16]. In the current study, all patients with treated ECMO were had unstable hemodynamic values and treated with vasoactive medications. Considering all these reasons; cerebral angiography, which is the gold standard in the diagnosis of brain death, was performed in patients who underwent ECMO. No complications were observed during the procedure.

Brain death is a clinical diagnosis, yet the diagnosis criteria vary from one country to another. In a study, it was pointed out that supplementary testing is imposed as a prerequisite for the diagnosis of brain death in 40% of all countries [17]. However, no study has been published on supplementary tests for patients going through ECMO, only with findings at a certain extent. Bronchard *et al*. [18], apnea testing was not performed on 62.5% of donors who went through ECMO before diagnosing brain death. In that study, 24 patients were subjected to apnea testing but the testing could not be finished with 10 of them. As for the supplementary testings' in brain death, 53% of the ECMO-treated patients went under computerized tomographic angiography and 39% experienced electroencephalography. By contrast, in the group which was not applied ECMO, it apnea testing was noted to be performed successfully by 71.9% [12]. In the present study, all of the subjects undergoing ECMO and diagnosed with brain death are patients who were hemodynamically unstable following cardiac surgery performed with veno-arterial ECMO. None of the patients were treated with apnea testing, without prejudice to cerebral angiography on all. The reason could be the motivation to prevent complications (hypoxia, arrhythmia, etc) in ECMO-treated patients with unstable hemodynamics as a result of apnea testing and, more importantly, the lack of a valid set of guidelines published on this matter.

In patients undergoing extracorporeal membrane oxygenation, concerns about the chance of being donors are at least as important as brain death diagnosis. In an example study investigating brain death in ECMO-treated patients in relation with eligibility for being donors, 10,869 of total 22,270 patients donated at least one organ, leading to being donors in ECMO-treated and the control group by 39.8% and 48.9%, respectively ($p = 0.02$) [18]. Additionally, it was reported that the transplantation team demanded organs from ECMO-treated patients less frequently. However, no difference was found between the groups after one year's follow-up in terms of renal graft survival and functions. It was added that ECMO-treated patients who were diagnosed with brain death can also make good donors [18]. In another study, Fan *et al*. [19] regarded ECMO as a step for preserving organs of patients diagnosed with brain death; consequently, they reported improved results on liver and kidney transplantation (an increase from 47.8% to 84.6%). Moreover, in case presentations by Lee *et al*. [20].

ECMO was performed on 3 patients diagnosed with brain death and 11 organs from these patients were transplanted on 10 patients successfully.

However, ethical issues might arise in connection with patients for whom organ donation consent could not be finished and who are currently under ECMO during brain death. In our study, only 2 (25%) of the ECMO-treated patients became donors. This figure corresponds to a lower level than the overall literature, which might be accounted for by cultural and religious beliefs among different nations as well as other attitudes.

CONCLUSION

As a conclusion, although several modified apnea testings were applied for brain death during ECMO, cerebral angiography remains a definite method in this regard. The use of modified apnea testing on patients undergoing ECMO could be proliferated if such tests are standardized and reliable guidelines are set. For this reason, we think that in addition to apnea test, cerebral angiography should be kept in mind, especially on ECMO-treated patients with unstable hemodynamics variable.

Authorship declaration

All authors listed meet the authorship criteria according to the latest guidelines of the International Committee of Medical Journal Editors, and all authors are in agreement with the manuscript.

Author contributions

SA, İBA, SÇ = conception and design; SA, SÇ, FKA, CÖÇ = data collection; FKA, CÖÇ = analysis and interpretation; SÇ, SA, İBA, JE = literature review and writer, and JE = critical review.

Conflict of interest

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The European Research Journal 2020;6(6):661-664 Original Article

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Is hospitalization necessary in isolated traumatic sternal fractures?

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ABSTRACT

Objectives: Clinical characteristics, follow-up and treatment outcomes of patients with isolated traumatic sternal fracture were presented and our clinical experiences on these patients were shared.

Methods: Between January 2010 and December 2017, a total of 87 patients with isolated traumatic sternal fracture who were admitted to the emergency department and were hospitalized, were included in this observational cohort study. Medical data of these patients were collected from hospital records and then were retrospectively reviewed.

Results: There were 65 (74.7%) male and 22 (25.3%) female patients. Mean age was 42.4 ± 13.7 years (range: 17-83). The most common etiological reason was traffic accident in vehicle. Sternal fractures were localized at corpus in 64 (73.6%) patients and at manubrium in 23 (26.4%) patients. The most common concomitant pathology was rib fracture with a ratio of 23% (20 patients). Mean length of hospital stay of patients was 3.1 ± 0.8 days. During the hospitalization period, no evidence of cardiac injury was observed in any patient.

Conclusions: In view of rising healthcare costs and increasing demand for acute hospital and intensive care beds, it is crucial to determine hospitalization criteria for cases with traumatic sternal fracture. We believe that the hospitalization is not necessary in isolated traumatic sternal fractures where there is no other major injury.

Keywords: Sternal fracture, trauma, treatment, hospitalization

Sternal fractures are encountered with a 3% to 8% prevalence in blunt thoracic trauma cases [1]. These fractures are seen frequently in passengers in the front seats of vehicles involved in accidents are increasingly observed in recent years since the compulsory usage of safety belts [2, 3]. Pain in the sternal area and extreme sensibility on palpation are the most frequent symptoms and their presence should prompt suspicion of a sternal fracture. Sternal fractures are usually on a transverse direction and usually upper and mid portion of the sternum is involved. Physical examination reveals sensibility at the fracture site, eccyhymosis and crepitation on palpation of the injured area. Usually a lateral chest x-ray is sufficient for radiological confirmation of a suspected sternal fracture. Thoracic CT which is a more sensitive radiological technique is resorted when the diagnosis is not certain or when accompanying pathologies are anticipated [4]. The localization and structural properties of the sternum lends itself as an important barrier for the vital organs like the heart, great vessels and the lungs. This said the injuries to these vital

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organs due to sternal fractures are more important than the sternum fracture itself.

Isolated sternum fracture is by definition a traumatic sternal fracture without accompanying cranial fracture, intracranial hemorrhage, hemothorax, pneumothorax, intra-abdominal organ damage or any other kind of major injury [5, 6]. There is no universally agreed upon consensus regarding the evaluation of the sternum fractures nor on the algorithm of the treatment of them [7]. Naturally the evaluation and treatment is dictated by each center's experience and their equipment which leads to a variety of approaches among different centers.

In this study, patients who were admitted to the emergency department with sternum fracture and hospitalized were analyzed and their clinical and demographic features, and follow up and treatment outcomes were presented, our clinical experiences with this patient group were shared. Also, in view of our experience with this patient population that we had hospitalized, the merits of hospitalization were discussed.

METHODS

Over an 8-year period between January 2010 and December 2017, a total of 87 patients with isolated sternum fractures who were admitted to the emergency department of a tertiary referral center in Turkey and later hospitalized are included in this retrospective observational cohort study. Patients who were diagnosed as isolated sternum fractures after physical examination, laboratory and radiological studies at the emergency room were admitted to the Cardiovascular Surgery intensive care unit and ward where the patients were observed and treated. Emergency room registrations, forensic registrations and clinical documents were used to determine the patients' age, gender, type of the trauma, localization of the fracture, symptoms, employed diagnostic tools and treatment modalities, treatment outcomes, hospitalization duration and other minor pathologies accompanying the traumatic sternal fracture. The patients' hemodynamic parameters as arteriel blood pressure and heart rate were monitorized and electrocardiogram (ECG) monitorization was performed, furthermore cardiac damage indicators such as creatinine kinase (CK), creatinine kinase-myocardial band (CK-MB) and troponin-I levels were all periodically measured. Patients with suspected cardiac damage were evaluated with echocardiography. The treatment mainly consisted of the alleviation of pain, bed rest and treatment of associated other diseases if present. Patients with hypovolemic or hemorrhagic shock, multi-organ trauma, a history of cardiac disease such as coronary artery disease and congestive heart failure and had severe comorbid conditions (malignancy, hepatic and renal insufficiency, etc.) were excluded from the study.

The study protocol was approved by the institutional ethics committee, and the study was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical Analysis

All statistical analysis of our study was done using Statistical Package for Social Sciences (SPSS) program (version 20.0, SPSS, Chicago, Illinois, USA). Continuous variables were shown as average ± standard deviation, while categorical variables were expressed as frequency and percentage.

RESULTS

The average age of patients was 42.4 ± 13.7 years, with an age range of between 17 and 83 years. Sixty five (74.7%) of the patients were male, while 22 (25.3%) of the patients were female. When the type of trauma and etiological causes of the sternum fractures were evaluated, the most common cause was found to be in-vehicle traffic accidents with 44 (50.6%) cases (Table 1). The remaining causes were falling in 30 (34.5%) cases, workplace accidents in 5 (5.7%) cases, animal induced trauma in 4 (4.6%) cases, vehicle induced trauma in 2 (2.3%) cases and assault (battering) again in 2 (2.3%) cases. While all patients had pain and sensitivity with palpation on sternum, only 19 (21.8%) patients displayed an eccyhymosis and/or crepitation with palpation. When the patients were evaluated radiologically; fractures were seen at the corpus sterni in 64 (73.6%) patients (Fig. 1) and at the manubrium of sternum in 23 (26.4%) cases. The most frequent pathology accompanying sternal fracture was rib fracture which was detected in in 20 (23%) cases.

Table 1. Clinical characteristics of patients

Characteristics	Data
Age (years) (mean ± SD)	42.4 ± 13.7
Gender, n (%)	
Male	65 (74.7)
Female	22 (25.3)
Etiological factors, n (%)	
In-vehicle traffic accident	44 (50.6)
Falling	30 (34.5)
Workplace accident	5 (5.7)
Animal induced trauma	4 (4.6)
Vehicle induced trauma	2 (2.3)
Assault	2 (2.3)
Fracture localization, n (%)	
Corpus sterni	64 (73.6)
Manubrium sterni	23 (26.4)

Average length of hospital stay was 3.1 ± 0.8 days. During the hospitalization, none of the patients displayed hemodynamic instability, major changes on ECG, cardiac enzymes levels, and echocardiographic finding to indicate cardiac injury.

DISCUSSION

The most important finding of this study was that in our study population none of the patients with isolated traumatic sternal fracture had apparent traumatic cardiac injury. Sternal fracture presents itself in a conscious patient with direct or indirect pain over the sternum. Physical examination may reveal irregularity and/ or step-up deformity of the sternum on palpation. Eccyhmosis, hematoma and contusion may be observed in inspection. Posterior-anterior, lateral and oblique sternum x-rays are essential in cases of suspected sternal traumas [8, 9]. In our study population 65 (74.7%) patients were diagnosed solely with direct radiography. In cases with uncertain diagnosis and/or suspected additional pathologies, computed tomography was performed and 22 (25.3%) patients in whom sternal fracture could not be verified by direct radiography were diagnosed as such.

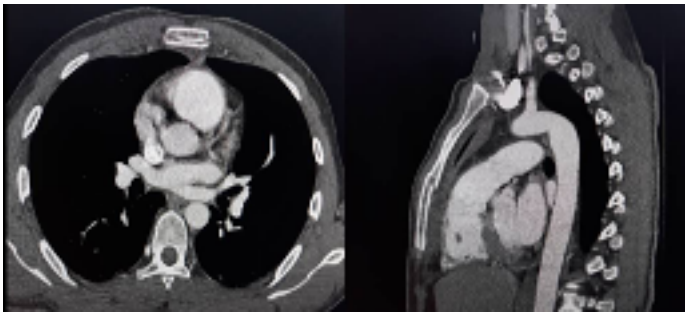

Fig. 1. Fracture on corpus sterni

Treatment of patients with sternal fracture consists of bedrest in supine position, pain relief and respiratory physiotherapy. Pain can persist for six weeks. Complete sternal stabilization with conservative management generally ensues in two months [10]. Sternal fracture rarely necessitates surgical treatment. If sternal stability has been disrupted sternal fixation is required. In some patients in which severe paradoxical respiration evolves stabilization can be performed surgically. Due to prominent visible compression surgical repair may be warranted on cosmetic grounds [11-13]. In any case no patient in our study required surgical stabilization nor correction. We recommended only bedrest and analgesic therapy to our patients on discharge.

Pericardial tamponade, mediastinal hematoma and abscess, osteomyelitis due to delayed healing can be seen in sternal fracture. Furthermore patients should be carefully evaluated for myocardial contusion and pulmonary parenchymal injuries. Upon admission ECG ,CK CK-MB values of the patients should be evaluated. In cases with suspected myocardial injury, the most convenient and time sparing diagnostic method is echocardiographic assessment [1, 14-16]. In our study group no patient had myocardial damage.

Duration of hospitalization in sternal fracture is related rather to the associated traumatic pathologies than to the sternal fracture itself. Isolated sternal fracture generally has a benign course and usually heals on its own. Analgesic therapy and respiratory physiotherapy are thought to be sufficient in isolated SF. If accompanying intrathoracic pathologies are present these pathologies determine the prognosis of the patients [2, 5, 15-18].

Limitations

The most important limitation of our study was that it was designed as a retrospective data analysis with a relatively small sample size in a single-center.

Another important limitation was the lack of the follow-up data.

CONCLUSION

The results of our study have demonstrated that the isolated sternum fractures are benign entity. Considering the ever increasing health care expenses and the demand for the hospital beds both of wards and intensive care units; establishing the criteria for hospitalization of patients with traumatic sternum fractures is very important. We believe that in patients diagnosed as isolated sternum fracture without any coexisting major injury in the emergency room do not warrant hospitalization. We suggest that this patient group can be treated at home with medical recommendations and organizing home therapy.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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