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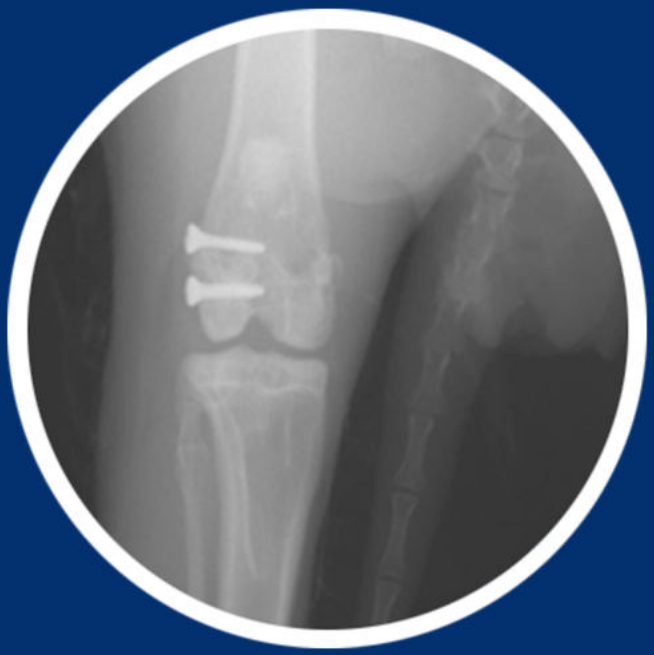

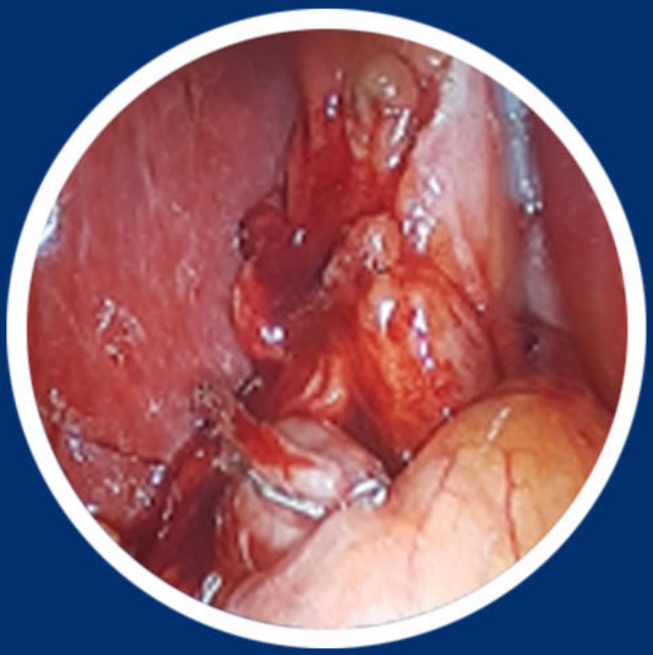

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Table of Contents

Original Articles

Case Report

The European Research Journal 2020;6(5):373-379 Original Article

DOI: 10.18621/eurj.554642 *Anesthesiology & Reanimation*

Comparison of neuroprotective effect of isoflurane and sevoflurane on cerebral ischemia

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ABSTRACT

Objectives: In this study, we aimed to evaluate the effects of isoflurane and sevoflurane on cerebral ischemia in patients undergoing intracranial tumour surgery by measuring protein S-100B in serum. Patients undergoing intracranial surgery are at risk for cerebral ischemia. The presence of S100-B in serum is an early and the most sensitive determinant of cerebral ischemia.

Methods: Twenty patients, scheduled for elective, intracranial tumor surgery were enrolled in this prospective and randomized study. Anaesthesia induction was performed with thiopental, fentanyl and vecuronium. In the maintenance, isoflurane or sevoflurane was administered in a minimum alveolar concentration of 0.8-1.2%. Peripheral blood samples were taken at 9 different times to measure protein S-100B levels.

Results: Demographic data, heart rate, systolic arterial pressure, mean arterial pressure and the deviations in the end-tidal carbon dioxide were similar in the study groups ($p > 0.05$), whereas diastolic arterial pressure was found to be significantly decreased in isoflurane group after the intubation, and there was an increase in the sevoflurane group ($p < 0.05$). Also there was no significant difference between the groups regarding protein S-100B levels.

Conclusions: It was concluded that isoflurane and sevoflurane have similar neuroprotective effects against cerebral ischemia and sevoflurane may be a good alternative to isoflurane.

Keywords: protein S-100B, intracranial tumor, inhalational anaesthetics

Patients undergoing intracranial surgery are at risk for cerebral ischemia. Therefore, the aim of neuro-anaesthetics is to provide adequate cerebral perfusion during surgery [1]. In patients with increased intracranial pressure (ICP), due to cerebral tumors it is accepted that volatile anaesthetic agents are effective in the protection of cerebral ischemia that may develop due to decrease in systolic arterial pressure (SAP) [2].

Isoflurane's cerebral protection mechanisms include reduction of cerebral metabolic rate (CMR) and metabolic suppression, inhibition of sympathetic activity, reduction of glutamate receptors which are prevent calcium flow, and suppression of excitotoxicity of calcium cascade [3]. It is thought that isoflurane may be secondary to direct vasodilatation or to reduction in CMR by the increase in cerebral blood flow (CBF) reduction [4]. Isoflurane has been reported to cause cerebral protection similar to barbiturates by depressing CMR, and it was shown that it reduce cere-

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bral energy metabolites more slowly than other anaesthetics during incomplete global ischemia and critical cerebral blood flow. In addition, isoflurane, similar to other volatile anaesthetics, reduces CMR in the cerebral cortex and electroencephalography (EEG) findings supports it is neuroprotective effects [4].

Sevoflurane's capacity to increase CBFwhile maintaining the cerebral autoregulation makes it an attractive agent for the preservation of neuronal function. In addition, the positive effect of sevoflurane to stabilize CBF and cerebral metabolism may be an important reason for choosing it as a neuroprotective agent when compared to other anaesthetics [5]. In protection against cerebral ischemia, sevoflurane is determined to decrease the damage that occurs with focal cerebral ischemia and the CMR, the reduction in local CBF has been found to be lower than isoflurane [6, 7]. Isoflurane and sevoflurane are shown to significantly reduce the cerebral infarct area and improve neurological function after cerebral ischemia [8].

Cerebral ischemic damage is a complex mechanism. Energy metabolism disorder, oxygen free radical damage, inflammatory reactions and apoptosis are the some of mechanism [9]. During cerebral ischemia, the cerebral blood barier (BBB) is damaged due to endothelial cell death. Cytosolic content released from damaged brain tissue has the potential to pass through the damaged BBB. This shows that proteins release from the brain into the plasma can be used to determine the onset and size of cerebral ischemia [10].

Ideal biochemical serum markers should be very specific and sensitive in central nervous system (CNS) injuries, and after the damage, they should only be released from the CNS tissues and mixed into the blood stream by passing through the BBB [11]. Different biochemical substances were investigated to find an spesific indicator of cerebral cell damage. S-100B is a protein that binds to calcium, mainly found in the brain grey matter, especially in astrocytes and Schwann cells [12].

There is very little information about how the S-100B passes through the BBB and is mixed into the blood. The molecular weight of the S-100B (21,000 Dalton) is high, which limits it to pass through the BBB. Therefore, the increase in S-100B is probably due to the combination of astroglial cell membrane integrity, BBB dysfunction and early brain edema [13, 14]. S100B has been shown in serum after brain injury [15, 16]. The presence of S100-B in serum is an early and the most sensitive determinant of cerebral ischemia, which is known today [16-18]. Some studies have evaluated serum S100B during surgical manipulation; after aneurysm surgery [19] and elective meningioma resection [20]; increased serum S100B levels were reported to correlate with poor neurological outcome and post-craniotomy brain damage.

The aim of this study was to compare the effects of isoflurane and sevoflurane on cerebral ischemia in patients undergoing intracranial tumor surgery by measuring protein S-100B levels.

METHODS

After obtaining approval from local ethics committee, 20 patients from ASA (American Society of Anaesthesiologists) I-III class, who were to be operated under elective conditions due to intracranial tumor were enrolled in our study. Exclusion criteria were refused to participate, previous intracranial mass surgery, emergency surgery, patients who developed air embolism during the operation or who had a history of chemotherapy, stroke, cardiopulmonary resuscitation and head trauma. Patients with chronic renal failure (creatinine >200 mmol/I) were also excluded due to potential interference. Patients were randomly allocated to receive either isoflurane (Group I, n = 10) or sevoflurane (Group S,. n = 10) using sealed enveloped. Peripheral arterial catheter was inserted after electrocardiography (ECG), peripheral oxygen saturation (SpO_2) and non-invasive blood pressure monitoring in the operation room. Following general anaesthesia induction and intubation, a central venous pressure catheter inserted.

Study data and medical records were collected prospectively. The demographic data of patients, heart rates (HR), SAP, diastolic arterial pressure (DAP), mean arterial pressure (MAP) and SpO_2 baseline values were recorded before induction of general anaesthesia. During surgery, HR, SAP, DAP, MAP, SpO_2, central venous pressure (CVP), end-tidal carbon dioxide (EtCO2) were monitored continuously. The measurements were made in 12 periods. They were recorded before induction of anaesthesia (BI), after intubation (AI), during skull clamping (SC), during skin incision (SI) during craniotomy (CT), during dura

incision (DI), during tumor resection (TR), during dura suture (DS), during the bone placement (BP), during skin suture (SS), before extubation (BE), and after extubation (AE).

In order to suppress sympathetic response, 2 $mg.kg^{-1}$ lidocaine and for sedation 0.03 $mg.kg^{-1}$ midazolam was given intravenously (i.v.). Anaesthesia induction was performed with sodium thiopental (3-5 $mg.kg^{-1}$), fentanyl (2 µg kg^{-1}) and vecuronium bromide (0.1 $mg.kg^{-1}$). In the maintenance, the volatile anaesthetic (0.8-1.2 MAC) was given in a mixture of 50% oxygen-air according to the study group. The need for additional doses of muscle relaxant agent was determined according the response to the train-of-four. Vecuronium bromide (0.02 $mg.kg^{-1}$) and fentanyl (1 $\mu g.kg^{-1}$) were administered i.v. as required .At the end of the operation, patients were extubated and transferred to Neurosurgical Intensive Care Unit.

S-100 B Measurement

Venous blood samples were taken at 24 hours before surgery (BS), before anaesthesia induction (BI), during tumour resection (TR), recovery room (RR) and at postoperative 3rd (P3), 6th (P6), 12th (P12), 24th (P24) and 48th (P48) hours. The blood samples were centrifuged for 10 minutes, the serum was separated and the samples were coded and stored at -50ºC for further processing. After all the samples were collected, serum was brought to room temperature to measure S-100B. For measurement, the Nexus DX™ S-100 test kit was used. Enzyme immunoassay (ELISA) technique was used to measure S-100B in serum with this kit. The Nexus DX™ S-100 test kit is highly specific for β subunit of the protein S-100 and is sensitive over 95%.

Statistical Analysis

Statistical analysis of the study data was performed with the Statistical program pack for the Social Sciences 21 (SPSS, Harmony, New York, Il, USA) by the Department of Biostatistics, all data were expressed as average ± SD. The Mann-Whitney U test was used for statistical analysis. Statistical analysis was performed using Wilcoxon series comparisons. In all statistical analyses, two-way hypothesis tests and a level of significance for $p < 0.05$ were accepted.

RESULTS

There was no statistically difference in terms of demographic data and duration of surgery between the two groups ($p > 0.05$), (Table 1). When we compared groups, there was no significant difference in terms of HR. When we compared the two groups, the changes in SAP and MAP were similar, while it was observed that the DAP only decreased Group I, and an increased Group S after intubation ($p < 0.05$) (Fig. 1). There was no significant difference between the groups regarding CVP and SpO_2 values.

In Group I, there was a significant increase in S-100B levels in all periods except the BI period ($p < 0.01$). In Group S, S-100B levels significantly increased in the periods of IA, RR, P3, P6, P12, P24 ($p < 0.05$, $p < 0.01$, $p < 0.05$, $p < 0.05$, $p < 0.01$, $p < 0.05$; respectively) (Fig. 2).

DISCUSSION

In intracranial tumor surgery, the anaesthetic

Table 1. Demographic data of the patients and duration of surgery

	Isoflurane Group (n = 10)	Sevoflurane Group (n = 10)
Gender (M/F)	3/7	4/6
Age (year)	54.6 ± 18.4	44.4 ± 15.0
Weight (kg)	67.4 ± 11.9	69.7 ± 12.4
Length (cm)	164.7 ± 11.0	169.2 ± 8.3
Duration of the surgery (min)	373.0 ± 102.8	300.0 ± 104.8

Data are shown as mean ± standard deviation or number. M = Male, F = Female

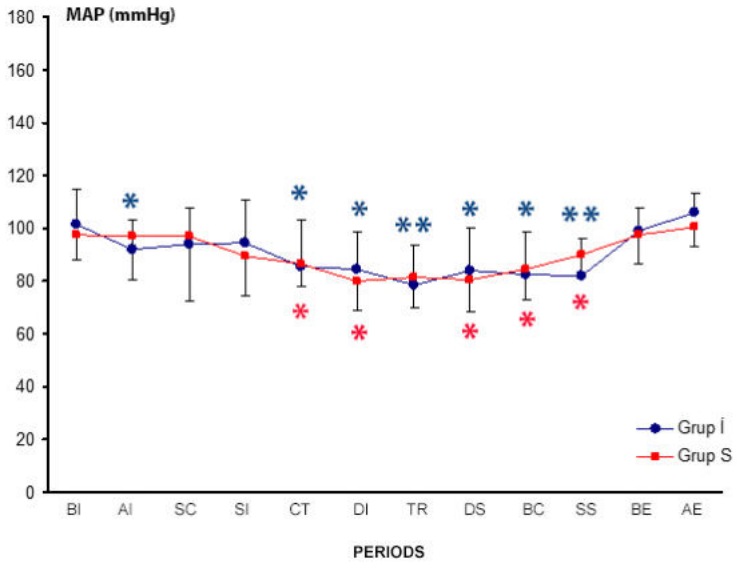

Fig. 1. Mean arterial pressure (MAP) values. Group I = Isoflurane group, Group S = Sevoflurane group, BI = before anaesthesia induction, AI = after intubation, SC = during skull clamping, SI = during skin incision, CT = during craniotomy, DI = during dura incision, TR = during tumor resection, DS = during dura suture, IB = during bone closure, SS = during skin suture, BE = before extubation, AE = after extubation. *$p < 0.05$, **$p < 0.01$
Group I = Isoflurane group, Group S = Sevoflurane group,

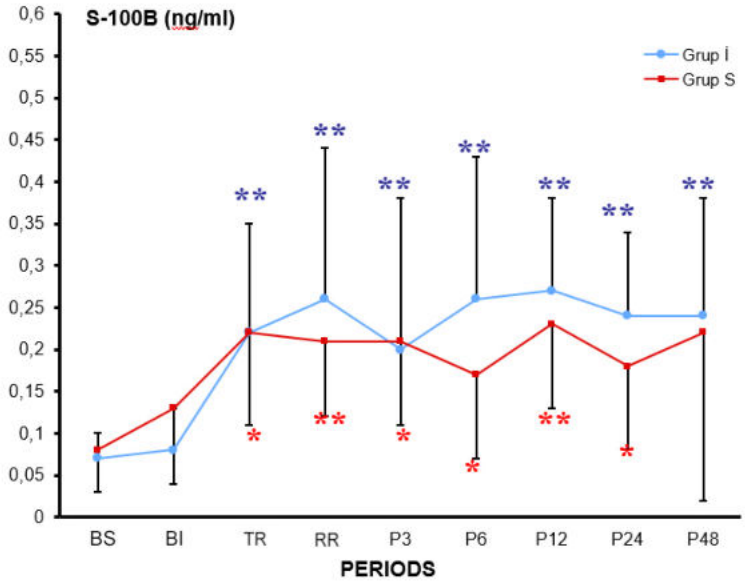

Fig. 2. Serum S-100B levels. Group I = Isoflurane group, Group S = Sevoflurane group, BS = 24 hours before surgery, BI = before anaesthesia induction, TR = during tumour resection, RR = in the recovery room, P3 = postoperative 3th hour, P6 = postoperative 6th hour, P12 = postoperative 12th hour, P24 = postoperative 24th hour, P48 = postoperative 48th hour. *$p < 0.05$, ** $p < 0.01$

approach involves certain objectives. These objectives are protecting the brain from ischemia, providing adequate hemodynamics to maintain the CPP maintaining the balance between oxygen delivery and the consumption of the brain. The cerebral autoregulation mechanism, is to keep CBF stable while SBP is between 50 and 150 mmHg, protects the brain against sudden changes in arterial pressure. Therefore, the effect of anaesthetic agents on cerebral autoregulation becomes important.

Regarding the hemodynamic parameters, Negargar *et al*. [21], in their study DAP, SAP and MAP in the sevoflurane group were significantly lower than the isoflurane group. This might have been due to the effect of sevoflurane on cardiorespiratory reflexes. Our study showed that there was significant difference only DAP after intubation between the two groups. We could not make sense of this differece. This difference was clinically unimportant because HR, SAP and MAP were on normal ranges.

Sevoflurane, isoflurane, as compared with that provide more rapid induction and recovery advantages provided similar hemodynamic stability and less airway is considered to be a significant clinical potential for doing irritation [22]. The direct vasodilator effect of sevoflurane is considered to be 20% of isoflurane. This weak and vasodilator effect of sevoflurane may help explain why cerebral vasculature remains stable in response to changes in CPP during anaesthesia of sevoflurane, but this is disturbed due to extensive cerebral vasodilatation in isoflurane anaesthesia at the same concentration [21]. In addition, because of this weak vasodilation effect, sevoflurane is likely to cause a marked increase in ICP. This is a preferred feature for agents used in neurosurgical anaesthesia [22]. According to Artru *et al*.'s study [23] there is no increase in ICP during 0.5-1.0 and 1.5 MAC sevoflurane anaesthesia. Therefore, the protection is less in isoflurane because of vasodilatation. Summors *et al*. [24], in their study, found that cerebellar autoregulation was better in 1.5 MAC sevoflurane than 1.5 MAC isoflurane anaesthesia, and as a result, sevoflurane could bebe a better neuro-anaesthetic agent.

The chain of events that occur during cerebral ischemia results in neuronal death. The most common of these is the release of exitotoxic neurotransmitters. Reduction of the release of transmitters constitutes the mechanism of the drugs acting as neuron preservatives. The effect of sevoflurane on the release of neurotransmitters such as dopamine, glutamate and aspartate has been investigated by Toner *et al*. [25] They reported that when sevoflurane was administered at an average concentration of 1.7 MAC, it reduced release of neurotoxic transmitter and thus had a neuroprotective effect [25].

Martens *et al*. [26] have studied the role of serum S-100B and (Neuron-specific enolase) NSE in the recovery of consciousness after global cerebral ischemia. They reported that serum S-100B, as an independent biochemical marker, could provide information about acute global cerebral ischemia after brain injury, and that the results of serum S-100B were valid and reliable [26].

The release of cytochrome enzymes from the brain as a result of global cerebral ischemia shows widespread neuronal damage. After the acute injury, the S-100B is found in the serum for 24 hours and for 48 hours in the cerebro spinal fluid (CSF) [26]. There are also correlations between the volumetric measurements of the parenchymal destruction of the central nervous system (CNS) and first sample of the S-100B after brain injury. Therefore, S-100B concentrations should be interpreted within the scope of in damage-time relationship [27].

Two pathways have been proposed in the S-100B's transition from glial cells in the CNS to peripheral blood. The first is the absorption of CSF from CNS the veins. The second way is directly from the extracellular area to the local capillary. In their study, De Vries *et al*. [28] suggested that the second pathway was the main entrance due to the absence of the relationship between the cysternal and serum S-100B. Thus, they suggested that the increased concentration of serum S-100B showed dysfunction of the blood-brain barrier [28].

In our study, the first significant increase in S-100B levels found during tumor resection due to neuron damage. Although S-100B levels were higher in isoflurane group compared to sevoflurane group, but there was no statistically significant difference between the two groups. At the postoperative 48th hour, significant increases were observed in the isoflurane group, whereas the high levels in the sevoflurane group were not statistically significant. it was concluded that sevoflurane may have a protective effect on cerebral ischemia due to low levels of S-100B compared to isoflurane group.

In our study, we found that the protein S-100B levels did not decrease as expected in the postoperative period, we considered that it might be due to the developing brain edema due to retraction, as Vries *et al*. [28] pointed out. In addition, if intracranial mass is large or if there is bleeding that will cause hypotension during the operation, may be responsible for the increase in S-100B.

Limitations

The limitation of our study wasthe small number of patients. In our study we used special laboratory kit. Because of our limited financial resource we could not increase the number of patients.

CONCLUSION

As a result, we consider that in addition to isoflurane, which is one of the preferred volatile anaesthetic agents for intracranial tumor surgery, sevoflurane has similar effects on cerebral ischemia and can be used as a good alternative to isoflurane.

Conflict of interest

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

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

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The AKT antagonist AZD5363 suppresses features associated with cancer progression in human larynx cancer cells

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ABSTRACT

Objectives: Larynx cancer (LCa) represents approximately 30% of all cancers seen in the head and neck region, with an unchanged overall survival rate over the last decades. Although several novel diagnostic and therapeutic options has been developed, an effective treatment strategy is not currently available due to the high metastatic and recurrent potential of LCa. In this study, we aimed at investigating the inhibitory potential of AZD5363 on the phenotypes associated with LCa progression *in vitro.*

Methods: The impacts of AZD5363 on the proliferation, colony formation, and apoptosis potentials of HEp-2 cells were tested using Cell Viability Detection Kit-8, soft agar assay and Annexin V-FITC Apoptosis assay, respectively. Migration features of cells were evaluated using scratch and transwell migration assays.

Results: We showed that AZD5363 increased phosphorylation of AKT and inhibited the phosphorylation of its downstream effector GSK3β in an *in vitro.* LCa model in line with the findings of previous studies carried out with different cancer types. Besides, AZD5363 successfully suppressed proliferative, clonogenic, and migratory features of HEp-2 cells through induction of apoptosis.

Conclusions: We revealed putative functions of AZD5363 *in vitro.* that points its potential to be used as an adjuvant agent against LCa. However, further comprehensive molecular and clinical research is needed to elucidate the potential use of AZD5363 in LCa therapy in detail.

Keywords: Larynx cancer, AZD5363, AKT, chemotherapy

Larynx cancer (LCa), as a common reason for cancer-related mortality worldwide, represents approximately 30% of all cancers seen in the head and neck region [1, 2]. Almost 40% of the LCa patients do not visit doctor until the last stage of the disease [3] and without treatment, patients have only 56.4% and 26.5% overall 1- and 2-year survival rates, respectively [4, 5]. Besides, although several novel diagnostic and therapeutic options such as surgery, radiotherapy, and chemotherapy are applicable for LCa patients, an effective treatment strategy is not present due to its high metastatic and recurrent potential [6]. Therefore, there is an urgent need to develop new treatment strategies and novel adjuvant therapies especially against advanced LCa to enhance the life expectancy and survival rates of LCa patients.

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Phosphoinositide 3-kinase/serine-threonine protein kinase B (also known as AKT)/mammalian target of rapamycin (PI3K/AKT/mTOR) signaling pathway is involved in numerous key processes including cell proliferation, apoptosis, and migration [7]. Abnormal activation of this signaling pathway in various types of cancers is quite common [8]. Loss of phosphatase and tensin homolog deleted on chromosome 10 (PTEN), the negative regulator of the PI3K/AKT/mTOR pathway, is a frequent event leading to activation of AKT signaling in various tumors such as breast, prostate, and larynx cancers [9-12]. AKT, which is the center of this signaling network, was reported to be overexpressed or activated in many malignancies [13, 14]. Abnormal activation of AKT was related to resistance to anti-cancer drugs, poor survival and advanced disease [15]. In LCa, AKT2 was found to be strongly positive in cancerous and pericancerous tissues, although no protein expression was reported in normal laryngeal epithelium. AKT2 positivity in protein level was also significantly related to tumor site, lymph node metastasis and clinical stage [16]. In another study, elevated AKT3 expression was related to shorter overall survival of LCa patients [17]. Therefore, PI3K/AKT/mTOR pathway might serve as a crucial axis for development of novel treatment strategies against LCa.

AZD5363 [(S)-4-amino-N -[1-(4-chlorophenyl)-3-hydroxypro pyl]-1-(7H-pyrrolo [2, 3-d] pyrimidin-4-yl) piperidine-4- carboxamide] is a novel new-generation drug with inhibitory potential against AKT 1, 2, and 3 [18]. AZD5363 was found to suppress phosphorylation of AKT substrates including GSK3β in various cell lines and rodent xenografts models [18]. It has been shown to suppress the progression of various human tumor xenografts when administered alone or in combination with chemotherapeutics [19]. AZD5363 is currently being investigated in phase I clinical trials in patients with advanced solid tumors [20, 21]. However, its activity against LCa cells is not yet documented.

We, therefore, examined the inhibitory potential of AZD5363 on the phenotypes associated with LCa progression *in vitro* We demonstrated that AZD5363 increases phosphorylation of AKT and inhibits its downstream effector GSK3β in an *in vitro* LCa model and AZD5363 successfully suppresses proliferative, clonogenic, and migratory features of HEp-2 cells through induction of apoptosis.

METHODS

Cell Culture

Hep2 cell line was received from SAP Institute, Turkey (Ministry of Food Agriculture and Livestock). Cells grown in RPMI (Gibco, Gaithersburg, MD, USA) medium containing 10% Fetal Bovine Serum (FBS, Gibco, Gaithersburg, MD, USA), 1% penicillin/streptomycin (Gibco, Gaithersburg, MD, USA) were cultured at 37°C in a humidified 5% CO2 incubator. Cell culture medium was changed when the cell density reached 80-90% confluency.

Preparation of AZD5363 Stock Solution

AZD5363 (MedChemExpres, NJ,USA) was solubilized in dimethyl sulfoxide (DMSO). Its stock solution was prepared at 10mM concentration and stored at -80°C until its use for *in vitro* experiments.

Cell Proliferation Assay

To determine the effect of AZD5363 on the viability of HEp-2 cells, Cell Viability Detection Kit 8 (CVDK-8, EcoTech Biotechnology, Turkey) was used. Briefly, cells seeded at 2.5×10^3 cells per well in 96 well plates were cultured overnight in the appropriate medium. Then AZD5363 was administered to cells at increasing concentrations for 24 and 48h. To measure changes in cell growth rate, CVDK-8 reagent was added to each well according to the manufacturer's instructions. After 1h incubation, absorbances were measured at 450nm with an Epoch 2 Microplate Spectrophotometer (BioTek, Winooski, VT, USA) to determine cell viability.

Soft Agar Colony Formation Assay

Soft agar assay was performed to investigate the effect of AZD5363 on HEp-2 cells' colonization potential. Cells at a concentration of 3×10^3/per well within 0.3% agar in RPMI were plated on a 0.6% base agar in 6-well plates. Cells within agar were exposed to varying concentrations of AZD5363 at 37°C for 21 days. Cells were fixed and stained with 0.01% crystal violet solution containing 10% ethanol to visualize and count the colonies.

Apoptosis Assay

Annexin V-FITC Apoptosis kit (BioVision, Milpitas, CA, USA) was used to observe the effect of AZD5363 applied at 1, 5 and 10 µM concentrations on apoptosis of HEp-2 cells cultured in 6 well plates at 2.5×10^5 cells per well. Following 48 hours of incubation of HEp-2 cells with AZD5363, cells were washed twice with cold PBS, harvested in 500 µL binding buffer and then incubated with 5µL Annexin V and PI for 15 minutes at room temperature. Finally, the apoptotic state of HEp-2 cells was determined by CyFlow® Cube 6 flow cytometry device.

Scratch Assay

HEp-2 cells were seeded in 6-well plates with a cell density of 5×10^5. After reaching %90-100 confluency, the areas where the cells are dense were detected and uniform wounds were opened with a sterile 100 µL pipette tip. Then, cells were treated with fresh medium containing the indicated concentrations of AZD5363 and the wounds were photographed with an inverted microscope (Leica, Wetzlar, Germany). After 24h, the migration potential of the cells was assessed by comparing the size of wound closure.

Cell Migration Assay

The transwell migration assay was performed to examine the chemotactic motility of HEp-2 cells. Firstly, serum-free medium containing 1×10^4 cells were placed in the migration chambers with a total volume of 250µL and the wells of the 24-well plate into which the chambers were placed were filled with 500µL of RPMI-1640 medium containing 10% FBS. The chambers were incubated for 24h in a humidified atmosphere at 37°C. Subsequently, cells remaining on the upper surface of the membrane were removed with a cotton swap, and the chambers were stained with crystal violet solution to count the migrated cells through the 8 µm pores to the lower surface of the membrane. Each membrane was photographed under an inverted microscope (Leica, Wetzlar, Germany) and cells at 3 randomly selected regions were counted.

Western Blot Analysis

Western blot analysis was performed to evaluate the Akt, phospho-AKT (pAKT), and phospho-GSK3β (pGSK3β) level in HEp-2 cells treated with AZD5363. Briefly, HEp-2 cells were plated at a density of $2.5\text{x}10^5$ cells per well in 6 well plates and cell lysates were prepared using RIPA Lysis Buffer (EcoTech Biotechnology, Turkey) from cells treated with AZD5363 for 48h at concentrations ranging from 1µM to 10µM. Cell lysates were mixed with same volume of Laemmli buffer (EcoTech Biotechnology, Turkey) and boiled at 100°C for 5 minutes. Samples containing equal amounts of protein were separated with 10% SDS/PAGE and then transferred to nitrocellulose membranes. Membranes were blocked with 5% non-fat dry milk (EcoTech Biotechnology, Turkey) in PBS-T buffer at room temperature and then incubated at 4°C overnight with one of the following primary antibodies: β-Aktin (1:200, Santa Cruz Biotechnology, Dallas, TX, USA), Akt (1:200, Santa Cruz Biotechnology, Dallas, TX, USA), pAkt (1:100, Santa Cruz Biotechnology, Dallas, TX, USA), and pGSK3β (1:500, Santa Cruz Biotechnology, Dallas, TX, USA). After washing with PBS-T containing 0.1% Tween-20 for 3 times, membranes were probed with anti-mouse (1:2000, Santa Cruz Biotechnology, Dallas, TX, USA) or anti-rabbit (1:2000, Santa Cruz Biotechnology, Dallas, TX, USA) horseradish peroxidase (HRP)-linked secondary antibodies for 1h at room temperature. Specific proteins were visualized using Pierce ECL Western Blotting Substrate (Thermo Scientific, Waltham, MA, USA) following the manufacturer's instructions.

Statistical Analysis

Numerical values were shown as mean ± standard error of mean. Differences between groups were tested using Student's t test. A p - value of < 0.05 was accepted as statistically significant.

RESULTS

AZD5363 induced phosphorylation of AKT and reduced phosphorylation of its downstream effector GSK3β

To investigate the effects of PI3K/AKT/mTOR pathway inhibition using AZD5363 in HEp-2 cells, we initially measured the relative protein levels of AKT, pAKT, and pGSK3β. AZD5363 increased the phosphorylation level of AKT, which is in parallel with the previous results (Fig. 1). To validate the inhibition of PI3K/AKT/mTOR pathway, we evaluated the phosphorylation of GSK3β and found that AZD5363 sup-

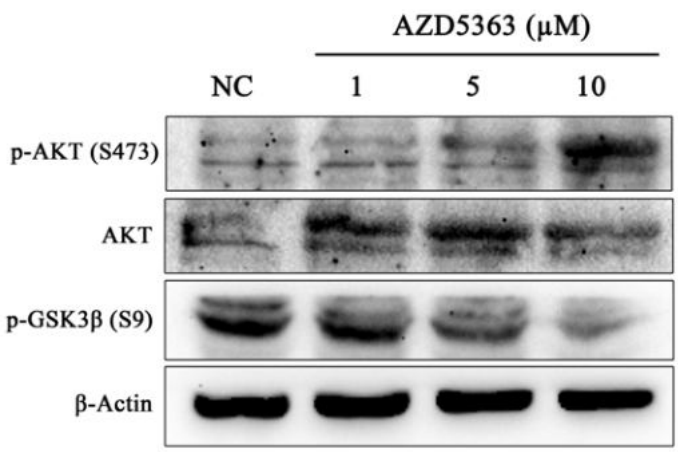

Fig. 1. A) Western Blot analysis of pAKT, AKT, and pGSK3β in cells treated with 1, 5, and 10 μM AZD5363. B-Actin was used as loading control.

pressed pGSK3β in a dose-dependent manner (Fig. 1).These data suggest that AZD5363 successfully inhibits PI3K/AKT/mTOR signaling pathway in the HEp-2 laryngeal cancer cell line, which has been previously demonstrated to possess a constitutively active PI3K/AKT/mTOR axis.

AZD5363 reduced the proliferative and anchorage dependent growth potential of HEp-2 cells in a dose and time dependent manner

To determine the anti-proliferative effect of AZD5363 on HEp-2 cells, varying AZD5363 concentrations ranging from 1 to 10 μM were exposed to cells for 24 and 48 hours. 24 hour treatment of AZD5363 did not affect the proliferative potential of HEp-2 cells, however, 5 and 10 μM AZD5363 significantly decreased the relative number of cells compared to control. On the other hand, 48 hour after treatment, the viability of the HEp-2 cells was reduced effectively at all concentrations (Fig. 2A).

In order to evaluate the effect of AZD5363 on anchorage independent growth features of cells, soft agar assay was performed. Soft agar evaluates the aggressiveness of the cancer cells through testing the ability of cells to form colonies under anchorage-independent growth conditions on a semisolid surface.AZD5363 was found to inhibit the colony formation capabilities of HEp-2 cells in soft agar in a dose-dependent manner (Fig. 2B, C).

AZD5363 effectively induced apoptosis of HEp-2 cells

To evaluate the underlying mechanism for decrease in proliferative potential of HEp-2 cells treated with AZD5363, we assessed apoptosis using Annexin V-FITC Apoptosis Kit. Flow cytometry analysis showed that both 24 and 48 hours treatment of AZD5363 did not markedly altered the early apoptotic cells in HEp-2 cells (Fig. 3E, J). However, exposure to AZD5363 for 24 or 48 hours significantly enhanced the late apoptotic cells (Fig. 3A-D, F-I).10 μM of AZD5363 increased the ratio of late apoptotic cells from 0.69% to 1.55% and from 0.89% to 2.17% at the end of 24 and 48 hours, respectively (Fig. 3A, D, E, F, I, J).These results suggest that one of the possible

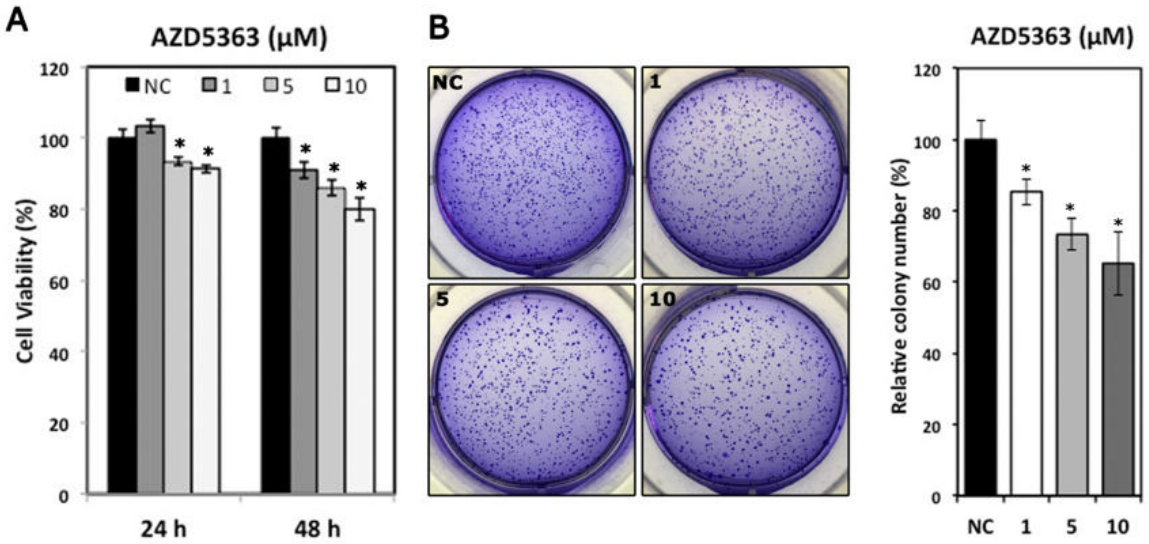

Fig. 2. A) Viability of cells exposed to1, 5, and 10 μM AZD5363 for 24 or 48 hours. B) Colony formation abilities of HEp-2 cells treated 1, 5, and 10 μM AZD5363. Mean ± Standard Error of Mean (SEM) is shown *$p < 0.05$; t-test.

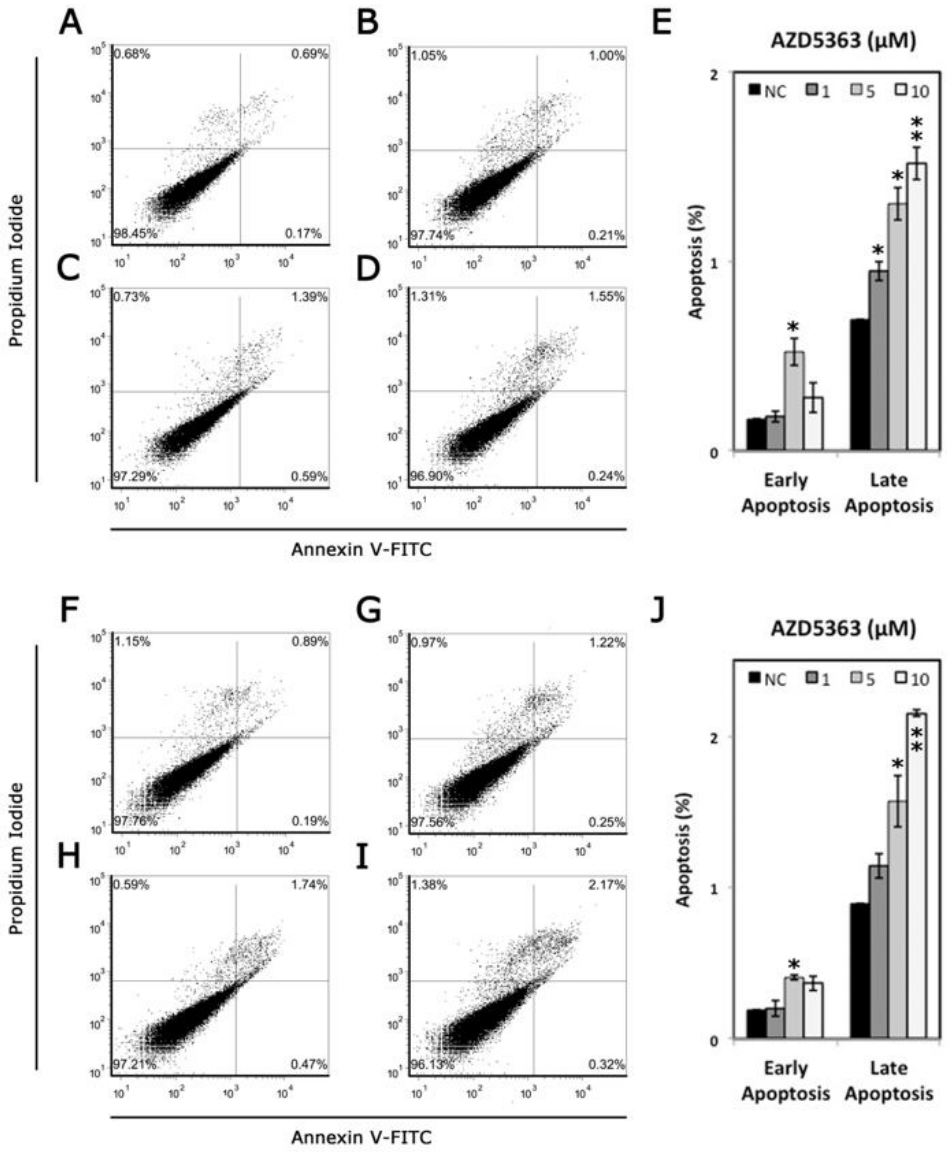

Fig. 3. The flow cytometry analysis of apoptosis in A) control, B) 1 μM, C) 5 μM, and D) 10 μM AZD5363 for 24 hours. E) Relative apoptosis rates of control, 1, 5, and 10 μMAZD5363 treated

mechanisms of cell proliferation inhibition stems from the potential of AZD5363 to induce apoptosis in a dose and time-dependent manner in HEp-2 cells.

AZD5363 reduced the ability of HEp-2 cells to migrate in a dose dependent manner

To investigate the effect of AZD5363 on the ability of HEp-2 cells to migrate *in vitro*, we initially tested the migratory potential of HEp-2 cells treated with 1-5-10μM AZD5363 with scratch assay. Results demonstrated that although the migration capabilities of HEp-2 cells exposed to 1 μM AZD5363 did not change, cells treated with 5 and 10 μM AZD5363 covered significantly smaller fraction of the wound in a dose dependent manner when compared to control cells (Figure 4A, C). Control cells could close more than 50% of the wound, whereas the HEp-2 cells treated with 5 and 10μM AZD5363 were able to close only 33% and 17%, respectively.

We used transwell migration assay to further confirm the effect of AZD5363 on the migration capabilities of HEp-2 cells.As the most effective result was obtained at 10 μM in the wound-healing assay, transwell migration assay was performed using this concentration. The migration rate of 10μM AZD5363 treated group decreased to 44% compared to the con-

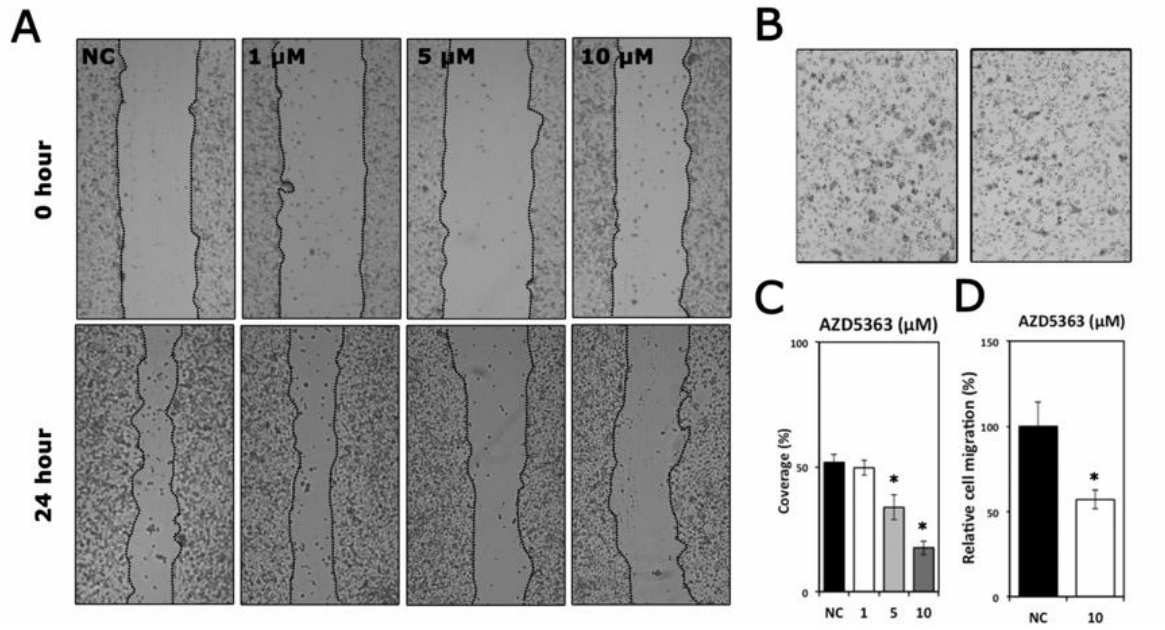

Fig. 4. A) The impacts of 1, 5, and 10 µM AZD5363 on HEp-2 cell migration evaluated with wound-healing assay, B) The mean relative wound closure of control, 1, 5, and 10 µM AZD5363 treated cells. C) The impacts of 1, 5, and 10 µM AZD5363 on HEp-2 cell migration evaluated with Transwell migration assay, D) The mean relative migration rates of control, 1, 5, and 10 µM AZD5363 treated cells. Mean ± SEM is shown *$p < 0.05$; t-test.

trol group cells (Fig. 4B, C). This result was consistent with the scratch assay. These findings indicate that AZD5363 can effectively decrease the mobility of human laryngeal cancer HEp-2 cells.

DISCUSSION

LCa is one of the most frequent cancer types localized in the head and neck region worldwide, with an unchanged overall survival rate over the last decades [25]. Considering recent developments in the diagnostic and therapeutic options for LCa patients, an effective treatment strategy could not be presented due to its high metastatic and recurrent potential.

Currently chemotherapy serves as a standard therapeutic option for LCa, for especially those with non-operable locally advanced disease, to reduce the systemic tumor burden and to sensitize cancer cells to radiotherapy [26, 27]. Nonetheless, with some success providing complete responses following chemotherapy in certain cases with LCa [27], present chemotherapy agents alone or together with other therapies cannot successfully provide complete clinical responses. This situation necessitates identification of adjuvant or neoadjuvant treatment modalities with potential to increase positive clinical outcomes.

Several recent evidences support deregulation of the PI3K-AKT-mTOR signaling pathway during carcinogenesis, metastasis, recurrence, and drug resistance [28]. Considering the potential involvement of PI3K-AKT-mTOR signaling pathway components in the acquisition of important hallmark capabilities of cancer, several novel small molecule inhibitors targeting PI3K, AKT or mTOR were developed [29].

Among those, inhibitors of AKT, which is the master regulator of this pathway, attract more attention to be used as therapeutic agents against various cancer types [30]. AKT has been found to be constitutively active in laryngeal HEp-2 cells, emphasizing its importance as a therapeutic target in LCa [24]. Besides, abnormal expression of AKT isoforms was associated with several clinico-pathological parameters including tumor site, lymph node metastasis, clinical stage, and shorter overall survival in LCa patients, which projects the potential clinical value of AKT inhibition as a putative adjuvant therapy [16, 17]. As one can expect, inactivation of AKT and thus its downstream effectors in a recent study utilized a potential AKT inhibitor, chamaejasmine, induced apoptosis in LCa cells both

in vitro and *in vivo* [24].

AZD5363, as a novel new-generation small molecule with catalytic inhibitor activity against all AKT isoforms, have potential to inhibit the phosphorylation status of AKT downstream targets, including that of GSK3β [19, 31]. In our study, we demonstrate increased pAKT in HEp-2 cells upon AZD5363 treatment, which is in parallel with the previous researches carried out with different types of cancer cells [22, 23]. AZD5363 treatment has been reported to cause its hyperphosporylation due to a secondary feedback mechanism activated as a result of inhibition of its kinase activity [19]. Being the only AKT inhibitor with the potential to induce AKT phosphorylation among many other well-known AKT inhibitors like, LY294002, MK-2206, and wortmannin, it reduces the pGSK3β level as other AKT inhibitors [32]. Our results demonstrated a concentration dependent inhibition of GSK3β, pointing successful inhibition of PI3K-AKT-mTOR signaling pathway in those cells treated with AZD5363.

We further evaluated the affects of AZD5363 *in vitro* on the phenotypes associated with cancer aggressiveness using HEp-2 cells. Our results demonstrated that AZD5363 significantly inhibited proliferative and anchorage independent growth potential of HEp-2 cells in a dose and time dependent manner through inducing apoptosis. Further analysis of migratory potential in cells treated with AZD5363 showed also inhibitory potential of AZD5363 on the migration capacity of HEp-2 cells. On a protein microarray analysis carried out with tumor samples, confirmed increased phosphorylated AKT levels and demonstrated decreased Ki67 staining pointing its anti-proliferative capacity *in vivo* [20]. In other studies, AZD5363 lead to cell-cycle arrest in all cells studied, through inhibition of cell cycle related genes such as Rb and Cyclin D, and induction of apoptosis via elevated levels of apoptosis related gene such as CASP9 [30].

Interestingly, a recent study demonstrated that AZD5363 treatment more effectively killed cells with activating mutations in PIK3CA and/or loss of PTEN function [30]. This implies an important point for targeting LCa tumors, where sequence analysis for PI3K/AKT/mTOR pathway components might increase the efficacy of a potential therapy utilizing AZD5363. Moreover, Choi *et al*. [32] demonstrated that co-treatment of LY294002 or MK-2206 with AZD5363 lead to a significant reduction in the level of phosphorylated AKT. Considering MK-2206 is a clinically used agent, they offered synergistic use of MK-2206 and AZD5363 as potential therapeutic options for patients with cancer [32]. The success of their synergistic use might be evaluated in further *in vitro* and *in vivo* studies to demonstrate the feasibility of AZD5363-based sensitization therapies in LCa.

CONCLUSION

In conclusion, further comprehensive molecular and clinical research may elucidate the potential use of AZD5363 in LCa therapy in detail. However, the current study has revealed putative functions of AZD5363 *in vitro* that might be utilized against LCa.

Conflict of interest statement

Fatma Şanlı, Neslişah Barlak, Ahsen Kılınç, Özel Çapık, and Abdülmelik Aytatlı declare that they have no conflict of interests. Ömer Faruk Karataş holds stocks in EcoTech Biotechnology. The terms of this arrangement have been reviewed and approved by Erzurum Technical University in accordance with its policy on objectivity in research.

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The European Research Journal 2020;6(5):388-394 Original Article

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Is ischemia modified albumin a good marker in acute exacerbation of chronic obstructive pulmonary disease?

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ABSTRACT

Objectives: Our aim is to compare the Ischemia modified albumin (IMA) in patients with acute exacerbation of Chronic Obstructive Pulmonary Disease (COPD) with a high oxidative and inflammatory biologic marker like C-reactive protein (CRP) and to investigate its employability in AECOPD and the relationship between arterial blood gas and pulmonary function parameters.

Methods: Forty-six patients diagnosed with acute exacerbation of COPD between March 2015 - September 2016 at Ufuk University School of Medicine were included. The 1st and 5th days of IMA and CRP levels were measured. Also, IMA levels were given in absorbance units (ABSU).

Results: Total 46 patients of COPD, 13 (28.3%) were females and 33 (71.76%) were males. The mean age of the patients was 71.39 ± 10.04 years. The 1st and 5th day values of IMA, ABSU and CRP were 1.08 ± 0.33 and 0.49 ± 0.24; 1.06 ± 0.34 and 0.49 ± 0.26; and 29.25 (3.10-288.00) and 6.35 (0.30-149.00), respectively ($p < 0.001$). No significant correlation was determined between IMA and CRP. Also, no correlation were determined between the parameters of arterial blood gas and pulmonary function.

Conclusions: Although IMA values showed significant increase during acute exacerbation of COPD and decreased after treatment, CRP still appeared more effective in evaluating the exacerbation status and following up of the treatment of patients with COPD.

Keywords: Chronic obstructive pulmonary disease, exacerbation, C-reactive protein, ischemia modified albumin

Chronic obstructive pulmonary disease (COPD) is one of the leading causes of morbidity and mortality worldwide [1]. It is well-established that COPD is not only associated with pulmonary inflammation, but also with systemic inflammation. The mechanistic basis underlying COPD involve recurrent inflammation, oxidative stress, protease/antiprotease imbalance, environmental insult, and host genetics [2]. Increased plasma pro-inflammatory cytokines, hypoxia and increased oxidative stress may cause endothelium damage [3]. Acute exacerbation of COPD is an important event in the natural history of COPD that negatively impacts health status, increases the rates of hospitalization, and disease progression. COPD exacerbations are associated with increased airway and systemic inflammation and physiological changes, especially the

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development of hyperinflation [4].

C-Reactive Protien (CRP) is an acute-phase protein produced by the liver in response to IL-6 stimulation. CRP is raised in most conditions associated with infection, inflammation, or tissue damage and it iswidely used for the assessment of acute exacerbation of COPD [5].

I schemia modified albumin (IMA) is a form of albumin modified by oxidative stress. The pathophysiological events of ischemia, including hypoxia and free oxygen radicals changed the N-terminus of albumin, and this molecule is called as IMA [6]. IMA was first discribed as a marker of myocardial ischemia, but now it has been shown that elevated in various states of noncardiac ischemia and oxidative stress conditions such as cerebrovascular diseases, inflammatory bowel diseases, chronic liver diseases, obstructive sleep apnea (OSA) and pulmonary embolism [7-13].Additionally, it was suggested to be a strong indicator of short-term mortality in patients with end-stage renal disease [14] and myocardial infarction with ST elevation [15].

Therefore the need for a useful biomarker to provide objective confirmation of exacerbation and predict the severity of COPD, we examinethe serum IMA, and compare between ABSU and CRP levels, in patients with acute exacerbation of COPD.

METHODS

The study was conducted on 46 acute exacerbation of COPD patients between March 2015- September 2016 applying to Emergency Unit of Ufuk University School of Medicine, Ankara, Turkey. Exclusion criteria were being under 18 years of age, the diagnosis of acute coroner syndrome, pulmonary emboli, acute or chronic renal failure and acute cerebrovascular disease and being reluctant to participate in the study, while inclusion criteria were being over 18 years old with diagnosis of COPD. Blood samples were taken from the patients on the first and the fifth day of IMA and CRP. CRP levels were measured by immunoturbidimetricassay (Abbott® Architect c8000, USA). Hemogram parameters were measured with autoanalyzer (Abbott Cell-Dyn Ruby-Serial Number 54507BG/Abbott Diagnostics). Electrolyte measurements and the other biochemical parameter assays were performed with autoanalyzer (Abbott® Architect c8000, USA). The Architect cSystems ICT (Integrated Chip Techonology) is used for the quantitation of sodium, potassium and chloride in human serum. The Quantita D-Dimer assay were performed by the quantitative determination of D-Dimer in human citrated plasme using the Abbott ARCHITECT cSystems. The cholesterol and the other lipid parameter levels were performed for the quantitation of lipids in human serum with Abbott® Architect c8000, USA. CK-MB and hsTnI levels were measured with autoanalyzer (Abbott® Architect i2000, USA). Architect STAT CK-MB and STAT High Sensitive Troponin-I assays are a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of the MB isoenzyme3 of creatine kinase (CK-MB) and cardiac troponin I (cTnI) in human serum.

Body mass index (BMI) was calculated as weight divided by the square of the height (kg/m^2). Modified British Medical Research Council (mMRC) Dyspnea Scale of patients were taken from patients' medical records at their latest polyclinic control during stable COPD period. The ethical commission approval was taken from the same hospital.

A 5 cc of venous blood sampling was performed on all patients before treatment (for ischemia-modified albumin assay) and to non-anticoagulant biochemistry tubes with gel. After waiting for about 30 minutes for coagulation of the blood in the tubes, they were centrifuged at 4000 rpm for 10 minutes. Serum samples were stored at -80°C deep freeze. Ischemia modified albümin levels were analyzed by the difference in the capacity of albumin cobalt binding. A decrease in this abovementioned binding capacity was evaluated by a rapid and colorimetric assessment method developed by Bar-Or *et al*. [6]. The analyses in the spectrophotometer (Human HumalyzerVR 2000, Germany) were performed at 470 nm for the detection of absorbance of the specimens, and the results were given as absorbance units (ABSU). For the accurate estimation of maternal IMA levels, correction formula [individual serum albumin concentration/median albumin concentration of the population × IMA] defined by Lippi *et al*. [16]. Results were proportioned with serum albumin levels and expressed in terms of absorbance unit (ABSU)/g albumin.

Pulmonary function tests (PFTs) results were taken from medical records of the patients at their latest polyclinic control. They were performed by ''VMAX''; ''Encore system (Germany)'' device. During PFTs, post-bronchodilator FEV1%, FVC% and FEV1/FVC values were recorded based on GOLD criteria. For COPD staging, patients with FEV1 values > 80% were considered to have mild, FEV1: 80-50% were considered to have moderate, FEV1: 50-30% were considered to have severe, and FEV1 < 30% were considered to have very severe COPD.

Arterial blood gase (ABG) analyses were performed by 2001 version of the "Instrumentation Laboratory-Synthesis 25" device. pH, partial oxygen pressure (PaO2), partial carbon dioxide pressure (PaCO2) and arterial oxygen saturation (SaO2) values were recorded.

Statistical Analysis

All statistical analyses were performed by SPSS for Windows version 18.0 (SPSS Inc, Chicago, Illinois, USA). Definitive statistics were expressed as number (N) and percentage (%) ratio. Proportions were compared with the chi-square test. Distribution of numeric values was evaluated by Kolmogorov Smirnov test. Data with non-normal distribution were expressed as median (minimum-maximum), while data with normal distribution were expressed as mean ± standard Deviation. In the presence of comorbid diseases, comparisons of groups in pairs was made with the Student's t-test for data with normal distribution and Mann Whitney U test for data with non-normal distribution. For comparisons of paired data, Paired-sample T-test was used for data with normal distribution, while Wilcoxon test was used for data with non-normal distribution. For correlation analyses, Pearson correlation coefficient or Spearman correlation coefficient was used in accordance with the status of data distribution. Reciever operator characteristics (ROC) curves were performed to evaluate the prognostic values of IMA and adj IMA for predicting acute exacerbation of COPD. A p value < 0.05 was considered statistically significant.

RESULTS

A total of 46 patients were included in the study, 13 (28.3%) females and 33 (71.76%) males. The mean age of the patients was 71.39 ± 10.04 years. The average package of cigarette smoked was 44.74 ± 33.38 (Table 1). IMA, ABSU and CRP values were

Table 1. Demographic characteristics, number of annual attacks, mMRC score and BMI.

Characteristics	Data
Sex (male/female), n (%)	33/13 (71.7/28/3)
Mean age, years	71.39 ± 10.04
COPD group, n (%)	
C	11 (23.9)
D	35 (76.1)
Smoking history (pack-year), n (%)	
never	11 (23.9)
current	3 (6.5)
Ex-smoker	32 (69.6)
Smoking history (pack-year)	44.74 ± 33.38
Disease duration (years)	11.59 ± 6.55
Number of annual attack, median (min-max)	1 (0-5)
mMRC	2.23 ± 0.82
BMI, kg/m^2	28.19 ± 5.41

Data are shown mean ± standart deviation or n(%). COPD = chronic obstructive pulmonary disease, mMRC = Modified Medical Research Council, BMI = Body mass index

Table 2. The values of CRP and IMA values on the first and the fifth days

	1st day	5th day	*p* value
CRP (mg/dL)	29.25 (3.10-288.00)	6.35 (0.30-149.00)	**< 0.001**
IMA (ABSU)	1.08 ± 0.33	0.49 ± 0.24	**< 0.001**
IMA/albumin ratio (ABSU/g/dL)	1.06 ± 0.34	0.49 ± 0.26	**< 0.001**

CRP = C- reactive protein, IMA = Ischemia modified albumin, ABSU = Absorbance units

Table 3. The relationship between CRP value and IMA on the first day, disease progression, disease duration, number of annual attacks, arterial blood gas, biochemical tests, Echocardiography and pulmonary function tests.

	R	*p* value
IMA (ABSU)	0.94	0.373
IMA/albumin ratio (ABSU/g/dL)	-0.078	0.606
Disease stage	-0.022	0.886
Disease duration (years)	0.215	0.15
Annual attacks (n)	0.30	0.84
BMI ($kg/m^{2)}$	-0.238	0.111
Arterial pO2 (mmHg)	-0.310	0.036
Arterial pCO2 (mmHg)	0.024	0.875
FEV1 (%)	0,034	0.823
FEV1/FVC (%)	-0.253	0.090
Troponin (ng/ml)	-0.079	0.604
CK (U/L)	-0.139	0.356
CK-MB (U/L)	-0.050	0.739
Albumin (g/dL)	-0.345	0.019
Total-C (mg/dL)	-0.077	0.610
LDL-C (mg/dL)	-0.048	0.749
HDL-C (mg/dL)	0.018	0.905
Triglycerides (mg/dL)	-0.007	0.964
EF (%)	-0.050	0.741
mPAP (mmHg)	-0.079	0.691

FEV1 = forced expiratory volume in 1 second, FEV1/FVC = Forced expiratory volume in 1 second /forced vital capacity, CK = creatin kinase, CK-MB = creatin kinase-MB, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, Total-C = total cholesterol, EF = ejection fraction, mPAP = Mean pulmonary arteial pressure.

shown on the first day and on the fifth day in Table 2. There was a significant decrease in all these values after treatment ($p < 0.001$). However, we could not find the same correlation for the admission day results of CRP and IMA among COPD stage, COPD years, and attack numbers of year (Table 3).

ROC analysis resulted a cut-off value for IMA level ≥ 0.845 as higher inflammation with sensitivity = 0.79 and specificity = 0.34 (Fig. 1A). Additionally, ROC analysis resulted a cut-off value for adj IMA level ≥ 0.838 as higher inflammation with sensitivity = 0.72 and specificity = 0.29 (Fig. 1B). ROC analysis showed that although the sensitivity of our test results was partially high their specificity to show exacerbation was low.

DISCUSSION

As our knowledge, our study is the first to investigate the IMA levels in patients with acute exacerbation of COPD before and after the treatment. The values of serum IMA, ABSU and CRP were assessed at first and fifth days and we observed that although there was no correlation between CRP and IMA and ABSU levels at the first day of hospital admission, a significant decrease was demonstrated for these values at the fifth day of treatment. The reason of this can be explained with IMA half-life being shorter than CRP and IMA is studied manually while CRP is studied with automatic standardized devices, leading to a possible discrepancy.

In COPD, especially in advanced stage and during exacerbations, various cytokines showing systemic inflammation, acute phase proteins, and chemokines are increased and abnormal changes in the circulating cells are observed [17]. Oxidative stress is defined as the decrease in anti-oxidant capacity against oxidants and/or the increase in oxidants. Oxidant matters form reactions with various biological molecules like protein, lipid, and nucleic acid and damage in the structure of extracellular matrix, biologic membranes, genetic cell structure by DNA damage, and ciliary function. Enzymatic events are affected, surfactant activity is decreased, and mucus production is increased along with the increase in the effectiveness of cytokines and proteases [18]. IMA is a modified serum albumin formed under conditions of oxidative stress and accepted as a biological marker of ischemia. Ischemic stress (like hypoxia, acidosis, and free radical damage), reactive oxygen radicals that appear during ischemia changes the N-terminal of the albumin and this results in the decrease of the binding ability of the albumin to nickel, cobalt and copper. It

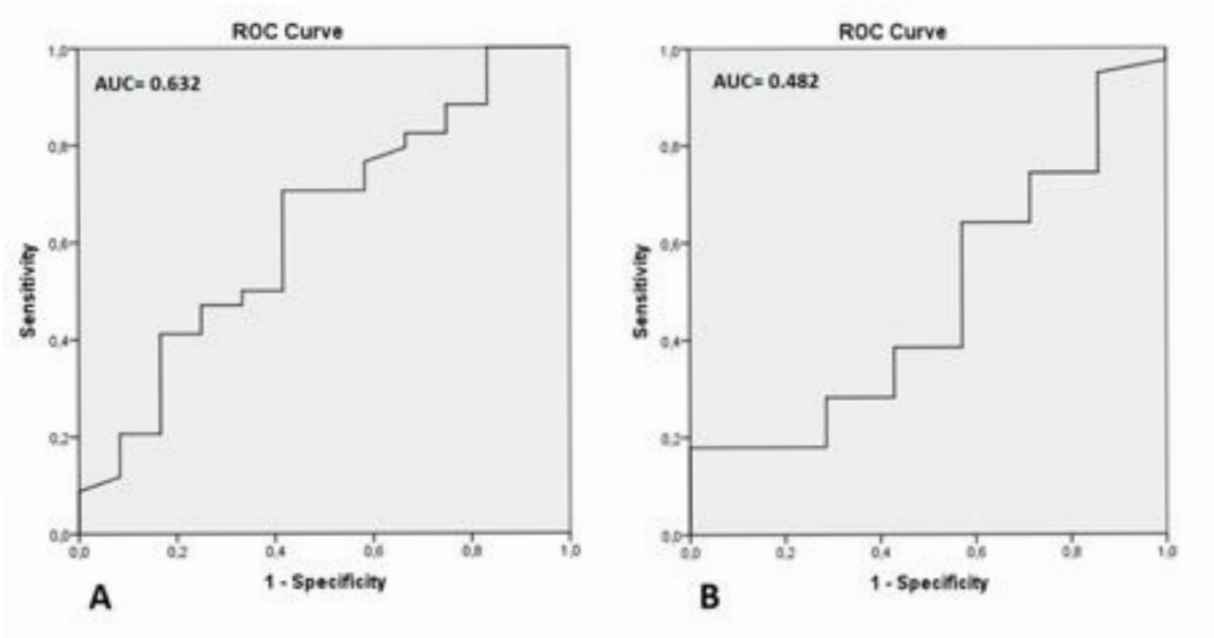

Fig. 1. ROC curve for IMA level (A), ROC curve for ABSU levels (B).

increases in the few minutes after the start of ischemia, rises for 6-12 hours, and returns to normal in 24 hours [18]. Many studies have been published about IMA in acute cardiac and noncardiac ischemic events, however only few studies were related to COPD [7-13]. Can *et al*. [19] compared serum IMA, ox-LDL (Oxidized Low-Density Lipoprotein), TAS (Total Anti-oxidant Status), and TOS (Total Oxidant Status) levels in 51 patients with stable COPD and 45 healthy cases. Their results revealed that TAS levels did not show any difference between two groups while IMA, ox-LDL, and TOS levels were significantly increased in the COPD group, and furthermore ox-LDL and TOS levels were high only in stage IV while IMA levels were high in GOLD stages I, III, and IV. The author suggested that this finding indicated IMA being a marker showing hypoxia and oxidative stress in COPD [19]. Also, a study by Yang *et al*. [20], showed a significant correlation between the severity of COPD as per GOLD criteria and increased serum concentrations of oxidative parameters such as IMA. In the present study, IMA levels of COPD patients were compared on the day of admission because of exacerbation and on the 5th day, which is relatively a more stable period, and it was found that IMA levels decreased along with the treatment response. This is indicative of decreased oxidative stress as the exacerbation is being controlled.

On the other hand, in the study of Roy *et al*. [21] with marathon runners, a decrease in IMA concentrations was observed immediately after exercise, and an increase after 24-48 h. Paulraj *et al*. [22] found low IMA values and positive correlation between the levels of IMA and the degree of smoking in their study. They explained decreased IMA levels are reason of a rise in lactate. Another reason could be low albumin levels that given falsely a low IMA value. Zapico-Muniz *et al*. [23] also revealed an immediate and transient decrease in IMA concentrations after the induction of forearm ischemia. In the present study, IMA and CRP levels at admission showed significant decrease as a response to treatment in patients with acute exacerbation of COPD. However, this correlation of CRP could not be detected with IMA and ABSU.

Limitations

One of the limitations of the present study is the limited number of patients. Second, no comparison with the stable period after discharge from hospital was made. Instead of this, control levels on the 5th day were measured because of the short half-life of IMA. Furthermore, no comparisons with healthy controls were studied.

CONCLUSION

IMA is studied in many diseases, however, data regarding COPD is very limited. In accordance with the present findings, it can be stated that as the present patients had advanced age, mostly positive smoking history, and had a high rate of comorbidities, especially cardiac problems, IMA can be indicative of oxidative stress and possible complications in patients with COPD. The short half-life of IMA will accelerate the patients' scanning phase in terms of determining treatment priorities. Nonetheless, as IMA increases in many ischemic, neoplastic, and traumatic events, CRP still seems to be a cheaper and more efficient marker when compared with IMA. Further investigations are required for determining the employability of IMA in following-up the prognosis of COPD acute exacerbations.

Conflict of interest

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

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The effect of pulmonary rehabilitation on dyspnea and factors related to dyspnea in lung transplantation candidates

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ABSTRACT

Objectives: Lung transplantation is the last treatment option when conservative treatment is not effective in individuals with terminal stage lung disease. Dyspnea is the primary symptom affecting quality of life in these patients. In our study, the effects of Pulmonary Rehabilitation (PR) on dyspnea and factors related with dyspnea were investigated in lung transplant candidates.

Methods: Patients who were in the lung transplant waiting list and completed the 3-month PR program were included in the study. Study result measurements: 6-minute walk test distance (6MWD), lung functions (FEV1, FVC), respiratory muscle strength (MIP, MEP), quadriceps femoris muscle strength as measured by digital dynanometer, hand grip force measured by hand dinanometer (HG) and modified Modified Medical Research Council (mMRC) dyspnea scale.

Results: A total of 47 patients were included in the study. After PR, 6MWD ($p < 0.0001$), MIP ($p < 0.0001$), MEP ($p < 0.0001$), HG ($p < 0.0001$) and mMRC ($p < 0.0001$) improvements were detected. There was no statistically significant relationship between the decrease in mMRC and the amount of change occurring in other outcome measurements ($p > 0.05$).

Conclusions: According to the results of our study, PR has a positive effect on exercise capacity, peripheral and respiratory muscle strength and dyspnea in lung transplant candidates. But there was no relationship between these positive developments and dyspnea. There is a need for studies investigating the effects of different clinical features on rehabilitation outcomes.

Keywords: lung transplantation, dyspnea, exercise, muscle strength, rehabilitation

In lung transplantation candidates, the advanced lung pathology causes dyspnea and exercise limitation [1]. Exercise intolerance and dyspnea cause prolonged inactivity and consequently peripheral muscle weakness [2]. In these patients, despite the optimal medical treatment given to relieve symptoms during the waiting period, the lung disease usually progress [3]. As a result, patients' clinical condition worsens and quality of life decreases [4].

Pulmonary Rehabilitation (PR) has an important role in the management of comorbidities and complications in the lung transplantation process [5]. Dyspnea in this patient population is the primary symptom affecting quality of life. In our study, the effect of PR

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on dyspnea and factors related with dyspnea were investigated in lung transplant candidates. We believe that the determination of primary factors affecting dyspnea will be guiding the development of PR program design and the data obtained from our study will guide clinicians in which or which different PR components such as respiratory muscle strength, peripheral muscle strength or medication should be focused. In this way, we believe that patients' quality of life will increase.

METHODS

Our study is a retrospective, single center, self-controlled clinical trial. The records of the patients who listed lung transplantation and enrolled in the exercise program of Pulmonary Rehabilitation Centre between 2017-2019 were restrospectively reviewed. The study was approved by the local ethics committee (Protocol no: 1665). Signed informed consent was obtained from each patient prior to commencing the PR program for routine clinical procedure. The study was carried out in accordance with the Helsinki Declaration. The exercise programme included of aerobic and strengthening training.

Aerobic Training

The aerobic exercise program had included treadmill walking (15 sc), cycle training, arm ergometer training . Group exercises were performed in sets of 15 minutes each with three exercise modalities. During the exercises, oxygen saturation, heart rate, Borg fatigue and dyspnea scores, and distance covered were recorded. It was based on the target heart rate method (Target Heart Rate = resting heart rate + (% Aerobic exercise intensity × heart rate reserve). The intensity of the exercise was progressively increased to 80% workload, starting with a 60% workload, with a limited symptom limit.

Strengthening/Resistance Training

It is recommended that resistance targets are set at loads equivalent to 20 to 40% of a 1-repetition maximum (1RM) maneuver and performed between 8 to 12 repetitions for 1 to 2 sets per session. Exercises focused on biceps, triceps, quadriceps, hamstring and hip muscles. Dumbbles and free weights were used in training sessions.

Outcome Measurements

Modified Medical Research Council (mMRC) Dyspnea Scale

Dyspnea perceptions during the activities of daily living were assessed with modified Medical Research Council scale [6].

Pulmonary Function Test

It was conducted by using the Sensor Medics model 2400 (Yorba Linda, CA, USA), and according to the American Thoracic Society (ATS) guidelines 7].

Six Minute Walking Test

The test was conducted in a 30-meter corridor in line with American Thoracic Society (ATS) guidelines. Before and after the test, oxygen saturation, heart rate, Borg fatigue rating, and walking distance were recorded [8, 9].

Maximum Inspiratory Pressure-Maximum Expiratory Pressure (MIP-MEP)

The mouth pressure measurement was performed with the Micro-RPM® instrument from SensorMEDIC. Patient placed a rubber mouthpiece with flanges, on the device, sealed their lips firmly around the mouthpiece, exhaled/inhaled slowly and completely, and then tried to breath in as hard as possible [10]. The aim is that the variability between measurements is less than 10 cm H2O. The maximum value was obtained [11].

Peripheral Muscle Strength Measurement

Handgrip measurements of the patient were performed. Upper and lower extremity muscle strength measurements were performed on the major muscles using a digital dinanometer (J-Tech Commander muscle testing device). Each measurement was repeated three times on the right and left extremities.

Statistical Analysis

Statistical analysis was conducted using SPSS (Statistical Package for Social Sciences Version IBM Statistic15.0. Chicago, IL, USA). The Shapiro-Wilk statistic was used to test the normality of the distribution of all variables. The Wilcoxon signed rank test was used to analyze the differences between the

parameters. Variables were expressed as median (minimum-maximum). "The Pearson correlation test" was done to determine the correlation dyspnea perception and the other parameters. A $p < 0.05$ was considered statistically significant.

RESULTS

The study included 47 patients with a mean age was 39 ± 14.56 years (range 15-68 years). The mean body mass index was 21.09 ± 5.98 kg/m2 and 34% (n = 16) patients were female (Table 1). The diagnostic distributions were predominantly bronchiectasis (38%) and chronic obstructive pulmonary disease (COPD) (11%), but there were different pulmonary pathologies.

After PR, there were statistically significant improvements in 6 MWD ($p < 0.0001$), MIP ($p < 0.0001$) and MEP ($p < 0.0001$), HG ($p < 0.0001$) and mMRC ($p < 0.0001$) (Table 2). There was no statistically significant correlation between the decrease in mMRC dyspnea score and in other outcome measures (Table 3).

Table 1. Demographic information and diagnostic distributions of patients

Age (years)	39.38 ± 14.56
Gender	
Male, n (%)	31 (66)
Female, n (%)	16 (34)
BMI (kg/m2)	21.09 ± 5.98
Diagnosis, n (%)	
Alveolarproteinosis	1 (2.1)
Bronchiectasis	18 (38.3)
Interstitiallungdisease	5 (10.6)
Kartagener	1 (2.1)
Cysticfibrosis	5 (10.6)
COPD	11 (23.1)
RA lunginvolvement	1 (2.1)
Sarcoidosis	2 (4.3)
Silicosis	3 (6.4)

Data are shown as mean ± Standard deviation or n (%). BMI = Body mass index, COPD = Chronic obstructive pulmonary disease, RA = Rheumatoid arthritis

DISCUSSION

Our study showed that PR has a positive effect on exercise capacity, peripheral and respiratory muscle strength and dyspnea in lung transplant candidates. But there was no relationship between these positive developments and dyspnea.

The dyspnea mechanism is not fully understood. In the studies conducted, it is reported that some neurological mechanisms may be responsible for this condition and may be caused by carbondioxide (CO2) reflex chemostimulation [12, 13]. The patient

Table 2. Changes in study outcome measurements after pulmonary rehabilitation

	Before PR	After PR	*p* value
6MWD (m)	337.51 (42-548)	419.40 (84-629)	**< 0.0001**
FEV_1(%)	33.47 (10-64)	34.56(10-98)	0.435
FVC (%)	41.04 (6-75)	42.64 (21-78)	0.267
MIP (cmH_2O)	77.53(12-129)	91.67(19-149)	**< 0.0001**
MEP (cmH_2O)	124.44(47-229)	142.40 (62-217)	**< 0.0001**
mMRC	3(1-4)	2(0-4)	**< 0.0001**
QF muscle strenght (N)	40.52 (14-74)	43.66(12-83)	0.094
HG	61.73(30-108)	66.93 (30-125)	**< 0.0001**

Data are shown as median (minimum-maximum). PR = Pulmonary Rehabilitation, 6MWD = 6-minute walking test distance, FEV1 = expiratoryvolume in 1st, FVC = Challenging vital capacity, MIP = maximal inspiratory pressure, MEP = maximal expiratory pressure, mMRC = Modified Medical Research Council dyspnoea score, QF = quadriceps femoris, HG = handgrip force. The Wilcoxon signed rank test was used for *p* values.

Table 3. Correlation analysis of exercise capacity, respiratory function, respiratory muscle strength and peripheral muscle strength changes with dyspnea scale

	Δ6MWD	ΔFEV$_1$ (%)	ΔFVC (%)	ΔMIP	ΔMEP	ΔQF muscle strenght	ΔHG
ΔmMRC							
r	-0.062	-0.201	-0.192	-0.133	0.171	-0.033	-0.108
***p* value**	0.679	0.186	0.206	0.379	0.249	0.827	0.476

6MWD = 6-minute walking test distance, FEV1 = expiratoryvolume in 1st, FVC = Challenging vital capacity, MIP = maximal inspiratory pressure, MEP = maximal expiratory pressure, mMRC = Modified Medical Research Council dyspnoea score, QF = quadriceps femoris, HG = handgrip force, Δ= difference before and after PR. Pearson correlation was used for *p* value, $p > 0.05$.

population in our study has terminal stage lung disease and it can be said at first glance that dyspnea is caused by CO2 increase. On the other hand, although there is no increase in lung functions as expected after the exercise program, the decrease in dyspnea severity of patients suggests that there may be different factors affecting this situation.

In a study examining the dyspnea mechanism in patients with interstitial lung disease and COPD, disease-specific differences in mechanical and respiratory muscle activity have been shown to not affect the relationship between the severity of dyspnea and the inspiratory neural drive to the diaphragm [14]. In our study, various patient groups with lung pathology were included. Similar gains were observed in all patients and dyspnea levels were decreased.

In all chronic respiratory diseases, the most important symptom with similar mechanisms is dyspnea [15]. In the literature, mMRC is widely used in dyspnea scoring. It has been reported that patients with dyspnea score between 3 and 5, who cause functional limitation should be referred to out patient PR programs [16, 17]. In studies comparing PR with standard treatment, PR has been reported to decrease dyspnea more than standad treatment [18]. In our study, when the initial mMRC scores were above 3, a significant reduction in dyspnea was achieved at the end of the program. This means that, before rehabilitation, patients experience shortness of breath when they walk for 100 meters or a few minutes, and at the end of the program they define a walking performance that is worse than their peers. Therefore, PR is an important treatment modality that normalizes the lives of these patients in the terminal period.

The rehabilitation practices during the waiting process take on the task of bridging the lung transpantation operation [19]. In a study examining the indicators of PR success in transplant candidates during the waiting process, it was demonstrated that short-term comprehensive exercise programs increase exercise capacity and quality of life regardless of the underlying disease [20]. In this particular and difficult population, exercise programs that are individualy planned, with symptom limited and appropriate exercise intensity should be arranged within the framework of the basic exercise program information [21]. The primary aim of our study was to determine the clinical characteristics affecting the level of dyspnea in individuals with advanced lung disease, and to determine the factors that have the potential to be improved by rehabilitation, and to guide the medication and exercise programs.

There are studies related to the increase of exercise capacity [22, 23], respiratory muscle strength [22] and peripheral muscle strength in lung transplant candidates [24]. Similarly, in our study, exercise capacity, respiratory and peripheral muscle strength were increased by PR. Considering that patients have terminal stage lung disease, it is not possible to expect an improvement in pulmonary functions. Our study results support this situation. Reduction of dyspnea of patients whose pulmonary function values do not change indicates that different factors may be effective. On the other hand, in our analysis, we could not detect any relationship between the decrease in dyspnea and the change in other outcome measures. Further studies are needed to investigate the effect of different clinical features on rehabilitation outcomes.

Limitations

Our limitations are the retrospective nature of the study and the relatively small number of patients. With more patients, prospective randomized controlled trials are needed.

CONCLUSION

As a result, PR improves exercise capacity, peripheral and respiratory muscle strength, and has a positive effect on dyspnea in lung transplantation candidates. Reduction of dyspnea, which is the most effective symptom of quality of life in this patient group, is an important gain. On the otherhand, there was no relationship between these positive developments and dyspnea in the clinical status of patients. Different factors that may affect dyspnea should be examined.

Authors' contributions

EP = designed study, performed study, collected data, analyzed data, wrote the paper; AB = performed study, collected data; and LK = wrote the paper

Conflict of interest

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

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The effect of teicoplanin coating on osteointegration of titanium screws: a biomechanical and histomorphometric study in a rabbit model

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ABSTRACT

Objectives: The aim of this experimental animal study was to make a biomechanical and histomorphometric evaluation of the effects of titanium screws covered with teicoplanin, which is wanted to prevent the development of infection, on osteointegration of the screw.

Methods: Twenty New Zealand white rabbits were randomly separated into 2 groups. In Group 1, 2 mini screws with teicoplanin coating were placed in the femoral condyles of the right knee and in Group 2, 2 mini screws with no coating. After 4 weeks, all the animals were sacrificed and prepared for biomechanical and histological examinations.

Results: In the pull-out test, the values of Group 1 were found to be higher and in the removal torque test, the values of Group 2 were higher. No positive correlation was found between the pull-out and removal torque tests (r = 0.88). The bone-implant contact value was found to be similar in both groups (p = 0.132).

Conclusions: The results showed that titanium screws with teicoplanin coating did not interfere with osteointegration process biomechanically andhistomorphometrically by comparison with screws having no coating so that teicoplanincoating can be considered for use in orthopedic devices and joint prosthesis to prevent the development of infection.

Keywords: teicoplanin, osteointegration, histomorphometry, biomechanics, titanium

Prevention of implant-associated infections are essential for orthopaedic surgeons which are devastating for the patients and difficult to treat for the surgeons. Incidence has been reported to be 0.7% to 4.2 and may reach 33% in cases of high-energy trauma injuries [1-5]. Treatment is extremely difficult with very high costs and despite long-term antibiotic use, complete recovery might not be obtained. If this is the case, it is necessary to remove the orthopedic device or joint prosthesis [1, 2, 5]. In the majority, the removal of the device and prosthesis, placement of antibiotic-loaded cement spacer, long-term antibiotic treatment, and multiple debridements and revision operations cause a great increase in the cost of treatment and labor force losses [1, 6]. In the treatment of these infections, systemic and local antibiotics are

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applied following the removal of the device and especially joint prosthesis [1-4, 6-11]. Since there are some side effects with the use of systemic antibiotics, the application of local antibiotics has become an area of more current research [12, 13]. In recent years, an experimental study showing the effects of teicoplanin and clindamycin coated titanium wires in the prevention of infection has been published [11]. Coating of the implant with antibiotic forms an important stage in the prevention of bacterial colonization of the implant surface starting from the moment of implantation and continuing with the expression of active substance [14]. Coating implants with antibiotics seems to be an effective method in the prevention of infections and successful results have been reported from experimental studies related to this method [8-11]. But, one might worry whether antibiotic coating interferes with osteointegration. There is a limited number of studies on the subject of the effect of the antibiotic coating of implants on osteointegration [5, 7, 9-11, 15]. We hypothesized that titanium implants with teicoplanin coating did not interfere with osteointegration process biomechanically or histomorphometric. So, we decided to conduct an experimental animal study in order to see the effect of teicoplanin coating on osteointegration.

METHODS

Animals and Surgery

Approval for this experimental study was granted by the Local Ethics Committee of Animal Experiments of Uludag University(decision no: 2009-11/01, dated 15.12.2009). The number of animals was decided with power analysis and a total of 20 adults, female, healthy, New Zealand White (Oryctolaguscuniculus L) rabbits weighing with a mean of 3.10 kg (range, 2.85-3.36) and with a mean age of 6 months with simple randomization were separated into 2 groups and included in the study. All procedures were performed into the Experimental Animal Research Center of Uludag University Veterinary Faculty. The nutrition and care of the animals were provided by the professionals of the relevant center.

Screws

In this study, a total of 40 titanium mini-screws of 2 mm in diameter and 7 mm in length were used. The surfaces of the screws were covered with 200 mesh silica at 6 bar pressure. The process of the antibiotic coating was applied directly without any supporting system as used in the same coating procedures in literature [1, 11, 12]. Separate methanol solutions containing 16 mg/mL teicoplanin were sprayed directly onto the sanded surfaces of the titanium mini-screws. The screws were left to dry at room temperature. Following this process, they were placed in sterilization packaging and were sterilized with cobalt-60 (Co-60) gamma rays at a dose of 25.2 kilograys (kGy).

Surgery

All the operations were performed by the same surgery team in a blinded fashion. Following general anesthesia induction with ketamine HCl 35 mg/kg, it was maintained with intramuscular (im) application of xylazine HCl and ketamine HCl 0.3 mg/kg. Prophylactic antibiotic administration was started 30 min-

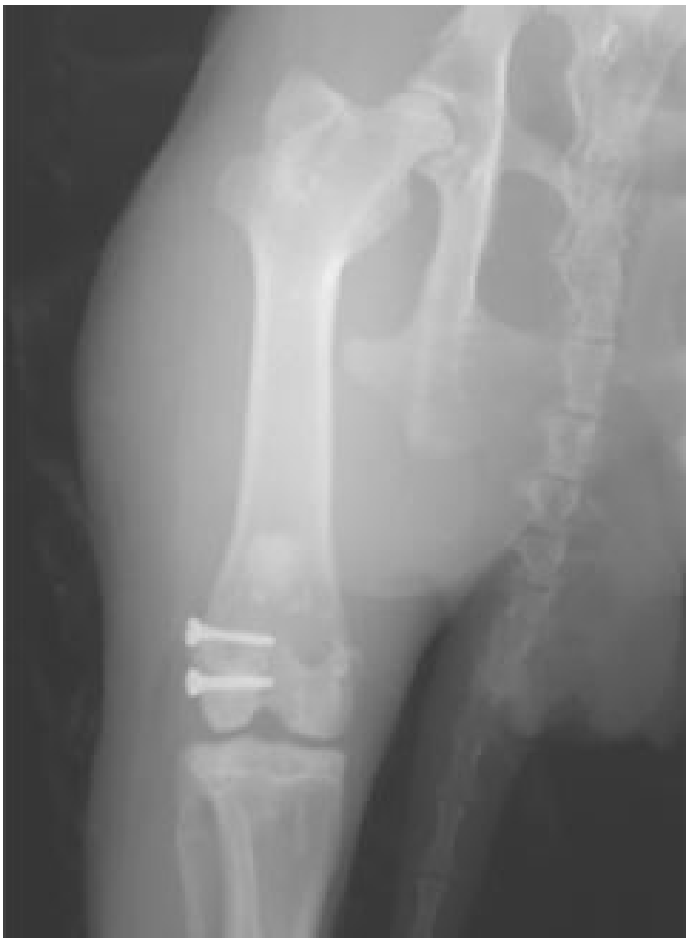

Fig. 1. Postoperative X-Ray of rabbit shows the 2 screws in the metaphyseal area of the femur.

utes (min) preoperatively with 40 mg/day cefazolin sodium im and was continued for 72 hours. Carprofen SC was administered as pain relief preoperatively and immediately postoperatively, then was continued for 3 days at a dose of 4 mg/kg. Preoperatively, the right hind leg of each rabbit was shaved and washed with benzalkonium chloride solution, then stained with 10% iodine-povidone solution (Batticon®). Following the necessary draping, a 3 cm straight skin incision was made from the lateral side of the right patella. Skin and subcutaneous tissue were dissected, then arthrotomy was made with a lateral parapatellar cut and the femoral lateral condyle was reached. The bed for the screw was prepared in the bone with a drill bit of 1.5 mm in diameter and irrigation with an isotonic solution to prevent overheating. Using a screw-driver, 2 screws were placed in the metaphyseal area of each femur at a distance of 10 mm from each other (Fig. 1). In Group 1 (n = 10) rabbits, 20 titanium mini-screws with teicoplanin coating were placed and in Group 2 (n = 10), 20 titanium mini-screws with no coating. The skin was sutured with 3/0 monofilament nylon. The wound was closed with a sterile dressing. Postoperatively, anterior-posterior and lateral radiographs were taken to check the position of the screws. All the screws were seen to be in the lateral side of the distal femoral region, with 1 screw distal and 1 proximal. At the end of 4 weeks, all the rabbits were sacrificed by im pentobarbital (100 mg/kg) injection. The distal femurs containing the implants were removed with en bloc resection and were prepared for testing.

Biomechanical evaluation and Histomorphometry

The biomechanical evaluation was applied to a total of 14 rabbits (randomly 7 rabbits from each group), with the pull-out test applied to 14 screws and the removal torque test to 14 screws. The remaining 6 rabbits (3 from each group) were prepared for histomorphometricanalysis by cleaning the surrounding soft tissues from the right femurs and then fixing in 10% formaldehyde. For the removal torque test, the implants were resected together with the surrounding bone. For this test, 1 of the 2 implants in the femur was prepared. The removal torque test was applied to 7 implants from each group. The resected samples were wrapped in saline-soaked sponges and were stored at -80°C until testing. For the test, the samples were thawed at room temperature, then fixed in standard testing equipment. After fixing the part with the implant into the testing device, removal force was applied manually, slowly and in a gradually increasing manner in an anti-clockwise direction with the digital torquemeter device probe. The process was halted when the bone with the implant started to turn within its bed. The highest torque value obtained at the moment of breaking was recorded on the digital screen as Newton/centimeter (N/cm) (Fig. 2). For the pull-out test, 1 of the 2 implants in the femur was prepared. An incision was made to the lateral femur. Without putting pressure on the screws, the bone was reached with careful dissection. The torque test was applied at a speed of 1.0 mm/min of the pull-out apparatus of the device (Lutron TQ-8800, Taiwan) (Fig. 3). The highest torque value obtained at the moment of breaking was recorded on a graphic and the digital screen as Newton units (N).

Histomorphometry allows evalution of the biological fixation of the implant at the microscopic level and provides quantitative data. With this method, metallic implants are cut in situ and implant- bone interface is evaluated while the damage during removing is prevented. This assesment method prolonged use with mechanical test in orthopedic implants, oral surgery

Fig. 2. Photograph shows the torque-meter device

Fig. 3. Photograph shows the pull-out apparatus.

and maxillofacial surgery, and in the evalution of osteointegration so determine long- term implant survival.

In 3 rabbits of each group (6 rabbits, 12 screws), the distal femoral metaphysis was resected as the screws together with the surrounding bone for histomorphometric evaluation. Sections were taken and stained without decalcification of the samples and histomorphometric evaluation was made for comparison between the groups at the microscopic level. Digital photographs of the prepared sections were taken under a light microscope at ×4 magnification and the images were recorded on the computer (Olympus DP 70, Tokyo, Japan). Measurements on the recorded images were calculated as Bone Implant Contact (BIC)percentages using a semi-automatic image analysis program (Image J 1.43u, Wayne Rasband, National Institute of Health, USA). The BIC calculation was made using the following formula:

$$BIC = \frac{\text{Bone-Implant contact length}}{\text{Surrounding length of the whole implant}} \times 100$$

Statistical Analysis

For statistical analysis, SPSS 13.0 (Windows v 13.0, SPSS Inc, Chicago, IL, USA) statistical software program was used. Pull-out torque, removal torque and BIC values were compared between the groups with and without antibiotics using the Mann Whitney U-test. Correlations of these values for the groups were examined with Pearson correlation analysis. Data were expressed as a mean, median, and standard deviation. A value of $p < 0.05$ was accepted as statistically significant. This experimental study was performed with the least number of animals because of ethical principals and animal rights. The biomechanical and histomorphometric evaluation was performed with a similar number of subjects in the literature. Power analysis was calculated according to the experimental animal study of Moojen *et al*. [15] and 20 rabbits were included in the study.

RESULTS

In Group 2 (not antibiotic-coated), 1 rabbit died due to diarrhea and was replaced by the addition of a new rabbit that had been operated on. No other problems were encountered throughout the experiment.

In the pull-out test, the values of the screws in Group 2 (not antibiotic-coated) were higher than those of the screws in Group 1 (antibiotic-coated) [290.90N (Newton) (238.50-330.60), 195.41N (129.20-281.50); $p = 0.009$). In the removal torque test, the values of the screws in Group 1 (antibiotic-coated) were higher than those in Group 2 (not antibiotic-coated) [10.07N/cm (6.3-11.7), 7.17N/cm (4.8-10.2); $p = 0.017$] (Table 1). No positive correlation was determined between the

Table 1. Bone implant contact percentages, pull-out test values and removal torque test values

	Antibiotic coated group median (min, max)	Control group median (min, max)	*p value*
Bone Implant Contact (%)	48.11 (36.90, 79.10)	57.66 (47.80, 73.50)	**0.132**
Removal Torque (N/cm[1])	10.07 (6.30, 11.70)	7.17 (4.80, 10.20)	**0.017**
Pull-out (N[2])	195.41 (129.20, 281.50)	290.90 (238.50, 330.60)	**0.009**

[1]Newton/centimeter, [2]Newton

pull-out test and the removal torque test (r = 0.88). From the histomorphometric results, osteointegration was determined in both groups. Following the 4weeks healing period, new bone formation was seen on the implant-bone interface under polarised light microscope imaging with toluidin blue staining. In Group 1, mean BIC was calculated as 48.11% (36.9-79.1) and in Group 2, mean BIC was 57.66% (47.8-73.5). In the general evaluation of the groups, although the BIC value of Group 1 was lower than that of Group 2, the difference was not determined as statistically significant (p = 0.132).

DISCUSSION

In this study, the effect on bone-implant osteointegration of a direct coating of teicoplanin on titanium screws was investigated and the results showed that teicoplanin coating did not interfere with osteointegration biomechanically or histomorphometrically. To the best of our knowledge, there are no other studies in the literature which have researched the effect of teicoplanin coating on osteointegration.

In an experimental study, the application of tobramycin to PA coated titanium foam on cylindrical titanium rods appeared to have a beneficial effect on implant fixationin a rabbit Staphylococcus aureus infection model histomorphometrically, which would result in improved longterm implant survival. Although the difference was not statistically significant (21 ± 3% for PA-tobra, 16 ± 3% for PA), it was reported that the effect of tobramycin and periapatite coating on osteointegration was relatively better. In the same study, it was reported that there was a high local antibiotic effect of tobramycin and periapatite coating as infection prophylaxis in uncemented implants and it was concluded that it could be useful for biological fixation which is important for the longterm survival of the prosthesis. However, due to the suppression of infections with local tobramycin coated implants of a group of rabbits in that study, the effect of the antibiotic coating on osteointegration could not be evaluated independently [15]. Gentamycin hydroxyapatite and gentamycin RGD (arginine-glycine-aspartate) coatings of wires expressed antibiotics for up to 2 days and new bone formation were not inhibitedas well [5]. In another experimental rabbit study in which antibiotic soaked microparticles were used, it was shown that the coating with these microparticles was both protectives against infection and did not affect osteointegration [9]. In our study, we used titanium screws in metaphyseal area of rabbit femurs which we think screws imitate the cementless femoral stems and acetabular cups because they actually have primary stabilization through the metaphyseal region and as if they lock in with increasing diameters. In addition, we did not use an infection model in the current study and eliminate the disadvantage of suppression of infection so that we could evaluate the effect of antibiotic coating just on osteointegration contrary to the abovementioned study.

In the current study, to evaluate the connecting force of the implant to the bone, the pull-out and removal torque tests were applied as biomechanical test methods. Using the removal torque test, three-dimensional evaluation is made possible of the interface between the bone and the implant and is accepted as an important test in the evaluation of osteointegration [16, 17]. This method focuses primarily on the properties of the conflicting forces related to the interface [13]. In cases where the removal torque test could be standardized, it has been reported to be a reliable method by many researchers [13]. There are a limited number of studies on the use of temporary implants such as mini implants in clinical studies as it is

an invasive test [14]. By creating shearing force in the implant-bone interface, this test aims to measure the breaking resistance of this connection. Forced rotation against the direction in which the implant has been tightened inside the bone results in increased tension in the interface and a break occurring at the point at which it exceeds maximum resistance strength of the implant-bone connection, which causes movement of the implant [18]. In studies where the removal torque test has been applied, force scales such as digital torquemeter and digital dynamometer have been used [13, 18].

One of the findings of the current study was that different results were obtained from the groups in the pull-out and removal torque tests. The removal torque test is known to be used in the measurement of biomechanical properties of the implant-bone interface, whereas the pull-out test shows the biomechanical properties of the bone surrounding the implant [19]. In the pull-out test evaluating the bone around the implant, the nonantibiotic coating group values were higher but these values were not as valuable as the removal torque test, as 4-week-time was early in terms of completely mature bone formation around the implant. Long periods of up to 24 weeks are required for the complete connection between the bone and the implant material and for bone remodeling to be fully completed [20]. Therefore, as can be understood from this, the results of the removal torque test are more useful in the evaluation of osteointegration. The lower values of the antibiotic coating group in the pull-out test can be considered to be because of the relatively minor percentage values in the bone-implant contact of the screws of this group in the histomorphometric analysis.

In our study, the connection between the bone and the implant was examined with the histomorphometric method. Histomorphometry presents data which provide the possibility of quantitative evaluation of the biological fixation of theimplant at the microscopic level [5]. With this method, metallic implants are evaluated in situ thus avoiding cutting and damage to the implant-bone interface when removing the implant. Therefore, osteointegration is an evaluation method which has been used for a long time together with mechanical tests in the determination of implant survival in dental surgery and maxillofacial surgery and in the evaluation of orthopedic implants [12, 19, 21].

One might argue that the mini-screws that we used in our study did not reflect all the orthopedic devices. But, we put the screws in metaphyseal area of the long bone of rabbit femur and this site is the primary stabilizing site of cementless femoral stems and acetabular cups in terms of arthroplasty. We think that this site reflects osteointegration of the cementless prosthesis. A Kirschner wire coated with phage and linezolid was used in one study and authors made conclusions about arthroplasty. Gentamycin hydroxyapatite and gentamycin RGD (arginine-glycin-aspartate) coatings of wires had similar amounts of new bone formation regarding the proximal and distal metaphyseal area of rabbit tibia [5]. It is hard to simulate the cementless prosthesis in animals since a wire which has been implanted intramedullary had been used in animal studies and biomechanical loading of these wires which is crucial for new bone formation is not an appropriate method. Because of those reasons, we studied the metaphyseal region for osteointegration which is important for primary stability of cementless femoral stems and acetabular cups in joint replacement. Additionally, we used screws and we think that it also imitates the acetabular prosthesis since it also locks into the acetabulum. Besides, the screws naturally reflect the other orthopedic fixation devices. We also studied the effect of direct teicoplanin coverage on bone ingrowth. One advantage of our study is the histomorphometric analysis showing the bone-implant contact directly and numeric comparison could be done easily between groups. In the study of Moojen *et al*. [15],tobramycin periapatite coating on titanium implantshas been shown to have better osteointegration histomorphometrically.

Teicoplanin is a glycopeptide antibiotic and has a broad spectrum, including gram-positive aerobic and anaerobic bacteria as well as methicillin-resistant S. Aureus (MRSA) [22-26]. The observation that teicoplanin can penetrate into muscle and bone tissues has made its use parenterally common in bone and joint infections [27]. It is very effective against all staphylococci, streptococci, enterococci, and pneumococci. In the current study, we used the technique described by Darouiche *et al*. [12] for antibiotic coating. In this technique, there is a direct antibiotic

coating. It has been shown to be effective in vitro and in vivo against Staphylococcus aureus infection in a rabbit model [11].

CONCLUSION

In conclusion, when it is considered that direct coating of titanium screws with teicoplanin did not inhibit osteointegration, which is important in the biological contact of prosthetic implants, this method can be recommended to be used especially in implants with expected long-term function. Further experimental and clinical studies are required on this subject.

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The relationship between postoperative atrial fibrillation after coronary artery bypass surgery and inflammation

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ABSTRACT

Objectives: Postoperative atrial fibrillation (PoAF) may occur up to 50% after coronary artery bypass grafting (CABG) operations. The most important problems related to this are prolonged hospitalizations, thromboembolic cerebrovascular events and new onset heart failure. In this study, we aimed to investigate the relation of high sensitivity C-reactive protein (hsCRP) and heat shock protein 70 (HSP70) levels and occurrence of PoAF in patients undergoing isolated CABG.

Methods: Patients who underwent elective isolated coronary artery bypass surgery between November 2008 and April 2009 in the Cardiovascular Surgery Clinic of Dıskapı Yıldırım Beyazıt Training and Research Hospital were prospectively included in the study.

Results: A total of 40 patients (20 Off-pump CABG (OPCABG), mean age: 59.3 ± 5.56 years) and 20 On-pump CABG (mean age: 60.7 ± 5.3 years)) were included in the study. PoAF ratio was 25% in on-pump CABG patients and 15% in OPCABG group ($p = 0.356$). Age and diameters of the heart cavities were statistically significantly higher in patients with POAF in both surgical groups. Left ventricular ejection fraction was significantly lower in patients with PoAF. Preoperative hsCRP, postoperative hsCRP, preoperative HSP70 and postoperative HSP70 levels were significantly higher in patients with PoAF who underwent OPCABG ($p = 0.018$, $p = 0.044$, $p < 0.001$ and $p = 0.047$; respectively).

Conclusions: As a result, PoAF is undesirable for CABG operations. PoAF can be predicted by evaluating HSP70 and hsCRP values before coronary bypass operations.

Keywords: Coronary artery bypass grafting, postoperative atrial fibrillation, inflammation, heat shock protein 70

Coronary artery disease (CAD) is the leading cause of mortality worldwide. Although technological advances in endovascular interventions have increased treatment options in recent years, coronary artery bypass grafting (CABG) surgery is still an effective and important treatment modality [1, 2]. CABG is a major surgical procedure and it is important to predict the possible morbid and mortal conditions after surgerical approach and to take the necessary precautions. Cardiac rhythm problems after these operations are important and the most common one is atrial fibrillation. Postoperative atrial fibrillation (PoAF) may occur up to 50% after CABG operations [3]. The most important problems related to this are prolonged hospitalizations, thromboembolic cerebrovascular events and new onset heart failure. The

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risk factors for PoAF development includes advanced age, history of cerebrovascular event, existence of preoperative heart failure, and chronic pulmonary disease [4].

The role of inflammatory parameters in the development and prognosis of cardiovascular diseases has been the subject of research. One of those, high sensitivity C-reactive protein (hsCRP), is a valuable parameter that is safe and easily detectable [5]. Blood hsCRP levels is found to be higher in patients suffering from atrial fibrillation (AF) than normal individuals in several studies [6, 7].

Heat Shock Protein 70 (HSP70), which is one of the heat shock protein family, can limit the damage in the cells exposed to stress. A study revealed that, intracellular HSP70 levels in the right atrial tissue were shown to be negatively correlated with PoAF [8].
In this study, we aimed to investigate the relation of hsCRP and HSP70 levels and occurrence of PoAF in patients undergoing isolated CABG.

METHODS

Patients

After obtaining local ethical comitee approval, 40 patients who underwent elective isolated coronary artery bypass surgery between November 2008 and April 2009 in the Cardiovascular Surgery Clinic of Dışkapı Yıldırım Beyazıt Training and Research Hospital were prospectively included in the study.

Patients who were scheduled forelective CABG between the ages of 40-70 years, left ventricular functions were not depressed (ejection fraction [EF] > 45%), had no cardiac valve pathology, left atrial and right atrial diameters were not increased, had no chronic obstructive pulmonary disease, had no preoperative arrhythmia and did not have ahistory of any antiarrhythmic drug usage were included in the study.

Patients with previous history of arrhythmia, advanced age (> 70) patients, heart failure patients, patients who suffered from an acute coronary syndrome attack in the last 60 days, patients who were operated under emergency conditions, patients with temporary or permanent pacemaker, patients who had proximal right coronary arterylesions, patients with inotropic need for more than 24 hours postoperatively and/or intra-aortic balloon pump implanted, patients whose preoperative echocardiogram revealed grade 2 or more mitral insufficiency or moderate and severe mitral stenosis or greater than 2 degree aortic insufficiency or with moderate and severe aortic stenosis or grade 2 or higher tricuspid regurgitation or moderate to severe tricuspid stenosis, patients of impaired renal function (serum creatine levels > 2mg / dl), patients who were diagnosed with pulmonary embolism in the last 60 days prior to CABG, and severe lung disease that may lead to right heart failure was excluded from the study group.

Age, gender, presence of diabetes mellitus, hypertension, hypercholesterolemia, preoperative EF, left ventricular end-systolic diameter (LVESD), left ventricular end-diastolic diameter (LVEDD), left atrial diameter (LAD), right atrial diameter (RAD), righ ventricular diameter(RVD), duration of total anesthesia time, postoperative inotropic drug support (5-15 µg / kg / min dobutamine infusion if low flow or hypotension persists noradrenaline infusion) and PoAF development were recorded for all patients. Also for On-pump patients, cross-clamp (CC) time and cardiopulmonary bypass (CPB) time were recorded.

Laboratory Test Analysis

Blood samples were collected on the day before the operation and on the postoperative 3rd day to measure hsCRP and HSP70 levels. Blood samples were taken after a fasting period longer than 12 hours. Platelet-poor plasma samples were obtained by centrifugation at 3500 rpm for 5 minutes at + 4 ° C. Samples were stored at -80 ° C. After all samples were prepared, HSP70levels were measured in the HSP70 ELISA Kit test system. In the study, the unit for HSP70 was taken as ng / ml. The lower limit of the Hsp 70 level that the test can detect is 0.039 ng / ml. Serum levels of hsCRP (Dade Behring, CardioPhase hsCRP) were studied by immunonephelometer (Dade Behring, Marburg GmbH, Germany). The normal value of hsCRP was < 3.19mg / dl.

Postoperative Cardiac Rhytm Analysis

All patients were fully monitored includingelectrocardiography (ECG) monitoring during intensive care follow-up and 12-lead ECG recordings were recorded daily during the postoperative hospital stay.In addition, 12-lead ECG was performed in patients with palpitation, sweating, dyspnea and chest

pain during the follow-up.

PoAF was defined as the absence of p wave and development of irregular ventricular rhythm before QRS complex in all of the ECG derivations.

Statistical Analysis

In this study, statistical analyzes were performed using SPSS 16.0 statistical package program. The p values obtained in the test results were evaluated at significance level below 0.05. Mean and standard deviations were calculated using descriptive methods for continuous and ordinal data. The distribution of normality were tested by "Kolmogorov-Smirnov test" and "Shapiro-Wilk test". "Student 's t test" was used for the data showing normal distribution and "Mann-Whitney U test" was used for the data that did not fit the normal distribution. Frequency and persentage analysis was performed for nominal data and Chi Square test was used to compare these data.

RESULTS

A total of 40 patients (20 OPCABG (mean age: 59.3 ± 5.56 years) and 20 On-pump CABG (mean age: 60.7 ± 5.3 years) were included in the study. There were no statistically significant difference between the two surgical groups in terms of hypertension, diabetes mellitus, hyperlipdemia, preoperative echocardiography findings, distal anostomosis numbers, total anesthesia duration and inotropic requirements. PoAF ratio was 25% in on-pump CABG patients and 15% in OPCABG group (p = 0.356). Demographic characteristics and peroperative data of both surgical groups are given in Table 1.

The parameters affecting the development of PoAF were evaluated separately for both surgical groups and the data are given in Table 2. Age, LVESD, LVEDD, LAD, RAD and RVD were statistically significantly higher in patients with PoAF in both surgical groups. Left ventricular ejection fraction was significantly lower in patients with PoAF. When the surgical groups were individually evaluated and PoAF and the rest were compared, there was no difference between the groups in terms of the number of distal anostomosis, inotropic requirement, and total anesthesia duration times. There were no significant difference between the patients of PoAF and the rest

Table 1. Demographic characteristics and peroperative features of the patients

Characteristics	Off-Pump (n = 20)	On-Pump (n = 20)	*p* value
Age (years)	59.3 ± 5.56	60.7 ± 5.3	0.424
Hypertension, n (%)	16 (80)	18 (90)	0.661
Diabetes mellitus, n (%)	10 (50)	13 (65)	0.523
Hiperlipdemia, n (%)	18 (90)	18 (90)	1.000
EF (%)	49.2 ± 2.6	50.2 ± 3.5	0.292
LVEDD (cm)	4.8 ± 0.5	4.7 ± 0.6	0.726
LVESD (cm)	3 ± 0.3	3.1 ± 0.4	0.404
LAD (cm)	3 ± 0.5	3.2 ± 0.5	0.135
RAD (cm)	3.54 ± 0.58	3.56 ± 0.59	0.893
RVD (cm)	2.2 ± 0.18	2.13 ± 0.21	0.273
Distal anostomosis number > 2, n (%)	10 (50)	15 (75)	0.102
Inotropic support, n (%)	9 (45)	12 (60)	0.342
Anesthesia time (min)	216.4 ± 26.8	241.8 ± 40.7	0.026
PoAF, n (%)	3 (15)	5 (25)	0.356

Data are shown as mean ± standard deviation or number (%). EF = Ejection fraction, LVESD = Left ventriculer end-systolic diameter, LVEDD = Left ventriculer end-diastolic diameter, LAD = Left atrial diameter, RAD = Right atrial diameter, RVD = Right ventriculer diameter, PoAF = Postoperative atrial fibrillation

Table 2. Comparison of demographic and peroperative characteristics of patients in two surgical groups according to PoAF status

Characteristics		Off-Pump (n = 20)	On-Pump (n = 20)	*p* value
Age (years)	PoAF (-)	58.2 ± 5.3[a]	59 ± 4.9[b]	[a]**0.038**
	PoAF (+)	65.3 ± 2.3[a]	65.8 ± 2.8[b]	[b]**0.010**
EF (%)	PoAF (-)	49.8 ± 2.2[a]	51.5 ± 3[b]	[a]**0.007**
	PoAF (+)	45.6 ± 0.57[a]	46.4 ± 1.5[b]	[b]**0.002**
LVESD (cm)	PoAF (-)	3 ± 0.36[a]	3 ± 0.38[b]	[a]**< 0.001**
	PoAF (+)	3.5 ± 0.1[a]	3.5 ± 0.15[b]	[b]**0.011**
LVEDD (cm)	PoAF (-)	4.77 ± 0.55[a]	4.59 ± 0.58[b]	[a]**0.009**
	PoAF (+)	5.26 ± 0.15[a]	5.36 ± 0.2[b]	[b]**0.012**
LAD (cm)	PoAF (-)	2.9 ± 0.45[a]	3.1 ± 0.45[b]	[a]**< 0.001**
	PoAF (+)	3.73 ± 0.11[a]	3.78 ± 0.13[b]	[b]**0.005**
RAD (cm)	PoAF (-)	3.44 ± 0.56[a]	3.36 ± 0.52[b]	[a]**0.002**
	PoAF (+)	4.1 ± 0.17[a]	4.18 ± 0.18[b]	[b]**0.003**
RVD (cm)	PoAF (-)	2.15 ± 0.15[a]	2 ± 0.15[b]	[a]**0.003**
	PoAF (+)	2.47 ± 0.05[a]	2.4 ± 0.07[b]	[b]**< 0.001**
Distal anostmosis > 2, n(%)	PoAF (-)	9 (52.9)[a]	11 (73.3)[b]	[a]1.000
	PoAF (+)	1 (33.3)[a]	4 (80)[b]	[b]1.000
Inotropic support, n(%)	PoAF (-)	8 (47)[a]	9 (60)[b]	[a]1.000
	PoAF (+)	1 (33.3)[a]	3 (60)[b]	[b]1.000
CC time (min)	PoAF (-)		58.9 ± 22.5[b]	[b]0.668
	PoAF (+)		54.2 ± 14.2[b]	
CPB time (min)	PoAF (-)		109.2 ± 42[b]	
	PoAF (+)		96.4 ± 23.9[b]	[b]0.517
Anesthesia time (min)	PoAF (-)	216 ± 27.2[a]	243.6 ± 43.8[b]	[a]0.879
	PoAF (+)	218.6 ± 29.9[a]	236.4 ± 33.2[b]	[b]0.740

Data are shown as mean ± standard deviation or number (%). EF = Ejection fraction, LVESD = Left ventriculer end-systolic diameter, LVEDD = Left ventriculer end-diastolic diameter, LAD = Left atrial diameter, RAD = Right atrial diameter, RVD = Right ventriculer diameter, CC = Cross-clamp, CPB = Cardiopulmonary bypass, PoAF = Postoperative atrial fibrillation. Evaluating patients with and without PoAF among OPCABG patients [a], Evaluating patients with and without PoAF among On-pump CABG patients [b]

in terms of CC time, CPB time in the On-pump CABG group.

Preoperative hsCRP, postoperative hsCRP, preoperative HSP70 and postoperative HSP70 levels were significantly higher in patients with PoAF who underwent OPCABG ($p = 0.018$, $p = 0.044$, $p < 0.001$ and $p = 0.047$; respectively). (Table 3).

Preoperative hsCRP, postoperative hsCRP, preoperative HSP70 and postoperative HSP70 values were significantly higher in patients with PoAF who underwent CABG with cardiopulmonary bypass ($p = 0.013$, $p = 0.009$, $p = 0.011$ and $p < 0.001$; respectively) (Table 3).

DISCUSSION

Atrial fibrillation is an atrial activation and rhythm disorder in which the atrium loses its mechanical functions. This is often accompanied by a rapid and irregular ventricular rhythm. This occurs frequently after CABG operations, and in several studies the inci-

Table 3. Preoperative and postoperative change of HSP70 and hsCRP values between patients with and without PoAF in two surgical groups

Characteristics		Off-Pump (n = 20)	On-Pump (n = 20)	*p* value
Preoperative hsCRP (mg/dL)	PoAF (-)	72.6 ± 10.6[a]	52.5 ± 25.8[b]	[a]**0.018**
	PoAF (+)	89.5 ± 7.5[a]	92.9 ± 36[b]	[b]**0.013**
Postoperative hsCRP (mg/dL)	PoAF (-)	97.4 ± 15.7[a]	125.6 ± 36.8[b]	[a]**0.044**
	PoAF (+)	122.9 ± 34.6[a]	176.8 ± 21.6[b]	[b]**0.009**
Preoperative HSP70 (ng/dL)	PoAF (-)	1.37 ± 0.2[a]	1.69 ± 0.58[b]	[a]**< 0.001**
	PoAF (+)	2.08 ± 0.1[a]	2.52 ± 0.55[b]	[b]**0.011**
Postoperative HSP70 (ng/dL)	PoAF (-)	1.73 ± 0.35[a]	2.13 ± 0.71[b]	[a]**0.047**
	PoAF (+)	2.23 ± 0.51[a]	3.91 ± 0.6[b]	[b]**< 0.001**

Data are shown as mean ± standard deviation. hsCRP = High-sensitivity C-reactive protein, HSP70 = Heat shock protein 70, PoAF = Postoperative atrial fibrillation. Evaluating patients with and without PoAF among OPCABG patients [a], Evaluating patients with and without PoAF among On-pump CABG patients [b]

dence rates are given in the range of 20-50% [9]. According to data from the United States, PoAF can increase hospital costs [10]. The occurrence after coronary bypass surgery increases postoperative mortality and morbidity. In this study, we demonstrated that preoperative and postoperative elevated hsCRP and HSP70 levels were associated with the occurrence of PoAF in on pump CABG and OPCABG patients.

Although CABG operations using cardiopulmonary bypass are proved to be safe and effective, side effects can be occurred due to blood and foreign surface contact. Additionally, ischemia-reperfusion injury due to cross-clamping of the ascending aorta may also adversely affect other vital organs such askidney, intestines and brain [11]. In OPCABG these CPB-related effects have been avoided, and postoperative complications have been shown to be less [12]. One of the undesirable and common problems after these operations is PoAF.

After on pump CABG, the inflammatory response increases due to the CPB system, posing a risk for the development of PoAF [13]. However, in a study conducted by Lin *et al*. [14] OPCABG and on pump CABG PoAF rates were found to be similar. In our study, PoAF rates were similar between the two surgical groups.

The relationship of C-reactive protein (CRP) levels, that predicts the inflammation and tissue damage, and cardiovascular diseases has been extensively investigated in recent years. However, hsCRP values have become more prominent since more sensitive measurements are needed for the cardiovascular system. Plasma CRP shows a proinflammatory effect by clustering with blood lipids and activating the complement pathway [15].

Inflammation also poses a risk for AF and also plays a role in prolonging AF periods [16]. Because arrhythmia occurs as a result of fibrosis in atrial muscle structures and conduction pathways [7]. The most important reason for this is inflammatory processes. Accordingly, in a study by Ucar *et al*. [17] 49 patients who underwent on pump CABG were prospectively examined and the relationship between PoAF and inflammatory markers was investigated. In this study, the authors reported PoAF rates as 28.5% and found that hsCRPvalues collectedpreoperatively and postoperatively on the first day were significantly higher in the group developing PoAF [17]. In our study, we studied preoperative and postoperative 3rd day hsCRP values and found significantly higher in patients who developed PoAF in both surgical groups.

Heat shock proteins (HSPs) are heat-permeable gene products that protect cells against stress. HSP90, HSP70 and HSP60 belong to the first group family of these proteins [18]. HSP70 plays important intracellular roles in facilitating protein transport, preventing misfolding of newly synthesized polypeptide chains and preserving protein denaturation [19]. Many studies have shown increased HSP levels in atrial tissue in patients with paroxysmal or persistent AF [20, 21]. In

a study by Mandal *et al*. [8], HSP 70 levels obtained from atrial tissue with AF found to be negatively correlated with PoAF development. In our study, HSP70 peripheral blood levels were found to be high in patients with PoAF in both surgical groups.

Although advanced age generally poses a risk in all surgeries, it is one of the most important risk factors for PoAF in patients undergoing CABG. Myocardial fibrosis and accumulating amyloid stores with increasing age lead to AF by causing reentries between the atria [22]. Studies have shown that advanged age is a risk factor for PoAF after cardiac surgery [23, 24]. In our study, patients over 70 years of age were excluded, but age was higher in the PoAF group in both surgical groups.

Increased left atrial diameter and low ejection fraction are also risk factors for PoAF. This means increased fibrosis in the heart muscle and therefore increased inflammation. As a result, the risk for AF increases [25, 26]. In our study, left atrial diameters, LVESD, LVESD and RVD were significantly higher and EF was significantly lower in patients with PoAF in both surgical groups.

Limitations

The most important limitations of our study includesmall number of the patients and single-centeredness. Another limitation is the fact that holter recording could not be used in rhythm follow-ups during the follow-up of the patients.

CONCLUSION

As a result, PoAF is undesirable for CABG operations. PoAF can be predicted by evaluating HSP70 and hsCRP values before coronary bypass operations. The importance of these blood parameters should be investigated by prospective and multicenter studies with high number of patients.

Conflict of interest

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The European Research Journal 2020;6(5):416-421 Original Article

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Examination of marital adjustment and sexuality in patients with schizophrenia

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ABSTRACT

Objectives: The main aim of the study is to compare marital adjustment and sexuality in schizophrenia patients.
Methods: The sample of the study consists of 48 outpatients with a diagnosis of schizophrenia according to DSM V by the Department of Psychiatry, Bursa Uludağ University School of Medicine and 48 healthy volunteers. Sociodemographic Information Form, Marriage Adjustment Scale (MAS), Arizona Sexual Experiences Scale (ASEX) - (Female and Male form) are applied to both groups.
Results: A significant difference was observed between individuals with schizophrenia and healthy group according to sexual dysfunction. The incidence of sexual dysfunction in schizophrenia patients is high. While the average values of the individuals' MAS were higher in the healthy group, no significant difference was found between the groups according to the MAS total score.
Conclusions: There is no difference in terms of compliance with marriage between the healthy group and schizophrenic patients, but there is a difference in terms of sexual function. Data from this study suggest that schizophrenia may not be a factor in marital adjustment but may be a factor that may cause sexual dysfunction. All schizophrenic patients in our study used drugs. It cannot be ruled out that drug use may cause sexual dysfunction.
Keywords: Schizophrenia, marital satisfaction, marital adjustment, sexual satisfaction

Schizophrenia is a mental disorder that begins at a young age, causing the individual to keep himself away from human relations and realities, to be attracted to his inner world, paving the way for significant disturbances in emotions, thoughts and behaviors, bringing along loss of work power [1]. It is a chronic disorder that is seen in both sexes, even though it is usually seen less in women than men, it follows a worse course in men. The age of onset is often in young adulthood, but it can also begin later, especially as in the case of women [1-3]. The progression and termination of the disease vary depending on the patients. This disease is a public health problem in which symptoms and signs can be seen in all areas of mental state [4]. Although schizophrenia is known to be multifactorial; genetic predisposition, environmental factors, and perinatal factors are thought to play a role frequently in its etiology [5]. Today, in its treatment, medical treatment and Electroconvulsive Therapy (EKT), being in the first place, and psychoynamic approaches such as, Cognitive Behavioral Therapy (CBT) and group therapy can be used as supportive therapy [6]. According to the general population, the rate of marriage is lower and divorce rates are higher in schizophrenia patients [7]. Despite the lack of evidence that marriages play a protective role, Eatol's study of ş schizophrenia and marriage en in 1975 found that morbidity was higher in never-married

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schizophrenic patients than in divorced patients [8]. Although the disease affects many aspects of sexuality, a number of patients can develop a meaningful close relationship [9]. Studies have reported that schizophrenia patients have an interest in sexuality but have difficulty in expressing it [10].

Table 1. Comparison of schizophrenic and healthy group in terms of sociodemographic data

	Schizophrenia (n = 48)	Healthy (n = 48)	*p* value
Age (years)	52.50 (21-65)	49 (30-67)	0.207
Gender (F/M)			
Female	20 (41.70%)	17 (35.40%)	0.529
Male	28 (58.3%)	31 (64.60%)	
Employment status			
Working	12 (25%)	38 (79.20%)	**< 0.001**
Not working	36 (75%)	10 (20.80%)	
Educational attainment			
Less than high school	33 (68.80%)	10 (20.80%)	**< 0.001**
High school	10 (20.80%)	26 (54.20%)	
Undergraduate and graduate	5 (10.40%)	12 (25%)	
Profession			
State servant	8 (16.70%)	22 (45.80%)	**0.004**
Worker	20 (41.70%)	18 (37.50%)	
Unemployed	9 (18.80%)	6 (12.50%)	
Retired	11 (22.90%)	2 (4.20%)	
Income (TL))			**< 0.001**
0-1,500	23 (47.90%)	8 (16.70%)	
1,501-3,000	23 (47.90%)	23 (47.90%)	
3,001-4,500	1 (2.10%)	9 (18.80%)	
> 4,500	1 (2.10%)	8 (16.70%)	
Lenght of marriage (year)	3 (1-3)	3 (1-3)	0.390
Cohabiting relatives			
Wife/husband	23 (47.90%)	28 (58.30%)	0.306
Wife/husband & other relatives & children	25 (52.10%)	20 (41.70%)	
Sexual dysfunction			
No	6 (12.50%)	33 (68.80%)	**< 0.001**
Yes	42 (87.50%)	15 (31.30%)	
Marriage adjustment			
Compatible	28 (58.30%)	35 (72.90%)	0.133
Incompatible	20 (41.70%)	13 (27.10%)	
MC total	41.92 ± 12.57	47.25 ± 7.88	**0.015**
ASEX total	20 (5-29)	10 (5-28)	**< 0.001**

Data are expressed as mean ± standard deviation, median (minimum- maximum) and n (%).

METHODS

The sample of the study consists of 48 outpatients with a diagnosis of schizophrenia according to DSM V by the Department of Psychiatry, Bursa Uludağ University School of Medicine and 48 healthy volunteers. Sociodemographic Information Form, Marriage Adjustment Scale (MAS), Arizona Sexual Experiences Scale (ASEX) - (Female and Male form) are are given to patients and healthy group.

Participants are between the ages of 18-65 and married, they are not mentally retarded and do not have physical disorders that cause sexual dysfunction. Those with any systemic disease (diabetes, coronary heart disease, prostate) that could prevent their sexuality were excluded from the study. The aim of the study is to see whether the disease affects sexual function. All schizophrenic patients included in the study are drug users.

Arizona Sexual Experiences Scale (ASEX)

It consists of five items of six Likert type, consisting of male and female forms. 11 and above points indicate sexual dysfunction [11].

Marital Adjustment Test (MAT)

Scoring in the scale increases from noncompliance to compliance. The lowest compliance score is found to be 2, the highest compliance score is 58, and the cut-off point is 43, 5. The lower total score in the scale points out mismatch in the marriage, the higher total score in the scale points out adjustment in the marriage [12].

Statistical Analysis

The conformity of the variables to the normal distribution is investigated by ShapiroWilk test. Continuous variables are expressed with median (minimum: maximum) and mean ± standard deviation values. Categorical variables are expressed in n (%). According to the results of normality test, Mann Whitney U and independent double sample t tests were used in the comparisons between the two groups. Pearson Chi - Square, Fisher's exact Chi - Square and Fisher - Freeman - Halton tests were used for the comparison of categorical variables. For statistical analysis, SPSS (IBM Corp. Released 2012. IBM SPSS Statisticsfor Windows, Version21.0. Armonk, NY: IBM Corp.) was used and $p < 0.05$ is considered statistically significant.

RESULTS

Table 1 shows the demographic characteristics of schizophrenic and healthy group. Table 2 presents questions that are unique to schizophrenia patients and patient characteristics. In Table 3, there is no difference between the groups in the schizophrenia group according to the marriage compliance scale. As a result of the classification made within the

Table 2. Disease characteristics of schizophrenia group (n = 48)

Did the disease start during marriage?	Data
Yes	34 (70.80%)
No	14 (29.20%)
Duration of treatment (years)	
0-5 years	12 (25%)
6-10 years	10 (20.80%)
>10 years	26 (54.20)
Hospitalization	
Yes	32 (66.70%)
No	16 (33.30%)
Number of hospitalizations	
0-5	27 (84.40%)
6-10	2 (6.30%)
>10	3 (9.40%)
Did he have an attack	
Yes	37 (77.10%)
No	11 (22.90%)
Number of attacks	
0-5	25 (52.10%)
6-10	3 (6.30%)
>10	9 (41.60%)
Duration of drug use (years)	
0-5	14 (29.20%)
6-10	9 (18.80%)
>10	25 (52.10%)

The data are expressed as n (%).

Table 3. Evaluation of marital adjustment in schizophrenia group

	Marriage Compliance:		*p* value
	Compatible (n = 28)	Incompatible (n = 20)	
Lenght of marriage	3 (1-3)	3 (1-3)	0.658
Drug Use (years)			
0-5	10 (35.70%)	4 (20%)	0.489
6-10	5 (17.90%)	4 (20%)	
>10	13 (46.40%)	12 (60%)	
Hospitalization			
Yes	18 (64.30%)	14 (70%)	0.679
No	10 (35.70%)	6 (30%)	
Number of children	1 (1-2)	1 (1-2)	0.809
Employment status			
Working	8 (28.60%)	4 (20%)	0.499
Not working	20 (71.40%)	16 (80%)	
Sexual dysfunction			
No	2 (7.10%)	4 (20%)	0.218
Yes	26 (92.90%)	16 (80%)	

Data are expressed as median (minimum- maximum), n (%).

schizophrenia group; there is no difference between the groups with and without sexual dysfunction according to the variables in table 4. In tables: Duration of marriage was 3 (1-3) 0-5, 6-10, 11 >; number of children 1 (1-2) 0-5 and 6 >.

Table 4. Evaluation of sexual dysfunction in schizophrenia group

	Sexual dysfunction		*p* value
	No (n = 6)	Yes (n = 42)	
Lenght of marriage	3 (3-3)	3 (1-3)	0.367
Drug Use (years)			
0-5	2 (33.30%)	12 (28.60%)	1.00
6-10	1 (16.70%)	8 (19%)	
>10	3 (50%)	22 (52.40%)	
Number of children	1 (1-1)	1 (1-2)	0.867
Employment status			
Working	0	12 (28.60%)	0.315
Not working	6 (100%)	30 (71.40%)	

Data are expressed as median (minimum: maximum), n (%).

DISCUSSION

The data we have obtained in this section will be reviewed in the light of the general literature. In our study, sociodemographic data obtained from schizophrenia patients were consistent with the data in the general literature [1, 3]. The fact that only 25% of patients in the study group are working, can be associated with low levels of general education and demolition caused by disease [13]. Parallel to this, income levels are also low compared to the general population [14]. As a result of disease and socioeconomic conditions, patients with schizophrenia need social support while continuing their lives [13, 14]. In our study (Table 2), disease characteristics such as hospitalization and number of attacks were also consistent with the general literature [15]. In our study, no difference was found between sexes according to treatment periods. Similarly, in the literature, it is thought that a number of possible differences have disappeared over time as patients have a very long disease process [16].

In the literature, there are very few studies on the sexual dysfunctions of schizophrenia patients. Our findings were also consistent with the literature [17-19]. The study conducted in Turkey by Hocaoglu *et al*. [17] with a group of 101 patients with schizophrenia and 89 healthy persons using ASEX scale, showed that 46% of male patients with schizophrenia and 68% of female patients with schizophrenia has sexual function disorder. Ghadirian *et al*. [19] found in their study with 55 patients with schizophrenia using antipsychotic drugs that 54% of males and 30% of females had sexual dysfunction. In a study of 7655 patients with schizophrenia, Dossenbach *et al*. [18] reported that a form of sexual dysfunction was present in approximately half of the patients. In a study performed by Uçok *et al*. [20], 826 patients with schizophrenia were found to have sexual dysfunction disorder in 52.6% of patients according to ASEX scale.

When the data of our study were examined, even though there is no statistically significant difference between the schizophrenia group and the control group in terms of marital adjustment, the total marital adjustment scores of the schizophrenic patients are lower than those of the healthy group. In our literature review, there was no study about marital adjustment and satisfaction among patients with schizophrenia. As a result of the classification of patients according to MAS, no difference is found between the groups with and without marital adjustment (according to the variables in Table 3). These variables may not have determined the harmony of marriage. Patients are thought to be more indicative of the more abstract understanding of marriage.

As shown in Table 4 within the schizophrenia group, it was found that the variables such as duration of marriage, duration of drug use, number of children and working status did not have any effect on sexual function.

CONCLUSION

There is no difference in terms of marriage adjustment, between the groups of healthy individuals and schizophrenic patients, but there was a difference in terms of sexual function. Data from this study suggest that schizophrenia may not be a factor in marital adjustment but may be a factor that may cause sexual dysfunction. All schizophrenic patients in our study used drugs. It cannot be ruled out that drug use may cause sexual dysfunction. However, there is no difference observed between the schizophrenia patients who have sexual dysfunction or not, due to the duration of drug use. The studies which will be performed with bigger numbers of patients will provide a more healthy evaluation of the data we have obtained.

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 European Research Journal 2020;6(5):422-428 Original Article

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Investigation of the relationship between atherosclerosis and interleukin-6 -174G/C gene polymorphism

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ABSTRACT

Objectives: Atherosclerosis is a chronic disease that causes various cardiovascular complications. The onset and progression of atherosclerosis depends primarily on genetic factors and life style, but the underlying cellular and molecular mechanisms are still unclear. In recent studies, circulating cytokines have been shown to play an important role in inflammatory events. Interleukin-6 (IL-6) plays an important role in the regulation of proinflammation. In this study, a single nucleotide polymorphism of IL-6 gene at position -174 was studied. Our aim was to investigate the relationship between IL-6 -174G/C polymorphism and atherosclerosis.

Methods: In this prospective randomized study, 104 patients were included in both groups. We used Polymerase Chain Reaction-Restriction Fragment Lenght Polymorphism (PCR-RFLP) method to amplify the polymorphism region.

Results: Allele frequency distributions of IL-6 -174G/C polymorphism in the study and control groups were evaluated. There were no statistically significant diversity between A and B allele frequencies.

Conclusions: The allele frequency and genotype distribution between the groups was not statistically different, which indicates another mechanisms on regulation of these cytokines. Single gene polymorphisms are generally not reproducible. Therefore, broad-based studies should be carried out considering suitable conditions and multi-factor features.

Keywords: Coronary atherosclerosis, IL-6 gene polymorphism, risk factors

Atherosclerotic coronary artery disease is the leading cause of cardiovascular morbidity and mortality in developed countries. Risk factors such as presence of family history, lipid disorders, diabetes mellitus, metabolic syndrome, obesity, hypertension, sedentary life, smoking, hyperhomocysteinemia and increased CRP level were determined [1-3].

Many risk factors have been mentioned in the pathogenesis of atherosclerosis and genetic studies have gained importance [4]. Proinflammatory and anti-inflammatory types of cytokines have been demonstrated that contribute to the pathogenesis of many infections, inflammations, autoimmune and malignant diseases. Genes affecting the structures which involved in the inflammatory process have been demonstrated to contribute directly and indirectly to the development of atherosclerosis [5].

Cytokines take an important role in the control and regulation of response versus external antigens and agents, and also play an important role in local and systemic inflammatory response by regulating intercellular relationships. A significant part of the

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cytokines secreted from the immune system are interleukins and their primary task is to stimulate immune system cells. Interleukin-6 (IL-6), which has an important role on cytotoxic T cells, is also involved in a system regulated by these genes [6].

IL-6 is an interleukin that synthesized by IL-1 stimulation by mononuclear phagocytic cells (MFH), endothelial cells, fibroblasts, monocytes, keratinocytes, mesangial cells and bone marrow stromal cells. IL-6 mainly involves in regulation of immune and inflammatory response, hematopoietic system and central nervous system [7]. IL-6, as a pleiotropic cytokine, induces T and B cell differentiation and proliferation and also induces vascular endothelial growth factor production [7]. IL-6 facilitates the adhesion of endothelial cells to lymphocytes by increasing the expression of E-selectin molecules, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in endothelial cells. [7].

The IL-6 gene is located in the chromosome '7p21-14'. To date, two major functional single gene nucleotide polymorphisms have been identified in the IL-6 gene region, namely -572G/C and -174G/C. This gene polymorphism may be effective in many inflammatory events including vascular pathologies by affecting plasma IL-6 and C-reactive protein (CRP) levels [8-10].

It has been declared that 174G⁄C polymorphism may be associated with atherosclerosis and coronary artery disease [11]. The aim of this study is to investigate the relationship between IL-6 -174G/C gene polymorphism, which is responsible for the regulation of the functions of IL-6 and coronary artery atherosclerosis and to contribute to the elucidation of etiologic factors.

METHODS

The Patients

In this prospective randomized study, patients were selected from patients who underwent coronary angiography with a preliminary diagnosis of coronary artery disease. In this study 104 patients with a decision of coronary bypass surgery were included as the study group (Group 1) and 104 patients with normal coronary flow were included as the control group (Group 2). The study was approved by the local institutional Ethical Committee of Cumhuriyet University (Ethical Committee number: 2011-109). Patients with preoperative cerebrovascular disease, presence of valvular heart disease, peripheral arterial disease, renal insufficiency were not included in the study. All data were recorded as age, gender, history of hypertension, diabetes mellitus, smoking.

Blood Sampling and Biochemical Analyses

DNA isolation

Fasting blood samples were taken from antecubital vein of patients who included study. The tubes with EDTA were used for automatic blood count according to the protocol of our hospital.
Total genomic DNA isolation from individuals was performed by making some modifications in the standard phenol-chloroform protocol described by Sambrook *et al*. [12].

Agarose Gel Electrophoresis

Approximately 5µl of the amplification product was taken and mixed with 1µl loading buffer (50% glycerol, 0.1 M EDTA, 0.1% bromophenol blue, Xylene cyano). 1.5% agarose gel was separated in Tris-Boric acid-EDTA (TBE) buffer (0.089 M Tris, 0.089 M Boric Acid and 0.011 M EDTA, pH: 8.3).In the preparation of agarose gel, 10X TBE, distilled water, 0.5µg / ml ethidium bromide and agarose (sigma) were used. After electrophoresis, DNA was visualized under ultraviolet light and Polymerase chain reaction (PCR) products were checked.

PCR Amplification

The region with 431 base pairs which containing the polymorphism region was amplified by polymerase chain reaction (PCR) using primers 5'CAGAAGAACTCAGATGACTG3 'and 5'GTGGGGCTGATTGGAAACC3.The PCR products which obtained were exposed to 2% agarose gel electrophoresis and the emerged bands were stained with ethidium bromide and imaged under UV.

Length Variability Reaction of Restriction Section (LVRS)

In the polymorphism studied in IL-6, genotypes of the individuals were determined by PCR based Length

Variability Reaction of Restriction Section (LVRS) method. Restriction cutting conditions using the enzyme for polymorphism are as follows.

Restriction of digestion:

- Distilled water: 3.5 µl
- Enzyme buffer: 1 µl
- Restriction Enzyme: 0.5 µl
- PCR product: 5 µl
- Total volume: 10

NlaIII restriction enzyme was used to determine the IL-6 locus and Codon -174G > C polymorphism. Enzyme buffer (Tris-acetate pH: 7.9, 10 mM magnesium acetate, 66 mM potassium acetate, 0.1mg / ml BSA, Fermentas) was used at a 10-fold concentration.

Amplification of the primer pair used in the detection of the IL-6 gene codon -174G > C mutation revealed the fragment sizes as follows; GG: 233,202,176,29 bp GC: 233,202,111,122,176,29 CC: 202,111,122,176,29 (total size 640 base pairs).

Sequence Analysis

In order to confirm the genotypes determined by LVRS method, 5% of the samples belonging to individuals with different genotypes (wild, heterozygous and variant genotype) were subjected to sequence analysis. By means of sequence analysis, the risk of partial digestion resulting from LVRS was eliminated. In this study, PCR products were purified from gel using DNA extraction kit (AXYGEN). PCR products of different genotype were run on 1% agarose gel. The bands of each sample in the gel were cut with a scalpel under UV and cut into small pieces on a clean surface and placed in 1.5 ml Eppendorf tubes. Each tube was tared on a precision scale and the weights of the gel pieces were measured. AXYGEN's DNA gel extraction Spin Protocol was used for DNA Isolation from the gel. The gel piece containing the PCR band was placed in the tube. Buffer DE-A (Gel solvent) was added to the gel pieces in each tube 3 times of each volume. Each tube was vortexed until suspended. Each tube was heated to 75 °C for 6-8 minutes to completely dissolve the gel. The tube was vortexed gently every 2-3 minutes and the dissolution of the gel was accelerated. Buffer DE-B (Binding Buffer) was added in as much as the half volume of Buffer DE-A used in the process. An equal amount of isopropanol was added to the sample volume. The filtration column was placed in Eppendorf tubes and centrifuged at 15 000 rpm for 1 min. The filtration column was removed and the liquid descending to the bottom of the tube was pipetted. Then the filtration column was placed again and 500µl of Buffer W1 (Wash Buffer) was added. It was centrifuged for 30 sec at 15,000 rpm. The filtration column was removed and the liquid at the bottom of the tube was pipetted and the column was placed again. 700µl Buffer W2 (Desalinized Buffer) was added and centrifuged at 15,000 rpm for 30 seconds.The filtration column was removed and the liquid descending to the bottom of the tube was pipetted. After placing the filtration column, 700µl of Buffer W2 was added once more and this time centrifuged at 15,000 rpm for 1 min. The filtration column was removed and the liquid descending to the bottom of the tube was pipetted. The filtration column was then placed again. The tube was once again centrifuged at 15,000 rpm for 1 minute and the filtration column was placed in a 1.5 ml centrifuge tube. 30µl of the solvent which was kept on at 65 °C for 1 minute was added to the tube. It was then allowed to stand at room temperature for 1 minute and centrifuged at 15,000 rpm for 1 minute. The filtration column was removed and 5 µl of the dissolved DNA descending to the bottom of the tube was run on 1% agarose gel. Their picture was taken and 25 µl of dissolved DNA samples were sent for sequencing with the primers.

Statistical Analysis

Statistical analysis data were analysed with the Statistical Package for the Social Sciences (IBM SPSS Statistic Inc. version 13.0, Chicago, IL, USA). Continuous variables were expressed as mean ± standard deviation and nominal variables were expressed as frequency and percentage. Kolmogorov-Smirnov test and Shapiro-Wilk tests of normality were used to identify distribution of variables. Student's t test was used to compare two groups for continuous variables with normal distribution. Chi Square test was used to compare two groups for nominal variables. The statistical significance of the IL-6 genotype between the study and controls and the risk coefficient (OR) were calculated using Pearson's X2 test. Fisher's Exact Test was used for values less than five. Probability (p) values less than 0.05 were considered statistically significant.

RESULTS

In the present study, 104 patients were enrolled into each group. The demographic and clinical characteristics of the participants are summarized in Table 1. Significant differences were found in the study group in terms of hyperlipidaemia, smoking, hypertension and diabetes mellitus. In this study, IL-6 -174G/C gene polymorphism has carried out on agarose gel and the results read under UV light (Fig. 1). Allele frequency distributions of IL-6 -174G/C polymorphism in the study and control groups are presented in Table 2. The A allele frequency for IL-6 -174G/C polymorphism was 78.36% in the control group and 81.25% in the study group. B allele frequencies were 21.64% in the control group and 18.75% in the study group. Although allele B frequency was higher in IL-6 -174G/C polymorphism for patients with coronary artery disease, it was not statistically significant. (p = 0.464 OR = 0.83; 95% Cl = 0.51-1.35).

Table 1. Demographic features of the patients

	Study group (n = 104)	Control group (n = 104)	*p* value
Age (years)	61.11 ± 11.86	58 ± 13.35	0.257
Male gender, n (%)	74 (71.15)	44 (42.3)	**< 0.001**
Hypertension, n (%)	72 (69.24)	37 (35.58)	**< 0.001**
Diabetes mellitus, n (%)	36 (34.62)	20 (19.23)	**0.012**
Smoking, n (%)	64 (61.53)	43 (41.34)	**0.004**
Hyperlipidaemia, n (%)	25 (24.04)	20 (19.23)	**0.031**

Table 2 presents the risk prediction for the genotype frequency of IL-6 -174G/C polymorphism between the study and control groups. There was no statistically significant result in comparing AA genotype with AB genotype for IL-6 -174G/C polymorphism (p = 0.257, OR = 0.71; 95% Cl = 0.39-1.28). Similarly, no statistically significant result was observed in comparison with BB genotype. (p = 0.565 (Fisher's Exact test p value), OR = 1.19; 95% Cl = 0.25-5.56).

DISCUSSION

Risk factors for coronary artery disease such as

Table 2. Allele and genotype frequency distributions and risk coefficients

	Study group (n = 104)	Control group (n = 104)	x*	*p* value	OR 95% Cl
Allele Frequency					
A	169 (81.25%)	163 (78.36%)			
B	39 (18.75%)	45 (21.64%)	0.537	0.464	0.83 (0.51-1.35)
Genotype Frequency					
AA	69 (64.35%)	62 (59.61%)			
AB	31(29.80%)	39 (37.50%)	1.283	0.257	0.71 (0.39-1.28)
BB	4 (3.85%)	3 (2.89%)	-	0.565	1.19 (0.25-5.56)

*Fisher's Exact test *p* value

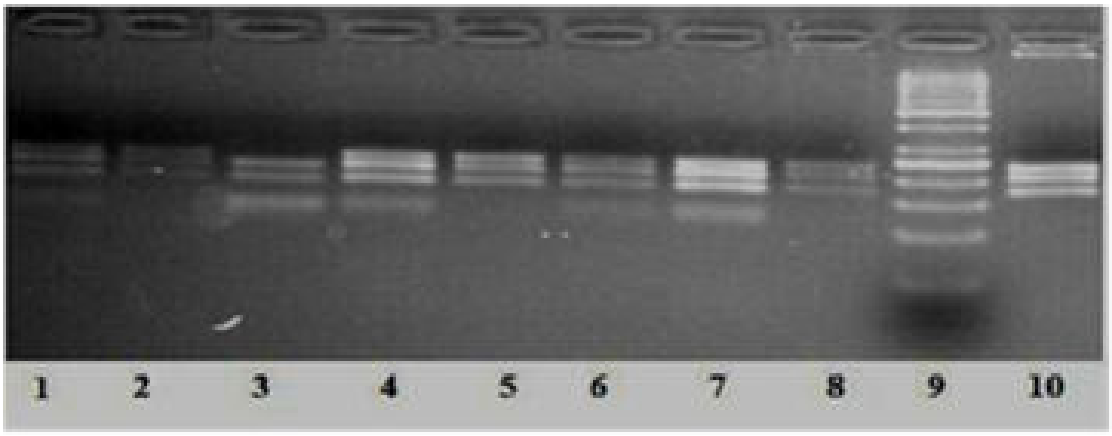

Fig. 1. Cutting results with NlaIII enzyme to determine IL-6 -174G/C change. GG: columns 2,5,8,10 GC: 1,4 columns 6,7 CC: column 3. Column 9 is marker 50bp fermentas.

age, gender, hyperlipidaemia, diabetes mellitus, hypertension, and active smoking were significantly different in the study group (Table 1). Although all these risk factor's distribution were significantly higher in the study group suggested that two groups were not similar in terms of risk factor, it should be remembered that these risk factors, which have been proven to be associated with severe atherosclerosis, are naturally more in the study group. Due to the association of all these risk factors with inflammation and the other immune mechanisms defined for atherosclerosis, it is considered that evaluation of the inflammatory events underlying atherosclerosis and the regulatory mechanisms, can achieve meaningful results. In atherosclerosis, ordinarily inflammatory cytokines tend to reduce receptor levels, whereas cytokines that stimulate macrophage development and differentiation increase receptor levels. Most T cells in the human atherosclerotic plaque are of the TH1 type, which causes macrophage activation and inflammation [13]. The most important TH1 cytokine is interferon gamma (IFN-γ) which has significant vascular activity. IFN-γ stimulates macrophages to increase major macrophage-activating cytokines and phagocytosis and this provides secretion of inflammatory cytokines such as TNF-α and IL-1. This leads to the release of proteolytic enzymes, resulting in the formation of large amounts of toxic oxygen and nitric oxide (NO) radicals [14]. Cytokines such as IL-1, IL-6, IL-10, TNF-α were investigated in these studies, where ischemia and reperfusion models were also frequently discussed [7, 15, 16]. Although these inflammatory events show similar characteristics, their individual ethnic or instantaneous different behaviours have increased efforts to reveal genes responsible for their regulation.

Genetic factors play an important role in atherosclerosis, and genome-wide association studies (GWAS) have identified several single nucleotide polymorphisms (SNPs) associated with increased risk of coronary artery disease. The most potent genetic association has been described for SNPs involved in trans regulation of IFNγ signalling at locus 9p21 [17]. Many of the identified loci have unknown risk mechanisms, but some are associated with lipoprotein metabolism or atherothrombosis [18].

Recently inflammation and genetics are accepted as two important mechanisms in atherosclerosis and arterial thrombosis. In vitro studies and animal experiments have shown that inflammatory markers are significantly increased in clinically stable angina, unstable angina and acute myocardial infarction [16]. Additionally, these studies support that genetic variation in the inflammatory system increases the risk of coronary artery disease. Differences in genetic regulation may explain why some people do not develop disease but some develop a more severe inflammatory reaction. In a British study of the IL-1 gene, IL-1 gene variants were not significantly associated with the presence or prevalence of the disease, but IL-1Ra 2 homozygotes were associated with single vessel disease [19]. In a cohort study, a statistically significant relationship was found between coronary restenosis and IL-1 gene mutation in patients underwent stenting [20]. IL-6 is another cytokine for which serum levels have been investigated. Serum IL-

6 levels were also found to be elevated in patients with unstable angina in parallel with CRP and high concentrations of IL-6 were associated with poor prognosis [21]. Atherogenic dyslipidaemia, diabetes as well as insulin resistance during obesity are important in the development of atherosclerosis. TNF-α and IL-6 released from adipose tissue independently of atherogenic dyslipidaemia and diabetes have been shown to stimulate the development of inflammation and atherosclerosis [22]. The IL-6 -174G/C gene is a gene site responsible for regulation of this cytokine. C allele was found to decrease plasma IL-6 levels in healthy individuals [23].

The phenotypic reflection of homozygous (CC or GG) or heterozygous (GC) genotypes and their effects on diseases have been discussed in some other studies. In a study of familial Mediterranean fever (FMF), it was claimed that high IL-6 production (G allele transport) may contribute to amyloid development in FMF patients by increasing SAA gene expression [24].

Although various studies have shown that plasma IL-6 concentrations are elevated and thrombopoietin concentrations are decreased in patients diagnosed with thrombocytopenic purpura, studies have not shown the efficacy of IL-6 - 174G/C gene polymorphism [25]. It is known that the release of proinflammatory cytokines such as IL-6 increases during the course of diseases such as FMF, amyloidosis, ITP, thrombosis, sepsis and pulmonary embolism. However, the number of individuals carrying the -174G/C polymorphism, which has been shown to be effective on IL-6 levels, is similar in the study and control groups. This suggests that increasing cytokine release as response to changing environmental conditions during the course of the mentioned diseases may be more important than genetic changes [8, 23-25]. In a study by Losito *et al*. [23] IL-6 -174G/C gene was suggested to be associated with left ventricular hypertrophy and hypertension in dialysis patients. Vakili *et al*. [9] reported that this gene polymorphism might be related to acute myocardial infarction and it was suggested that detailed investigation of IL-6 -174G/C gene polymorphism would be appropriate in these patients. Ma *et al*. [10] reported that this gene polymorphism may be effective in ischemic stroke and coronary artery disease. In this study, guided by all these results, we investigated the relationship between coronary atherosclerosis and IL-6 -174G/C gene polymorphism. In this study, IL-6 -174G/C gene polymorphism was carried out on agarose gel and the results were read under UV light. The A allele frequency for IL-6 -174G/C polymorphism was 78.36% in the control group and 81.25% in the study group. B allele frequency distribution was 21.64% in the control group and 18.75% in the study group. Allele B frequency in IL-6 -174G/C polymorphism in the patients with coronary artery disease was higher than the control group, but it was not statistically significant (p = 0.464 OR = 0.83; 95% Cl = 0.51-1.35). No statistically significant result was found after comparing AA genotype with AB genotype in IL-6 -174G/C polymorphism (p = 0.257, OR = 0.71; 95% Cl = 0.39-1.28). No statistically significant result was observed in comparison with BB genotype. (p = 0.565 (Fisher's Exact test p value), OR = 1.19; 95% Cl = 0.25-5.56) (Table 2).

Limitations

There are several limitations in our study. Firstly, the sample size was relatively small. Secondly, risk factors for coronary artery diseasewere significantly different in the study group. And we focused on the gene mutations and did not measure plasma IL-6 and C-reactive protein (CRP) levels.

CONCLUSION

This study demonstrates that; Individuals with AB genotype do not differ in risk of developing Coronary Artery Disease compared to individuals carrying AA genotype. On the other hand, individuals with BB genotype had 1.19 times higher risk of developing coronary artery disease than individuals with AA genotype. However, this was not statistically significant. The fact that the allele frequency and genotypic distribution between the groups does not differ statistically suggests that this cytokine's regulation, which is shown to be effective, may be influenced by mechanisms such as environmental, racial and instantaneous organism response other than IL-6 -174G/C gene regulation. Single gene polymorphisms are often not reproducible. Racial and genotypic features may not always produce phenotypic reflections. For this reason, broad-based

studies should be carried out considering appropriate conditions and multifactorial features.

Conflict of interest

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

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Evaluation of sleep quality and perceived stress of nursing students who are engaged in clinical practice based on their sleeping habits

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ABSTRACT

Objectives: Sleep quality and perceived stress of nursing students affects both their personal health and nursing care. The aim of this study is to identify nursing students' sleep quality and perceived stress and the sleeping habits affecting them.

Methods: This descriptive cross-sectional study was carried out with 446 nursing students. The data were collected through a questionnaire involving some questions aiming to identify sleeping habits and perceived stress, Pittsburgh Sleep Quality Index (PSQI) and Perceived Stress Scale for Nursing Students (PSSNS).

Results: The average score of PSQI was 7.71 ± 3.27 while scale of PSSNS was 63.50 ± 26.65. According to PSSNS, female students perceived stress level more than males ($p = 0.005$). However, there was no statistically significant difference between female students and male students in terms of total PSQI scores ($p = 0.113$). Students with poor sleeping quality showed some signs of sleep deprivation during the day. There was a weak relationship between sleeping quality and perceived stress ($p = 0.01$). Nevertheless, delaying sleep due to academic workload affects students' perceived stress.

Conclusions: Nursing students should be encouraged to develop healthy sleeping habits and skills to deal with stress.

Keywords: nursing student, perceived, sleep quality, stress

Sleep plays a significant part in terms of health at every stage of life. Significant bodily functions and brain activities take place during sleep [1]. Adolescence can be considered the phase in which sleeping routines are changed the most in human life [2]. Sleeping durations in adolescence and in adulthood are different [1]. In adolescence, the intensity of academic life, increased social activities, and age-related traits may result in sleeping/waking up late and daytime sleepiness [3].

University students are also among the risk groups who suffer from sleeping disorders [4]. They have an irregular sleep hygiene. Their time for going to bed and waking up, and sleeping periods are different on weekdays and weekends [5]. Various personal and environmental factors affect sleeping habits. Among such factors, anxiety and stress are known to cause sleeping problems [6]. It is very hard to avoid stress in academic life [7]. Students may be under stress due to in-class presentations, midterm and final exams [8]. As the academic level of students increases, the way they perceive stress and the intensity of the stress change as well. Having stress may ruin students' sleeping quality after a while [9, 10].

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Nursing students and other university students are exposed to similar stressors [11]. However, different from other students, nursing students go through an intense and exhausting nursing education program. Such programs provide theoretical and practical classes together [3]. In clinical practices, one can be exposed to intense stressors which have a lot of physical and psychological effects [12]. Lack of confidence and fear of making a mistake during clinical practices, approaches of instructors and clinical nurses, attitudes of patients and reports that are issued after the clinical practice cause students to experience stress [13-15]. Such intense and exhausting education process may also cause students to sleep less [3, 14].

Recent studies show that stress caused by clinical practices leads to various physiological diseases and sleeping disorders in nursing students. However, among studies conducted with nursing students, there is a small number of studies evaluating sleep and stress together. Examining the issue in detail will facilitate a) identification of the stressors affecting sleep quality of students and b) development of strategies to reduce such stressors.

The aim of the study is to answer the following questions: (1) How is the sleeping quality of nursing students? (2) How do the students engaging in clinical practice perceive stress and what types of stress do they perceive? and (3) What is the effect of students' sleeping quality and perceived stress on their sleeping habits?

METHODS

Design and sample of the study

This is a descriptive cross-sectional study. The population of this study consists of second, third and fourth grade students studying at a nursing school and performing clinical practice (n = 482). First grade students are excluded from the study since they have not engaged in clinical practice yet. The study was conducted with the participation of 446 voluntary students.

Data collection tools and implementation

The study was implemented in classrooms between December 2017-January 2018 in the fall term. The data were collected through a data collection form developed based on a literature review, Pittsburgh Sleep Quality Index (PSQI) and Perceived Stress Scale for Nursing Students (PSSNS). The aim of the study was explained to the students before the implementation. The questionnaires were distributed to the students who accepted participating in the study. The students were given time until they completed the questionnaires.

Student Information Form

This form consists of two parts. The first part includes socio-demographic questions regarding the age, gender, grade, school success, number of siblings, education level of mother and father of the student whereas the second part consists of questions that were created based on a literature review and are about the factors which are thought to affect sleeping habits [3, 6, 16].

Pittsburgh Sleep Quality Index (PSQI)

It is an index developed by Buysse *et al*. [17] in 1989. It provides a quantitative measure of sleep quality to define good and poor sleep and consists of 24 questions 19 of which are self-assessment questions and 5 of which are answered by the partner or a roommate of the person. The questions which were answered by the partner or a roommate were not included in the calculation of the index score. Each item was evaluated with a score between 0-3. A study was carried out by Ağargün *et al*. [18] in 1999 to identify the validity and reliability of the index in the Turkish culture and the Cronbach's alpha value was found to be 0.79. One can get a total score ranging between 0-21 from the index which has 7 sub-dimensions. 0-5 refers to "good" sleep quality while a score above 5 means "poor" sleep quality. The Cronbach's alpha value of the index in this study was found to be 0.80.

Perceived Stress Scale for Nursing Students (PSSNS)

This scale was developed by Sheu *et al*. [19] in 2002 and adapted into Turkish by Karaca *et al*. [20]. The scale is consisting of 29 items and 6 sub-dimensions. Total score is 0-116 (total scores of sub-dimensions: 12, 32, 20, 24, 12, 16). High score means higher stress levels. The Cronbach's alpha value of the index in this study was found to be 0.90.

Ethical Statement

The study was reviewed and approved by Human Studies Ethics Commission (No: 2017/315). Then, necessary written approvals were obtained from the institution where this study was to be conducted. Before the distribution of the questionnaires, the participants were reminded that it is a voluntary participation and the answers will be kept confidential.

Statistical Analysis

First, the number and distribution of the missing data within the variables were identified in the analysis. Missing data were excluded from the data set for nonrandomly distributed variables ($p < 0.5$). As to the randomly distributed variables, the averages were calculated and the analysis was performed ($p > 0.5$). 15 questionnaires were not included in the evaluation. Thus, analyses were performed on a total of 431 questionnaires. In interpreting the demographic data, number and percentage distribution was used for categorical variables and arithmetic mean and standard deviation was used for continuous data. The analysis was performed at 4 stages. First, the scale scores were calculated with demographic variables and skewness and kurtosis values ±2.5 were taken as reference for normal distribution. Then, scale sub-scores were calculated. At the third stage, perceived stress levels and sleep quality of students were compared based on their gender through independent samples t- test. At the fourth stage, sleep quality of students were examined according to various sleeping habits through chi-squared test. Then, perceived stress score of students and their habit of delaying sleep due to academic reasons were compared through the independent samples t-test. Finally, the relation between PSQI and PSSNS scores was compared through Pearson correlation. Statistical significance was calculated to be $p < 0.05$ in all of the analyses.

RESULTS

The analyses were performed on the questionnaires filled out by 431 students who participated in the study (women: 349, man: 82). The average of age of the participants was 21 ± 0.12 yeras. Distribution of participants by their grade was close to each other. 127 (29.5%), 136 (31.6%) and 168 (39.0%) students from second, third and fourth grades participated in the study respectively.

The score averages of PSQI was determined to be min 1 and max 19. The average PSQI score was found to be 7.59 ± 3.22 for women and 8.23 ± 3.42 for men. It is obvious that students' general sleep quality was poor. Perceived stress scores of students ranged between minimum 0 and maximum 116 which were 64.04 ± 27.41 for women and 56.91 ± 22.10 for men, respectively (Table 1).

The highest score was observed to be at the "sleep duration" sub-dimension in the PSQI (6.37 ± 1.81). In PSSNS, on the other hand, sub-dimensions of "stress caused by instructors and nurses" (14.42 ± 6.04) and "stress experienced while caring for patients"(16.57 ± 7.97) had higher scores compared to other sub-dimensions (Table 2).

PSSNS scores of women (65.04 ± 27.41) were found significantly higher than the average value of perceived stress level of men (56.91 ± 22.10) [t (2.504): 145.696, $p = 0.005$]. However, there was no statistically significant difference between women and men in terms of total PSQI scores [t (-1.586):117.011, $p = 0.113$] (Table 3).

There was a week positive relationship between waking up as rested in the morning ($p = 0.001$ phi cramer's v = 0.369), feeling sleepy/tired during the day ($p = 0.001$, Phi Cramer's v = 0.256) and sleep quality. There was a weak negative relationship between having sleeping problems in ($p = 0.001$, Phi Pramer's

Table 1. Distribution of PSQI and PSSNS scores by gender

	PSQI Scores		PSSNS scores	
Gender	**Mean ± SD**	**Min.- Max.**	**Mean ± SD**	**Min.- Max.**
Women (n = 349)	7.59 ±3.22	1-7	65.04 ± 27.41	0-116
Men (n = 82)	8.23 ±3.42	1-19	56.91 ±22.10	0-107
Total (n= 431)	7.71 ±3.27	1-19	63.50 ± 26.65	0-116

PSQI = Pittsburgh Sleep Quality Index, PSSNS = Perceived Stress Scale for Nursing Students

Table 2. Average scores of sub-dimensions of PSQI and PSSNS

Total PSQI Score and Average Scores of Sub-components		Total PSSNS Score and Average Scores of Sub-components	
PSQI Sub-dimensions	**Mean ± SD**	**PSSNS Sub-dimensions**	**Mean ± SD**
Subjective Sleep Quality	1.67 ± 0.71	Stress caused by lack of occupational knowledge and skills	6.02 ± 3.51
Sleep Onset Latency	1.52 ± 0.88	Stress during patient care	16.57 ± 7.97
Sleep duration	6.37 ± 1.81	Stress caused by homework and workload	12.07 ± 5.02
Accustomed Sleep Efficiency	0.39 ± 0.83	Stress caused by instructors and nurses	14.42 ± 6.04
Sleep Disorder	1.41 ± 0.59	Stress caused by environment	6.77 ± 3.05
Usage of Sleeping Pills	0.15 ± 0.52	Stress caused by peers and daily life	8.10 ± 4.54
Daytime Dysfunction	1.62 ± 0.90		
PSQI total	7.71 ± 3.27	PSSNS total	63.50 ± 26.65

PSQI = Pittsburgh Sleep Quality Index, PSSNS = Perceived Stress Scale for Nursing Students

Table 3. Comparison between PSSNS scores and PSQI scores of students

Variable	Women	Men	Test		
	Mean ± SD	Mean ± SD	t*	df	*p* value
Total PSSNS score	65.04 ± 27.41	56.91 ± 22.10	2.501	145.696	**0.005**
Total PSQI score	7.59 ± 3.22	8.23 ± 3.42	-1.586	117.011	0.113

PSQI = Pittsburgh Sleep Quality Index, PSSNS = Perceived Stress Scale for Nursing Students
*Independent samples t-test

v = -0.346) and out of the school (p = 0.013, Phi Cramer's v = -0.119) and sleepquality. Students with a poor sleep quality did not wake up as rested in the morning and had sleep problems in and out of the school. Also, students who sleep quality was poor, feeled sleepy and tired during the day (Table 4).

A significant relationship was found between the effect of school-related efforts and using PC/tablet on delayed sleep and PSSNS scores ($p < 0.05$). PSSNS scores of the students who delay sleeping because of their homework (67.90 ± 28.58) was significantly higher than those who did not (59.94 ± 26.11) [t(2.874):355.58, p = 0.004]. Average PSSNS scores of the students who delay sleeping to do homework (72.72± 82.40) and play games on a notebook computer (68.87 ± 27.90) were higher, which was statistically significant [t (4.151):270.91, p = 0.001 and t (2.846): 424.60, p = 0.005] (Table 5).

The relationship between students' PSSNS scores and PSQI scores was examined. There was a weak relationship between their PSSNS scores and PSQI scores (R:0.195, p = 0.01) (Fig. 1).

DISCUSSION

This study presents important findings regarding students' sleep quality and perceived stress. In the study, the average PSQI score used to evaluate the sleep quality was found to be higher than 7 for both female and male students. The index score in other studies which used PSQI ranged between 4.65 and

Table 4. Evaluation of the sleep quality of students identified through PSQI based on various sleeping habits

		Good sleep quality	Poor sleep quality	Test		
				x^{2*}	Phi, Cramer's V	*p* value
Waking up rested in the morning	Yes	52	33	58.562	0.369	**0.001**
	No	68	278			
Not having sleeping problem at school	Yes	75	284	51.681	-0.346	**0.013**
	No	45	27			
Not having sleeping problem out of the school	Yes	95	275	6.108	-0.119	**0.001**
	No	25	36			
Feeling sleepy and tired during the day	Yes	80	275	28.223	0.256	**0.013**
	No	40	80			

PSQI = Pittsburgh Sleep Quality Index
*Chi-squared test

Table 5. The relationship between delayed sleep due to students' school-related activities and PC/Tablet use for fun and the PSSNS score

		PSSNS scores		Test		
		n	Mean ± SD	t*	df	*p* value
School-related activities						
Before exam	Delaying sleep	342	65.44 ± 27.68	0.706	128.85	0.482
	Not delaying sleep	84	63.01 ± 28.96			
While doing daily homework	Delaying sleep	143	72.72 ± 28.40	4.151	270.91	**0.001**
	Not delaying sleep	286	61.06 ± 26.91			
While doing homework	Delaying sleep	270	67.90 ± 28.58	2.874	355.58	**0.004**
	Not delaying sleep	159	59.94 ± 26.11			
Notebook computer use for entertainment						
While using a notebook computer	Delaying sleep	208	68.87 ± 27.90	2.846	424.60	**0.005**
	Not delaying sleep	221	61.25 ± 27.50			

PSSNS = Perceived Stress Scale for Nursing Students
*Independent samples t-test

20.08 [21-25]. As other studies, the results of this study also revealed that most of the nursing students have a poor sleep quality [3, 12, 14, 21, 24, 26].

Nurses who are healthy and have the required practice knowledge/skills are the essence of a health care of good quality. Nurses who do not meet their basic needs such as sleep might have troubles in both the patient care and personal care. Poor sleep quality brings about various problems. Previous studies revealed that poor sleep quality causes depression and

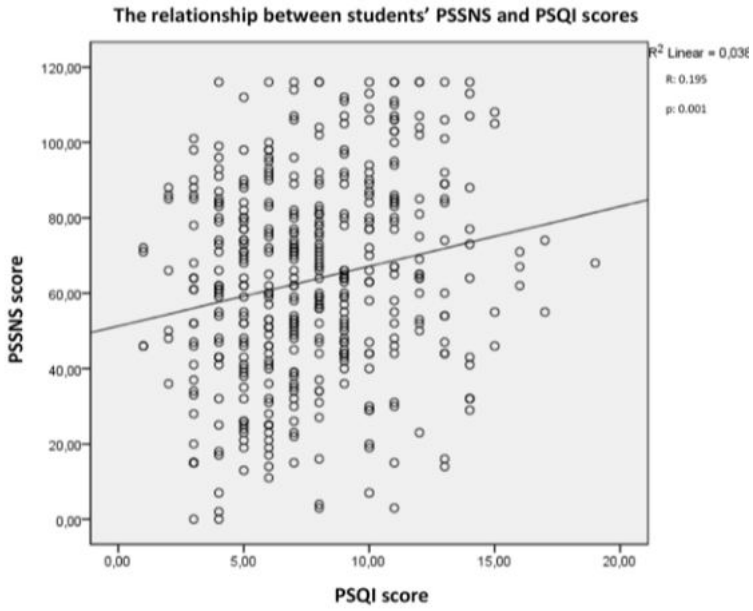

Fig. 1. The relationship between students' PSSNS and PSQI scores.

affects nursing students' mental health negatively [6, 11]. In addition, school life and practices of a student having a poor sleep quality are also affected. Another important finding of the study was that students whose sleep quality was poor did not wake up rested in the morning and were sleepy during the day both in and out of the school. This situation may lead to fatigue and attention deficit in students and negatively affect their both academic and social life [27]. As a matter of fact, there is a risk for these students of attaining lower academic success compared to their peers, making mistakes in clinical practices and having occupational accidents. If suffering from lack of sleep becomes constant, students may encounter many problems in the future as to the patient care, communicating with colleagues, and medical mistakes. Thus, before starting their professional life, students should attain a healthy sleep routine for both their career and health.

Various research on the sleeping habits of students revealed that there are several reasons to their poor sleep quality. One these reasons is the students' experienced and perceived stress [6, 11, 25, 27, 28]. Studies suggest that majority of students experience stress [12, 29-31], which affects nursing students' sleep quality and performance in clinical practices. Occupational practices, learning environment, educators, nurses and patient-related factors may play a part in the stress experienced by students. In this study, students' perceived stress scores were found to be average as in the study carried out by Labrauge *et al*. [32] and it was revealed that students did not experience an intense stress. However, the literature suggests that students are under a lot of stress [30, 31, 33]. Students' perceived stress sub-scores varied by studies. Some studies revealed that homework and workload are the main causes of stress for students [32, 34]. This study, on the other hand, was highly consistent with the study carried out by Al-Gamal *et al*. [35]. In both studies, the sub-dimensions "stress experienced in patient care", "stress caused by instructors and nurses" and "stress caused by homework and workload" had the highest scores. Differences regarding the stressors identified in these studies were thought to stem from the difference in the curricula of nurse education adopted by countries and the cultural differences. As a matter of fact, another study which was carried out in Turkey showed that instructors and nurses were the primary stressors for students [36] because their expectations of students were either not explicit [37, 39] or high and they were judgmental when communicating with students [39].

In a study carried out to identify the stressors affecting students' sleep quality, the students who had a poor sleep quality, had troubles in vocational education and failed to properly manage their time were found to have a higher stress level [40]. In a study carried out by Labrague (2018), lack of

occupational knowledge and skills was shown to be one of the fundamental stressors [41]. In this study, that students who cannot manage their time properly due to the duration of study and notebook computer use would have a higher level of stress was an expected finding. However, since it was not always the case, it did not have a large effect on sleep quality.

It is known that stress varies by gender in youth [29, 42]. Women are more emotionally sensitive than men. Thus, they perceive and react to stress more intensely than men do. Previous studies had proved that this was also the case in the field of nursing [23,29]. The findings of this study put forth the same fact and showed that female students were more stressful than males. Although males have been increasingly represented in nursing, it is still a female-dominated occupation [34, 43]. Another reason why female nursing students perceive stress more than males is that the profound interaction and experience sharing taking place among women in the clinical environment. Students want to be supported during clinical practices. However, due to the high number of students, they cannot get sufficient support from their instructors. Thus, they become inclined to expect to be supported by clinical nurses with whom students may sometimes have various problems [38]. Students share the problems they had with a medical personnel at the clinic with their friends or relatives rather than talking to the relevant person to settle the dispute. This experience sharing process further increases students' stress [37, 44]. As the stress level increases, the likelihood for nursing students to deliver a poor performance in clinical practices increases also [12]. In addition, a student whose sleep quality is poor is likely to respond to the busy pace of the clinic in a more nervous and stressful manner. The retardation in cognitive processes caused by lack of sleep may lead to misunderstanding doctors' request, losing time and making errors in the practices requiring skills, and thus experiencing stress in the patient care and communication with clinical nurses [29, 45]. This study suggests that although the relationship between the sleep quality and the perceived stress of nursing students is weak, it is still statistically significant. A similar result was obtained through a study conducted with psychology students, which suggests that 15% of poor sleep quality can be explained by the perceived stress of students [46]. Although this rate is not very high it is still indicative of the fact that to improve their sleep quality, students need to be prevented from experiencing stress. This is not only necessary for the protection of students' personal health but also for the improvement of the quality of nursing practices and for a healthy communication between medical personnel at the clinic.

Limitaions

One of the limitations to this study is the evaluation of students' sleep quality based on their perceived stress. The other factors (cigarette/alcohol consumption, internet/social media addiction, etc.) that may affect the sleep quality was not taken into consideration. Another limitation is that first grade nursing students were not included in this study since they did not engage in clinical practice yet. Future studies may include first grade students who do not engage in clinical practice and evaluate the relationship of the sleep problems with the clinical practice.

CONCLUSION

This study, which was conducted in order to evaluate the sleeping quality and perceived stress of the nursing students based on their sleeping habits, revealed that the sleep quality of nursing students is very poor. Students' poor sleep quality causes them to have sleep problems in classes during the day. There was not a strong relationship between students' sleep quality and perceived stress. However, it can be said that both can be affected by some of the sleeping habits of the students. Future studies having a larger sample size and adopting different research designs (qualitative research, experimental research, etc.) can provide a more in-depth examination of the students' stress and sleep.

Conflict of interest

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

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The European Research Journal 2020;6(5):438-448 Original Article

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The impact of testosterone levels on J-wave patterns observed in healthy Turkish males

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ABSTRACT

Objectives: Early-repolarization (ER) and Brugada-type-ECG-patterns (BTEP) have recently been grouped under a common terminology called "J-wave patterns" (JWP) and have been associated with an increased risk of sudden-cardiac-death. Scarce data is present about the male dominance in JWP and the probable effects of gonadal hormones on cardiac ion-channel functions. We sought to evaluate the relationship of testosterone-levels and the presence of JWP in healthy Turkish-males.

Methods: One hundred eighty-five healthy male volunteers between ≥18 to ≤50 years old without any cardiac disorders were evaluated. ECG, blood biochemistry and total testosterone levels were obtained together with thorough physical examination. Subjects with complete-bundle-branch-block, non-sinus-rhythms and any abnormality on cardiac examination were excluded from the study. BTEP was searched according to the EHRA/HRS 2016 Consensus Conference on V1-V3. ER on ECG was defined as J-point elevation of ≥ 0.1 mV in ≥ 2 leads in the inferior (II, III, aVF) (Inferior ER), lateral (DI, aVL, V4-6) (Lateral ER) or both (Inferolateral ER).

Results: A total of 179 subjects (mean age 34.9 ± 7.9 years) were included in our analyses. Three BTEP (1.7%) and 45 ER (26%) were detected. 22 were lateral (49%), 13 inferior (29%) and 10 were (22%) inferolateral ER. JWP (+) subjects (n = 48, 27%) were demonstrating significantly lower basal heart rates (73.9 ± 11bpm vs 68.4 ± 10.3 bpm, $p = 0.001$) and longer PR intervals (153.9 ± 20.3 ms vs 163.3 ± 21.6 ms, $p = 0.01$). JWP (+) subjects had significantly higher testosterone levels compared with the ones without (485.5 ± 128.3 ng/dl vs 559.3 ± 167.7, $p < 0.001$). In the subgroup analyses, BTEP and inferior/inferolateral ER patterns were significantly associated with higher testosterone levels compared with the JWP (-) population, while testosterone levels of subjects with lateral ER was not significantly higher. Electrolytes and blood chemistry values were non-significant between JWP + and - subjects. In the ROC analysis, the cut-off value for predicting the presence of a JWP on ECG was 629 ng/dl with a sensitivity of 44% and specificity of 86% [AUC = 0.66 (95% CI: 0.56-0.75) $p = 0.001$]. In multivariate analysis, total testosterone level > 629 ng/dl was significantly predicting a JWP on ECG, even outperforming age and hs-CRP levels with an OR of 4.57 (95% CI 1.910-10.9, $p = 0.001$).

Conclusions: Testosterone might be associated with the male predominance observed in the JWP. More malignant inferior/inferolateral ER seems to be mainly associated with the high testosterone levels in Turkish male population. This finding might be attributed to the previously demonstrated effects of testosterone on cardiac ion-channel functions, especially outward-K channels.

Keywords: Electrocardiogram, j-wave pattern, male, testosterone

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Electrocardiographically the point where the QRS complex ends and a prominent ST segment starts is called the J point [1]. In 1953, Osborn *et al*. [2] defined J points in the form of a positive deflection from the isoelectric line in experimental hypothermia which were clinically referred to as "*Osborn Waves*" and then as "*J waves*". The fact that such changes are frequently observed in the young male population also led to the association of J waves with ST-segment elevation as a benign electrocardiographic finding called "Early Repolarization Variant/ Pattern (ER) [3–5]. In recent years, several population-based studies in which J waves such as inferolateral ER and Brugada type ECG patterns (BTEP) were determined to be associated with an increased sudden cardiac death (SCD) and lethal ventricular arrhythmias (VA) have been published worldwide [6-11]. Although they have different diagnostic criteria, clinical phenotypes such as Brugada Syndrome (BS) and Early Repolarization Syndrome (ERS) which are associated with specific J wave findings / J wave patterns with different dispersion of repolarization mechanisms in different regions of the heart and causing life-threatening arrhythmias, have been grouped under a new terminology and concept as "*J Wave Syndromes*" in recent years [12, 13]. National and international research on the etiology, mechanisms and frequency of J wave syndromes are increasing and new studies are designed to recognize this assumingly rare but fatal syndrome earlier and reveal its physiopathology [13-16]. In these studies, the frequencies of observed J wave patterns (JWP) were highly heterogenous between 3-5% to 0.1% in the general population according to the race, age and comorbidities of the study population, however constantly and significantly found to be highest in the male sample groups irrespective of the other features of the study population [10, 12, 13]. Even though, the repolarization theory is the most popular explanation about the observed transmural epi-endocardial voltage gradient in JWPs, contemporary findings in electroanatomic mapping/ablation studies state that JWPs might also occur because of delayed depolarization on the related segments [13, 17].

Although J wave syndromes such as autosomal dominant BS genetically demonstrate an equal transition in males and females, it is extremely rare to have a phenotypic appearance in women compared with men (1:10 ratio) [10, 12, 18]. The presence of more Ito channels in the male right ventricular outflow tract and right ventricular epicardium is shown to be one of the reasons, but some aspects of the BS clinic cannot be fully explained in this way, such as its clinical preponderance after the puberty and attenuation of the malignant clinical features after the 6th decade in males [10, 18, 19]. In research studies, testosterone and dihydrotestosterone levels effected the Na, Ca and K (especially Ito) channels forming the action potential dome leading to a repolarization dispersion similarly observed in J-wave syndromes [20]. It was also shown that the Brugada pattern was lost in men who underwent orchiectomy due to prostate cancer [21]. Regarding the ER patterns, young athletic male predominance might give a clue about the probable contributing effects of higher testosterone levels to the ER phenomenon which share similar ionic and clinical characteristics with BS [12].

Hence, we aimed to evaluate the relationship of testosterone-levels and the presence/distribution and clinical features of JWP in healthy Turkish-males and compared the effects with other clinical and laboratory parameters which might contribute to the frequency of JWP such as age and inflammation parameters.

METHODS

One hundred eighty-five healthy male volunteers between ≥ 18 to ≤ 50 years old without any diagnosed cardiac/systemic history and no family history of SCD were prospectively and consecutively evaluated for inclusion to the study. The study complies with the Declaration of Helsinki, patients provided signed informed consent and the local ethical committee approval obtained. Supine 12-lead surface ECG, complete blood count, high sensitivity (hs)-CRP, renal/thyroid function tests and total testosterone levels obtained together with thorough physical examination. Subjects with complete bundle branch blocks on ECG, non-sinus rhythms and any abnormal findings on cardiac examination were excluded from the study.

Electrocardiographic Assessment

The 12-lead ECGs were recorded at 25 mm/s with a calibration of 10 mm/mV (Nihon Kohden, Tokyo, Japan) and uploaded on the hospital ECG database at

300 DPI. These images were amplified x10 and then baseline heart rates, PR, QRS, QTc (Bazett) intervals were manually measured by electronic calipers. The presence of a lateral (I, aVL, V5-V6), inferior (II, III, aVF) or inferolateral (II, III, aVF, I, aVL, V5-V6) ER was defined as an evident J-point elevation of at least 1 mm (0.1 mV) above the isoelectric line in at least two consecutive leads with either QRS slurring (i.e. a smooth transition from the end QRS to the beginning of ST-segment) or notching [positive deflection (J-wave) occurring immediately after a positive QRS complex at the onset of the ST-segment] according to the most recently proposed terminology from the latest international consensus documents for the ECG definition of ER [13, 18], and "J peak" was accepted as "J point" denoting the peak of a notch or onset of a slur. BTEPs were searched according to the EHRA/HRS 2016 Consensus Conference on V1-V3 leads [10, 18]. All the ECGs were analysed by the author in a blinded fashion for the laboratory findings of the patients with borderline results being reassessed by another experienced cardiologist.

Blood Tests and Analysis

Venous blood samples were drawn with patients after they rest supine for about 15 min prior to sampling. Samples were drawn atraumatically without venous stasis through a 21-gauge cannula inserted into an antecubital vein using ethylenediamine tetraacetic acid containing monovettes (Sarstedt, Nuembrecht, Germany), and transferred immediately to the laboratory to be centrifuged. Hs-CRP level was measured on Cobas Integra 400 Plus using a latex particle-enhanced immunoturbidimetric assay following the manufacturer's instructions (Roche Diagnostics, Indianapolis, IN). Testosterone levels were determined using electrochemiluminescence immunoassay method and an auto-analyser (Cobas 6000, E 601 Roche Diagnostics, GmbH, Mannheim, Germany). The complete blood counts were evaluated using an auto-analyzer Sysmex XT-1800i Haematology Analyzer (Sysmex Corporation, Kobe, Japan). The remaining routine biochemistry parameters have been determined by the core laboratory.

Statistical Analysis

Continuous variables are expressed as mean ± SD (if the parameter is normally distributed) or standard error of mean (SEM ± SD) whichever is suitable. If appropriate, they were compared using the Student's t-test. Categorical variables are expressed as numbers

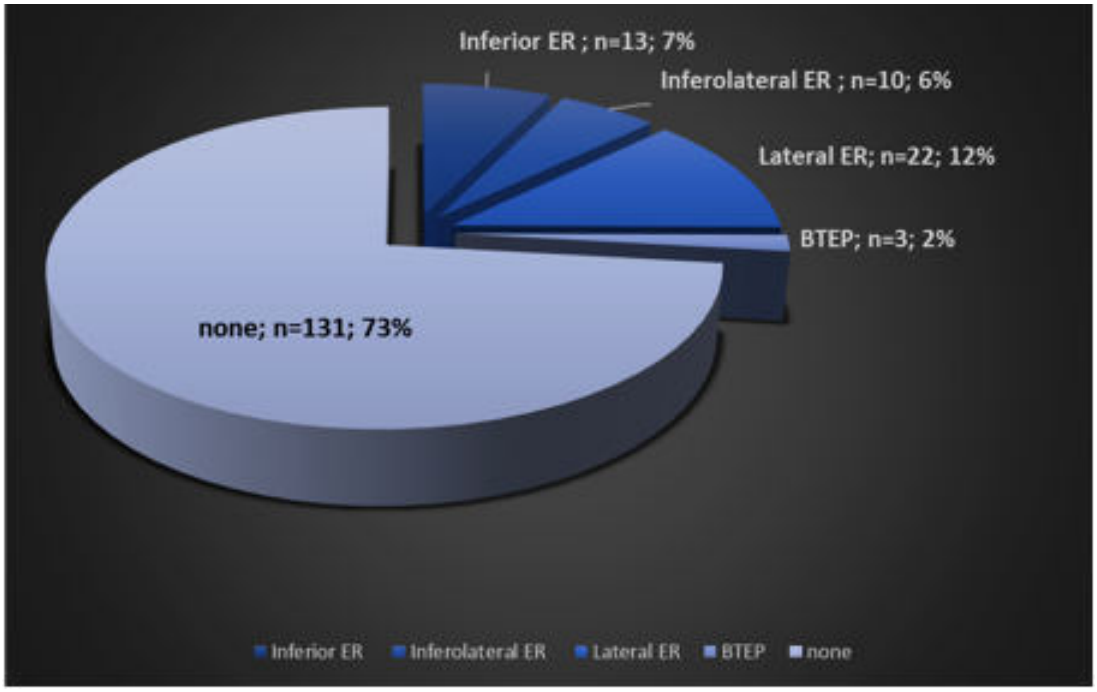

Fig. 1. Prevalence of the J-wave patterns in study population. ER = Early repolarization pattern, BTEP = Brugada type ECG pattern.

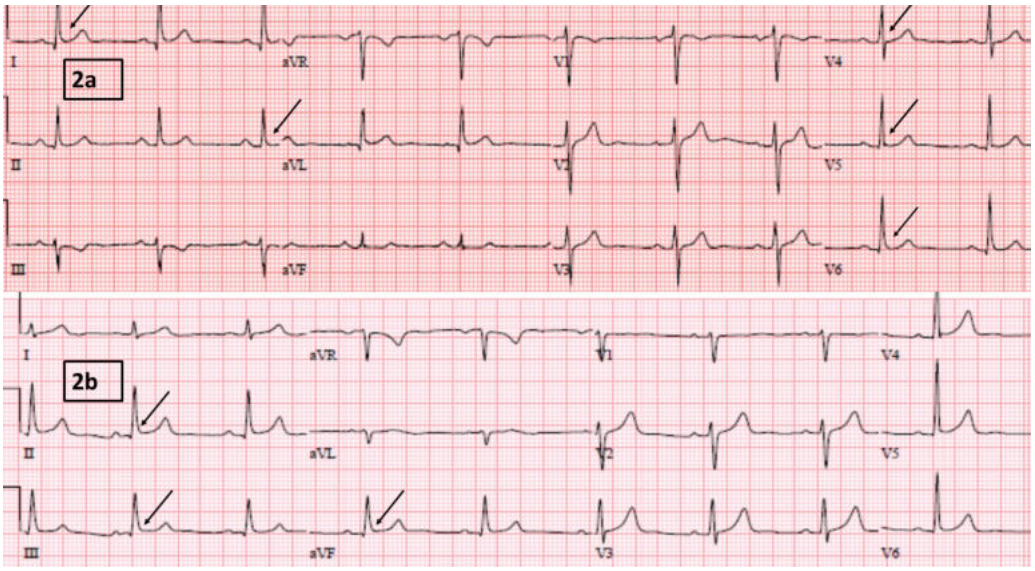

Fig. 2. (a) Lateral early repolarization pattern example. Arrows indicate QRS slurring with around 2 mm J point elevation followed by upsloping benign ST segment changes. (b) Inferior early repolarization pattern example. Arrows indicate QRS slurring with around 1 mm J point elevation and a malignant type horizontal ST segment elevation is followed.

and percentages and, if appropriate, were compared with the Chi-square analysis. Univariate and later multivariate analysis was performed to determine the predictive value of significant and predetermined confounders on the JWP observations using the logistic regression model. A Receiver Operating Characteristic (ROC) curve was plotted in order to determine the diagnostic accuracy of a certain laboratory value. A p value < 0.05 was accepted statistically significant. Statistical analysis was performed using SPSS 16.0 (IBM Inc., Armonk, New York, USA).

RESULTS

Of 185 volunteers, 179 male subjects (mean age 34.9 ± 7.9 years) were included in our analyses after

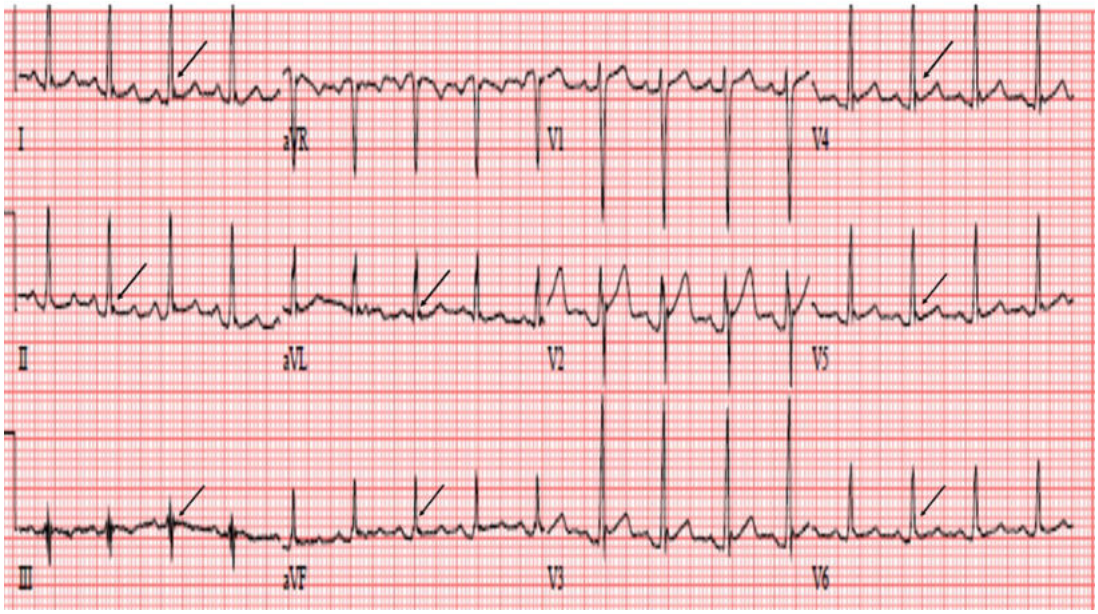

Fig. 3. Inferolateral early repolarization pattern. Arrows indicate QRS slurring with around 1 mm J point elevation and a benign type upsloping ST segment elevation is followed in inferior leads and QRS notching on lateral leads followed by upslope ST elevations most evident on DI, DII, aVF, aVL, V4 to V5.

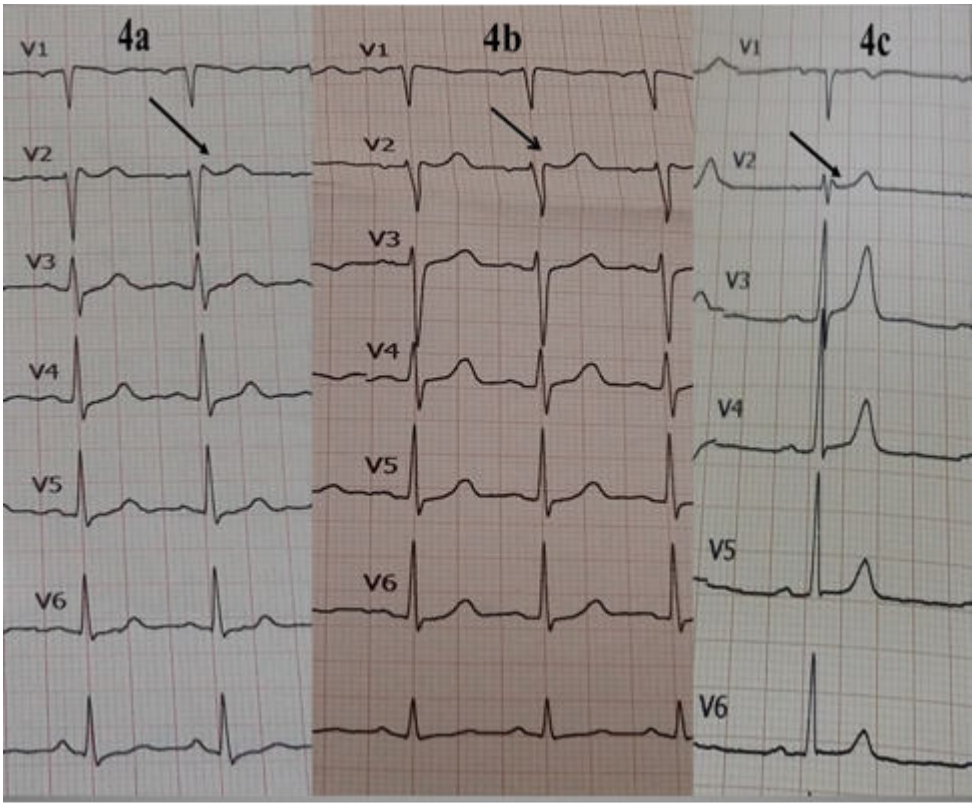

Fig. 4. Brugada-type-ECG-patterns revealed in our study. 4a: Type-2 Brugada pattern, 4b and c: Type-3 Brugada pattern. Arrowheads denote the diagnostic j-waves. No type-1 pattern was found.

the application of inclusion criteria. In total 48 JWPs (26.8%) were detected on surface ECG; consisting of 3 BTEPs (1 Type-2, 2 type-3) and 45 ER patterns (22 lateral, 13 inferior and 10 of them were inferolateral ER) (Figs. 1 to 4). JWP (+) subjects were significantly younger, with a lower basal heart rate, longer PR interval and had significantly higher hs-CRP and testosterone levels compared with the ones without (Table 1). However, no significant association could be demonstrated with ESR, white blood cell count,

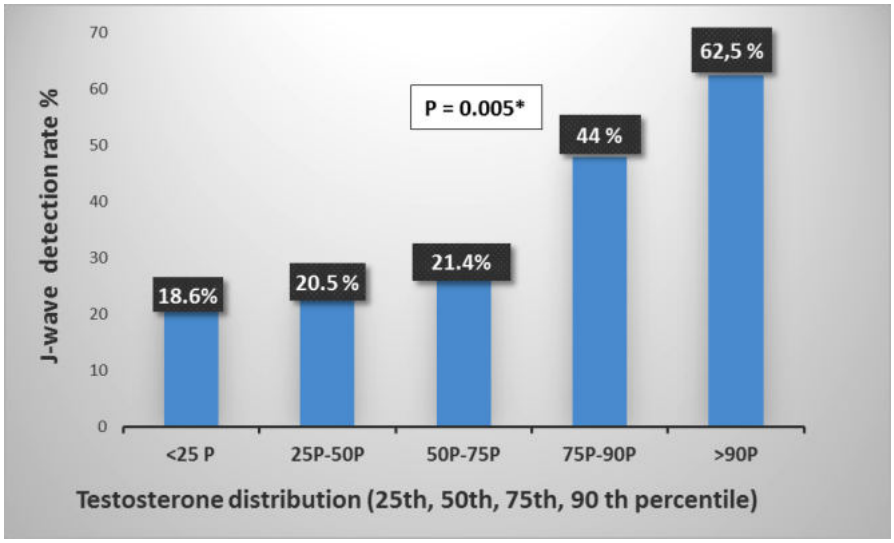

Fig. 5. The frequency of J wave patterns according to the testosterone percentiles Asterix denote a statistically significant and evidently higher J wave patterns in the groups higher than 50th percentile when compared with the low testosterone levels

Table 1. Baseline characteristics of the subjects with and without J-wave patterns

	JWP (-) (n = 131)	JWP (+) (n = 48)	*p* value
Age (years)	35.5 ± 8.1	33 ± 5.8	**0.048**
Weight (kg)	83.1 ± 11.6	81.5 ± 10.6	0.42
Height (cm)	176.2 ± 5.6	176.3 ± 4.8	0.82
Body mass index (kg/m^2)	26.7 ± 3.3	26.2 ± 3.5	0.40
ECG parameters			
Heart rate (bpm)	74 ± 11.5	68 ± 10	**< 0.001**
QTc duration (ms)	404.1 ± 18	404 ± 14.3	0.89
PR duration (ms)	153 ± 20.3	163.5 ± 22	**0.01**
QRS duration (ms)	90.2 ± 11	92 ± 10	0.30
Laboratory results			
Glucose (mg/dL)	87.1 ± 11.1	85.9 ± 10.5	0.51
BUN (mg/dL)	29.2 ± 7.2	29.5 ± 6.7	0.75
Creatinine (mg/dL)	0.91 ± 0.12	0.89 ± 0.10	0.28
Uric acid (mg/dL)	5.3 ± 1.01	5.4 ± 1.1	0.53
Sodium (mmol/L)	143.1 ± 2.5	143.1± 2.4	0.94
Potassium (mmol/L)	4.5 ± 0.3	4.5 ± 0.3	0.96
Hemoglobin (g/dL)	14.5 ± 0.9	14.4 ± 0.9	0.57
Platelet count (10^3/µL)	232.9 ± 57.9	235.1 ± 50.2	0.81
WBC (10^3/µL)	5.97 ± 1.4	5.63 ± 1.1	0.13
Neutrophil (10^3/µL)	3,15 ± 1.03	2.86 ± 0.88	0.08
Lymphocyte (10^3/µL)	2.02 ± 0.46	1.891 ± 0.52	0.17
NLR (%)	1.56 ± 0.47	1.63 ± 0.68	0.50
hsCRP (mg/L)	1.6 ± 1.7	3.0 ± 2.9	**0.004**
ESR (mm/h)	6.7 ± 4.6	8.4 ± 6.2	0.07
Total cholesterol (mg/dL)	182.5 ± 34.3	184.2 ± 33.8	0.78
HDL (mg/dL)	41.5 ± 8.7	42.1 ± 9.8	0.75
LDL (mg/dL)	112.2 ± 27.5	112.1 ± 26.3	0.99
Triglycerides (mg/dL)	140.9 ± 78.4	143.0 ± 78.6	0.87
TSH (mIU/L)	1.7 ± 0.9	1.8 ± 0.96	0.73
Vitamin D, 25-Hydroxy (ng/mL)	12.8 ± 6.03	13.2 ± 5.08	0.73
Vitamin B12 (pg/mL)	270.5 ± 118.9	305.9 ± 113.6	0.18
Total Testosterone (ng/dL)	481.6 ± 125.7	556.4 ± 165.3	**0.002**
LDH (U/L)	346.8 ± 49.9	341 ± 62.1	0.57
Iron (Fe) (µg/dL)	102.6 ± 35.8	89.2 ± 57.5	0.35
Ferritin (mg/L)	92.1 ± 70.8	13.2 ± 5.08	0.79
Folate (ng/mL)	7.58 ± 1.8	7.83 ± 2.05	0.45
Fibrinogen (mg/dL)	270.3 ± 89.4	274.6 ± 96.01	0.83

BMI = body mass index, BUN =blood urea nitrogen, ECG = electrocardiography, ESR = erythrocyte sedimentation rate, HDL = high density lipoprotein, hsCRP = high-sensitive-C-reactive peptide, JWP = J-wave patterns, LDH = lactate dehydrogenase, LDL = high density lipoprotein, NLR = neutrophil-to-lymphocyte ratio, TSH = thyroid stimulating hormone, WBC = white blood cell count

Table 2. Testosterone levels among the J-wave patterns

J-wave Pattern	Testosterone levels			*p* value*
	Mean ± SD	95% Confidence Interval		
Inferolateral ER	600.2 ± 163.3	483.3	717.1	**0.005**
Inferior ER	593.4 ± 201.6	471.6	715.3	
Lateral ER	520.6 ± 145.5	456.1	585.1	
BTEP	627.6 ± 206.5	114.6	1140.7	

ER = early repolarization pattern, BTEP = Brugada type ECG pattern, *P value is for the statistical significance of the higher testosterone levels observed in inferior/inferolateral ER and BTEP compared with the lowest testosterone levels observed in lateral ER group.

neutrophil to lymphocyte ratio and uric acid levels. For the subgroup analyses and to efficiently define a probable dose-response effect with the testosterone levels, we divided the testosterone levels into 25th, 50th, 75th and 90th percentiles and showed that significantly more JWPs were demonstrated after the 50th percentile and significance continues as the testosterone percentiles rises, when compared with each other (Fig. 5). Among the JWPs, BTEPs have the highest mean testosterone value and it was significant when compared with JWP (-) population and it was followed by the inferior and inferolateral ER patterns with significantly higher testosterone levels (Table 2). However, lateral ER patterns did not demonstrate any significant difference regarding the mean testosterone levels when compared with the JWP (-) subjects. In the ROC curve, the Area Under the Curve was determined 0.66 (95% CI: 0.56-0.75) (p = 0.001) and a cut-off value for testosterone was determined as 629 ng/dl with a sensitivity of 44% and a reasonable specificity of 86% to predict the presence of JWP (Fig. 6). In multivariate analysis, total testosterone level > 629 ng/dl was significantly predicting a JWP on ECG, even outperforming age and hs-CRP levels with an OR of 4.57 (95% CI 1.910-10.9, p = 0.001) (Table 3).

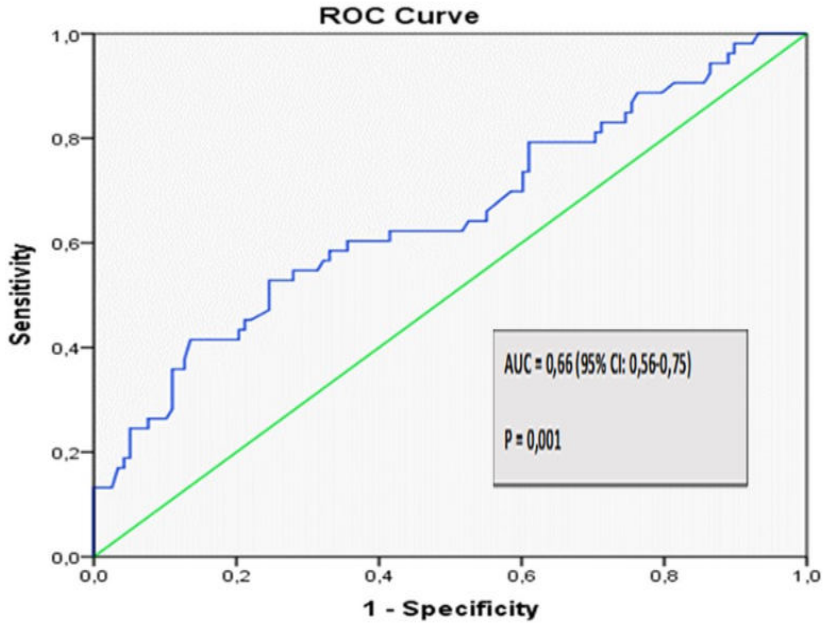

Fig. 6. ROC analysis to define the predictive value of higher testosterone levels and the presence of J wave pattern on ECG. ROC = Receiver Operating Characteristics, AUC = Area Under the Curve, Cutt off value for total testosterone level : 629 ng/dL (sensitivity 44%, specificity 86%)

Table 3. Multivariate regression model for the testosterone cut-off value determined in ROC analysis predicting the odds that the subject might demonstrate a JWP on ECG.

Parameters	Odds ratio	95% confidence interval	*p value*
Age	0.95	0.90-1.02	0.950
hsCRP	**1.26**	1.08-1.47	**0.004**
BMI	0.97	0.85-1.11	0.662
Vitamin D	1.01	0.93-1.08	0.653
TSH	1.11	0.73-1.68	0.620
Testosterone value > 629 ng/dL	**4.57**	**1.910-10.9**	**0.001**

hsCRP = high-sensitive-C-reactive peptide, BMI = Body mass index, TSH = thyroid stimulating hormone

DISCUSSION

In our study, a JWP prevalence of 27% and a BTEP percentage of 1.7% was consistent with the findings of the previous similar age and gender matched population based studies [12]. No type-1 BTEP was found. The ones with JWP were significantly demonstrating a higher vagal tone (relatively low basal heart rate, longer PR interval) compared with the ones without JWP, a finding also compatible with the previous population based studies and proposed mechanisms[12, 13, 22]. Our healthy male population gave out evidently higher prevalance than the general population for these relatively rare ECG findings however, it was the result of our design to increase the expected number of cases in order to perform significant statistical calculations. Testosterone demonstrated a dose response effect on the prevalence and type of JWPs. Significantly more JWPs were being observed in the higher levels of testosterone and suggestively high-risk patterns such as BTEP and inferior/inferolateral ER patterns were related with the highest percentile of testosterone distribution. In the ROC analysis a cut of testosterone value of 629 ng/dl were significantly found to be predictive of a JWP on ECG with a good specificity and a reasonable sensitivity. In the multivariate regression model, predetermined testosterone level of 629 ng/dl were evidently and significantly predicting the presence of JWP on the surface ECG and even outperforming previously known predictors of JWP and cardiac risk including young age and inflammation.

In J wave syndromes, main mechanism leading to ERs and Brugada pattern is explained by an outward shift in cardiac action potential repolarizing current due to a decrease in Na+ or Ca+2 channel currents or an increase in outward currents (Ito, IK-ATP, IK-ACh, or other) leading to a more negative intracellular ionic balance on the related myocardial sites giving rise to a transmural voltage gradient between endocardium and epicardium on partial regions of the heart leading to lethal ventricular arrhythmias [12].

Clues about the possible role of the gonadal hormones on electrocardiogram

It has formerly been demonstrated that, on average, adult men have shorter QT intervals than do women, but gender differences become apparent after the onset of puberty [23]. This gender difference is absent at birth and in young children [24]. Throughout puberty, the QTc interval in males shortens by 20 ms, whereas the QTc of females remains unchanged, resulting in a 6% shorter QTc in males compared to females [25] Pecori-Geraldi *et al*. [26] compared the QTc in 26 men with hypogonadism with the QTc in 26 age-matched controls. They reported a higher prevalence of a prolonged QTc in hypogonadal men (15%) than in controls (0%) ($p < 0.05$) and demonstrated normalization of the QTc after testosterone therapy. Similarly, a case-control study with 27 orchiectomized men with no exogenous testosterone therapy and 53 non-orchiectomized controls demonstrated that orchiectomized men had significantly longer JTc intervals (from the start of the J wave to the end of the T wave) than non-orchiectomized men [27]. In fertile women it was demonstrated that menstrual cycle influences QT interval and responses to drugs. During the follicular phase, when oestradiol rises, there is a greater

sensitivity to potassium channel blockers (Ibutilide) resulting in a greater QT prolongation. During luteal phase progesterone seems to shorten QT interval. Furthermore, there are changes in autonomic tone resulting in higher level of noradrenaline (but not adrenaline or vagal tone) in luteal phase in respect with follicular phase [28]. Males manifest a greater transient outward potassium current (Ito)-mediated phase 1 notch in the right ventricular epicardium than females [29], however after puberty, the J point amplitude gets higher and the ST segment angle becomes steeper suggesting additive role of the ever-changing hormonal status on the JWPs observed apart from the microstructural differences observed in males [30, 31]. It has also been demonstrated that diurnal changes in the testosterone levels effect the augmentation or disappearance of the BTEPS observed in BS patients, even in the same day and night.

Cellular effects of gonadal hormones on cardiac ion channels

It has previously been demonstrated that sex steroid hormones exert their effects via transcriptional regulation and in order to do that, they bind to sex hormone receptors and then translocate into the nucleus leading to regulation of gene expression [32]. That means that, it was formerly thought that gonadal hormones need several hours or days to establish their effects. However, in the recent literature, new research challenged this idea and has shown that sex steroids might also acutely affect the cardiac ion channel activity PI3K/Akt/eNOS pathway. It has been shown that testosterone induced phosphorylation of the Ser/Thr kinase and endothelial nitric oxide (NO) synthase leads to NO -synthase-3 activation and production of NO [33]. NO leads to s-nitrosylation of cysteine residues on the channel underlying the slow delayed rectifier K+ current (IKs) [34]. L-type Ca2+ current (ICa,L) is conversely suppressed by NO via a cGMP dependent pathway. Regulation of IKs and ICa,L by testosterone is dose-dependent and leads to shortening of action potential duration and QT intervals [32, 33]. In the contemporary literature emerging evidence accumulates about the non-genomic acute effects of sex steroids such as directly binding to IKs channel to modify its functions directly to a special site on channel and effecting its gating functions suggesting that even non-genomic actions of testosterone and progesterone on cardiac ion channels are likely to contribute to the gender differences in cardiac repolarization processes [35]. In a letter by Juhani-Junttila *et al*. [36], authors demonstrated a higher prevalence of ER patterns on the highest tertile of the testosterone levels consistent with our findings however they demonstrated a higher incidence of the lateral ER patterns with benign type upsloping ST elevations in their Finnish male population. Oppositely, we demonstrated that in Turkish male subjects, highest testosterone tertiles were associated with the inferior/inferolateral ER patterns or BTEPs which theoretically have the highest risk characteristics according to the former population-based studies [13].

In our work, subclinical inflammatory parameter (hs-CRP) was also associated with an increased frequency of JWPs with a borderline significance as a secondary finding of our work and it was also consistent with the previously published data on the effects of inflammation and JWPs [37], yet testosterone levels outweighed this significance in the multivariate regression analysis revealing itself as a significantly more dominant risk factor.

Limitations

In this study we only considered the admittance surface ECG and the well-known dynamic character of the JWPs [12] might be the leading limitation for a probable underestimation. This aspect of the J-wave phenomenon will always be there in the clinical studies conducted on this concept because of its susceptibility to the ever changing vagal/hormonal tonus and environmental factors like temperature, diurnal vagal and hormonal changes and even by the food intake [12, 13]. We tried to overcome this underestimation problem by trying to conduct our study on young and male volunteers and hypothetically increasing our expected JWP prevalence. Because of the highly influential JWP changes in the menstrual cycle period of women [29], we did not include female subjects in our study however it would be good to compare the dose-response effects of male and female gonadal hormones on the ECG manifestations by a more complicated study design. It would also be better to look for the Na and K channel mutations in the JWP group for causal

and additive definitions in spite of the fact that recent data shows sex hormones failure to effect the functions of mutant Na channels [38]. Even though our study design was cross-sectional, it might be good to follow the patients with highest percentile testosterone and JWP in the long term for any arrhythmic event and changes in JWP.

CONCLUSION

The prevalence of JWPs observed in young health Turkish males are consistent with the frequencies observed in other Caucasian population studies revealing more lateral ER and similar BTEP prevalence. Testosterone levels seemingly influence the prevalence of JWP observed in young healthy males with a dose-response relationship demonstrating the highest frequency and suggestively highest JWPs. This finding might be attributed to the experimentally demonstrated effects of sex-hormones on various cardiac ion channel functions taking part in the cardiac action potential and might be related to non-genomic direct effects on different races and geographical areas [32, 35]. Larger, population-based studies with a long term follow up might be designed to elucidate the mechanistic pathways between the gonadal hormones and JWPs.

Conflict of interest

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

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The European Research Journal 2020;6(5):449-456 Original Article

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Retrospective review of children with vertigo: a 3-year experience

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ABSTRACT

Objectives: To evaluate the age, sex, accompanying symptoms, etiologic factors and laboratory findings in children with vertigo in a tertiary research hospital.

Methods: A total of 183 children (65 boys and 118 girls), aged 3-18 (median:14), who presented with complaints of vertigo between November 2016 and September 2019 in the pediatric neurology department were examined retrospectively. Systemic and neurological examination findings, laboratory findings including complete blood count and biochemical tests (fasting blood glucose, electrolytes, liver-kidney function tests), iron, iron-binding capacity, ferritin, vitamin B12 level and thyroid function test results, electroencephalography (EEG) and magnetic resonance imaging findings were examined.

Results: The frequency of vertigo complaints were found to be higher in female gender ($p = 0.008$). The frequency of admission was significantly higher in adolescents (> 12 years) (67%) compared to other age groups ($p < 0.001$). The most common cause of vertigo was benign paroxysmal vertigo of childhood (BPVC) (23%) and orthostatic hypotension (22%) was the second. When the relationship between the etiology of vertigo and age was examined, the most common cause was BPVC under 12 years of age, where as orthostatic hypotension was significantly more frequent in adolescents ($p < 0.001$). Headache (41%), syncope (27%) and nausea-vomiting (10%) were the most common accompanying symptoms with vertigo. Epileptiform disorder was detected in 7% of patients who underwent EEG. Of 171 patients who underwent neuroimaging, 85% reported as normal and 10% had non-specific findings.

Conclusions: In children presenting with a complaint of vertigo, a detailed history including the age at when the complaint began and the accompanying symptoms, physical examination, blood pressure measurement, laboratory tests, and EEG and neuroimaging (if necessary) should be performed with a multidisciplinary approach.

Keywords: vertigo, children, dizziness, differential diagnosis

Vertigo is defined as a sense of spinning dizziness and is not a common complaint in childhood [1]. The prevalence of childhood vertigo was reported to be 5.7% in a meta-analysis [2].

Patients with vertigo may experience nausea, loss of balance, sensory or visual problems, and headache in addition to the sense of dizziness [3]. The frequency of some symptoms accompanying vertigo and the un-

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derlying causes may be different in children than in adults. For example, migraine-associated vertigo (MAV) is common in children, whereas, Ménière's disease is more commonly observed in adults [4]. The studies to date have reported benign paroxysmal vertigo of childhood (BPVC), MAV, and psychogenic vertigo as the most common causes of vertigo in children [2].

Despite recent advances in medicine, the diagnosis of childhood vertigo is still based on a detailed medical history and physical examination. However, the pathogenesis of vertigo in children is different than in adults. Children have a better response to therapy and heal faster. Besides, pediatricians, otorhinolaryngologists, and pediatric neurologists often face challenges in establishing the diagnosis. The inability of the affected young children in particular, to explain the characteristics of symptoms, duration of episodes, and the provoking or accompanying factors may pose an obstacle in reaching a diagnosis [5]. Furthermore, vestibular tests are not clinically or neurophysiologically safe in young patients. However, the critical factor for the misdiagnosis and a delay in the diagnosis is the awareness threshold of clinicians about the nuances in symptomatology and treatment algorithms [6].

There is a paucity of data in the literature regarding childhood vertigo, although the first case of pediatric vertigo in the modern medical literature was described in 1962. The aim of the present study is to perform a retrospective review of pediatric patients presenting with vertigo, and thus, contribute to the literature.

METHODS

The medical records of 691 patients, aged younger than 18 years who presented to the Pediatric Neurology Outpatient Clinics at Bursa Yüksek İhtisas Training and Research Hospital (a tertiary healthcare facility) with the complaint of vertigo between November 2016 and September 2019, were retrospectively reviewed after obtaining the approval of local ethics committee (2011-KAEK-25 2019/09-09). Patients with an accompanying chronic condition, patients with a history of long-term drug use of any reason, patients older than 18 years, patients who could not be etiologically classified, and those with inaccessible or missing data were excluded from the study.

One hundred and eighty-three eligible patients were evaluated for age, gender, date of admission, and the symptoms accompanying vertigo. Systemic and neurological examination findings, as well as laboratory data including complete blood count, biochemical tests (fasting blood glucose, electrolytes, liver and kidney function tests), serum iron, iron-binding capacity, ferritin, vitamin B12 levels, and thyroid function tests, were examined. The results of consultations with pediatric cardiology, pediatric endocrinology, otorhinolaryngology, pediatric psychiatry, and the results of cranial CT/MRI (Computerized tomography/Magnetic resonance imaging), EEG (electroencephalography), and MR angiography were evaluated in case a need raised.

Statistical Analysis

Categorical variables were expressed as number (%), and continuous variables were expressed as mean ± SD for normally distributed variables and as median (minimum-maximum) if the data is not normally distributed. A chi-square test or Fisher's exact test was used to compare the frequency of qualitative variables. SPSS (Statistical Package for Science Studies) 21.0 software package was used in data analysis. A p-value less than 0.05 was considered statistically significant in all statistical tests.

RESULTS

Of 183 patients aged 3-18 years (with a median age of 14 years) who were included in the study, 65 (36%) were male, and 118 (64%) were female (Fig. 1). The frequency of vertigo in the present study was higher in females ($p = 0.08$). It was observed that the rate of presentation increased during adolescence (>12 years) when compared to other age groups ($p < 0.001$). The rate of presentation among girls was significantly higher in the 14-18 years age group (61%) compared with that of other age groups ($p < 0.001$).

The most common cause of vertigo was BPVC (23%), followed by orthostatic hypotension (22%) (Table 1). When the relationship between vertigo etiologies and age was examined, the most common cause in patients aged 12 years and younger was

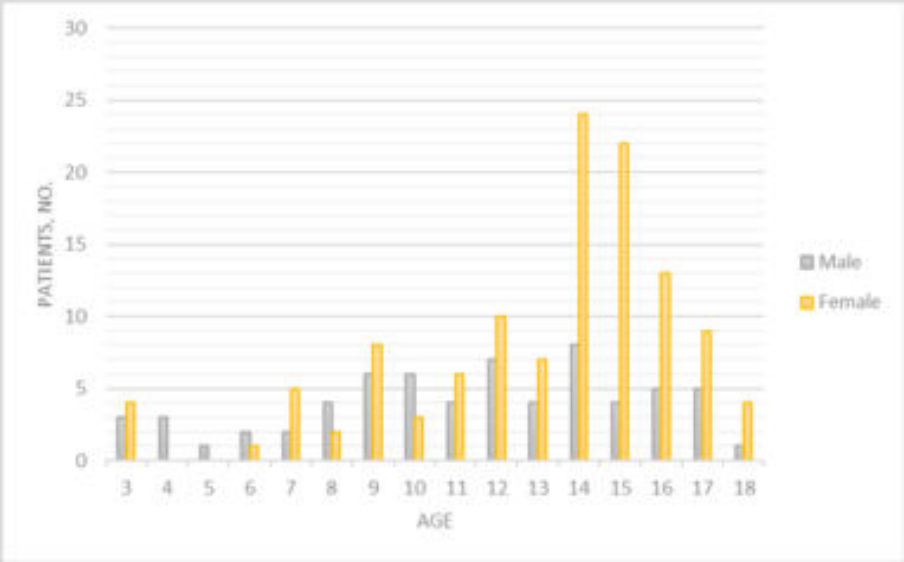

Fig. 1. Distribution of vertigo cases in terms of age and sex.

BPVC, whereas, orthostatic hypotension was significantly more common among adolescents ($p < 0.001$) (Fig. 2). The most frequently encountered symptoms accompanying vertigo were headache (41%), syncope (27%), and nausea and vomiting (10%) (Table 2).

The laboratory data revealed autoimmune thyroiditis in seven (4%) patients, vitamin B12 deficiency in nine patients (5%), and iron deficiency anemia in three (2%) patients.

Of 153 patients that underwent electroencephalography, eleven (7%) were found to have an epileptiform disorder, and these patients were diagnosed with epilepsy. Neuroimaging studies revealed normal findings in 144 (85%) out of 171 patients who underwent such investigations, whereas 17 (10%) patients had non-specific findings (arachnoid-pineal gland cyst, corpus callosum

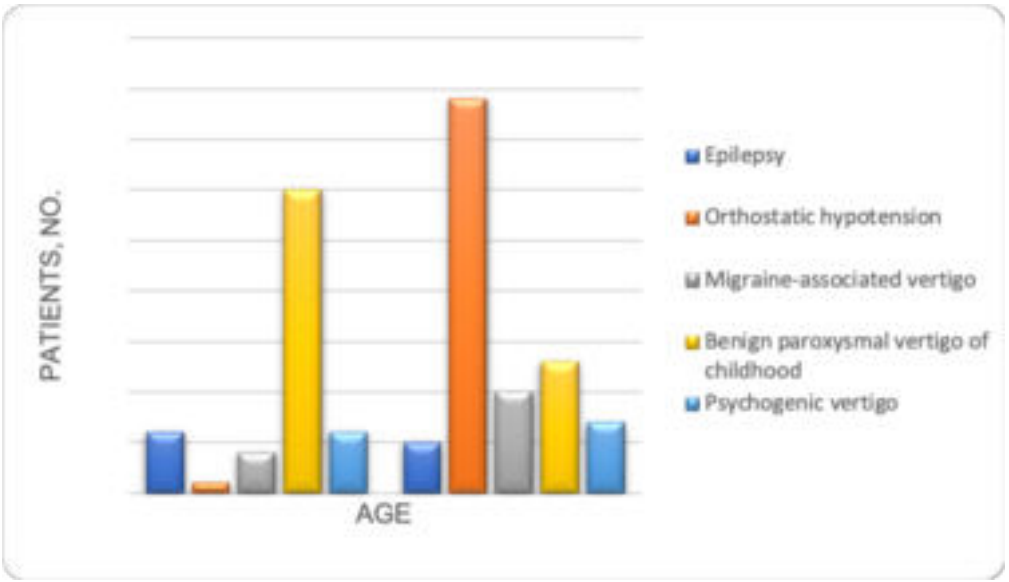

Fig. 2. The most common causes of vertigo by age group.

Table 1. Diagnosis of 183 children with vertigo

Diagnosis	n (%)	Male/Female	Age (median [min-max])
BPVC	43 (23)	18/25	6 (3-14)
Orthostatic hypotension	40 (22)	9/31	15 (11-18)
BPPV	15 (8)	6/9	15 (12-18)
Migraine-associated vertigo	14 (8)	5/9	14 (7-17)
Psychogenic vertigo	13 (7)	3/10	11 (7-16)
Epilepsy	11 (6)	5/6	12 (9-18)
Refractive errors	10 (6)	5/5	12 (3-18)
Vitamin B 12 deficiency	9 (5)	6/3	15 (10-17)
Thyroiditis	7 (4)	0/7	15 (11-17)
Sinusitis	6 (3)	4/2	10 (4-17)
Otitis media	5 (3)	2/3	8 (3-14)
IDA	3 (2)	0/3	13 (12-14)
Mastoiditis	2 (1)	0/2	4.5 (3-6)
Subclavian steal syndrome	2 (1)	0/2	11.5 (9-14)
Posttraumatic vertigo	1 (1)	1/0	14
Postoperative vertigo	1 (1)	0/1	16
Multiple sclerosis	1 (1)	1/0	17
Total	183 (100)	65/118	13 (3-18)

BPVC = Benign paroxysmal vertigo of childhood, BPPV = Benign paroxysmal positional vertigo, IDA = Iron deficiency anemia.

agenesis, choroid plexus papilloma, pituitary microadenoma, arachnoid granulation), four (2%) patients had sinusitis, two (1%) patients had mastoiditis, one patient had venous angioma and another patient had demyelinating plaques. Two (1%) patients were reported to have a thin-caliber unilateral vertebral artery. Patients evaluated with MR angiography were diagnosed with subclavian steal syndrome.

DISCUSSION

The current study presents a retrospective review of clinical and laboratory data of pediatric patients who admitted to the pediatric neurology outpatient clinics of our tertiary healthcare center with the complaint of vertigo during a 3-year period. Vertigo is uncommon in childhood, but it represents a broad differential diagnosis comprising a wide gamut of causes [7].

Benign positional vertigo of childhood is a heterogeneous disease characterized by recurrent short episodes of vertigo attacks that occur without prior warning. It is regarded as a common cause of vertigo in children with a prevalence of 2.6% [2]. The onset of BPVC is typically before the age of four. Disease onset after eight years of age is extremely rare [8]. The

Table 2. Symptoms accompanying vertigo

Symptoms	n (%)
Headache	45 (41)
Syncope	31 (27)
Nausea and vomiting	11 (10)
Convulsion	6 (6)
Tremor	5 (5)
Hypotension	3 (3)
Blurred vision	3 (3)
Numbness in hands	2 (2)
Tinnitus	2 (2)
Ataxia	1 (1)
Total	**109 (100)**

diagnosis is established on the basis of age and typical clinical appearance after ruling out other causes of vertigo. Headache can occur in time in patients with BPVC, and the diagnosis in these patients can be changed to MAV [9]. In our study, BPVC was found to be the most common (23%) cause of vertigo. In line with the literature, the median age of these patients was six years. In addition, BPVC was also found to be the most common cause of vertigo in the studied patients younger than 12 years. Examining more than 2,000 pediatric patients presenting with the complaint of vertigo, Wiener-Vacher [10] reported MAV (25%) and BPVC (20%) as the most common causes in the etiology of vertigo. Similarly, Ravid *et al*. [11] reported MAV (39%), BPVC (16%), psychogenic vertigo (13%), and orthostatic hypotension (9%) as the most frequent causes of vertigo. Consistent with the literature, the present study reports BPVC (23%) and orthostatic hypotension (22%) as the most prevalent causes of vertigo.

Orthostatic hypotension is frequently observed in children who undergo rapid growth (before and during adolescence), and it represents a temporary weak adaptation of the cardiovascular system to abrupt changes in body position. Vertigo often occurs in the morning after wake-up (while trying to get up rapidly) or while standing for an extended period. Vertigo can be accompanied by the feeling of dizziness, fainting, and rarely loss of consciousness. Vestibular tests prove normal. The diagnosis of orthostatic hypotension is established by demonstration of a change in arterial blood pressure when measured upon standing up quickly after having remained in the lying position for 20 minutes [10]. Orthostatic hypotension was found to be the second most common (22%) cause of vertigo in the present study, and even higher rates were noted in the 14-16 years age group in line with the literature.

Differential diagnosis requires a detailed medical history, physical examination, and scrutinizing accompanying symptoms. Headache, tinnitus, blurred vision, and loss of balance are the most common symptoms accompanying vertigo in pediatric patients [1, 12]. Headache (41%), syncope (27%), and nausea and vomiting (10%) were the most common symptoms accompanying vertigo in the present study. Epileptic vertigo and MAV should be consired in the presence of signs accompanying vertigo such as photophobia, nausea, vomiting, smell disorders and hallucination. Migrainoid headaches are more frequent in childhood than in adults. The studies reported prevalence rates as high as 35%, whereas the prevalence rate was only 6% in adults [13]. In addition to headaches, it can be seen with concomitant phonophobia, photophobia, nausea, vomiting, and smell disorders. The complaints often have an onset in childhood and are more common in girls [14]. In vertigo patients with accompanying headache, the rate of migraine-associated vertigo was 8% and more common in the girls (64%) that was consistent with the literature. Epileptic vertigo is usually associated with signs such as complex movement hallucinations (more than simple rotational vertigo) that provoke an epileptic seizure, auditory hallucinations, symptoms of neurological localization, and loss of consciousness. These kinds of complaints absolutely require neuroimaging studies and a consultation with a pediatric neurologist [15]. Epileptiform disorders were detected in 11 (7%) out of 153 patients undergoing EEG, and all these patients were diagnosed with epilepsy in our study.

Vertigo can occur secondary to infections in childhood. It can result from otitis media or middle ear effusions that are commonly seen in this age group. It is believed that vertigo occurs as a result of pressure changes in the middle ear and labyrinthitis [16]. The rate of otitis media in the present study was 3%. Infrequently, an association can be observed in childhood between vertigo and sinusitis. In these patients, vertigo can be accompanied by headache,

loss of balance, and nausea and vomiting [17]. In our study, six (3%) patients were found to have sinusitis, and mastoiditis was the underlying etiology in two (1%) patients.

Vertigo is often a subjective finding and it can be accompanied by psychiatric disorders, such as anxiety disorders, depression, and behavioral disorders [18]. In a study involving only children aged younger than 16 years, Manrique Lipa *et al*. [19] detected psychogenic vertigo in 10% of children with vertigo. In a study including 37 pediatric patients with vertigo, Gruber *et al*. [3] reported psychogenic vertigo in eight (22%) patients. In our study, 13 (7%) patients were diagnosed with psychogenic vertigo, mostly originating from anxiety and behavioral disorders.

Approximately 10% of cases of vertigo in the 5-6-year-old age group are associated with visual disturbances. Usually vertigo recovers when the visual disturbances are corrected. Visual disturbances can be related to refractive errors (myopia, hypermetropia or astigmatism) or ocular vergence anomalies. Vertigo perception involves the sense of spinning or rolling, can last shortly but is recurrent and often associated with fatigue [20]. The rate of vertigo associated with refractive error was 6% in the present study.

S uch peripheral vestibular disorders as benign paroxysmal positional vertigo (BPPV), vestibular neuritis, and Ménière's disease are uncommon in adults than in children [21]. The rate of BPPV was found to be 8% in the present study, whereas no patient had vestibular neuritis and Ménière's disease. Likewise, a history of trauma and vertigo after an operation that is considered in the differential diagnosis of vertigo was detected only in one patient. In this study, two patients with vertigo of childhood were found to have accompanying subclavian steal syndrome, which is an uncommonly reported condition, and one patient was found to have multiple sclerosis.

Autoimmune thyroiditis is the most common cause of goiter and acquired hypothyroidism among children living in iodine-sufficient areas. Patients may present with such symptoms as weakness, dry skin, constipation along with extrathyroidal symptoms, including alopecia, vitiligo, atopy, and depression [22]. In the present study, autoimmune thyroiditis was determined as the likely cause of vertigo in seven (5%) patients. Vitamin B12 deficiency often results in hematological and neurological problems in children. Aside from anemia, infants often present with the symptoms related to the brain development and a delay in overall growth and development including hypotonia, poor feeding, glossitis, lethargy, tremor, irritability, and coma. On the other hand, older children might have peripheral neuropathy, symmetrical paresthesia, ataxia, gait disorders and even psychiatric disorders involving depression and psychosis [23]. Iron deficiency anemia in children may cause symptoms such as palpitation, fatigue, exercise intolerance, headache, irritability, and sensory disorders [24]. In our study, seven (4%) patients had autoimmune thyroiditis, nine (5%) had vitamin B12 deficiency, and three (2%) patients had iron deficiency anemia.

While considering whether to perform neuroimaging studies and neurophysiological laboratory tests in patients with vertigo of childhood, potential effects of radiation must be taken into consideration along with the fact that the child may show limited cooperation in neuroimaging studies, vestibular tests and evoked potential tests that require cooperation for optimal results [3]. For example, CT has a very limited place in evaluation of vertigo in children. However, the use of MRI as a neuroimaging study has become a more common practice owing to lack of radiation exposure and its superiority in delineating posterior fossa and inner ear structures. Neuroimaging studies still contribute little to the diagnosis in patients presenting with vertigo alone. Raucci *et al*. [7] performed neuroimaging studies in 20.8% of 616 children who presented to the pediatric emergency room with the complaint of vertigo and detected serious neurological problems only in 2.5%. Also, they noted significant neurological symptoms accompanied vertigo in all of these latter patients.

Similarly, a retrospective study of 87 pediatric patients presenting with vertigo reported new findings on neuroimaging studies in 23 patients, however, 19 out of 23 patients had neurological deficits in addition to vertigo [25]. The authors particularly emphasized that neuroimaging studies do not contribute significantly to elucidating the etiology of vertigo. In a study from Turkey, Erdoğan *et al*. [1] performed MR imaging of the brain in 13 out of 30 pediatric patients with vertigo and reported no pathological findings in any of these patients. Neuroimaging studies showed

normal findings in 144 (85%) out of 171 patients, whereas 17 (10%) were found to have nonspecific findings (arachnoid-pineal gland cyst, corpus callosum agenesis, choroid plexus papilloma, pituitary microadenoma, arachnoid granulation), four (2%) had sinusitis, two (1%) had mastoiditis and subclavian steal syndrome, one patient had venous angioma and another patient had demyelinating plaques. Consistent with the literature, these results suggest that neuroimaging studies have a very limited role in the assessment of children with vertigo.

CONCLUSION

This study shows that orthostotic hypotension and BPPV are the most common causes of vertigo. Autoimmune thyroiditis, vitamin B12 deficiency, iron deficiency anemia weredetected in approximately one-tenth of patients, and epilepsy was diagnosed in 7% of the patients. This shows that as in all diseases, it is important to evaluate patients systemically in vertigo. It should be kept in mind that vertigo can be an epileptic symptom. As in other studies, it was seen that neuroimaging studies did not contribute much to vertigo.

Conflict of interest

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

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The impact of transcutaneous posterior tibial nerve stimulation in patients with premature ejaculation

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ABSTRACT

Objectives: Approximately 30-40% of men worldwide are affected by premature ejaculation. Despite much research on this subject, there is still little information about the cause and treatment of premature ejaculation. The aim of this study was to evaluate the therapeutic aspect of transcutaneous posterior tibial nerve stimulation (PTNS) in patients with premature ejaculation.

Methods: The study included 60 PE patients, aged 20-50 years, divided into 2 groups as the treatment group (n = 30) and the control group (n = 30). Transcutaneous PTNS was applied to patients in the treatment group for 30 minutes once a week for 12 weeks. In the control group, a stimulation probe was placed on the posterior tibial nerve without giving any stimulation, to provide a placebo effect. The Arabic Index of Premature Ejaculation (AIPE) scale and Intravaginal Ejaculation Latency Time (IELT) measurements were used before and after the procedure.

Results: In both groups, the mean IELT duration and AIPE scale scores were statistically significantly increased after the procedure ($p < 0.05$). The percentage change in the AIPE scale scores after the procedure was found to be higher in the treatment group than in the control group ($p = 0.007$).

Conclusions: The results of the study showed a statistically significant increase in the duration of the time to ejaculation with PTNS, and a significant improvement was obtained in the post-treatment AIPE scale scores. There is a need for further research to achieve more robust results to contribute to premature ejaculation treatment.

Keywords: premature ejaculation, posterior tibial nerve stimulation, sexual disorder, male

Premature ejaculation (PE) is the most common male sexual disorder and is estimated to affect up to 30%-40% of men worldwide [1-3]. Despite much research on the subject, there is still limited knowledge about the etiology, diagnosis, pathophysiology and treatment of PE. The International Society for Sexual Medicine (ISSM) adopted a new definition of PE in 2014 [4]. According to this definition, the main problem is the inability to delay ejaculation (short intravaginal ejaculation latency time (IELT), defined as the time between the start of vaginal intromission and the start of intravaginal ejaculation, frequently used as definition of PE with IELT < 1 min), resulting in distress, frustration, and/or the avoidance of sexual intimacy.

Pelvic floor muscle training has been shown to have a role in the treatment of many urological problems (e.g. lower urinary tract symptoms, urinary in-

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continence, erectile dysfunction and premature ejaculation). These studies have revealed that contraction of the male pelvic floor muscles (i.e., bulbospongiosus and ischiocavernosus) via neuromuscular electrical stimulation can be safely performed for several minutes, with good perception and without discomfort. As ejaculation involves rapid stereotyped rhythmic contractions of the bulbospongiosus and ischiocavernosus muscles, inhibiting this type of contraction may have a beneficial effect in the treatment of PE [5].

The aim of this study was to evaluate the therapeutic aspect of transcutaneous posterior tibial nerve stimulation (PTNS) in patients with PE.

METHODS

This prospective study included patients aged 20-50 years, diagnosed with PE between June 2015 and June 2016. Patients with cardiac failure, arrhythmia, skin scarring, sexual dysfunction other than premature ejaculation, urogenital anatomic disorder, oncology patients, and those unwilling to participate in the study were excluded. All participants provided written informed consent. The patients were divided into 2 groups as the treatment group and the control group. Demographic data of the patients were recorded, then the Arabic Index of Premature Ejaculation (AIPE) scale was used to evaluate both the diagnosis and the severity of PE. The AIPE is a 7-item questionnaire that evaluates sexual desire, hard erections for sufficient intercourse, time to ejaculation, control, satisfaction for the patient and partner, and anxiety or depression. The scale has been validated for use in the Turkish population [6, 7]. Patients were informed about how to perform the IELT measurement using a chronometer and the patients were asked to record the ejaculation times before and after treatment using the chronometer. Transcutaneous posterior tibial nerve stimulation was applied to the 30 patients in the treatment group, for 30 minutes once a week for 12 weeks. Electrical stimulation was applied with the Pagani ET10® electrotherapy device (Pagani Elettronica, Italy) using 200 µsec pulses with a pulse rate of 20 Hz (Fig. 1). In the control group, a placebo effect was provided by a stimulation probe placed on the track of the posterior tibial nerve without giving any stimulation. All data on the AIPE forms and the recorded IELT times measured using a chronometer were compared before and after the treatment procedure.

This prospectively designed study complied with the Declaration of Helsinki, regulations involving patient's rights and ethical guidelines and was approved by the Local Ethics Committee (approval number: 2011-KAEK-25-2015/11-01).

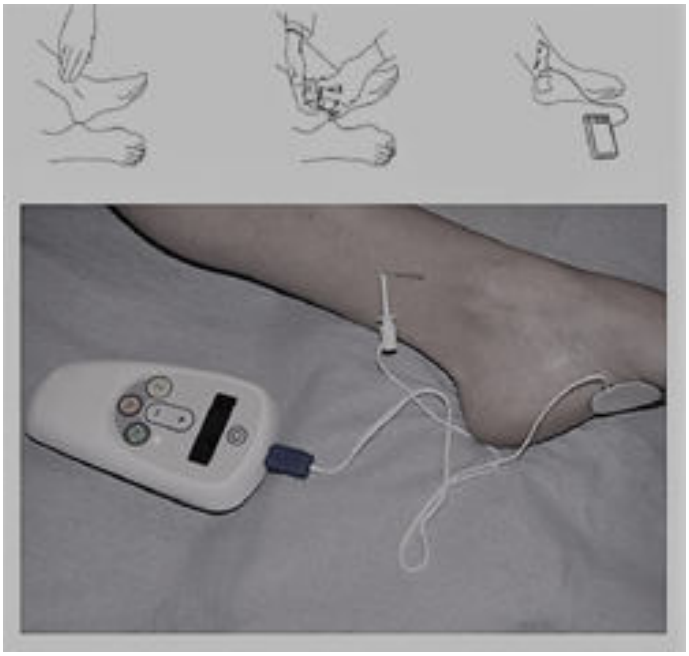

Fig. 1. Treatment with electrical stimulation

Statistical Analysis

Data were analyzed using SPSS (Statistical Package for the Social Sciences) v.23 software (SPSS Inc, Chicago, IL, USA). The descriptive statistics for the numerical variables were expressed as mean ± standard deviation, and as number and percentage for categorical data. After assessment of the conformity of the data to normal distribution, the paired t-test was used to compare the dependent samples and the Mann-Whitney U-test was used to compare the independent variables. The results were evaluated at 95% confidence interval and a value of $p < 0.05$ was considered statistically significant.

RESULTS

Eighty-two patients with PE included in the study. However, because of noncompliance to transactions,

Table 1. Evaluation of mean AIPE scores and IELT before and after treatment in the patient and control groups

	AIPE		*p* value	IELT (sec)	
	Pre-treatment	Post-treatment		Pre-treatment	Post-treatment
Treatment Group (n = 30)	16.55 ± 4.74	21.4 ± 4.98	**0.001**	40.4 ± 13.21	51.25 ± 10.5
Control Group (n = 30)	18.2 ± 5.2	20 ± 4.65	**0.001**	37.9 ± 11.81	42.5 ± 11.64

Data are shown as mean ± standard deviation. AIPE = Arabic Index of Premature Ejaculation, IELT = Intravaginal ejaculation latency time

22 patients were excluded. The mean age of the patients was 34.85 ± 6.29 years in the treatment group (n = 30) and 31.85 ± 6.02 years in the control group (n = 30), with no statistically significant difference determined between the groups ($p = 0.132$). In the treatment group, the AIPE mean score was 16.55 ± 4.74 pre-treatment and 21.4 ± 4.98 after the procedure. This increase in AIPE mean scores from pre to post treatment was statistically significant ($p < 0.001$). In the control group, the AIPE mean score was 18.2 ± 5.2 before the procedure and 20 ± 4.65 after the procedure and this increase was determined to be statistically significant increase ($p = 0.001$) (Table 1). The percentage change in the pre to post-procedural AIPE scores was 0.33 ± 0.26 in the treatment group and 0.13 ± 0.16 in the control group. There was a statistically significant difference between the treatment and control groups in terms of percentage change in AIPE scores after the procedure ($p = 0.007$) (Table 2).

In the patient group, the mean IELT was 40.4 ± 13.21 sec pre-treatment and 51.25 ± 10.5 sec post-treatment. The increase in mean IELT from pre- to post-treatment was statistically significant ($p < 0.001$). In the control group, the mean IELT was 37.9 ± 11.81 sec before the procedure and 42.5 ± 11.64 sec after the procedure, and the increase was statistically significant ($p = 0.030$) (Table 1). The percentage change in pre- to post-procedural IELT scores was 0.38 ± 0.47 in the treatment group and 0.23 ± 0.67 in the control group. No statistically significant difference was determined between the patient and control groups in terms of percentage change in IELT scores from pre to post-procedure ($p = 0.415$) (Table-2).

Table 2. Comparison of mean AIPE scores and mean IELT durations before and after treatment in the patient and control groups and assessment of the percentage change rates of AIPE scores and IELT after treatment

	Treatment Group (n = 30)	Control Group (n = 30)	*p* value
AIPE scores before treatment	16.55 ± 4.74	18.2 ± 5.2	0.301
% change of AIPE scores after treatment	0.33 ± 0.26	0.13 ± 0.16	**0.007**
IELT before treatment (sec)	40.4 ± 13.21	37.9 ± 11.81	0.532
% change of IELT after treatment	0.38 ± 0.47	0.23 ± 0.67	0.415

Data are shown as mean ± standard deviation. AIPE = Arabic Index of Premature Ejaculation, IELT = Intravaginal ejaculation latency time

DISCUSSION

PE with a prevalence of 4% to 39% in all populations is ranked first among male sexual dysfunctions, with a negative impact on the social life of both the individual and their partner. Psychological, environmental, endocrine and neurobiological factors have been shown to be effective in the etiology. However, the highly variable and complicated etiology has not yet been fully clarified [1, 8].

Age is undoubtedly a risk factor for erectile dysfuction, whereas for PE the opposite is true. PE is observed more frequently in younger men, and the risk decreases with age. Researchers have explained that this reduction is achieved through more relevant experience, and learning appropriate techniques and positions, and it has been emphasized that young men may not be able to sufficiently control ejaculation [9]. Therefore, the mean age of 34.85 ± 6.29 years for the 30 PE patients in treatment group of this study was found to be consistent with these published data.

PE leads to a decrease in general well-being, loss of self-esteem, feelings of shame and inferiority, depression and anxiety. In a previous study, self-esteem related to PE was found in 68% and anxiety in 36% of patients [10]. Dunn *et al*. [11] also reported that the relationship between PE and anxiety affected the man's quality of life. Patrick *et al*. [12] reported sexual satisfaction at 31% and personal stress at 64% in men with PE and these rates were determined to be statistically significantly higher than those of non-PE cases. The AIPE scale includes key items related to both the mental status of the patients and erectile dysfunction. In a study of 1,608 applications made to a urology polyclinic due to urological complaints, 20.8% of the complaints were reported to be related to PE. It was also stated that 16.2% of these cases did not want treatment for reasons such as not considering this to be a problem, feelings of shame or for economic reasons [13, 14]. However, with the decline in quality of life, patients begin to seek treatment, such as focusing on another topic during the relationship, stopping for a while and resuming the relationship, pre-relationship masturbation, more frequent relationships, drinking alcohol pre-relationship or taking pleasure from other items. Current treatments for PE include behavioral treatments (stop-start and squeeze methods), topical treatments, PDE5 (phosphodiesterase type 5) inhibitors, tramadol, selective serotonin reuptake inhibitor (SSRI), dapoxetine, oxytocin, hyaluronic acid application, dorsal penile nerve cryoablation, intracavernosal injection, acupuncture, botulinum neurotoxin, surgical treatment options and pelvic floor rehabilitation [15-17].

According to current guidelines, the first step in PE treatment consists of oral antidepressants and topical anesthetics. Over time, SSRI and clomipramine in particular have become the first choice for PE treatment [16, 17]. Abdel-Hamid *et al*. [18] reported that antidepressants (clomipramine, sertraline, paroxetine) are equally effective in themselves and paroxetine is more effective than the "stop-squeeze" method and other antidepressants. However, SSRIs have a risk of accumulation, and when the dose increases, the side effects also increase. Side-effects can be very dangerous because of serotonin syndrome, myoclonus, hyperreflexia, sweating, impaired coordination and changes in mental status. Sexual side-effects including erection loss and decreased libido can be seen. An increase in suicide and self-harm tendency has been reported in SSRI users. Since PE is not a life-threatening event, benefit and risk analysis should be performed for SSRI treatment [17]. In a randomized, placebo-controlled study, Busato and Galindo [19] applied topical anesthetic cream (lidocaine-prilocaine solution) to 42 PE patients and as a result they found that mean IELT time (8.49 min) was significantly higher than mean IELT time (1.49 min) before application. However, topical treatments can cause vaginal numbness and anorgasmia if the glans penis is not washed well, and can cause drowsiness and erection of the penis when it is expected to be over 45 minutes after application. Skin reactions can develop on the penis and vagina [17]. Tramadol is not recommended for long-term treatment of PE. A decrease in the efficacy of tramadol has been reported after 12 weeks of treatment, which causes a need to increase the dose, and there is the risk of opioid addiction [20]. Treatments such as dorsal penile nerve cryoablation increases IELT but is an invasive and irreversible treatment [17]. The continuous use of these medical treatments results in restriction of activities, often not being able to meet expectations, low patient compliance, persistence, side-effects and even irreversible effects, and therefore

the necessity for alternative medicinal treatment modalities has increased in recent years [16]. Thus, the aim of this study was to investigate the efficacy of transcutaneous posterior tibial nerve stimulation as a placebo-controlled treatment as an alternative treatment option in patients with PE.

In a meta-analysis by Waldinger *et al*. [21], 79 studies (total 3034 cases) were evaluated in respect of methodology by searching PE in the scientific literature. It was emphasized that studies that measured IELT duration with the stop-watch method fulfilled the criteria more effectively [18]. In the current study, the stop-watch method was used to measure real-time IELT, as recommended by Waldinger *et al*. [21].

Glans penis hypersensitivity and hyperexcitability have been reported repeatedly in patients diagnosed with PE, which has been confirmed in somatosensory evoked potential studies. It is believed that this leads to organic consequences for ejaculation and PE, which cannot be controlled in patients. It is known that ejaculation occurs predominantly on the basis of stimulating spinal pathways, but inhibitor spinal inputs are also present [22]. To date, many electrical stimulation techniques such as sacral and pudendal neuromodulation, pelvic wall rehabilitation, electroejaculation, and acupuncture have been used for various sexual dysfunctions [23-25]. Tibial nerve stimulation, which was applied in the current study, involves many anatomic similarities to the technique used in somatosensory evoked potential studies. To date, in addition to use in the treatment of overactive bladder syndrome, transcutaneous electrical nerve stimulation (TENS) has been widely used for the treatment of back and neck pain, increased motor function and reflex movements, hemiplegia therapy, osteoarthritis pain treatment, rheumatoid arthritis treatment, and some opioid peptides in CSF have been reported frequently in the literature [26]. In these studies, TENS was found to be clinically effective and superior to placebo [27-29]. In the current study, transcutaneous posterior tibial nerve stimulation was applied similar to the use of TENS in the treatment of overactive bladder syndrome.

Many studies in the literature have described the use of acupuncture in sexual dysfunction treatments. Sunay *et al*. [30] applied medical treatment (paroxetine), acupuncture, and a placebo effect in the form of acupuncture by dividing the PE patient into 3 groups. The increases in IELT durations of the paroxetine, acupuncture and placebo groups were 82.7 sec, 65.7 sec, and 33.1 sec, respectively. Although the increase in IELT was most prominent in the medical treatment group, the increase in IELT in the acupuncture group compared to the placebo group was statistically significant ($p < 0.001$) [30]. The presence of anterior tibial muscle, metatarsal bone, dorsal pedis artery, calcaneal tendon and medial malleolus in selected aqueduct points are remarkable in terms of anatomic application areas similar to those of the current study. Although the physiological mechanism of acupuncture is not very clear, it can accommodate the spinal segmental and suprasegmental inhibition theory. It is a known fact that electrical peripheral / posterior tibial nerve stimulation is based on the traditional Chinese practice of acupuncture [31]. In a prospective study, Geirsson *et al*. [32] compared the results of stimulation of the tibial nerve with acupuncture and TENS methods. This is important as through the results of that study, the data of the current study can be compared with the data obtained from acupuncture studies because there is no comparable study published in the literature related to PTNS treatment in PE patients.

Although we did not provide any electrical current on the control group in both IELT and AIPE index scores improved significantly. We think that the contact of the PTNS probe to the body procuded a placebo effect.

The current study can be considered of value as the first study in literature to have applied transcutaneous posterior tibial nerve stimulation as a treatment option in PE-diagnosed patients. Nevertheless, there is a need for further studies involving comprehensive and larger sample groups to obtain results which will contribute to the treatment of PE.

CONCLUSION

Currently, none of the recommended PE therapy options alone can satisfy the patients and their effectiveness remains limited. Therefore, more effective, reliable, easily applicable, long-lasting, and well-tolerated therapy options are being sought. The

results of this study demonstrated that a statistically significant increase in the time to ejaculation was achieved by transcutaneus posterior tibial nerve stimulation. Furthermore, a significant improvement was obtained in scale scores after the procedure in patients diagnosed with PE. There is a need for further research with larger study groups to achieve more robust results to make an additional contribution to PE treatment.

Conflict of interest

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The effect of prenatal classes on pregnant women when deciding the delivery type and coping with labor pain

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ABSTRACT

Objectives: The purpose of the study is to determine the impacts of the prenatal classes on pregnant women while determining the type of delivery and coping with pain during delivery.

Methods: This study is descriptive. It involves analysis of 247 participants that were selected as samples from a known population of 653 pregnant women who participated in prenatal classes of our hospital. The prenatal classes took place for 3 weeks and 16 hours in total. They filled the forms regarding the class activities before and after the class. The data were evaluated by using SPSS 16.0 packaged software.

Results: The mean age of the pregnant women was 27.50 ± 4.60 years. The women, who had their first pregnancy, were 73.3%. Before the class, 62.8% declared that they were planning to have a vaginal delivery. The ones, who stated that they were afraid of pain, were 78.9%. In interviews after the classes, 89.5% of the participants stated that the classes affected their choice of delivery type. The rate of participants that found classes relieving for their anxiety and concerns was 94.70%. The rate of participants that had a vaginal delivery was 81.80%. The influence of the prenatal classes while determining the type of delivery was statistically significant. ($p < 0.001$ by Mc Nemar test).

Conclusions: Participation with the prenatal class removes the anxiety of the pregnant women, encourages them to have vaginal delivery and contributes to decreasing the rate of cesarean sections.

Keywords: Prenatal class, pain during delivery, cesarean section

The pregnancy carries risks for the mother and the baby so that it creates concerns even if they are considered as physiological by society [1]. Parallel to the advancement in healthcare, the pregnant women become more interested in getting informed about the pregnancy and delivery [2]. The classes, which are done to ensure a healthy pregnancy, to inform and answer the questions, to educate about the delivery and postpartum period, are important to reduce the cesarean section rates that are gradually increasing in the world and in our country [3]. The pregnant women choose to have a cesarean section particularly because they are concerned that they will not be able to deal with the pain [4, 5]. Acquiring information from competent people on pregnancy and delivery increases their confidence and provides active participation during delivery as they become aware of their body [6]. Prenatal classes that started in the 1930s continue to grow becoming more disciplined. In our hospital, this program is implemented as a pregnant school by ex-

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panding education, counseling and exercise programs related to pregnancy, delivery and postpartum periods and by further improving the physical properties of the environment [7]. Prenatal class program is conducted in order to raise awareness of mother candidates and to enable them to take an active role in birth and baby care.

The purpose of the study is to determine the impacts of the prenatal classes on pregnant women, who participated the prenatal classes that have been given in our hospital since June 2015, while determining the type of labor and coping with labor pain.

METHODS

This study is descriptive. The population is 653 pregnant women who participated in prenatal classes of our hospital and earned their certificate between in June 2015-December 2018. The minimum sample size is calculated as 242 per sampling formula for cases with a known population. As some of the participants might be excluded from the study, 260 individuals were selected from a simple table of random numbers. The actual sample size of this study was 247. The responses of these 247 participants were evaluated. Within the rules of admission to the prenatal classes, all participants filled a consent form both for attending the classes and for use of their data in this study.

Data were collected by "data collection form before the prenatal class" (1st part is composed of 8 questions on socio-demographic properties, 2nd part is composed of 26 questions on obstetric history), "data collection form after the prenatal class" that had 5 questions and "prenatal class training activity questions form" that had 21 questions.

The pregnant women, particularly the ones that were followed in polyclinics of our hospital or the ones that we were able to reach, who fulfilled the requirements of participation, were accepted to the class. After their heart rates were detected, they were invited to the class regardless of their week in pregnancy. The ones that had closer weeks in pregnancy with each other were put in the same group. The ones with medical conditions that would prevent their attendance to the classes and exercises or the ones that had risky pregnancies were not admitted to the class. The ones that satisfy all the criteria but previously had a cesarean section were not included. The classes took place during 2 half days for 3 weeks and 16 hours in total. The subjects were determined by the Republic of Turkey Ministry of Health that was centered on vaginal delivery. The subjects include anatomical and physiological changes during pregnancy, diet, frequently encountered problems, overall care, exercises, vaginal delivery, newborn care and breastfeeding. The participants that attended all classes were awarded a certificate. In our study, the participants who got certificates were included.

For the study, permission of the clinical research ethics committee of the University of Health Sciences, Erzurum Regional Training and Research Hospital was obtained. (Decision No: 17.09.2018/14-147).

Statistical Analysis

The data is analyzed by SPSS 16 packaged software and Mc Nemar test for descriptive statistics such as counts, percentages, mean values and standard deviation. The significance level is determined to be *p* - value smaller than 0.05.

Table 1. Socio-demographic properties

Property		n	%
Age	< 25	69	27.9
	25-35	165	66.8
	> 35	13	5.3
Education	Illiterate	1	0.4
	Elementary school	14	5.7
	Middle school	21	8.5
	High school	58	23.5
	University and postgraduate	153	61.9
Employment	Yes	71	28.7
	No	176	71.3
Health insurance	Yes	240	97.2
	No	7	2.8
Income	High	57	23.1
	Mediocre	141	57.1
	Low	49	19.8
Usage of internet and social media	Yes	228	92.3
	No	19	7.7

RESULTS

The socio-demographic information of the pregnant participants was as follows: Ages ranged between 19 and 40 (mean 27.50 ± 4.60) years, 61.9% was college graduate, 28.7% was currently employed. The participants that indicated their income levels as mediocre (income equals to expenses) were 57.1%. Almost all of them had health insurance (97.2%). The participants that used the Internet and social media were 92.3% (Table 1). The results from their obstetric history were as follows: For 73.3%, it was the first pregnancy; 13.8% had one and 12.9% had two or more deliveries with living children. Most of the participants (60.3%) were less than 28-weeks pregnant. The ones that planned the type of delivery were 65.6% whereas 34.4% stated that they did not have any plans (Table 2).

When the participants were asked whether they got any prenatal classes before, 9.7% answered positive and 8.5% of the ones, who got classes before, took them in Family Health Centers (Table 3). The percentage of participants, who stated that they were afraid of pain during pregnancy, was 78.9%. The percentage of participants, who indicated that they did not have any plans to cope with pain during delivery, was 5.3%, 41.7% stated that walking and moving would be the most efficient way to cope with the pain (see Table 3).

In the interviews after the classes, 100% of the participants stated that they were satisfied with the classes; 89.5 % stated that the classes affected their choice of delivery type; 94.7% of them said that prenatal classes contributed to relieve their anxiety and concerns related to the delivery (Table 4). This was statistically very significant ($p < 0.001$ by McNemar test). The rate of vaginal delivery was 81.8%.

Before the classes, 62.8% of the participants indicated that they preferred vaginal delivery. After the classes, this rate significantly increased to 97.2% ($p < 0.001$ by McNemar test) (Fig. 1).

Table 2. Pregnancy history

Questions	Responses	n	%
Number of pregnancies	First	181	73.3
	2	34	13.8
	3	22	8.9
	4 and more	10	4.0
Number of deliveries	0	190	76.9
	1	34	13.8
	2	17	6.9
	3 and above	6	2.4
Week of pregnancy (during labor)	≤ 28	149	60.3
	29-36	79	32.0
	37-40	19	7.7
	> 41	0	0
Problems encountered during pregnancy	Yes	71	28.7
	No	176	71.3
Pregnancy method	Spontaneous	237	96.0
	By treatment	10	4.0
Planned labor type	Vaginal	155	62.8
	Cesarean section	7	2.8
	No plan	85	34.4
Labor type	Vaginal	202	81.8
	Cesarean section	45	18.2

DISCUSSION

Labor and pain during delivery have always been scary for the woman whose fear and anxiety were built up by labor stories and experiences of others [8]. Advancement of technology and developing world view increase the desire and opportunity to reach knowledge [2]. Prenatal classes provide easy information and skills on pregnancy and delivery by interactive exercises [9].

With the help of the Internet, information on pregnancy and delivery becomes more accessible [10]. The studies suggest that the pregnant women that had higher education and worked in healthcare had a higher rate of attending prenatal classes [11]. In our study, the highest percentage of the participants was college graduates. Although the pregnant women are

Table 3. **The evaluation before the class**

Questions	Responses	n	%
Did you take prenatal classes before?	Yes	24	9.7
	No	223	90.3
How did you decide to attend the classes?	My doctor suggested	21	8.5
	Nurse	118	47.8
	Poster, brochure, internet	22	8.9
	Other	77	31.2
	Other	9	3.6
Why did you participate the classes?	To learn about the process	60	24.3
	To learn the exercises	5	2.0
	To learn easy delivery methods	22	8.9
	To reduce my anxiety	20	8.1
	All	140	56.7
Are you afraid of the pain during labor?	Yes	195	78.9
	No	52	21.1
In your opinion, what is the best way to cope with pain during labor?	I don't know	0	0
	Medication	17	6.9
	Listening to music	2	0.8
	Massage	49	19.8
	Walking	103	41.7
	Warm shower	49	19.8
	Other	27	10.9
Do you have any plan to cope with pain during labor?	Yes	234	94.7
	No	13	5.3

Table 4. **Prenatal class activity questions and changes in knowledge and behavior**

Questions	Responses			
	Yes		No	
	n	%	n	%
Did you gain new information and skills?	247	100.0	0	0
Did your fear and anxiety regarding the labor decrease?	234	94.7	13	5.3
Did your confidence increase?	237	96.0	10	4.0
Would you consider vaginal delivery if there are no complications?	240	97.2	7	2.8
Did class have any influence on you to change your decision on delivery type?	221	89.5	26	10.5
Are you satisfied with the training?	247	100.0	0	0

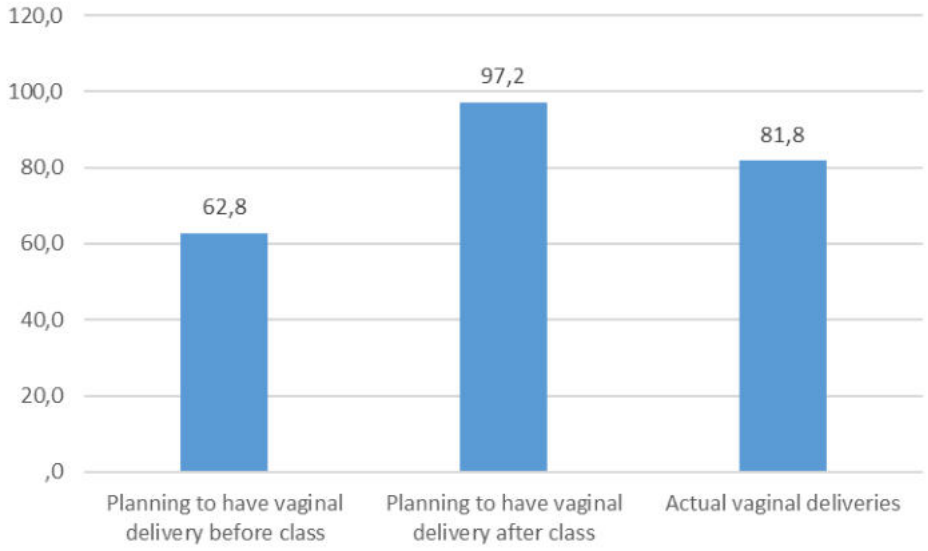

Fig. 1. Vaginal delivery rates after taking the class.

invited to the classes regardless of their education level, the higher demand by pregnant women with higher education indicates that we should focus more on the less educated. The rate of women that were working was stated as 37.10% by Turgut *et al*. [12] and in our study, the rate was 28.7%. The same study stated that women with their first pregnancy participated more (72.9%) and the results were similar in our study (73.3%). The participants that were actively using the Internet and social media were 92.3%. In a study carried out in Sweden, the rate was reported as 95% [13]. In our study, 57.1% of the participants declared their income to be mediocre (income equals the expenses), which was similar to the results from the study of Okumuş *et al*. [14].

The biggest concern of the pregnant about the delivery is not being able to cope with the pain, which unnecessarily leads them to opt for cesarean section [4]. In order to reduce the labor pain, in addition to the traditional methods, non-pharmacological methods are applied in health centers. Relaxation therapies such as music, aromatherapy, acupuncture, acupressure, yoga, hypnosis or dermatological stimulation methods such as massage (especially back and effleurage), intradermal water injection, transcutaneous electric neuron stimulation, and heat are among the applications [15]. In our study, before the classthe pregnant women were asked in a data collection form how they planned to deal with labor pain and then which of the choices that we offered they would choose. Most of them (94.7%) stated that they had a plan and they thought walking/moving would reduce the pain the most. In another study, breathing exercises were preferred the most by the participants [12]. Before the class, the participants were hesitant about the vaginal delivery but after the class, the rate of preference for vaginal delivery increased to 97.2%. In their postpartum histories, it is detected that 81.8% of them had a vaginal delivery. In another study, antenatal education is reported to be significantly effective to come over the fear [16]. In a study by Masoumi *et al*. [6] no significant change between the ones, who took the class and who did not, was reported and the rate of cesarean section was still high. The pregnant women prefer cesarean section because of the fear of being alone and the negative experiences that they heard from the family members, close friends, their circle and social media. One-to-one communication with the participants and the introduction of the emergency and delivery rooms of our hospital during the classes made them feel safe and encouraged them to have a vaginal delivery. Occasionally, nurse midwives took the same classes in order to be able to empathize and communicate with the pregnant women. This application removed the prejudice of the pregnant women about delivery and the care providers in the delivery room by ensuring a hospitable environment. Similarly, the approach of the nurses towards the patients was positively affected.

CONCLUSION

Labor requires physical and psychological cooperation of the mother with the healthcare providers. Participation with the prenatal classes reduces the concerns of the prospective mothers, prevents misguidance due to second-hand information, provides a fun environment where they feel secure and get educated with their peers. The participants are also encouraged by the certificates that they earn at the end to complete the classes where they are incentivized to have vaginal delivery reducing the rate of cesarean section.

Authors' contribution

BGK = Data collection and literaturere view, analysis, writing; EÇT = Data collection; and EBG = Data collection

Conflict of interest

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Should anaesthesia method for prostate biopsy be the same for every patient? A randomised prospective study to determine the risk factors for pain

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ABSTRACT

Objectives: To evaluate the risk factors for pain occurring during prostate biopsy.

Methods: This study included 123 patients were applied with prostate needle biopsy under transrectal ultrasonography. The patients were randomly separated into 3 groups of 41 individuals. For periprostatic nerve blockage, 10 cc 2% lidocaine was applied to Group 1, 10 cc 0.25% levobupivacaine to Group 2, and 10 cc 0.25% bupivacaine to Group 3. A 10 cm Visual Analogue Scale (VAS) was used to evaluate patient pain. The pain of the patients was evaluated in 4 stages. VAS 1: Pain score during the injection of the anaesthetic agent; VAS 2: Pain score during the biopsy when half the procedure was completed; VAS 3: Pain score following removal of the rectal probe immediately after the biopsy; and VAS 4: Pain score at 1 hour after the biopsy.

Results: There were significant negative correlations between VAS 3 pain scores and age in group 1, group 3 and for entire cohort ($p = 0.013$, $p = 0.031$ and $p = 0.033$, respectively). In group 1 both total and free PSA showed significant negative correlations with VAS 3 pain scores ($p = 0.020$ and $p = 0.010$, respectively). In group 2 VAS 4 pain scores of the patients with suspicious digital examination findings were found to be significantly higher than those of the patients with benign digital examination findings ($p = 0.025$).

Conclusions: Of all patients to be applied with prostate biopsy, those of a younger age, with a lower PSA level, with suspicious digital rectal examination findings constitute a relatively higher risk group in respect of pain.

Keywords: biopsy, pain, prostate, risk factors

The current standard method used to determine prostate cancer is prostate biopsy applied under transrectal ultrasonography (TRUS) guidance. When automatic biopsy instruments started to be used in prostate biopsy under TRUS guidance, patient comfort increased as the procedure became quicker and the needles are finer [1]. However, despite these developments, several studies have reported that the majority of patients feel discomfort because of pain felt during the biopsy [2, 3]. Many different protocols have been used in an attempt to control pain, ranging from minimally invasive methods such as the use of non-steroid anti-inflammatory drugs or rectal administration of an anaesthetic agent, to relatively more invasive methods such as periprostatic nerve blockage, or pudendal block. At this point, it is important to identify which anaesthesia methods will be more effective on which patient groups, or in the selection of how invasive an anaesthesia method will be in a specific patient group, the risk factors that could cause pain. The aim of the

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current study was to evaluate the risk factors for pain occurring during prostate biopsy.

METHODS

Approval for the study was granted by the Local Ethics Committee (decision no: 2012/9/3) and signed informed consent was obtained from all the patients. This study included 123 patients were applied with prostate needle biopsy under transrectal ultrasonography (TRUS) guidance because of suspected prostate cancer. The patients included in the study comprised those with indications for prostate biopsy of abnormal rectal examination findings and/or serum PSA levels > 2.5 ng/mL. Patient age, total and free PSA levels, prostate volume, education level, digital rectal examination findings, pathology and biopsy-related complications results were recorded.

In respect of the level of education of the patients, they were separated as 8 years of compulsory education or less (primary school and below) and more than 8 years of compulsory education (above primary school). The digital rectal examination findings of the patients were evaluated as benign or suspicious. Patients with findings of hardness, nodule, irregularity or eradication of the sulcus in the digital rectal examination were classified as suspicious. The pathology results of the patients were recorded as benign or malignant.

By adding new patients to the subsequent group, the patients were randomly separated into 3 groups of 41. For periprostatic nerve blockage, 10 cc 2% lidocaine was applied to Group 1, 10 cc 0.25% levobupivacaine to Group 2, and 10 cc 0.25% bupivacaine to Group 3.

The patients were positioned in the left lateral decubitus position with the hips and knees in flexion. For the TRUS imaging, a ultrasound device was used with a 6.5 mHz rectal probe of the widest diameter of 23 mm (LOGIQ 100 PRO Series). Following rectal placement of the probe, the prostate was visualised in the sagittal and transverse planes and prostate volume was automatically calculated with the ellipsoid formula in the ultrasound machine.

Following aspiration to prevent intravascular injection, the anaesthetic agents were injected slowly using a 30 cm 18 gauge (G) spinal needle, as two separate 5 cc doses between the prostate floor and the seminal vesicle in the sagittal plane to the area where both neurovascular bundles are. When the periprostatic nerve blockage was obtained, biopsy samples were taken from each patient as a standard 12-core biopsy from the posterolateral region of the peripheral zone, in accordance with the European Association of Urology (EAU) guidelines, using a 30 cm 18G fully automatic biopsy needle. As this was the first biopsy for all the patients in this study, transitional zone sampling was not applied. In all the patients, all the 12-core biopsy samples were taken following the same anatomic sequence.

Starting 1 day before the biopsy procedure and continuing for 4 days after, all patients were administered oral 500 mg ciprofloxacin twice a day. To clean the intestines, Fleet enema was administered intrarectally on the morning of the biopsy.

A 10 cm Visual Analogue Scale (VAS) was used to evaluate patient pain. The scale was explained to the patients and they were instructed to mark the scale to represent their pain where 0 = no pain and 10 = the most severe pain ever experienced. Data obtained by measuring in millimeters the marks made on the scale by the patient were recorded as the pain scores.

The pain of the patients was evaluated in 4 stages. VAS 1: Pain score during the injection of the anaesthetic agent; VAS 2: Pain score during the biopsy when half the procedure was completed; VAS 3: Pain score following removal of the rectal probe immediately after the biopsy; and VAS 4: Pain score at 1 hour after the biopsy. Explaining VAS to patients, recording VAS scores and digital rectal examination findings, and all biopsies were performed by the same physician (SA).

Patients were monitored for 1 hour after the procedure and any complications were recorded. Those with no complications were discharged. Second evaluations related to complications were made during the follow-up visits for the pathology results. Complications without any medical or surgical interventions were evaluated as minor complications. The opposite was evaluated as major complications. Complications were also evaluated according to Clavien-Dindo classification.

Statistical Analysis

As the variables did not conform to normal

distribution, comparisons were made with non-parametric statistical tests. In the comparisons between groups, the Mann-Whitney and Kruskal-Wallis tests were used, and for categorical variables, the Chi-square and Fisher tests. Correlations between VAS values and quantitative data were evaluated with Spearman analysis. As non-parametric tests were used, the results were stated as median, minimum and maximum values. A value of $p < 0.05$ was accepted as statistically significant.

RESULTS

The median, minimum and maximum values of patient age, total PSA, free PSA and prostate volume of the groups are shown in Table 1. No statistically significant difference was determined between the groups in respect of age, total PSA, free PSA and prostate volume ($p > 0.05$) (see Table 1). The education level, digital rectal examination findings and pathology results of the groups are shown in Table 1. No statistically significant difference was determined between the groups in respect of education level, digital rectal examination findings and pathology results ($p > 0.05$). The median, minimum and maximum values of the VAS scores calculated according to the education level, digital rectal examination findings and pathology results are shown in Table 2.

In Group 1 and Group 3, and for entire cohort, a statistically significant negative correlation was determined between age and the VAS 3 pain scores (correlation coefficients: -0.388, -0.337, -0.192, respectively, $p < 0.05$) (Table 3). In Group 2, no statistically significant correlation was determined between age and any of the VAS scores ($p > 0.05$). A statistically significant negative correlation was determined between total PSA and the VAS 3 score in Group 1 (correlation coefficient: -0.367, $p = 0.020$). In Group 2, Group 3, and for entire cohort, no statistically significant negative correlation was determined between total PSA and pain scores ($p < 0.05$) (see table 3).

In Group 1, a statistically significant negative correlation was determined between free PSA and

Table 1. The comparisons between the groups of the age, total PSA, free (PSA) and prostate volume of the patients

	Group 1	Group 2	Group 3	*p* value
Age, years	66.5 (50-79)	62 (50-83)	64 (49-82)	0.057
Total PSA	7.3 (0.9-100)	7 (1.4-49)	11.2 (3.4-314)	0.202
Free PSA	1.5 (0.2-50)	1.3 (0.3-6.4)	1.6 (0.3-66)	0.127
Prostate volume	65.5 (21-160)	57 (20-122)	63 (13-240)	0.233
Education level				
Primary school and below (n = 95)	30 (73%)	32 (78%)	33 (80%)	0.376
Primary school and above (n = 28)	11 (27%)	9 (22%)	8 (20%)	
Digital rectal examination				
Benign (n = 69)	25 (61%)	25 (61%)	19 (46%)	0.147
Suspicious (n = 54)	16 (39%)	16 (39%)	22 (54%)	
Pathology result				
Benign (n = 94)	32 (78%)	30 (73%)	32 (78%)	0.862
Malignant (n = 24)	9 (22%)	11 (27%)	9 (22%)	

Data are shown as median (minimum-maximum) or n (%). The *p* values was calculated with the Kruskal Wallis test. The *p* values of the comparisons with the Chi-square test betwen the groups and of the numerical distribution of the education level, digital rectal examination findings and pathology results. PSA = prostate specific antigen

Table 2. The visual analogue scale (VAS) scores according to the education level, digital rectal examination findings and pathology results of the groups

	Group 1				Group 2				Group 3			
	VAS 1	VAS 2	VAS 3	VAS 4	VAS 1	VAS 2	VAS 3	VAS 4	VAS 1	VAS 2	VAS 3	VAS 4
Education level												
Primary school and below	16 (2-83)	17 (2-83)	5 (2-84)	2 (1-72)	28 (3-94)	20 (3-63)	6 (2-69)	2 (1-6)	19 (2-92)	15 (2-94)	8 (2-97)	3 (1-18)
Primary school and above	7 (5-61)	10 (2-30)	7 (4-60)	2 (1-5)	27 (4-82)	35 (5-52)	27 (3-90)	1 (1-5)	40 (6-92)	28 (8-70)	20 (4-58)	3 (1-4)
Digital rectal examination												
Benign	15 (2-83)	16 (2-83)	8 (2-84)	2 (1-72)	27 (3-87)	23 (3-59)	9 (2-90)	1 (1-5)	33 (4-92)	22 (4-91)	27 (2-79)	3 (1-18)
Suspicious	18 (5-57)	17 (3-72)	5 (2-22)	2 (1-48)	29 (4-94)	13 (5-63)	9 (2-69)	2 (1-6)	24 (2-94)	15 (2-94)	6 (2-97)	2 (1-7)
Pathology												
Benign	14 (2-61)	16 (2-81)	6 (2-66)	2 (1-72)	25 (3-94)	15 (3-48)	6 (2-60)	2 (1-5)	33 (4-92)	21 (4-94)	12 (2-97)	3 (1-18)
Malignant	20 (15-83)	17 (3-83)	5 (2-84)	2 (1-48)	40 (8-82)	47 (8-63)	22 (2-90)	2 (1-6)	14 (2-44)	11 (2-24)	5 (2-26)	2 (1-3)

Data are shown as median (minimum-maximum). The values was calculated with the Mann Whitney test. VAS = visual analogue score

VAS 3 pain scores. For entire cohort, a statistically significant negative correlation was determined between free PSA and VAS 2 pain scores (correlation coefficients: -0.401, -0.185, respectively). In Group 2 and Group 3, no statistically significant finding was recorded between free PSA and any of the pain scores. No statistically significant result was obtained in any of the groups between prostate volume and any of the pain scores. The p values calculated for the correlations between pain scores and age, total PSA, free PSA and prostate volume values of the groups separately and together are shown in Table 3.

When the groups were evaluated separately and together, education level was not determined to have significantly affected the pain scores. No statistically significant effect on the pain scores was seen of the digital rectal examination findings in Group 1, Group 3 and for entire cohort. In Group 2, the digital rectal examination findings were observed to have a significant effect on the VAS 4 score. The pain scores of the group with suspicious examination findings were found to be significantly higher than those of the group with benign examination findings.

In Group 1 and for entire cohort, the pathology results were not observed to have had a significant effect on the pain scores. In Group 2, the pathology results were determined to have had a significant effect on the VAS 2 pain scores, and in Group 3 on all the pain scores. In Group 2, the pain scores of those with malignant pathology results were significantly higher than those of the patients with benign results. In Group 3, the pain scores of those with benign pathology results were significantly higher than those of the patients with malignant pathology results. The p values calculated for the effects on pain scores of education level, digital rectal examination findings and pathology results of the groups separately and together are shown in Table 3.

As minor complications, rectal bleeding was seen in 35 patients and hematuria in 10 patients. The only major complication was orchitis observed in 1 patient. Rectal hemorrhage and hematuria resolved spontaneously without any surgical or medical intervention so they were classified as grade 1 according to Clavien Dindo classification. Because of medical treatment orchitis was classified as grade 2 according to Clavien Dindo classification. The distribution of complications according to the groups

Table 3. **The Spearman Correlation Analysis of the relationships between the visual analogue scale (VAS) scores and the age, total prostate specific antigen (PSA), free prostate specific antigen (PSA) and prostate volume values of each group separately and of all the patients together.**

	p values														
	Group 1				**Group 2**				**Group 3**				**All patients**			
	VAS 1	VAS 2	VAS 3	VAS 4	VAS 1	VAS 2	VAS 3	VAS 4	VAS 1	VAS 2	VAS 3	VAS 4	VAS 1	VAS 2	VAS 3	VAS 4
Age	0.639	0.687	**0.013**	0.294	0.689	0.773	0.932	0.213	0.244	0.170	**0.031**	0.226	0.432	0.513	**0.033**	0.926
Total PSA	0.950	0.368	**0.020**	0.117	0.692	0.439	0.532	0.815	0.226	0.245	0.192	0.834	0.531	0.146	0.054	0.561
Free PSA	0.999	0.287	**0.010**	0.066	0.844	0.129	0.814	0.152	0.369	0.338	0.290	0.707	0.527	**0.040**	0.060	0.936
Prostate volume	0.109	0.128	0.092	0.683	0.860	0.240	0.800	0.300	0.284	0.913	0.865	0.333	0.056	0.110	0.335	0.930
Education level	0.379	0.050	0.569	0.818	0.728	0.517	0.051	0.960	0.198	0.097	0.663	0.809	0.886	0.881	0.169	0.774
Digital rectal examination	0.361	0.912	0.074	0.619	0.440	0.747	0.790	**0.025**	0.886	0.283	0.073	0.188	0.366	0.397	0.141	0.390
Pathology	0.065	0.778	0.829	0.475	0.221	**0.003**	0.158	0.770	**0.032**	**0.024**	**0.049**	**0.049**	0.731	0.682	0.833	0.537
Complication	0.896	0.464	0.379	0.619	0.572	0.827	0.551	0.578	0.409	0.483	0.491	0.247	0.371	0.860	0.264	0.387

The *p* values calculated using the Kruskal Wallis test of the effects on VAS scores of complications and the p values calculated using the Mann Whitney test of the effects on the VAS scores of the education level, digital rectal examination findings and pathology results of each group separately and all the patients together.

is shown in Table 4. In the paired comparisons of the groups, no significant difference was determined in respect complications (p values are shown in Table 4). When the groups were evaluated separately and together, no significant correlation was seen between pain and complications (p values are shown in Table 3).

When the pain score results of all the groups were evaluated together according to the education level, digital rectal examination findings and pathology results and the pain score results in each evaluation according to complications were not statistically significant, they are not shown in Table 2. The p values related to the above-mentioned evaluations are shown in Table 3.

There was no statistically significant difference between the groups for VAS 1, VAS 2 and VAS 3 pain scores. Since there was a statistically significant difference between the groups for VAS 4 pain scores, pairwise comparisons were examined between the groups (p values are shown in Table 5 and Table 6).

DISCUSSION

Prostate biopsy applied under transrectal ultrasonography (TRUS) guidance remains the current standard method used in the diagnosis of prostate cancer. Many studies have been conducted to reduce the pain that occurs associated with this procedure and the necessity for the application of anaesthesia before prostate biopsy has been included in the guidelines. However, few studies have evaluated the risk factors for pain. In the European Association of Urology (EAU) guidelines there is no mention of in which patient groups pain may develop in particular and the same anaesthesia method is recommended for all patients.

In the current study, a statistically significant negative correlation was found between age and the pain scores measured immediately after the procedure (VAS 3) in Group 1, Group 3 and for entire cohort. According to this, the pain scores were significantly higher in younger patients. This negative correlation showed a similarity with several studies in literature [1, 4-7]. Djavan *et al*. [4] stated that significantly greater pain was felt by patients aged < 60 years compared to older patients. This can be considered to

Table 4. Distribution of complications in the groups

	Minor complication (Clavien Dindo group 1)						Major complication (Clavien Dindo group 2)		
	Rectal bleeding			Hematuria			Orchitis		
	Absent	Present	p value	Absent	Present	p value	Absent	Present	*p* value
Group 1 n (%)	27 (30.7)	14 (40)	G1 vs G2 0.453	36 (31.9)	5 (50)	G1 vs G2 0.356	41 (33.6)	0 (0)	G1 vs G2
Group 2 n (%)	31 (35.2)	10 (28.5)	G1 vs G3 0.622	39 (34.5)	2 (20)	G1 vs G3 0.675	40 (2.8)	1 (100)	1.000 G2 vs
Group 3 n (%)	30 (34.1)	11 (31.5)	G2 vs G3 1.000	38 (33.6)	3 (30)	G2 vs G3 1.000	41 (33.6)	0 (0)	G3 0.494
Total n (%)	88 (100)	35 (100)		113 (100)	10 (100)		122 (100)	1 (100)	

The p values of the paired comparisons made using the Chi-square test in respect of complications. G1 = group 1, G2 = group 2, G3 = group 3

Table 5. p-values of VAS scores between groups calculated by Kruskal-Wallis test.

	VAS 1	VAS 2	VAS 3	VAS 4
p values	0.152	0.178	0.323	**0.039**

Table 6. *p* values for pairwise comparisons of groups for each visual analog scale score

	p values		
	Group 1 - Group 2	Group 1 - Group 3	Group 2 - Group 3
VAS 2	0.345	0.470	0.872
VAS 3	0.508	0.114	0.346
VAS 4	**0.049**	0.233	**0.001**

be related to anal tonus and relatively greater anxiety before the procedure in younger patients. In studies by Peyromaure *et al*. [8] and Zisman *et al*. [9], a significant correlation was found between anxiety before the procedure and pain occurring during prostate biopsy.

When studies in literature that found no significant relationship between age and pain were examined, Hossack *et al*. [10], patients who had undergone biopsy with local anaesthesia were questioned about their preference for the same procedure or general anaesthesia/sedation for a potential second biopsy and it was reported that those who expressed a preference for general anaesthesia/sedation were younger patients. Zisman *et al*. [9] reported that even if there is no correlation between age and pain, those with pain persisting on the seventh day were significantly younger patients. In the current study, the latest pain score was measured at 1 hour after the procedure (VAS 4) and there was no significant relationship with age. This finding was attributed to the pain having been reduced to a great degree in the first hour with the effect of the anaesthesia applied (mean VAS 4 pain scores for Group 1, Group 2, Group 3 were 6, 1.9, 3.2, respectively). In a study by Inal *et al*. [11], although a negative correlation was reported between age and pain, it was not statistically significant. In that study, 6-12 cores sampling was applied depending on the

prostate volume and the mean core number was 8.8. Bastide *et al*. [12] also found no significant correlation between age and pain and the median core number was 7 (range: 4-10 cores). In the current study, a standard 12-core biopsy was applied to all patients. The difference between previous studies and the current study in the relationship between age and pain could be related to the number of cores taken.

In patients with prostate volume > 40cc, Yun *et al*. [13] reported that pain scores during the procedure and at 20 mins after the procedure were significantly higher. The mean prostate volume of the 71 patients in that study was 42.2 cc, whereas in the current study the mean volume was 66.6 cc. However, no significant relationship was determined between prostate volume and any of the VAS scores in the current study when the groups were evaluated separately or together. Unlike the study of Yun *et al*. [13], our result was consistent with several studies in literature [4, 5, 7, 9, 12, 14].

From a scan of literature, no studies could be found that have reported a significant relationship between PSA levels and pain [4]. In the current study, a significant negative correlation was determined between both total and free PSA levels and the VAS 3 pain scores in Group 1. For entire cohort, although a similar negative correlation was seen for VAS 3, it was not statistically significant. A significant negative correlation was determined between the VAS 2 scores and free PSA for entire cohort (Table 3). To the best of our knowledge, this is the first study in literature to have shown a significant relationship between PSA levels and pain. However, as this relationship was not observed in all the groups there can be considered a need for further studies to investigate the relationship between PSA levels and pain.

In studies by Kaygisiz *et al*. [15], it was stated that however great the pain during digital rectal examination, then the pain occurring during probe placement and biopsy would be of the same degree. As far as we know, that is the only study that has evaluated the relationship between digital rectal examination and pain. In the current study, it was aimed to contribute to literature in a different aspect by evaluating the relationship between pain and digital rectal examination in respect of the examination findings. Accordingly, the VAS 4 pain scores of Group 2 patients with suspicious digital rectal examination findings were seen to be higher than those of the patients with benign findings (Table 3). When the results of the study by Kaygisiz *et al*. [15] are evaluated together with those of the current study, it can be concluded that it should be kept in mind that patients who experience greater pain during the digital rectal examination and have suspicious examination findings could feel more pain during the prostate biopsy.

The lower PSA value and the suspicious rectal examination findings as risk factors for pain may be considered as two opposite conditions. However, it is a clinically known fact that those may not always be in a relationship. We think that this result may be related to an underlying prostatitis in these patients, considering that suspicious digital rectal examination findings are not always associated with malignancy but may also be related to inflammation in the prostate. The increased risk of pain may also be due to this inflammatory condition in the prostate. However, we believe that this topic which is beyond the scope of this study should be evaluated with new studies.

In only one study that evaluated the relationship between pathology results and pain, no significant relationship was found [16]. In the current study, two contradictory results were seen related to the correlation between pain and the pathology results. In Group 3, the pain levels of those with benign pathology results were significantly higher than those of the patients with malignant pathology results in all the pain scores and the reverse of this was seen in Group 2 only in the VAS 2 score, suggesting that pain associated with the biopsy procedure was greater in those with benign pathologies compared to those with malignant pathologies (Table 2, Table 3). Nevertheless, despite this finding, when it is considered that the pathology results are unknown before the procedure, it is debatable whether the pathology results should be evaluated as a risk factor for pain occurring associated with the prostate biopsy. Djavan *et al*. [4] stated that patients with rectal bleeding experienced a significantly more uncomfortable procedure. Hossack *et al*. [10] reported that vasovagal syncope attacks were seen more in the group with higher pain scores compared to the other groups. However, no direct evaluation was made between pain and complications in either of these two

studies. In the current study, when the groups were evaluated separately and together, no significant correlation was seen between pain and complications.

As much as we know, the relationship between education level and the pain occurring during prostate biopsy has not been previously evaluated. In the current study, education level was not seen to affect the pain scores when the groups were evaluated separately or together. However, values of borderline statistical significance were determined in Group 1 and Group 2 (Table 3). According to this, the pain scores during the procedure (VAS 2) of Group 1 patients with an education level of primary school and below, and the pain scores immediately after the procedure (VAS 3) of Group 2 patients with an education level of primary school and above, were seen to be higher. As these results showed borderline significance as a result of the evaluation of the relationship between pain and education level and because the results were contradictory, there can be considered to be a need for further studies on this subject.

There was no significant difference between the groups in terms of VAS 1, VAS 2 and VAS 3 pain scores (Table 5). VAS 4 pain scores were significantly lower in group 2 than in group 1 and group 3 (Table 6). We conclude that this result is due to the fact that levobupivacaine is longer effective than other agents.

Limitations

In our study, the small number of patients and the absence of all complications due to prostate biopsy were considered as limiting factors in our study.

CONCLUSION

The results of this study suggested that of all patients to be applied with prostate biopsy, those of a younger age, with a lower PSA level, with suspicious digital rectal examination findings and benign pathology results constitute a relatively higher risk group in respect of pain. The use of analgesia and/or anaesthesia methods which could be personalised beyond the routine protocols should be considered for patients with these risk factors. However, as the risk factors for pain occurring during prostate biopsy have been evaluated in a limited number of studies and as some of these risk factors have only been examined in this study, there is a need for further studies including a greater number of patients.

Conflict of interest

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

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The European Research Journal 2020;6(5):479-484 Original Article

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Safe use of vascular stapling devices during laparoscopic cholecystectomy in cases with enlarged cystic canal

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ABSTRACT

Objectives: Bile duct injuries and fistulas due to bile leakage are observed at certain rates in laparoscopic cholecystectomy. In complicated cases, in patients with cholelithiasis cystic duct edema, the cystic canal may be enlarged to the extent that it cannot be closed with a clip in a standard operation. In this study we evaluated the eficiency of stapler closure of cystic canal.

Methods: In this retrospective study the cases who had laparoscopic cholecystectomy and cystic canal was closed with stapler between August 2016 and December 2018 were reviewed. Patients' hospital stay and complications were noted.

Results: Thirty-three patients who were electively operated and cystic canal closure was performed with vascular staples because of a wide cystic canal during the operation were included in this study. Low bile drainage was observed in only one case and this patient had spontaneous regression with conservative follow-up. Mean duration of hospital stay was 3.7 days. Patients were followed up from 2 months to 28 months postoperatively.

Conclusions: Endo-vasculer stapler can be used safely during laparoscopic cholecystectomy with a history of stones in the main bile duct. In this group of patients, it is advisable to make preparations in this direction before the operation.

Keywords: Wide cystic, endostapler, laparoscopic cholecystectomy, cholelithiasis

The gold standard surgical treatment for cholelithiasis is now laparoscopiccholecystectomy [1]. The cholelithiasis operation can be recommended prophylactically in asymptomatic patients because of the risk of developing cancer, causing biliary pancreatitis, and the complications of long life expectancy.

Acute cholecystitis, chronic cholecystitis, biliary pancreatitis, biliary colic, stone in the main bile duct, cholangitis, Mirizzi syndrome, gallstone ileus and gallbladder cancer may arise from cholelithiasis or might be the complications of gallbladder stones and laparoscopic cholecystectomy should be performed under appropriate conditions [2, 3]. During laparoscopic cholecystectomy, there are technical difficulties that can make operations hard. Some of the technical difficulties include embedded gallbladder, short and thick cystic duct, anatomical anomalies, Mirizzi syndrome, fibrosis in the Calot triangle (frozen calot), empyema, gallbladder edema and inflammation secondary to intraabdominal adhesions [4].

The encountered situations might change the

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course of the operation and may require interventions, including transforming the conversion to open, T tube application into choledochus, percutaneous transhepatic bile drainage, tube duodenostomy, choledochoduodenostomy, hepaticojejunostomy and other interventions to decrease mortality and morbidity.

The cystic canal is sometimes edematous and the fibrous structure is thickened, or due to the expansion of the lumen, management of the operation becomes complicated. Various techniques in the literature for ligation of cystic channels include intra-corporal knots, titanium clips, endo-loop use and endo-stapler.

In this study, data were collected from 33 patients that, Endo-vasculer stapler were used for enlarged cystic canal ligation, during elective laparoscopic cholecystectomy.

METHODS

Of the 2573 laparoscopic cholecystectomy cases performed between August 2016 and December 2018, 441 cases were accepted as complicated (choledocholithiasis, acute biliary pancreatitis, acute cholecystitis, acute cholangitis, etc.).

Emergency laparoscopic cholecystectomy was performed in 47 patients for acute cholecystitis, and laparoscopic conversion to open surgery was performed in 16 patients. Four patients were treated with the endo-vasculer stapler because of acute cholecystitis due to a wide cystic duct. Fourteen patients underwent elective laparoscopic cholecystectomy conversion to open surgery.

The number of elective cases with wide cystic channels that Endo-vasculer stapler was used was 33. Our choice of stapler for cystic canal closure was the Endo-GIA 45mm – 2.5 mm with vascular (white) stapler. (Medtronic California USA).

Patient files were retrospectively obtained. Age, gender, preoperative diagnosis, additional pathologies, morbidity and treatment management, length of hospital stay and physical examination findings, imaging findings, operation notes, epicrisis and ERCP datas were recorded.

Within 33 patients, 14 had previous ERCP and stone extraction in their history. Complicated cases were consulted to the hepatobiliary surgeon peroperatively, before the decision of using the Endo-vasculer stapler. After agreeing on the biliary anatomy,the stapler indication was planned.

Operations were performed with 4 trocar techniques under general anesthesia. Following posterior dissection, Calot Triangle dissection was revealed by leaving cystic artery and cystic duct seperate. When the cystic canal diameter was not enough to be safely closed with titanium ML or Large and with a Polymer Large, XLclip that was the case in the patients with channel above one centimeter, the operations and the cystic stump's safety was questionable. That's why a vascular white 45 Endo-GIATM stapler was used in these patients.

After inspecting and manually controlling the cystic duct, and endovascular stapler was loaded with a white cartiridge and fired in being sure of no sxtones

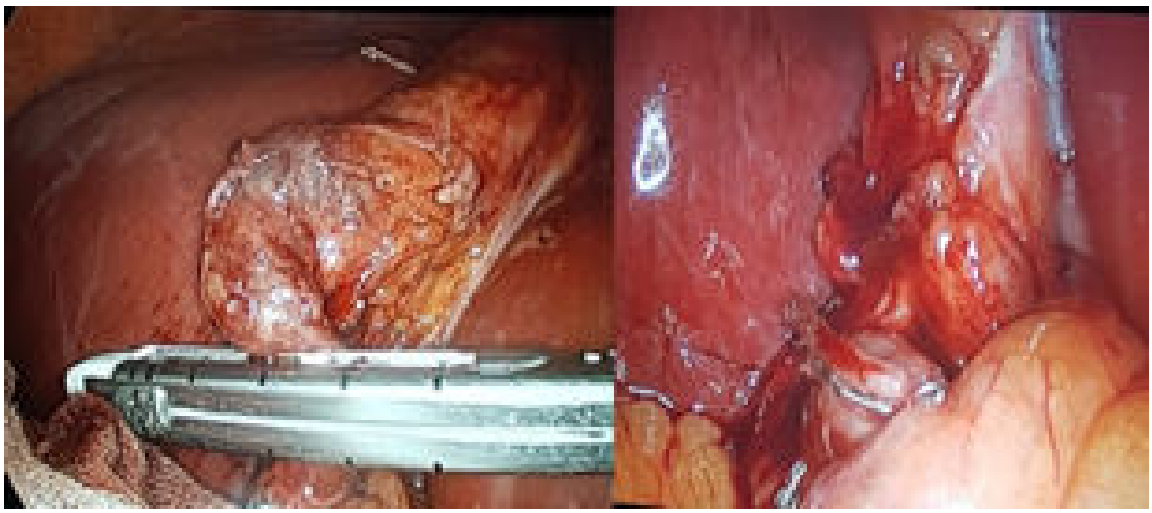

Fig. 1. Endostapler placement on large ductus cysticus and the view of the cystic stump after firing.

in the lumen (Fig. 1).

Technically in the operation, in order to protect the main biliary tract, the gallbladder was first released from the liver bed or the hepatobiliary anatomy was revealed by reducing the gallbladder from the fundus to the Hartmann pouch. The cystic artery was seen in all patients and was cut with a titanium clip or ligasure. The gallbladder was excised from the liver bed using electrocautery and ligasure, and hemostasis was achieved.

Hemostatic matrix application was required in 3 cases for bleeding from the liver bed (Floseal®). The gallbladder was removed from the epigastric incision outside the abdomen by taking it into the Endo-Bag inside the abdomen. Liver bed, operation area and the cystic duct stump was carefully reviewed. In all patients a soft abdomen drain extending for Winslow were placed from the 5-millimeter trocar site. The gallbladder was examined in the operating room before the operation was terminated.

Statistical Analysis

Data were analyzed with the SPSS 21 statistical program. Continuous data are given as mean ± standard deviation where applicable, and categorical data as percentage (%).

RESULTS

Between August 2016 and December 2018, 33 patients underwent elective laparoscopic cholecystectomy in our General Surgery Clinic, and cystic canal closure was performed with 45 white loaded Endo-vasculer stapler. Nineteen (57.6%) of the patients were males and 14 (42.4%) were females. The age range was from 24 to 67 years and the median age was 48 years.

In the preoperative diagnosis of the patient group, there were 11 (33.3%) patients with symptomatic gallbladder, 8 (24.3%) with medically treated acute cholecystitis and 14 (42.4%) with ERCP history (1 cholangitis, 9 biliary pancreatitis, 4 with obstructive jaundice). All patients were operated laparoscopically. In addition to these patients, 4 patients with acute cholecystitis that were diagnosed preoperatively were excluded from the study despite of using the Endo-stapler peroperatively. The mean hospital stay was 3.7 days. The follow-up period of the patients ranged from 2 months to 28 months.

Only one (3.03%) patient in the study group had low flow (50 cc per day) bile leakeage in the postoperative period, and regressed totally spontaneously after 3 days. It was thought that this patient hadan aberrant bile duct (lushca) from the gallbladder bed as the cause of leakage. Three patients with wound infection were treated with antibiotics. A periumblical site hematoma was observed in one patient. No mortality was detected in the study group.

DISCUSSION

Laparoscopic cholecystectomy is the gold standard treatment for both acute cholecystitis and elective cholecystectomy [5]. Laparoscopic cholecystectomy may be a good choice for a given group after the first 72 hours after acute cholecystitis [6]. Delayed surgical treatment in patients with acute cholecystitis complicates laparoscopic cholecystectomy due to increased inflammation [7]. Early laparoscopic cholecystectomy in acute cholecystitis has been shown to be more advantageous in some publications [8-10], but in practice, patients with additional comorbidities have been given an interval because of not fulfilling the appropriate conditions. The general approach to the treatment of acute cholecystitis is not having an additional condition (associated hepatobiliary pathology choledocholithiasis, suspicion of malignancy, use of anticoagulants, etc.) and preferably laparoscopic cholecystectomy within 72 hours.However, if the patient does not have appropriate conditions for surgical intervention, a conservative approach, including cool down, antibiotherapy and minimally invasive procedures (such as percutaneous transhepatic cholecystostomy) can be applied. After 6-12 weeks, elective cholecystectomy should be planned [6, 11].

Nowadays, many techniques have been defined to prevent major biliary tract injuriesin laparoscopic cholecystectomy, but in some patients it is a problem that the cystic stump cannot be safely closed due to the large size, regardless of the technique. The biggest problem during elective laparoscopic cholecystectomy in patients with cooled down is the dissection

difficulty of the critical calot triangle area [12]. After overcoming this difficulty and encountering a large cystic canal in the next step makes the operation even more difficult [13]. In the laparoscopic cholecystectomy operation, after revealing the Calot triangle and revealing the ductus cysticus in posterior dissection, it is essential to clearly reveal the biliary anatomy by separating the gallbladder from the liver bed [5].

The ductus cysticus is the connection between the gallbladder and the main bile duct and the diameter of this channel is known to be around 5 millimeters. When the diameter of the cystic channel increases, especially when it is above one centimeter, the laparoscopic cholecystectomy procedure becomes more difficult in acute cholecystitis and elective patients.

Multiple clips can be dangerous and may increase clip-related complications. According to Brooks *et al*. [14], in a series of 650 patients,laparoscopic cholecystectomy patients with 9 bile leakages were observed and three of the leakage patients were observed to be due to clip dysfunction (0.46%). In some articles, with obstructive jaundice patients and patients with main bile duct formation examinations,it was observed that the metallic clips placed during cholecystectomy caused migration into the bile duct. Therefore, it is not recommended to put and cut multiple clips consecutively [15, 16]. Intracorporeal knot or suture is technically difficult, requiring advanced laparoscopic experience and skill [17]. Ultrasonic coagulation and bipolar electrothermal sealers are effective in the dissection of concentrated tissues caused by hard and acute inflammation, however, this is dangerous because it might cause bile leakage [18]. Although ultrasonic coagulation was as effective as a clipin a study of the cystic canal [19], it is controversial since it will have the same security as in a wide cystic channel. An endoloop may be an alternative to the closure of a wide cystic canal but in practice, either the gallbladder is separated from the liver bed and crossed over the endo-loop pouch and seated in the cystic canal, or after the cystic channel is divided, the cystic canal can be connected to the endoloop. However, in this method, after the cystic channel is divided, it may be difficult to process as the cystic stump retracts towards the hilus [20].

The Endo-vascular stapler is used to cut and closethe cystic channel at the same time, but it is a highly debated and fearful condition in terms of overlooking main bile duct injuries. Another disadvantage is that it can only be entered through 12 mm diameter ports, larger than standard ports.

Endo-vascular stapling is safe and easy to use. In patients with clear cystic duct and anatomy, one must be sure that there is no stone in the cystic duct before stapler firing. It should be clearly considered that the main bile duct does not enter the stapler line and is not constricted. Before the Endo-vasculer stapler is fired, the contents of the cystic canal should be stroked by the gallbladder and the residual stone should not remain in the stapler line and should not be pushed in the direction of the main bile duct [21].

In a 7-year period (2008-2014) in France, when approximately 800,000 cholecystectomy procedures were examined, the rate of cholecystectomy due to gallbladder stones increased. When total number of cases increase, complicated cases are being expecting more frequently [22].

Although the vascular endo-vasculer stapler (2.5 mm) has been preferred in most of the publications in stapled preference for cystic duct in laparoscopic cholecystectomy, staplers with reticulator 4.8 mm and 3.5 mm can be preferred [13].

The only patient with minimal bile leakage was preoperatively ERCP and sphincterotomy performed patient, bile leakage regressed spontaneously on the third day operation. ERCP applications (stent, sphincterotmy, nasobilier stent application) are effective for the management of leakage in patients with bile leak after cholecystectomy [23].

In patients that were consulted to ERCP before cholecystectomy, the surgeon feels more confident because the pressure of the bile duct decrease and leaks may heal more easily. The surgeon feels more confident with patients with a history of ERCP who underwent use of the endo-vasculer stapler for enlarged cystic channels during laparoscopic cholecystectomy.

Laparoscopic cholecystectomy is accepted as safe in elderly patients [24]. However, complication management is more difficult in these patients due to additional diseases and comorbid conditions, therefore, in the operation the surgeon should be sure of the cystic channel stump.

Although the closure of the cystic stump with

vasculer stapler adds an additional cost on surgery, in complicated situations usage of stapler, it is obvious that it is cost-effective and should not be avoided because it might have a positive impact on decreasing postoperative complications such as biliary leakage.

The complication rate was low in our study, the complications were treatable. The duration of hospital stay was 3.7 days, which was higher than the duration of hospital stay for normal laparoscopic cholecystectomy procedures. The reason for this is the fear of developing complications. After the 10th patient, the hospital stay decreased to two days.

Limitaions

Limitaions of our study are, firstly the number of the patients in our study is low and this might affect the final results and analysis. Secondly; our study design was not prospective, retrospectively designed study. Another limitation is as the study group is a small group, there were no comparison in different groups like intra-corporeal knotting versus stapling.

CONCLUSION

In conclusion, the Endo-vasculer stapler method in elective laparoscopic cholecystectomy in complicated cases is a safe and easy method to use,especially in wide cystic duct. In the use of Endo-vascular stapling, it is important to note the isolation of the cystic canal and the clear presentation of the anatomy of the biliary tract.

In centers in which complicated cases undergo elective laparoscopic cholecystectomy, especially in patients with a history of stones in the main bile ductand history of ERCP, it is recommended that if a wide cystic duct is encountered while planning an operation, it may be necessary to use the Endo-vasculer stapler and it is recommended to be prepared preoperatively.

Conflict of interest

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Streamlined percutaneous atrial septal defect closure in adults

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ABSTRACT

Objectives: The aim of the study to evaluate the safety and efficacy of the transthoracic echocardiography (TTE) guided secundum atrial septal defect (ASD) closure without balloon sizing, sedation or general anesthesia in adults.

Methods: We retrospectively evaluated 200 secundum ASD closure patients in the tertiary cardiology center. Transesophageal echocardiography (TEE) was performed to all the patients at least one day before the intervention by the procedure operators. The patients who were closed with a cribriform device, using more than one device, with insufficient rim (<5 mm) (other than the anterior superior rim (aortic rim)), totally flail, and complex interatrial septum anatomy were excluded from the analysis.The size of the ASD closure device was chosen according to the largest diameter measured by TEE. ASD device was selected as 4 mm larger in patients without anterior superior rim and 2 mm larger in other patients than the largest diameter measured in 2D-TEE.

Results: In the remaining 166 patients, the procedure was performed with TTE and fluoroscopy guidance without balloon sizing, sedation or general anesthesia. The procedure was performed through right femoral vein. The patients age: 38.56 ± 14.72, gender: 57 male, 109 female, ASD size: 18.88 ± 5.99 mm, anterior superior rim: 5.30 ± 4.04 mm, anterior inferior rim: 14.22 ± 6.46 mm, posterior superior rim: 17.16 ± 4.96 mm, posterior inferior rim: 16.67 ± 7.48 mm. ASD device size: 23.74 ± 6.59 mm. The procedure success rate was 98,1% (163 patients). The complications; 1 patient device embolised, 2 patients device was not placed in the correct position by TTE.

Conclusions: TTE and fluoroscopy-guided secundum ASD closure without balloon sizing, sedation or general anesthesia by experienced operators is a safe and effective procedure.

Keywords: Atrial septal defect, transthoracic echocardiography, transesophageal echocardiography, closure, sedation

In adults, the atrial septal defect (ASD) is the most seen congenital heart disease after bicuspid aortic valve. Four different types of ASDs have been described. Secundum ASD is the most common type and is seen in 80%. Percutaneous ASD closure, which was first performed in 1974, has become the standard treatment in appropriate cases today [1]. At the present, surgical treatment of secundum ASD patients is performed only in cases not suitable for percutaneous treatment [2]. The procedure of percutaneous ASD closure is generally performed with general anesthesia and TEE or ICE guidance. Selection of the appropriate device size for ASD closure is done by the balloon sizing method. But, in recent years, percutaneous ASD

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closure is performed with TTE and sedation in suitable cases [3].

In this study, we aimed to evaluate the safety and effectiveness of percutaneous ASD closure under local anesthesia, concomitant TTE and without balloon sizing.

METHODS

We retrospectively evaluated 200 patients who were performed percutaneous closure procedure in our clinic and we included 166 patients who underwent the percutaneous closure procedure with TTE. Patients with complex Secundum ASD, need general anesthesia, need sedation and TEE were not included our study group.

Secundum ASD which closed with TTE and patients with adequate rims except the aortic rim are inclusion criteria for our study. Using more than one device, cribriform ASD, inadequate rims except aortic rim, secundum ASD which closed with TEE, the procedure performed with balloon sizing or under general anesthesia and complex atrial septum are the exclusion criteria for our study. General anesthesia was performed in patients with complex ASD closure procedure (using more than one device, insufficient rims with large ASD, etc.). If the patients need TEE guidance for ASD closure we used sedation. We performed the procedure with local anesthesia for all other patients.

Echocardiography procedure

TTE was performed to all ASD patients before the procedure. Echocardiographic measurements of the patients were done with X5-1 probe (EPİQ 7, Philips,

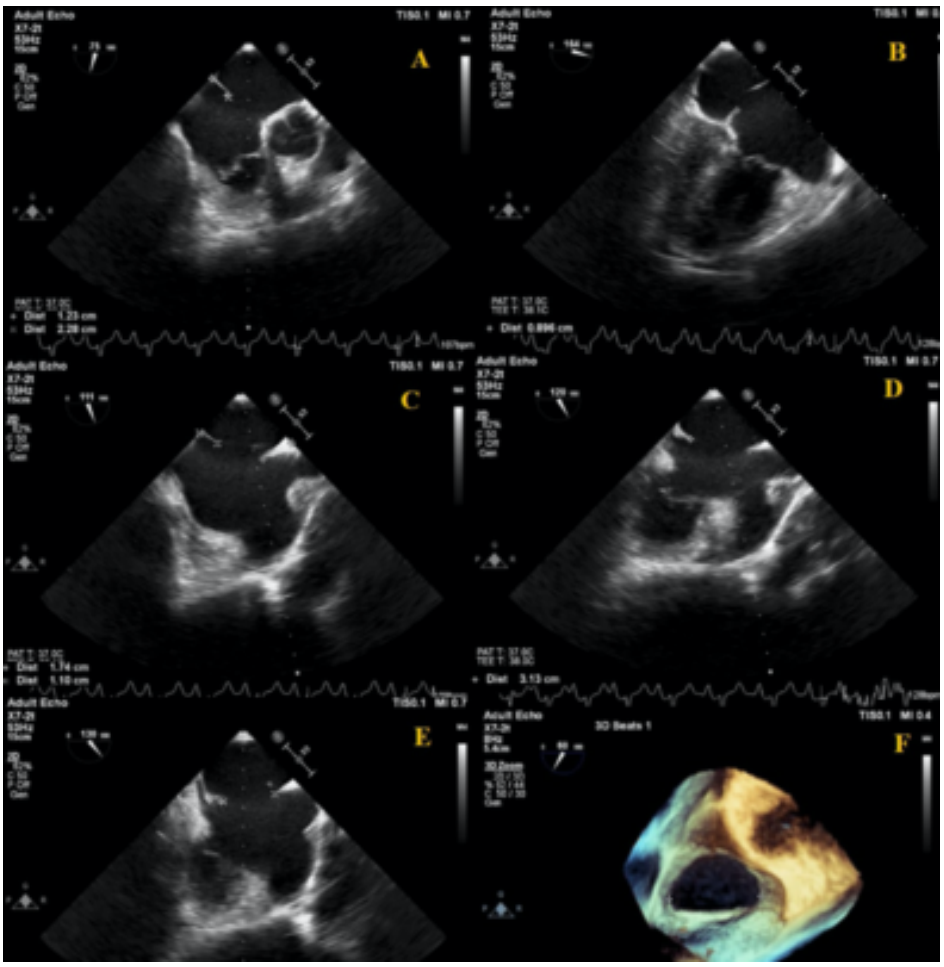

Fig. 1. (A) Short axis image of ASD in TEE, anterior superior rim (aortic rim); (B) Four chamber image of ASD in TEE, anterior inferior rim; (C) Bi-caval image of ASD in TEE, posterior süperior and posterior inferior rim; (D) Maximal ASD diameter in TEE; (E) ASD diameter and coronary sinus rim; (F) 3D image of ASD in TEE.

Nederland). Heart diameters and functions were measured. Qp/Qs value calculated. One day before the procedure, TEE was performed to all the patients with X7-2t probe (EPİQ 7, Philips, Nederland). In this process, ASD diameter and rims were measured, and their suitability for closure was evaluated. In the short axis image ASD diameter, anterior superior rim, posterior rim and narrowest atrial septum diameter, in the bicaval image ASD diameter, posterior superior rim and inferior rim, in four chamber image ASD diameter, anterior inferior rim were measured (Fig. 1). The widest diameter of ASD was measured at atrial end-diastole.

Closing procedure and device selection

The patients were taken to the hemodynami laboratory. Sheat was placed in the right femoral vein after local anesthesia. Fluoroscopy and TTE-guided, MP catheter was passed from ASD to the left atrium and the catheter was advanced to the pulmonary vein. 0,035-inch guidewire was placed into the pulmonary vein through the catheter. The delivery catheter, suitable for the ASD device was advanced to the left atrium over this guidewire. After that, ASD device which determined according to TEE measurements performed one day before the procedure was placed in the appropriate position under the guidance of TTE and fluoroscopy and the device was released. ASD device size was determined according to the largest ASD diameter which measured by 2D TEE. If the aortic rim was adequated together with other rims of the patient and septum was not flail, 2 mm wider device than the maximal ASD diameter which measured by TEE was selected, whereas 4 mm wider device was selected in patient with the insufficient aortic rim. If the atrial septum is flail and aortic rim is sufficient, we chosed 4 mm wider device than the measured defect diameter. We chosed 6-8 mm wider device, in patients with flail atrial septum and insufficient aortic rim. We considered the narrowest diameter of the septum while choosing the device size.

One day before the procedure, aspirin 100 mg 1×1 and clopidogrel 75 mg 1×1 treatment were started and this treatment was used for six months. Before the procedure, 2 grams of ampicillin were given to the patients. Endocarditis prophylaxis was recommended for patients who underwent an interventional procedure for 6 months after ASD closure.

Statistical Analysis

Statistical analysis was performed with SPSS (Statistical Package for the Social Sciences ver. 23, SPSS Inc, Chicago, Illinois, USA) computer program. Descriptive analysis was used to calculating to mean value of the continuous variables. Frequency analysis was used for calculating the categorical variables. Numerical variables were expressed as mean ± standard deviation and categorical variables were expressed as mean percentages.

RESULTS

Two hundred patients were evaluated for this retrospective study and 34 of them were excluded. Cribriform ASD device was used in 2 of these 34 patients. Two devices were used in 2 patients due to two ASD defects. 30 patients were excluded from the

Table 1. Demographic and biochemical characteristics of the patients

Variable	Data
Age (year)	38.56 ± 14.72
Gender, n (%)	
Female	109 (65.6)
Male	57 (34.4)
BMI (kg/m2)	24.82 ± 4.87
Heart rate (ppm)	73.50 ± 9.80
SBP (mmHg)	112.93 ± 20.31
DBP (mmHg)	69.27 ± 11.56
Rhythm, n (%)	
SR	157 (94.6)
AF	9 (5.4)
Laboratory parameters	
WBC (103/dL)	7.18 ± 2.31
Hemoglobin (gr/dL)	13.02 ± 1.71
Platelet (103/dL)	224.07 ± 42.62
Glukoz (mg/dL)	96.20 ± 30.43
Urea (mg/dL)	25.57 ± 9.81
Creatinine (mg/dL)	0.76 ± 0.17

Data are shown as mean ± standard deviation or n (%). AF = Atrial fibrillation, BMI = Body mass index, DBP = Diastolic blood pressure, SBP = Systolic blood pressure, SR = Sinus rhythm, WBC = White blood cell.

study because of their rims were insufficient. The reason for the exclusion of these complicated ASD patients was that the percutaneous ASD closure performed with TEE guidance.

Remaining 166 patients were evaluated. Percutaneous closure was performed successfully in 163 of 166 patients (98.19%) by TTE.

TEE had to be performed in 2 patients, because correct position could not be provided or could not be sure that it was in the correct position with TTE. ASD device was successfully placed in both patients with TEE. ASD device was embolized to right ventricle in one patient due to flail rim.

Our patients were between 14-72 years old and ASD device diameters were between 12-36 mm. Demographic and laboratory features of our patients are shown in Table 1. TTE, TEE data and ASD closing device features of our patients are shown in Table 2.

DISCUSSION

We found that in adult population TTE and fluoroscopy-guided percutaneous secundum ASD closure without balloon sizing, sedation or general anesthesia at experienced operators is a safe and effective proce-

Table 2. Echocardiographic and device parameters of the patients

Variable	Data
TTE parameters	
EF (%)	62.68 ± 5.61
LVEDD (mm)	42.94 ± 4.48
LVESD (mm)	25.43 ± 3.60
RV end-diastolic diameter, mm	40.89 ± 4.27
Left atrium (mm)	36.36 ± 6.23
Right atrial mediolateral diameter (mm)	38.49 ±7.57
sPAP (mmHg)	38.65 ± 9.90
Qp/Qs	2.2 ± 0.6
TEE parameters	
Maxilmal ASD diameter (mm)	18.88 ± 5.99
Narrowest septum diameter (mm)	39.83 ± 7.40
Anterior superior rim (mm)	5.30 ± 4.04
Anterior inferior rim (mm)	14.22 ± 6.46
Posterior uperior rim (mm)	17.16 ± 4.96
Posterior inferior rim (mm)	16.67 ± 7.48
Device parameters	
Type, n (%)	
MemoPart	65 (39.15)
Amplatzer	47 (28.31)
Lifetech Cera	37 (22.28)
OtherS	17 (10.24)
Device size (mm)	23.74 ± 6.59
Device size / septum size ratio	0.58 ± 0.15

Data are shown as mean ± standard deviation or n (%). ASD = Atrial septal defect, EF = Ejection fraction, LVEDD = Left ventricular end-diastolic dimension, LVESD = Left ventricular end-systholic dimension, sPAP = Systholic pulmonary artery pressure, TEE = Transesophageal echocardiography, TTE = Transthoracic echocardiography

dure.

Previously, especially in pediatric patients, percutaneous ASD closure was performed with TTE [4]. There are some study showing that ASD closure was performed with TTE in adults [3]. Today, success in ASD closure is over 98% [5]. Success in this case has been achieved concomitant with TEE and balloon sizing. In our cases, this success was achieved with TTE.

Standard recommendation in ASD closing processes in < 11 mm defects, it is the closure of the defect with a 2-4 mm wider device than the defect diameter measured by TTE, without balloon sizing. These types defects are generally seen in pediatric patients and TTE imaging is usually enough. For 11-24 mm sized defects, the defect can be closed with a device at the measured maximum defect diameter by balloon sizing method (fluoroscopy; LAO at 15° and TEE). For 24-32 mm defects, the defect can be closed with 2 mm wider device than stretched defect diameter by measurement balloon sizing method (fluoroscopy; LAO at 15° and TEE). Defects with inadequate aortic rim can be closed with 4 mm wider device than the stretched defect diameter by measurement balloon sizing method and in these defects, atrium roof rim and posterior rim should be sufficient.

The ratio of device size and septum size is important for ASD closure [6]. If the ratio of device size to the septum size is < 0.58, complications that may occur after the procedure will be less common [6]. If this rate increases, the risk of developing arrhythmia increases after the procedure (such as AF, SVT, AV block). In our study, the ratio device to defect diameter is 0.58. Choosing a large device also creates a large device area in the heart by making a mushroom effect and that prolongs the epithelialization time of the device. These problems occur in devices with two standard discs and one waist part. Today, different shaped and more elastic devices are available. With these devices, possible complications are tried to be minimized [2, 7].

Our patients consisted of the adult population and there was no ASD smaller than 10 mm. In our patients, we performed TEE the day before the procedure and we selected the device according to this TEE measurement. TEE imaging performed by operator who had done ASD closure procedure. ASD diameters change in the systolic and diastolic phase, for this the ASD diameter should be measured at the atrial end-diastole and at the widest defect size [8]. We determined the device size according to the widest ASD diameter which measured atrial end-diastole with TEE. One of the factors in the choice of device size is the narrowest diameter of the atrial septum in the end-diastole. The diameter in the short axis imaging is the narrowest diameter. The rims of ASD closure devices are 7-8 mm and the device size is 14-16 mm wider than the waist of the device in total. That is, it should be evaluated whether the ASD devices to be placed will fit into the narrowest atrial septum, taking in to account the wing lengths.

As seen from our successful procedure results, it is seen that our device selection is correct. The important thing is that operator should be experienced in TEE imaging, and the pre-procedure imaging should be performed by the operator. We must say that 2D images provide more accurate information when choosing the device size. Because 3D images are reconstructed images and defect boundaries may not be clearly visible especially in flail and thin septum, they should not be used in device size selection. However, 3D images provide very clear information for understanding the shape of the ASD and anatomy of the septum. This imaging modality will be very helpful in percutaneous ASD closure procedures, especially in ASD patients with complex anatomy. Two-plane modality in 3D probes also facilities the evaluation of ASD.

Operator experience is important for the correct device and diameter selection in ASD closure. Positioning the device parallel to the septum during the closing procedure will provide to settle the device correctly. Some devices and delivery systems have made changes in the delivery system, like moving head for this purpose [7]. In standard devices, the operator tries to provide a parallel position to the septum by changing the delivery angles. Various maneuvers, techniques, and assistive devices can be used to position the device in the correct position. These maneuvers, techniques and assistive device are used especially in large and complex ASD.

Maneuvers and techniques are clockwise rotation maneuvers, tulip bud technique, Greek maneuver, right and left upper pulmonary vein technique, left atrium roof technique. Assistive device techniques are Hausdorf sheath technique, SSH technique or Kutty's method, Boosfeld-Spies technique, wire assisted tech-

nique, dilatator or catheter asisted technique, balon asisted technique and Nounou technique.

Clockwise rotation maneuvers; the device, which is advanced to the left atrium, is directed to the right upper pulmonary vein with clockwise rotation, after that opening the left disk of ASD device in the left atrium and then pulling the delivery system to open the right disk of ASD device in right atrium. It is the first maneuver to make the ASD device parallel to the septum in which patients without aortic rim [9].
Tulip bud technique; the device is protruded to form a tulip bud in the left atrium. It aligns adjacent to and in-plane of ASD. The delivery system is withdrawn in quick succession [9].

Greek maneuvers; the left disc and 2/3 of the right disc of the device are opened in the left atrium, the delivery system and ASD device are positioned by pulling back to the septum and the right disc is opened. With this maneuver, the left disc protrusion is prevented from into the right atrium [10].

Right and left upper pulmonary vein technique; this technique is applied by opening the left atrium disc in the upper left or upper right pulmonary vein then extending the device, after that opening the right atrium disc in the right atrium, the device is pulled back to bring the left atrium disc to the correct position. If the left disc which opened in the pulmonary vein can not be opened by pulling, the left disc can be released by pushing forward with the right atrial disc delivery cable (contrarian technique) [9]. The left upper pulmonary vein technique is applied in patient with large ASD who without aortic rim and other rims are flail. The right upper pulmonary vein technique is applied in patients with inadequate aortic and posterior rim, with aneurysmatic inter-atrial septum, with flail rims, and patients with a not too big ASD diameter [9].

Left atrium roof technique; the left disc is opened between the interatrial septum and the upper left pulmonary vein in the left atrium roof, the device is extended and the right atrial disc opens in the right atrium then the device is pulled and placed at the interatrial septum from the left atrium roof. It is used in patients with small left atrium and with both aortic and posterior rim are inadequate [9].

Hausdorf sheath technique; it is the use of this delivery system (Hausdorf sheath), the end of which is shaped. This technique uses for large ASD with deficiency of anterior and/or aortic rim [11].

Straight Side-Hole sheath technique or Kutty's method; Mullin sheath end of side is cut to be atraumatic and angled (Uses Mullins Transeptal sheath, 1 to 2 F sizes larger than the minimum recommended sheath for ASD device). This method is used in inadequate inferior or anterosuperior (aortic) rims [11, 12].
Boosfeld-Spies technique; in this technique, 12F Cook Mullins-type sheath (Cook Medical) end-side of it is cut to be atraumatic and angled. This sheath is used as a delivery system. This technique is used in large and inadequate rim ASD [13].

Wire assisted technique; a wire is used to prevent left atrial disc to protrude into the right atrium. The wire can be inserted from the same delivery sheath or another sheath. It can be performed from the same sheath using thinner wire like 0,018-inch. This wire must be pulled back before the device is released [14].

Dilatator or catheter assisted technique; a multipurpose/JR/dilator catheter is placed in the left atrium across the ASD to prevents prolapse left atrial disc to the right atrium. This technique is used in large ASD and inadequate anterior, anterior süperior (aortic), and/or posterior rims [15].

Ballon assisted technique; this method needs two entries; first entry is for the delivery system and the second entry is needed to send balloon over the guidewire in which located in the right or left upper pulmonary vein. Firstly, the left disk of the ASD device is opened in the left atrium then the balloon is inflated, and after the left disk remains in the proper position with the balloon, the delivery system is pulled back and the right disk is opened. Balloon prevents the prolapse of the left atrial disc into the right atrium. After the device is positioned, the balloon is deflated and pulled. This technique is used for large ASD with flail and inadequate rims [16].

Nounou technique; the device is positioned using Agilis catheter. 20 mm and smaller ASD devices can be used with this technique. The reason is that catheter diameter is not suitable for larger devices [17].

Limitations

The limitations of this study are that the study is done retrospectively and not included a group to compare. However, this study contains a detailed examination and has been done with sufficient number of patients, for this, it is capable of guiding operators

who will perform a closure procedure.

CONCLUSION

In suitable cases of adult secundum ASD, percutaneous ASD closure can be performed with TTE and fluoroscopy guidance without balloon sizing and general anesthesia, with expert operators.

Conflict of interest

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

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The role of myocardial perfusion imaging in the identification of the obstructive coronary artery lesions: a tertiary cardiology center experience

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ABSTRACT

Objectives: Because of the moderate accuracy of treadmill electrocardiogram and fear of a claim if a diagnosis is missed, cardiologists usually order Myocardial Perfusion Imaging (MPI) as a first-step test in most of patients with chest pain admitted to cardiology department. The performance of the MPI in diagnosing obstructive CAD depends on the population studied. Thus we aimed to assess the agreement between MPI and coronary angiography in the identification of the obstructive coronary lesion.

Methods: A total of 231 patients who underwent MPI due to suspicion of coronary ischemia and had a coronary angiogram within the last three months were included in this retrospective study. MPI and coronary angiography findings were analyzed to weigh the performance of MPI in determining obstructive coronary lesion.

Results: The mean age was 63.9 ± 8.9 years, 54.5 % being males. MPI showed a sensitivity of 0.86 in determining patients who had a significant (> 70%) coronary lesion. While evaluating the ability of MPI to detect ischemia in the left ventricle region which is supplied by the lesioned vessel, the sensitivity was found to be; 60% in determining anterior ischemia associated with significant LAD lesion, 77.4% in determining inferior ischemia associated with significant RCA lesion, and 44.4% in determining lateral ischemia associated with significant CX lesion.

Conclusions: Our findings have shown that MPI with visual assessment has 86% sensitivity for detecting significant coronary artery stenosis. However, the sensitivity of MPI in determining ischemia in the left ventricle region which is supplied by the lesioned coronary artery was found to be 44.4 to 77.4%.

Keywords: myocardial perfusion imaging, coronary angiography, obstructive coronary artery lesion

Coronary artery disease (CAD) has remained the leading causes of morbidity and mortality all over the world during the last five decades [1]. The initial diagnostic evaluation of patients presenting with chest pain and suspected obstructive CAD generally consists of an exercise electrocardiogram that has a sensitivity of 68% and a specificity of 77% in diagnosing obstructive CAD [2]. Nevertheless, the information obtained from the myocardial perfusion imaging (MPI) is more precise with respect to localization, extension, and severity of ischemia; thus it becomes a dominant non-invasive modality, usually a

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gatekeeper test before coronary angiography, for the assessment of patients with suspected CAD in daily practice [3-4].

The performance of the MPI in diagnosing obstructive CAD depends on not only the prevalence of disease in the population studied but also individual characteristics of patients (i.e. likelihood of obstructive CAD) [4]. Thus clinical registries which weigh the performance of MPI among national cohort of patients provide important insights into the real-life health care.

The purpose of the present study was to assess the agreement between MPI and coronary angiography in the identification of the obstructive coronary lesion in patients with suspected CAD. Additionally, we intended to analyze the patient characteristics in whose MPI test was insufficient to detect the obstructive coronary lesions.

METHODS

Study Population

In this retrospective study, we initially searched the hospital database to find patients who had undergone both MPI and coronary angiography at our hospital between January 2018 and January 2019. The study protocol was approved by the local ethics committee. Patients who had previously undergone heart surgery, including coronary artery bypass grafting or prosthetic valve replacement and with a prior diagnosis of; heart failure, end-stage renal failure were excluded. Additionally, patients who experienced acute myocardial infarction or undergone revascularization within this period (January 2018 and January 2019), and who had insufficient data on patient cards were excluded. Finally 231 patients who underwent MPI because of clinical suspicion of CAD and were subsequently subjected to a coronary angiogram (owing to ischemia on MPI or ongoing symptoms) were found to be eligible. At this time, MPI records of recruited patients were analyzed to categorize those according to localization of ischemia. Afterwards angiographic findings were analyzed and categorized with respect to lesion localization and being significant (> 70%) or not. Then data were analyzed to weigh the performance of MPI in determining obstructive coronary lesion. The sensitivity of the MPI was defined as the percentage of any level of ischemia in patients with evidence of obstructive (> 70%) lesions in one or more coronary arteries. Specificity of the MPI was determined as the percentage of normal images (without ischemia) in patients with normal angiography or with less than 70% stenosis in any coronary arteries.

Data with respect to demographic and clinical characteristics and laboratory parameters were collected from the medical records. The definition of hypertension was specified as a systolic pressure ≥ 140 mmHg and/or a diastolic pressure ≥ 90 mmHg or if the participant was taking an antihypertensive medication [5]. Diabetes mellitus was determined as a fasting glucose level > 126 mg/dl and/or if the patient was taking an anti-diabetic medication [6]. Participants who were recorded as smoker at the patient card were classified as smokers. Hyperlipidemia was defined as taking a lipid-lowering therapy. A history of CAD was determined as the existence of prior acute coronary syndrome, percutaneous coronary revascularization, and/or at least one attested coronary stenosis ≥ 50% luminal diameter on angiography [7].

Coronary Angiography Data

Angiographic data with respect to index coronary angiogram was conducted from the cardiac catheterization laboratory records. Angiographic images were visually assessed by two cardiologists who were blinded to the MPI data. Coronary slow flow phenomenon was defined with respect to corrected TIMI frame count, an objective and quantitative index of coronary flow [8].

Myocardial Perfusion Imaging Data

Stress Protocols

Exercise (treadmill) was the favored stress modality in patients who could exercise and reach sufficient exercise goals. Modified Bruce protocol was used in all of those. Pharmacologic stress with adenosine, dobutamine and dipyridamole was used in patients who could not complete or tolerate sufficient exercise, those with limited heart rate response, or left bundle-branch block. Patients were charged to discontinue taking nitrates for 6 hours, calcium channel blockers for 24 hours, and ß-blockers for 48 hours prior to the stress protocol. Tc-99m sestamibi was given through a venous access when the patient's heart rate achieved

85% of calculated maximum heart rate (both in exercise treadmill or pharmacologic stress). Exercise or pharmacological induction was continued for two minutes after the Tc-99m sestamibi injection.

Gated Single-photon Emission Computed Tomography (SPECT) Protocol

All patients underwent a Gated SPECT protocol according to current guideline [9]. MPI images were interpreted based on a 17-segment model [10]. All stored images were analyzed by a single, blinded, experienced observer. Images were categorized as either normal or ischemic. A score model was used to define reversible defects. According to this modality, the total score at stress, Summed Stress Score (SSS), reflected the extent and severity of the abnormality including ischemia and infarction. The difference between the SSS and Summed Rest Score (SRS) was defined as Summed Difference Score (SDS) that reflected a reversible defect. Semi-quantitative parameters were classed as follows; A SSS ≤ 3 was defined as a normal result, while a score of 4-8 as a mild defect, 9-12 as a moderate defect and > 12 as a severe defect. A SDS of 1-3 reported mild ischemia, 4-7 moderate ischemia and > 7 severe ischemia [11].

Statistical Analysis

The SPSS 22.0 (IBM Corporation, Armonk, New York, USA) was used for statistical analysis. Categorical variables were demonstrated as number and percentage. All quantitative data were expressed as mean ± SD unless otherwise stated. We compared continuous variables using student t-test or Mann-Whitney U test between groups. Categorical variables were summarized as percentages and compared with the Chi-square test. Diagnostic concordance with coronary angiogram and MPI data was analyzed was by using cross tabulations. A p value < 0.05 was considered as statistically significant.

RESULTS

Two hundred thirty-one patients were included in the present study. Baseline characteristics of the recruited patients are shown in Table 1. The mean age was 63.9 ± 8.9 years (55% males). 123 (53.2%) patients had non-significant coronary lesions, 54

Table 1. Baseline characteristics of the study patients

Parameters	n = 231
Age (years)	63.9 ± 8.9
Males	126 (54.5%)
History of CAD	127 (54.9%)
Diabetes mellitusy	97 (41.9%)
Hypertension	208 (90%)
Atrial Fibrillation	16 (6.9%)
Hyperlipidemia	125 (54.1%)
Laboratory parameters	
Hemoglobin (g/L)	13.8 ± 1.6
Low-density lipoprotein (mg/dl)	124.3 ± 44.7
Creatinine (mg/dl)	0.95 ± 0.57
Ejection Fraction on the Echocardiography (%)	56.6 ± 9.2
Indication of the Myocardial Perfusion Imaging	
Angina	164 (70.9%)
Searching ischemia (e.g. left branch bundle block, any symptom thought to be an equivalent of angina)	67 (29%)

Data are expressed as mean ± standard deviation for normally distributed variables and percentage (%) for categorical variables. CAD = coronary artery disease

Table 2. Diagnostic concordance between coronary angiography and myocardial perfusion imaging with respect to left anterior descending artery lesions

	Patients without significant LAD stenosis (< 70%) at angiography (n = 171)	Patients with LAD significant stenosis (> 70%) at angiography (n = 60)	*p value*
No ischemia at anterior wall	78 (45.6%)	24 (40%)	0.397
Mild ischemia at anterior wall	57 (33.3%)	22 (36.6%)	
Moderate ischemia at anterior wall	32 (18.7%)	10 (16.6%)	
Severe ischemia at anterior wall	4 (2.3%)	4 (6.6%)	

Data are expressed as percentage (%) for categorical variables. LAD = left anterior descending artery

(23.3%) patients had a significant stenosis in only one coronary artery, 38 (16.4%) patients had significant coronary lesions in two different coronary arteries, while 16 (6.9%) patients had three-vessel coronary artery disease. There were 14 (6%) patients with coronary slow flow who had not a significant lesion in any coronary arteries. A diaphragmatic attenuation was reported in 20 (8.6%) patients. Overall, MPI could identify 86% of patients who had a significant coronary lesion among our study population.

In the present study, coronary angiography showed a significant (> 70%) stenosis of LAD in 60 patients. However, MPI did not report any level of ischemia at the anterior wall in 24 (40%) of those, while mild ischemia at the anterior wall was reported in 22 (36.6%) of those. Moderate and severe ischemia at the anterior wall were found to be in 10 (16.6%) and 4 (6.6 %) of those, respectively. On the other hand, coronary angiography revealed a no or non-significant stenosis of LAD in 171 patients. MPI did not report any level of ischemia at the anterior wall in 78 (45.6%) of those. But, mild, moderate and severe ischemia at the anterior wall were reported in 57 (33.3%), 32 (18.7%), and 4 (2.3%) of those, respectively (Table 2). Thus, sensitivity and specifity of MPI in identifying anterior ischemia associated with critical LAD lesion were found to be 60% and 45.6%, respectively.

Coronary angiography showed a significant (> 70%) stenosis of RCA in 62 patients. However, MPI did not report any level of ischemia at the inferior wall in 14 (22.5%) of those, while mild ischemia at the inferior wall was reported in 7 (11.2%) of those. Moderate and severe ischemia at the inferior wall were found to be in 29 (46.7%) and 12 (19.3 %) of those, respectively. On the other hand, coronary angiography revealed a no or non-significant stenosis of RCA in 169 patients. MPI did not report any level of ischemia

Table 3. Diagnostic concordance between coronary angiography and myocardial perfusion imaging with respect to right coronary artery lesions

	Patients without significant RCA stenosis (< 70%) at angiography (n = 169)	Patients with significant RCA stenosis (> 70%) at angiography (n = 62)	*p value*
No ischemia at inferior wall	72 (42.6%)	14 (22.5%)	< 0.001
Mild ischemia at inferior wall	25 (14.7%)	7 (11.2%)	
Moderate ischemia at inferior wall	68 (40.2%)	29 (46.7%)	
Severe ischemia at inferior wall	4 (2.3%)	12 (19.3%)	

Data are expressed as percentage (%) for categorical variables. RCA = right coronary artery

Table 4. Diagnostic concordance between coronary angiography and myocardial perfusion imaging with respect to circumflex artery lesions

	Patients without significant CX stenosis (< 70%) at angiography (n = 177)	Patients with significant CX stenosis (> 70%) at angiography (n = 54)	*p value*
No ischemia at lateral wall	127 (71.7%)	30 (55.5%)	0.001
Mild ischemia at lateral wall	17 (9.6%)	3 (5.5%)	
Moderate ischemia at lateral wall	31 (17.5%)	15 (27.7%)	
Severe ischemia at lateral wall	2 (1.1%)	6 (11.1%)	

Data are expressed as percentage (%) for categorical variables. CX = circumflex artery

at the inferior wall in 72 (42.6%) of those. But, mild, moderate and severe ischemia at the inferior wall were reported in 25 (14.7%), 68 (40.2%), and 4 (2.3%) of those, respectively (Table 3). Thus, sensitivity and specifity of MPI in identifying inferior ischemia associated with critical RCA lesion were found to be 77.4 % and 42.6%, respectively.

Additionally, coronary angiography showed a significant (> 70%) stenosis of CX in 54 patients. However, MPI did not report any level of ischemia at the lateral wall in 30 (55.5%) of those, while mild ischemia at the lateral wall was reported in 3 (5.5%) of those. Moderate and severe ischemia at the lateral wall were found to be in 15 (27.7%) and 6 (11.1 %) of those, respectively. On the other hand, coronary angiography revealed a no or non-significant stenosis of CX in 177 patients. MPI did not report any level of ischemia at the lateral wall in 127 (71.7%) of those. But, mild, moderate and severe ischemia at the lateral wall were reported in 17 (9.6%), 31 (17.5%), and 2 (1.1%) of those, respectively (Table 4). Thus, sensitivity and specifity of MPI in identifying lateral ischemia associated with critical CX lesion were found to be 44.4% and 71.7%, respectively.

While evaluating overall (regardless of the relationship between the vessel and its feeding area) sensitivity, MPI could identify; 85% of patients who had a significant LAD lesion, 88% of patients who had a significant RCA lesion, and 83% of patients who had a significant CX lesion.

While assessing patients with moderate-severe ischemia at the anterior wall (n = 50), there were not any significant difference on baseline characteristics with respect to lesion severity in LAD. However among patients with moderate-severe ischemia at the inferior wall (n = 113), subjects who had a significant lesion on RCA were more likely to have a prior history of diabetes mellitus, coronary artery disease and be male (48.7 vs. 29.1; $p = 0.042$, 78% vs. 56.9%; $p = 0.024$, 9.7% vs. 38.8%; $p < 0.001$, respectively).

Coronary angiography demonstrated a significant (> 50%) stenosis of LMCA in 4 patients. One of them displayed moderate-severe ischemia at the anterior wall on the MPI; one of them displayed moderate-severe ischemia at the lateral wall while the other two patients revealed moderate-severe ischemia at the inferior wall.

DISCUSSION

The present study was an evaluation of patient characteristics and the agreement between MPI and coronary angiography in the identification of the obstructive coronary lesion (>70%) in patients with suspected CAD admitted to a tertiary referral hospital. The main results of the present study were as follows: 1) MPI showed a sensitivity of 0.86 in determining patients who had a significant coronary lesion; 2) The overall sensitivity was found to be 0.85 in determining significant LAD lesion while anterior ischemia associated with significant LAD lesion MPI showed a sensitivity of 0.6; 3) The overall sensitivity was found to be 0.88 in determining significant RCA lesion while inferior ischemia associated with significant RCA lesion MPI showed a sensitivity of 0.77; 4) The over-

all sensitivity was found to be 0.83 in determining significant CX lesion while lateral ischemia associated with significant CX lesion MPI showed a sensitivity of 0.44).

Modern cardiology has a broad range of clinical exploration methods available to assist the diagnosis and to stratify the risk of patients with suspected coronary artery disease. Basic (first-line) testing in those includes standard laboratory biochemical testing, a resting ECG, possible ambulatory ECG monitoring, and structural tests including resting echocardiography, and, in selected patients, cardiac magnetic resonance (CMR) [4]. In patients, with an intermediate probability of CAD and need to be tested noninvasively, an exercise ECG is the most widely used non-invasive method [12]. Although exercise ECG has advantages, easy to access and cheaper test comparing with the other non-invasive test options (stress CMR or stress echocardiography or MPI), because of the moderate diagnostic accuracy of it, clinicians usually could not be sure while excluding CAD with the result of the exercise ECG alone. Moreover, The National Institute for Health and Care Excellence (NICE) has recommended that exercise ECG testing should not be used alone for the diagnosis (or exclusion) of stable angina in patients without known CAD [13]. This recommendation has been followed in part by the latest European Society of Cardiology guideline on the management of chronic coronary syndromes, which suggests the use of functional imaging (stress CMR or stress echocardiography or MPI) over exercise ECG testing in patients with suspected CAD, in whom the results of exercise ECG are inadequate to diagnose or exclude CAD [4]. Although Stress CMR has some technical advantages, it is not easily accessible, it is contraindicated in patients who either have a pacemaker or severe renal failure, it is very sensitive to the rhythm of the heart thus it may not be interpretable in patients with atrial fibrillation [14]. On the other hand results of the stress echocardiography is usually challenging in patients with left bundle branch block or septal dyssynchrony [15]. As a result of those, cardiologists usually order MPI as a first-step test, even it is not recommended at this stage, in most of patients with chest pain admitted to cardiology department [12]. Thus the performance of the MPI, not only in recognizing coronary artery disease but also in detecting severe lesion, is important in daily practice. Previous studies evaluating the concordance of findings between coronary angiography and MPI reported different accuracy rates which were between 41.7% and 93.3% [16-17]. Hasbek *et al*. revealed a slightly low, 41.7%, diagnostic concordance between coronary angiography and MPI, in which treadmill test was the favored stress modality. However, they reported a 75.8% concordance rate in patients whom stress modality was a pharmacologic stress with adenosine [16]. Thus diagnostic accuracy of MPI depends on studied population and design of the works. Many of the previous studies defined culprit coronary lesion as 50% or more stenosis responsible for the symptoms while evaluating the concordance between coronary angiography and MPI. In other words, these studies were design to evaluate the power of MPI in detecting a coronary stenosis of 50% or above [16-20]. However current data has clearly revealed that only an estimated diameter stenosis > 70% in a coronary artery is classified as severe lesion and predicted to cause symptoms. Thus in the present study we aimed to evaluate the performance of MPI in determining a coronary stenosis > 70%.

In the present study, MPI could identify 86% of patients who had a significant coronary lesion that was very similar to previous studies [18, 21]. However while determining the lesion associated ischemic zone, sensitivity rates were fallen (e.g. 77% for RCA associated inferior ischemia, 60% for LAD associated anterior ischemia, and 44% for CX lesion associated lateral ischemia). This means that seven of every 100 patients with critical stenosis in RCA had an ischemia on another wall than inferior, 26 of every 100 patients with critical stenosis in LAD had an ischemia on another wall than anterior, and 42 of every 100 patients with critical stenosis in CX had an ischemia on another wall than lateral. The possible explanations for these findings are: I) existing collateral circulation; II) variation in coronary anatomy; III) heterogeneity of coronary hyperemia with pharmacologic stress [22]; IV) technical issues (As example; in normal SPECT images, the lateral wall often may appear brighter than the contra-lateral septum because of the camera is physically closer to the lateral myocardial wall (in proximity to the lateral chest wall) than to the septum. So that, the acquisition is associated with

more efficient count capture [22] - this may explain why MPI much more deviated in revealing lateral wall ischemia associated with CX lesion).

One of the remarkable findings of the present study was that patients who had a ischemia at the inferior wall, even if moderate, the possibility of critical stenosis in RCA was found to be considerably decreased if they were female or had not a prior history of diabetes mellitus or coronary artery disease. This finding is important because, physicians are everyday faced with such cases. The possible explanations for this finding are; I) breast attenuation; II) it is plausible that females who have a diabetes mellitus or coronary artery disease are more likely to be overweight and have large breasts that could affect the performance of MPI, in particular in evaluating inferior wall perfusion. Further studies need to be carried out in order to validate this finding.

Limitations

Our study has several limitations. First, we analyzed MPI images with visual analysis. Indeed published data has not clearly demonstrated improved sensi¬tivity or specificity of automated quantitative analysis systems programs over visual analysis. Moreover the visual analysis data are derived from experienced readers in laboratories have been found to be an excellent quality control [22]. However it would be better if every single patient were analyzed with an automated quantitative analysis program. Second, we did not analyze the results with respect to sub-group according to modality of stress (pharmacologic or treadmill) on MPI. Third, the present work is a single-center analysis; therefore, the generalisability of these findings is limited. Fourth, we did not evaluate the outcome of patients. Finally, we cannot exclude the possibility of unmeasured confounding factors.

CONCLUSION

In conclusion, the present study is an evaluation of patient characteristics and the agreement between MPI and coronary angiography in the identification of the obstructive coronary lesion (> 70%) in patients with suspected CAD. Our findings have shown that MPI with visual assessment has 86% sensitivity for detecting significant coronary artery stenosis. However, the sensitivity of MPI in determining ischemia in the left ventricle region which is supplied by the lesioned coronary artery was found to be 44.4 to 77.4%.

Conflict of interest

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

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The European Research Journal 2020;6(5):500-507 Original Article

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The functional and radiological comparison of the surgical treatment results of forearm diaphyseal fractures in adults treated with open reduction internal fixation and intramedullary locking nail

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ABSTRACT

Objectives: The results of two different methods applied in the surgical treatment of forearm fractures in adults were evaluated.

Methods: Thirty-nine patients who applied to our clinic between 2016-2018 and were treated surgically were included in the study. Twenty-three patients out of these were treated with plate osteosynthesis (group 1), and 16 patients were treated with intramedullary locking nail (group 2). While 14 of the patients in group 1 were male, 9 were female, and the average age was 39.8 years (range; 19-74 years); and 11 of the patients in group 2 were male, 5 were female, and the average age was 36.6 years (range; 18-68 years). Patients were called for monthly check-ups until fracture union. Then, radiographic evaluation was done at 3, 6 and 12 months. The average follow-up time was 26 months (range;12-36 months) for group 1 and 25 months (range;12-35 months) for group 2. The loss of the line of fracture through radiographic imaging of trabeculations or callus formation in the cortex on the anteroposterior and lateral radiographs, and clinically loss of sensitivity on fracture were considered fracture union. In the last controls, while the elbow was at 90 degrees of flexion, the amount of rotation of both forearms was measured by using the goniometer. In the functional evaluation, the system described by Grace and Eversmann and used to evaluate fracture union and forearm rotation was used. Patient satisfaction was evaluated by using the DASH (Disabilities of the Arm, Shoulder and Hand) method.

Results: While the union duration in group 1 was 12.3 weeks (range; 8-18 weeks), the union duration in group 2 was 12 weeks (range; 9-16 weeks). There was no statistical difference in terms of union durations ($p > 0.05$). In Group 1, according to the Grace-Eversmann evaluation, 19 (82.6%) patients had excellent and good results, three (13.1%) patients had acceptable results, and 1 (4.3%) patient had poor results. Forearm pronation of the patient with poor results was less than 60% but his bone union was complete. In group 1, the average DASH score was 15.04 (range; 3-28). In group 2, Grace-Eversmann evaluation showed excellent and good results in 13 (81.3%) patients and acceptable results in 3 (18.7%) patients. Average DASH score was found to be 14.6 (range; 2-34). When Grace-Eversmann criteria and DASH values were compared, no significant difference was found between the two groups ($p > 0.05$). Vascular nerve injury, tendon injury, radioulnar synostosis, and compartment syndrome were not observed in any patient.

Conclusions: The results of the two fixation methods in terms of functional recovery and patient satisfaction

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were similar in the surgical treatment of forearm double fractures in adults.
Keywords: Adult, forearm diaphyseal fractures, intramedullary locking nail, plate osteosynthesis

The aim of the treatment of forearm diaphyseal fractures in adults is to provide axial and rotational stability [1]. Due to its functional and anatomical features, forearm diaphyseal fractures are different from long bone diaphyseal fractures, they should be evaluated as intra-articular fractures and treatment planning should be done accordingly [2]. The deforming muscle strength, continuity of the radial slope, and intraosseous membrane damage are important factors affecting the stability and reduction continuity. Open reduction and rigid internal fixation with plate-screw have been suggested by many authors. However, this technique has disadvantages such as causing soft tissue damage, damaging the periosteal circulation due to the discharge of the broken hematoma and plate compression, especially irritating the skin around the ulna [3]. Another disadvantage of the technique is that the fracture is seen again between the rates of 11% and 20% after plate removal [4]. The method of fixation with intramedullary unlocking nail causes less soft tissue damage and does not impair the extramedullary blood circulation; however, it may be inadequate to control rotational stability, especially in segmental and segmented fractures.However, intra-canal implants share the load on the bone and provide the formation of peripheral periosteal callus. The superiority of intramedullary locking nail that it can prevent shortcomings that may occur in diaphyseal fractures of the forearm fragments, segmental fractures and metaphysio-diaphyseal fractures [5]. In this study, the results obtained from patients with open reduction and plate-screw fixation, and from patients with closed reduction and intramedullary locking nailwere compared retrospectively.

METHODS

Thirty-nine patients who applied to our clinic between 2016 -2018 and were treated surgically were included in the study. Patient's medical information and demographic distribution are shown in Table 1. Twenty-three patients out of these were treated with plate osteosynthesis (group 1), and 16 patients were treated with intramedullary locking nail (group 2). While 14 of the patients in group 1 were male, 9 were female, and the average age was 39.8 years (range; 19-74 years); and 11 of the patients in group 2 were male, 5 were female, and the average age was 36.6 years (range; 18-68 years). In the fractures classified according to AO classification, 10 patients were type A, 8 patients were type B, 5 patients were type C in group 1. In Group 2, 8 patients were type A, 5 patients were type B, 3 patients were type C. Patients with additional injuries, pathological fractures, open fractures, and patients without pineal plate were excluded. Patients were called for monthly check-ups until fracture union. Then, radiographic evaluation was done at 3, 6 and 12 months. The average follow-up time was 26 months (range from 12 to 36 months) for group 1 and 25 months (range from 12 to 35 months) for group 2. The loss of the line of fracture through radiographic imaging of trabeculations or callus formation in the cortex on the anteroposterior and lateral radiographs, and clinically loss of sensitivity on fracture were considered fracture union.In the last controls, while the elbow was at 90 degrees of flexion, the amount of rotation of both forearms was measured by using the goniometer. In the functional evaluation, the system described by Grace and Eversmann and used to evaluate fracture union and forearm rotation was used [6]. Complete union of the fracture and providing at least 90% of the forearm rotation is considered excellent; union of the fracture and providing at least 80% of the forearm rotation is considered good; and union of the fracture and providing at least 60% of the forearm rotation is considered acceptable result. Non-union of the fracture or less than 60% forearm rotation was considered as a poor result. Patient satisfaction was evaluated by using the DASH (Disabilities of the Arm, Shoulder and Hand) method. In this scoring where the functional state of the upper extremity is questioned, while 0 points indicates a perfect extremity, 100 points indicate that the upper extremity is completely unusable.

Surgical Technique

For both patient groups, surgery was started under general anesthesia or axillary block, in the supine position, following the necessary treatment and covering. Before the operation, patients received 1 g of cefazolin for prophylaxis. The plate was applied by inflating the tourniquet, the nail was applied without inflating the tourniquet under control.

Radial styloid and tuberositas radii were determined for radius in patients undergoing osteosynthesis with plate. Between the two, along the fracture line, the incision from the volar was carried out along the fracture line (Henry approach). After the incision was made, the lateral cutaneous nerve of the forearm was isolated and preserved in the superficial adipose tissue. The incision was continued until fascia. The fascia incision was made at the ulnar end of the brachioradial muscle. The brachioradialis muscle was moved and lifted. Dissection was continued by preserving the radial artery and radial nerve. Radius was exposed. For distal extending fractures, the place where the pronator muscle was held was dissected from the shaft and the fracture was exposed. For the proximal fractures, the radial sensory nerve was followed up to the point where it bifurcated with the posterior interosseous nerve and the supinator muscle was dissected from the ulnar side. After the fracture reduction was done with reduction clamps, a 3.5 mm locked compression plate was placed.

Olecranon and ulna styloid were determined for ulna. An incision was made along the line of fracture between the two (posterior approach). The incision was deepened into the fascia. Ulna was seen distally between the extensor carpiulnaris and flexor carpiulnaris muscles, more proximally between the extensor carpiulnaris and ancaneus muscles. 3.5 mm locked compression plate was placed after reduction was carried out with reduction clamps.

First of all, screws close to the fracture were placed for both bones. Interfragmanter screws were placed in patients who were deemed necessary. Then, screws were placed on the proximal and distal sides of the fracture and fixation was completed. According to the type of fracture and severity of injury, the length of the plate to be used and the number of screws were determined, but as a general principle, 3 screws were placed on both sides of the fracture line. After the tourniquet was loosened and bleeding was checked, drains were placed in both surgical sites and the layers were closed according to the procedure (Fig. 1).

In patients with intramedullary locking nail, a 1 cm incision was made over olecronon while the elbow was at 90 degrees of flexion. Under the control of the scopy, 2 mm K wire was sent into the canal after closed fracture reduction. Then the channel entrance was carved up to 2 cm distance with a 6 mm reamer. The channel diameter was extended with hand carvers. The guide wire was left in the channel.

For Radius, while the forearm was in prone position, a 2 cm incision was made by the radial side of the Lister tubercle. The entrance was made under the extensor carpiraldias brevis tendon at a distance of 5 mm from the joint surface. Afterwards, 2 mm K wire was sent into the canal after closed reduction of radius

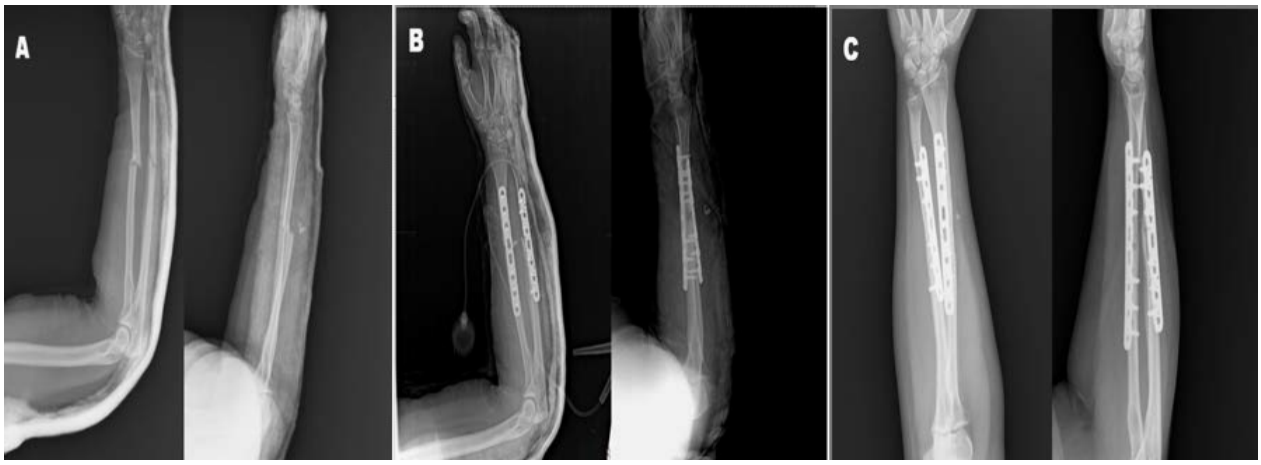

Fig. 1. Plate osteosynthesis. (A) Preoperative radiograms; (B) Postoperative radiograms; (C) Follow-up radiograms taken at 12th month.

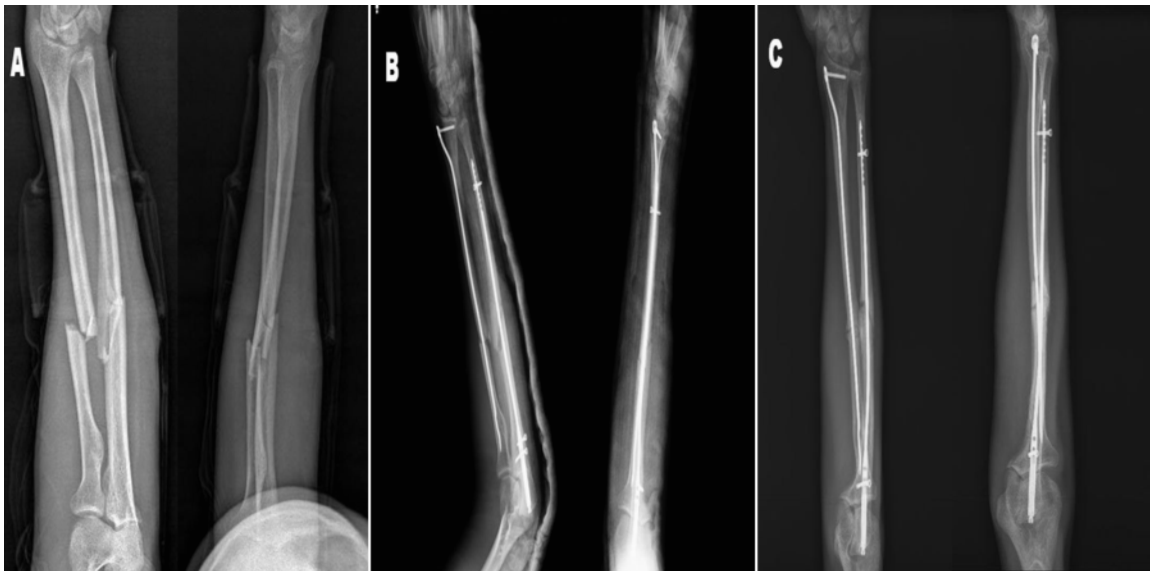

Fig. 2. Intramedullary locking nail. (A) Preoperative radiograms; (B) Postoperative radiograms; (C) Follow-up radiograms taken at 12th month.

fracture under the control of the scopy. Then the channel entrance was carved up to 2 cm distance with a 6 mm reamer. The channel diameter was extended with hand carvers. The guide wire was left in the channel. Nails of suitable length and width for both bones were placed in the canal and guide wires were drawn. For both bones, the side close to the fastening apparatus was first locked with a fully grooved 2.7 mm self-tapping screw, then the static locking screw was not used in any patient after stability control (Fig. 2).

Statistical Analysis

Statistical analysis was performed by using chi-square and t-student tests using SPSS 21 version for

Table 1. Patient's medical information and demographic distribution

	Group 1 (n = 23)	Group 2 (n = 16)
Average age (years) (range)	39.8 (19-74)	36.6 (18-68)
Average follow-up (month) (range)	26 (12-36)	25 (12-35)
Fractured forearm, n (%)		
Right	13 (56.5)	9 (56.2)
Left	10 (43.4)	7 (43.7)
Gender distribution, n (%)		
Female	9 (39.1)	5 (31.2)
Male	14 (60.8)	11 (68.7)
Trauma etiology, n (%)		
Traffic accident	3 (13)	3 (18.7)
Sports injury	3 (13)	3 (18.7)
Work injury	4 (17.3)	2 (12.5)
Fall	13 (56.5)	8 (50)
AO/OTA fracture type, n (%)		
A	10 (43.4)	8 (50)
B	8 (34.7)	5 (31.2)
C	5 (21.7)	3 (18.7)

Group 1 = Plate osteosynthesis group; Group 2 = Intramedullary locking nail group, OTA = Orthopaedic Trauma Association

Windows. Numerical variables were expressed as mean ± standard deviation and categorical variables were expressed as mean percentages. A p value < 0.05 was considered as statistically significant.

RESULTS

While the union duration in group 1 was 12.3 weeks (8-18 weeks), the union duration in group 2 was 12 weeks (9-16 weeks). Grace-Eversmann evaluation and DASH score statistical analysis are given in Table 2. There was no statistical difference in terms of union durations (p > 0.05). In Group 1, according to the Grace-Eversmann evaluation, 19 (82.6%) patients had excellent and good results, three (13.1%) patients had acceptable results, and 1 patient (4.3%) had poor results. Forearm pronation of the patient with poor results was less than 60% but his bone union was complete. In group 1, the average DASH score was found to be 15.04 (range 3-28). In Group 2's Grace-Eversmann evaluation, excellent and good results were obtained from 13 patients (81.3%) and acceptable results from three patients (18.7%). Average DASH score was found to be 14.6 (range 2-34). When Grace-Eversmann criteria and DASH values were compared, no significant difference was found between the two groups (p > 0.05). In group 1, two patients developed superficial infections; both patients were treated with one week of parenteral antibiotics followed by one week of oral antibiotics. Deep infection was not observed in either group.Implants were removed in 3 patients in group 1 and 1 patient in group 1 after union. Vascular nerve injury, tendon injury, radioulnar synostosis, and compartment syndrome were not observed in any patient.

DISCUSSION

There is consensus that the treatment of adult forearm double diaphyseal fractures is surgical. The results of these fractures with insufficient implants or conservative treatment led to high complication rates [7].The traditional surgical method is open reduction and fixation with plate [8].As the treatment complications with plate developed, alternative treatment methods were constantly sought in the historical treatment process [9]. Intramedullary application was first performed in 1913 by using K wire and Steinmann nails (1st generation) [10]. However, the necessary rotational stability was not achieved with these materials and it caused high non-union rates. The first nail design considering the forearm anatomy was made in 1959 (2nd generation) [11]. However, since these nails did not have mechanisms to prevent locking and rotation, a union rate as good as plate could not be achieved.Despite this, changes in nail design continued and 3rd generation nails which are locking mechanisms that also provide the rotational stability we use todaywere produced.

Anatomical or close reduction is obtained in fractures where osteosynthesis is applied with plate. However, impaired fracture hematoma and periosteal integrity are factors that may have negative effects on union [3]. During the surgical intervention, it is expected that there will be more bleeding than nail application. The risk of future fractures increases due to cortical atrophy occurring in the screw application areas. Cosmetic problems related to the surgical approach and more soft tissue damage are other unde-

Table 2. Statistical analysis: Union period, DASH score and Grace-Eversmann ratio

	Grup 1 (n = 23)	Grup 2 (n = 16)	*p value*
Union Period (weeks) (mean ± SD)	12.3±2.72	12.0 ± 2.19	0.710
DASH Score (mean ± SD)	15.04±6.05	14.6 ± 7.84	0.850
Grace-Eversmann Ratio (Perfect/Acceptable/Poor)	19/3/1	13/3/0	0.640

Group 1 = Plate osteosynthesis group; Group 2 = Intramedullary locking nail group, SD = standard deviation

sirable aspects of plate osteosynthesis. Intramedullary locking nail causes less bleeding and less soft tissue damage. Cosmetically, it provides superiority to osteosynthesis with plate. However, in nail applications, ensuring proper rotation and anatomical reduction can be more difficult thanosteosynthesis with plate [4]. Also, exposure to more radiation is one of the disadvantages of nail application [12].

Anderson *et al*. [13] treated 330 forearm fractures of 258 patients by using compression plates and achieved 96.3% union in ulna fractures and 97.8% in radius fractures.In other studies, union rates have been reported between 87% and 98% [14]. Kose *et al*. [15, 16] reported that they achieved 100% union in all cases with intramedullary locking nail. Visna *et al*. [17] reported that 78 patients had union in the treatment of 118 forearm fractures, while Gao *et al*. [18] achieved nail union in all 18 patients. In our study, rates of union with plate and rates of union with nail were similar, and union was achieved in all patients. Anderson *et al*. [13] achieved a union at an average of 7.4 weeks in patients with open reduction and plate screw fixation. Stevens *et al*. [14] observed union in their forearm fractures for an average of 22 weeks in patients using dynamic compression plates, and 33 weeks in patients who used locked compression plates. Gao *et al*. [18] who used intramedullary locking nail in the treatment reported that closed fractures united within an average of 10 weeks and open fractures within 14 weeks. In addition, in the studies comparing nail and plate applications; the average union duration with plate was found to be 10 weeks by Lee *et al.* [19] , 11.1 weeks by Kim *et al.* [21], 14 weeks by Özkaya *et al.* [22], and 13.1 weeks by Köse *et al.* [20]. In the same studies, the average union duration with nail was found to be 14 weeks by Lee *et al.* [19], 13.1 weeks by Kim *et al.* [21], 10 weeks by Özkaya *et al.* [22], and 10.8 weeks by Köse *et al.* [20]. In our study, the duration of union with plate was found to be 12.3 weeks, and the duration of union with nail was found to be 12 weeks.

Another criterion we compared in our study is the DASH (The Disabilities of the Arm, Schoulder and Hand) scores between both groups [23]. The average DASH score in patients who were treated with plate was found to be 9.8 by Köse *et al.* [20], 15.3 by Lee *et al.* [19], 7.1 by Kim *et al.* [21], and 15 by Özkaya *et al.* [22]. In the same studies, the average DASH score in patients who were treated with nail was found to be 18.3 by Köse *et al.* [20], 12.8 by Lee *et al.* [19], 15.1 by Kim *et al.* [21], and 13 by Özkaya *et al.* [22]. In our study, the fact that the average DASH value was 15 in the plate group and 14.6 in the nail group, and that the results were close to each other made us consider that there is not any difference between the two techniques in terms of patient satisfaction provided that it was performed according to the technique. Another method in functional evaluation is the system described by Grace and Eversmann, in which fracture union and forearm rotation are evaluated. In this staging system, complete union of the fracture and providing at least 90% of the forearm rotation is considered excellent; union of the fracture and providing at least 80% of the forearm rotation is considered good; and union of the fracture and providing at least 60% of the forearm rotation is considered acceptable result.Non-union of the fracture or less than 60% forearm rotation was considered as a poor result. As a result, when both our study and other studies are evaluated through Grace Eversmann evaluation, the similarity of the results between the plate and nail application is remarkable.

If intramedullary locking nail are not chosen in proper length and diameter, complications may develop during surgery. Incompatibility of nail diameter and canal width can lead to rotational movements in cases where the length of the nail is short, and if the length of the nail is long, the fracture may break more [5]. Careful planning is essential before surgery. An iatrogenic bone injury was not observed in patients who underwent surgery in our clinic. Iatrogenic posterior interosseous nerve injury is rarely seen in forearm surgical treatment. It has been reported that this risk can be minimized especially in nail operations by keeping the proximal locking screw of the radial nail at least 30 mm from the radius head and keeping the forearm in neutral rotation.As the radius fracture approaches to proximal in patients who receive plate, more attention must be paid in terms of nerve damage [24]. In our study, attention was paid to these issues in patients using both plate and locked nails, and therefore no iatrogenic posterior interosseous nerve injury was observed. The disadvantage is that the duration of union is longer than plate

osteosynthesis and more limited allowance for mobilization until bridge callus is seen in the fracture line. However, the use of a mini-incision, the peeling of the periosteum is the greatest advantage of the locked intramedullary nail when it is necessary to remove it again with a mini-incision [25].

CONCLUSION

In our study comparing two techniques used in the treatment of forearm fractures in adults, close results were obtained with both techniques in terms of patient satisfaction and functional evaluation.Therefore, we think that both techniques can be used in the treatment of forearm fractures in adults, provided that the rules are followed.

Conflict of interest

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

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Complications of labial minor salivary gland biopsy and comparison of complications in patients with and without primary Sjögren's syndrome

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ABSTRACT

Objectives: Labial minor salivary gland biopsy (MSGB) is a procedure in which complications such as bleeding, hematoma, numbness, wetness can be seen after the procedure. It is not known whether these complications have changed in patients with primary Sjögren's syndrome (PSS). The aim of this study is to investigate the frequency of complications after the labial MSGB and to investigate whether these complications has changed in PSS.

Methods: Participants with a preliminary diagnosis of PSS who underwent a labial MSGB without any suture were included in the study. One month after the procedure, the complication screening questionnaire was administered face-to-face interview or by telephone. All complications were compared between PSS and non-PPS groups.

Results: Complications screening questionnaire was applied to 99 participants (face to face with 79 participants and by telephone with 20 participants). After the procedure, 17.2% of the participants had uncomfortable bleeding, 2.0% had persistent numbness which continues more than 1 week and 1.0% had wetness and hematoma. Bleeding duration was more than 1 hour in 11.1% of the participants. Complications were not different between PSS and non-PSS groups ($p > 0.05$).

Conclusions: After the labial MSGB procedure without suturing, uncomfortable bleeding is frequent and the duration of bleeding is long, but complications other than bleeding are rare. The incidence of complications after labial MSGB procedure was not different in participants with PSS than in those without PSS.

Keywords: Complications, labial minor salivary gland biopsy, primary Sjögren's syndrome

Primary Sjögren's syndrome (PSS) is a chronic autoimmune inflammatory disorder characterized by diminished lacrimal and salivary gland function. Saliva has many useful functions in the mouth, such as; antimicrobial effect, tissue repair and protection [1]. Degreased saliva may cause traumatic lesions and may increase oral mucosal infections due to decreased antimicrobial effect [2, 3]. Thrombocytopenia and acquired hemophilia may occur in PSS, which may cause bleeding tendency [4, 5]. Minor salivary gland biopsy (MSGB) is a widely used procedure with high diagnostic value in the classification of PSS [6]. Minor

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salivary gland biopsy is usually performed from the labial region, but can also be done from the sublingual region [7]. Labial MSGB can be performed with different methods at different centers, for example, the procedure can be completed with or without suture, or incision can be performed vertically or horizontally [8, 9]. In literature, there are few studies evaluating complications after labial MSGB [7, 8, 10, 11]. Although the incidence of complications after the procedure is low in these studies, complications such as persistent paresthesia, hematoma, local wetting may be seen [10, 11]. Lip is a tissue with intense blood circulation and bleeding can be expected after incisions. There is no information in the literature about how long the bleeding continues after labial MSGB procedure. There may be clinical conditions in SS that may have the potential to increase the complications of labial MSGB. There is no study in the literature comparing the complications of labial MSGB in patients with and without SS. In this study, we aimed to determine the biopsy complications in which the bleeding time is evaluated in detail following labial MSGB and we aimed to compare biopsy complications between patients with and without SS.

METHODS

Ethics committee approval of the study was obtained from Necmettin Erbakan University (NEU) Clinical Research Ethics Committee. The study included volunteers from the NEU medical faculty of rheumatology department who were planned to undergo a labial MSGB procedure to investigate the etiology of sicca symptoms and subsequently underwent the procedure. Demographic data and preexisting diseases of all participants after the labial MSGB procedure were recorded.

Labial minor salivary gland biopsy

In our center, LSGB procedure is applied to all patients with a standard procedure. All labial MSGB procedures were performed by two investigators. The procedures of all participants were performed in semi-

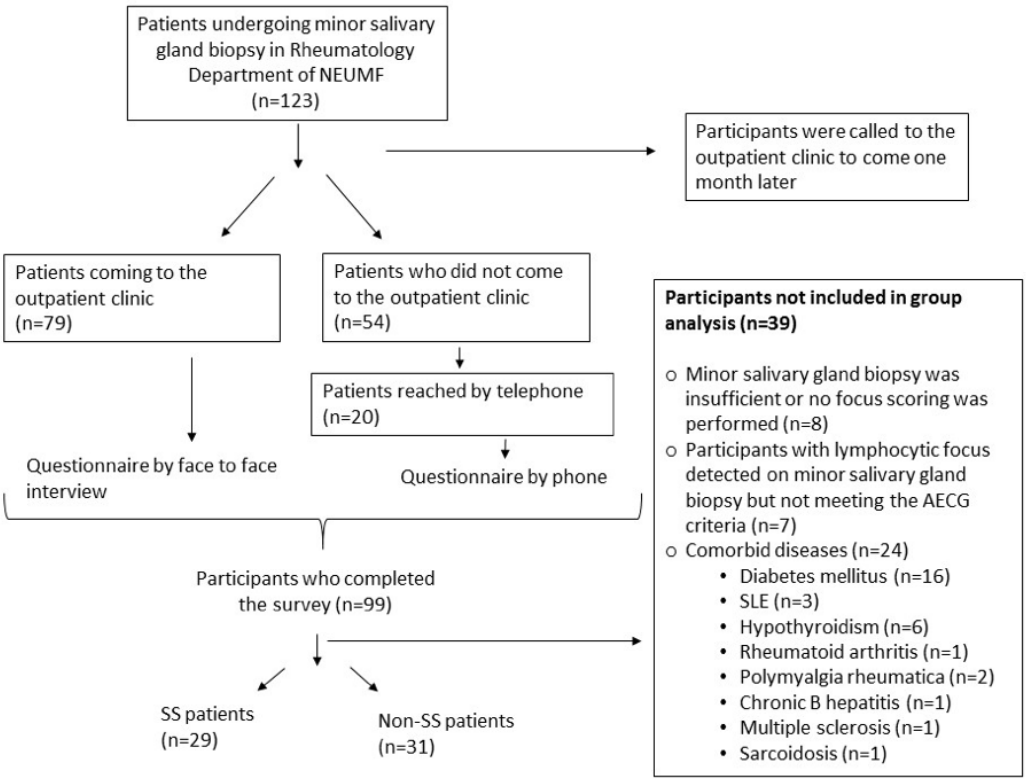

Fig. 1. A flow chart showing the number of the participants included in the study at all stages and the exclusion reasons of the participants excluded from the study.

fowlers position and local anesthesia was applied to all participants before the procedure. The incisions of the participants were applied vertically to the 2 cm square area on the right or left side of the inner lip midline with a length of 5-10 mm. Forceps was used for blunt dissection and excision of the minor salivary glands of all participants. In each labial MSGB procedure, at least 2 samples of 1-3 mm3 were collected, each with several minor salivary gland tissues. Biopsy wounds were left to heal spontaneously without any sutures. After the procedure was completed, the participant was instructed to apply 10 minutes of compression with a sterile sponge.

Histopathology of minor salivary gland biopsy

Labial MSGB specimens were consulted with the pathology department. Of the 99 samples included in the study, 1 was reported as insufficient material, 7 had no lymphocytic focus scoring, and all other samples' lymphocytic focus scorings were reported as described by Chisholm *et al*. [6].

Complication screening survey

After the labial MSGB procedure, participants were called to the outpatient clinic for 1 month later. Complications screening questionnaire (CSQ) was applied face to face to the participants who came to outpatient clinic control. Participants who underwent biopsy but did not come to the outpatient clinic control after 1 month were called by telephone and the CSQ was performed on the telephone. The CSQ consists of 13 questions. In 8 of the questions, the participants were questioned about the presence of complications after labial MSGB procedure and the participants were asked to answer the questions as yes, no or I don't remember. In 5 of the questions, how long the complication after MSGB procedure persists was asked to the participants. The questions of the CSQ are shown in Table 1.

Group analysis

Symptoms of dry mouth and dry eye, presence of objective dry eye, ANA, anti-Ro (SSA) and anti-La (SSB) test results of all participants with lymphocytic focus (grade 3-4) on MSGB were recorded from patient charts [6]. Patients diagnosed with SS according to the American-European Consensus Group (AECG) classification criteria were included in the PSS group [12]. Participants without lymphocytic focus on minor salivary gland biopsy were included in the non-PSS group. Participants with systemic disease, diabetes mellitus, hypothyroidism, chronic hepatitis, and those who did not have lymphocytic focus scoring in the salivary gland biopsy and who had insufficient MSGB material were not included in the group analysis. The number of the participants included in the study at all stages and the exclusion reasons of the participants excluded from the study are summarized in a flow chart in Fig. 1.

Statistical Analysis

SPSS Version 22.0 for Windows (SPSS Inc, Chicago, IL, USA) was used to process all data statistically. Variables are presented as mean, median, standard deviation (SD), minimum-maximum or frequency. To compare quantitative variables, Mann-Whitney U-test was used. For the comparison of qualitative data, $\chi 2$ test or Fischer's exact χ^2 test was used. All tests were two-tailed, and p values < 0.05 were considered to indicate statistical significance.

RESULTS

Complication screening questionnaire was applied to 99 participants (12 male, 87 female). Demographic data, pathological scoring of minor salivary gland biopsies and comorbid diseases of the participants who have completed the CSQ are summarized in Table 2. Answers of the participants who have completed the CSQ are shown in Table 1. Sixty participants were included in the group analysis, 29 of them were included in the PSS group and 31 were included in the non-PSS group. Sicca symptoms, ocular signs, autoantibody tests and MSGB histopathology results of participants included in PSS group are shown in Table 3. The answers of the participants in the PSS and non-PSS groups to the questions in the CSQ and the comparison of the answers between the groups are shown in Table 4.

DISCUSSION

In this study, we investigated the incidence of

Table 1. Answers of the participants who have completed the complication screening questionnaire

Questionnaires	Data
1) Did you feel pain during the procedure? (yes/no/don't remember)	21/78/0
2) Was there any bleeding that would disturb you after the biopsy? (yes/no/don't remember)	17/82/0
3) How long was the bleeding period?, n (%)	
< 10 minutes	56 (56.6)
10-30 minutes	19 (19.2)
31-60 minutes	13 (13.1)
61 minutes-3 hours	9 (9.1)
> 3 hours	2 (2.0)
4) Did you have trouble eating after the biopsy? (yes/no/don't remember)	16/81/2
5) How many days did the trouble persist?, n (%)	
< 7 days	14 (87.5)
7-14 days	1 (6.25)
≥ 14 days	1 (6.25)
6) Was there any persistent numbness around the lip after the biopsy? (yes/no/don't remember)	10/89/0
7) How many days did the numbness persists around the lip?, n (%)	
< 7 days	8 (80.0)
7-14 days	2 (20.0)
≥ 14 days	0 (0.0)
8) Was there a feeling of wetness in the mouth after biopsy? (yes/no/don't remember)	1/98/0
9) How many days did the wetness in the mouth persist after the biopsy?, n (%)	
< 7 days	1 (100.0)
7-14 days	0 (0.0)
≥ 14 days	0 (0.0)
10) Was there any blood collection (hematoma) in the lip after the procedure? (yes/no/don't remember)	1/98/0
11) Was there an infection at the incision site after the procedure? (yes/no/don't remember)	0/99/0
12) How many days did the incision heal?, n (%)	
< 7 days	88 (88.9)
7-14 days	7 (7.1)
≥ 14 days	3 (3.0)
Don't remember	1 (1.0)
13) Is there any scar after the incision has healed? (yes/no/don't remember)	6/93/0

Table 2. Demographic data, results of minor salivary gland biopsies and comorbid diseases of the participants who have completed the complication screening questionnaire

Demographic characteristics	Data
Age (years), mean ± SD	49.76 ± 13.12
Female, n (%)	87 (87.9)
Pathological scoring of minor salivary gland biopsy, n (%)	
Grade 0	12 (12.1)
Grade 1	18 (18.2)
Grade 2	17 (17.2)
Grade 3	21 (21.2)
Grade 4	23 (23.2)
No Chisholm scoring	7 (7.1)
Insufficient material	1 (1.0)
Comorbid diseases, n (%)	
Primary Sjögren's syndrome	33 (33.3)
Secondary Sjogren's syndrome	1 (1.0)
Systemic lupus erythematosus	4 (4.0)
Rheumatoid arthritis	1 (1.0)
Polymyalgia rheumatica	2 (2.0)
Multiple sclerosis	1 (1.0)
Sarcoidosis	1 (1.0)
Chronic B hepatitis	2 (2.0)
Diabetes mellitus	18 (18.2)
Thyroid disease	6 (6.1)

ASA = acetyl salicylic acid, SD = standard deviation

Table 3. Sicca symptoms, ocular signs, autoantibody tests and MSGB histopathology results of participants included in primary Sjögren syndrome group

(n = 29)	Positive	Negative	Not known/not performed
Oral symptoms, n (%)	26 (89.7)	3 (10.3)	0 (0.0)
Ocular symptoms, n (%)	23 (79.3)	6 (20.7)	0 (0.0)
Ocular signs[1], n (%)	22 (75.9)	3 (10.3)	4 (13.8)
ANA, n (%)	20 (69.0)	9 (31.0)	0 (0.0)
Anti-Ro (SSA), n (%)	10 (34.5)	12 (41.4)	7 (24.1)
Anti-La (SSB), n (%)	6 (20.7)	16 (55.2)	7 (24.1)
Histopathology[2], n (%)	29 (100.0)	0 (0.0)	0 (0.0)
Grade 3	15 (51.7)		
Grade 4	14 (48.3)		

ANA = anti-nuclear antibodies, Ro/SSA = anti-Sjögren's syndrome antigen A, La/SSB = anti-Sjögren's syndrome antigen B, MSGB = minor salivary gland biopsy, [1] Schirmer's test or Rose–Bengal or other ocular dye test, [2]lymphocytic focus on minor salivary gland biopsy [6]

Table 4. Demographic data of PSS and non-PSS groups and their answers to the questions in the complication screening questionnaire

	Non-PSS (n = 31)	PSS (n = 29)	*p* value
Age (years), median (mimimum-maximum)	48.00 (24-81)	48.00 (25-72)	0.739
Female, n (%)	24 (77.4)	27 (93.1)	0.148
Complication screening questionnaire			
1) Did you feel pain during the procedure? (yes/no/don't remember)	9/22/0	8/21/0	1.000
2) Was there any bleeding that would disturb you after the biopsy? (yes/no/don't remember)	8/23/0	2/27/0	0.082
3) How long was the bleeding period?, n (%)			0.775
10 minutes	15 (48.4)	18 (62.1)	
10-30 minutes	7 (22.6)	6 (20.7)	
31-60 minutes	5 (16.1)	3 (10.3)	
61 minutes-3 hours	3 (9.7)	1 (3.4)	
>3 hours	1 (3.2)	1 (3.4)	
4) Did you have trouble eating after the biopsy? (yes/no/don't remember)	6/25/0	5/23/1	0.574
5) How many days did the trouble persist?, n (%)			
< 7 days	5 (83.3)	5 (100)	
7-14 days	1 (16.7)	0 (0.0)	
≥ 14 days	0 (0.0)	0 (0.0)	
6) Was there any persistent numbness around the lip after th biopsy? (yes/no/don't remember)	2/29/0	2/27/0	1.000
7-) How many days did the numbness persists around the lip?, n (%)			
< 7 days	2 (100.0)	2 (100.0)	
7-14 days	0 (0.0)	0 (0.0)	
≥ 14 days	0 (0.0)	0 (0.0)	
8) Was there a feeling of wetness in the mouth after biopsy? (yes/no/don't remember)	1/30/0	0/29/0	1.000
9) How many days did the wetness in the mouth persist after the biopsy?, n (%)			
< 7 days	1 (100)	0 (0)	
7-14 days	0 (0.0)	0 (0.0)	
≥ 14 days	0 (0.0)	0 (0.0)	
10) Was there any blood collection (hematoma) in the lip after the procedure? (yes/no/don't remember)	1/30/0	0/29/0	1.000
11) Was there an infection at the incision site after the procedure? (yes/no/don't remember)	0/31/0	0/29/0	1.000
12) How many days did the incision heal?, n (%)			0.750
< 7 days	27 (87.1)	25 (86.2)	
7-14 days	3 (9.7)	2 (6.9)	
≥ 14 days	1 (3.2)	1 (3.4)	
Don't remember	0 (0.0)	1 (3.4)	
13) Is there any scar after the incision has healed? (yes/no/don't remember)	1/30/0	2/27/0	0.606

PSS = primary Sjögren's syndrome, ASA = acetyl salicylic acid, SD = standard deviation

complications following a labial MSGB procedure and compared the incidence of complications in a group of patients with and without PSS. According to the results we obtained, the most common complaint of the participants was that they felt pain during the procedure and 21.2% of the participants had pain during the procedure even though all the participants had local anesthesia before the procedure. In our study, we do not have any information about the amount of local anesthetic injected into the area before the procedure and how long to wait and perform the procedure after local anesthesia. This result may be related to the lack of effective local anesthesia. The second most common problem the participants complained of was bleeding, which was disturbing the participants after the procedure, which was seen in 17.2%. In addition, 11 of 99 participants (11.1%) who underwent labial MSGB procedure had post-operative hemorrhage lasting longer than 1 hour and 2 of them had bleeding lasting longer than 3 hours. Moreover, hematoma developed in one patient after labial MSGB. In the study of Caporali *et al*., hematoma was reported in 8 (1.6%) of 502 participants who underwent labial MSGB procedure, suture was performed after procedure and the frequency of hematoma was similar to that in this study (%1.0) [11]. In the study of Friedman *et al*. [8], Minor bleeding was detected in 2 of 118 participants who underwent labial MSGB, but no information was given about whether suture was performed or not in the participants with minor bleeding. To the best of our knowledge, there is no data in the literature regarding the duration of bleeding after labial minor salivary gland biopsy and, according to the information obtained from this study, bleeding after the labial MSG procedure may cause a significant proportion of patients to feel uncomfortable. Wound healing after the labial MSGB procedure can be completed with or without sutures. However, in the procedures completed by suturing, complications other than bleeding such as local wetness, local infection, and cheloid formation can be seen [8]. Another common complaint in this study was the difficulty in eating after the procedure and was present in 16.2% of the participants. Although the complaints of the majority of the participants who had difficulty eating were less than 1 week (87.5%), 1 of 99 participants stated that his complain lasted longer than 2 weeks. There is little data on the difficulty of eating after the labial MSGB procedure. In the study of Saruhanoğlu *et al*. [9], labial MSGB procedure was performed with vertical incision and suture was performed after the procedure. Thirty-five (43.2%) of 81 participants had eating difficulties after the procedure and in 4 of them, it was found that the problem of eating lasted longer than 5 days [9]. In this study, the frequency of difficulty in eating food after the procedure was lower than that of Saruhanoğlu *et al*. [9]. This may be related to the absence of sutures after the procedure in this study. In this study, 10.1% of the participants had persistent numbness after the procedure. However, this complaint lasted less than 1 week in 8.1% of the participants, 1-2 weeks in 2.0% of the participants, and numbness did not persist in any of the participants for more than 2 weeks. In the study of Richards *et al*. [10], 58 participants underwent labial MSGB procedure and 2 participants had decreased sensation in the lips, and one patient (1.7%) continued to have this complaint after one year. In the study of Berquin *et al*. [7], 16 labial MSGB procedures revealed 1 permanent lip or tongue anesthesia. In the study of Saruhanoğlu *et al*. [9], paresthesia was detected in 4 of 81 participants who underwent labial MSGB with vertical incision. In 1 (1.2%) of these, paresthesia continued between 0-2 months after the procedure and in 3 (3.7%) of them 2-12 months. In all these studies, postoperative paresthesia was evaluated long after the procedure and some participants had permanent paresthesia. In this study, long-term paresthesia has not been developed in anybody and this may be related to performing the procedure without suturing. Wet sensation after the procedure was detected in only one patient in this study, this complaint did not persist after 1 week and None of the participants had post procedure-infection. In the study of Friedman *et al*., the complications of the sutured and non-sutured participants after the labial MSGB procedure were compared. Of the 56 sutured participants, 5 (8.9%) had local wetting and 2 (3.6%) had local infection, whereas none of the 62 non-sutured participants had local wetness or local infection. These findings from the study of Friedman *et al*. are consistent with the findings of this study, and suturing may increase local complications such as wetness, infection and numbness.

In this study, the frequency of biopsy complications after labial MSGB was not different between

participants with PSS and those without PSS. In addition, postoperative bleeding times were not different between the two groups. However, although not statistically significant, the presence of bleeding to disturb participants after the procedure was less common in patients with PSS (25.8% vs 6.90%; p = 0.082). For some reason in PSS, participants may be less affected by the disturbing effect of bleeding. Peripheral nervous system involvement is common in PSS and varies between 2-25% [13-15]. Peripheral cranial nerve involvement can also be seen in PSS, and even a case of glossopharyngeal and vagal nerve involvement in Sjögren syndrome has been reported in the literature [16, 17]. In patients with PSS, less affected by the disturbing effect of bleeding may be due to the involvement of nerves involved in sensory innervation of the oral mucosa in participants with PSS. There is not enough information about this subject in the literature and further research is needed to clarify this issue.

Limitations

This study has some limitations. The number of participants included in the study was not sufficient to determine the frequency of complications of labial MSGB. Participants' self-descriptions were evaluated when evaluating post-procedure bleeding complications. In order to evaluate the amount and duration of bleeding more objectively, it would be more appropriate to observe and note the patient's bleeding time simultaneously by the investigator after the procedure. Labial MSGB procedures of the participants were performed by 2 investigators, so there may be minor differences between the two investigators in performing the procedure and this may have influenced the results.

CONCLUSION

Although postoperative complications are not common in labial minor salivary gland biopsy, some patients may have difficulty in eating after the procedure, bleeding and numbness. Bleeding may be more common in procedures performed without suturing, but complications such as infection, numbness, and wetness are very rare. The presence of underlying PSS has no effect on the complications after labial MSGB.

Authors' contribution

KA, AD, AK, RT designed the study. KA and AK performed minor salivary gland biopsy procedure. AD applied the screening questionnaires to the participants. KA transferred the data to SPSS package program and performed statistical analysis. KA conducted a literature review and wrote the preliminary draft text. AD, AK, RT reviewed the draft text.

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 prolactin and adipose tissue and metabolic parameters in patients with polycystic ovary syndrome

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ABSTRACT

Objectives: Polycystic ovary syndrome is a reproductive endocrinopathy, predominantly accompanied by insulin resistance, obesity, and metabolic disorder. In this study, we aimed to investigate the possible relationship between prolactin and adipose tissue and metabolic parameters in patients with polycystic ovary syndrome (PCOS).

Methods: A total of 58 patients with PCOS and 34 body mass index (BMI)-matched healthy controls between September 2018 and March 2019 were included in the study. Visceral and subcutaneous adipose tissues were measured using ultrasonography. Serum prolactin, fasting blood glucose, insulin, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglyceride, total cholesterol, luteinizing hormone (LH), total testosterone, dehydroepiandrosterone sulfate (DHEA-S), and 17-hydroxyprogesterone (17-OHP) levels were measured.

Results: The median BMI ($p = 0.001$), waist circumference ($p = 0.002$), hip circumference ($p = 0.003$), waist-to-hip ratio ($p = 0.013$), LH ($p = 0.012$), total testosterone ($p = 0.004$), DHEA-S ($p = 0.049$), 17-OHP ($p = 0.001$), insulin ($p = 0.001$), minimum preperitoneal fat thickness (p = 0.001), maximum preperitoneal fat thickness ($p = 0.048$), and intraperitoneal fat thickness ($p = 0.018$) were significantly higher in the PCOS group compared to the control group. However, there was no significant correlation between prolactin levels and adipose tissue parameters and insulin levels in the patients with PCOS.

Conclusions: Although there was an increase in the preperitoneal and intraperitoneal fat thickness in the PCOS group compared to the control group, no significant correlation was observed between prolactin and visceral and subcutaneous adipose tissues and metabolic parameters.

Keywords: Prolactin, polycystic ovary syndrome, adipose tissue

Prolactin (PRL) is a versatile hormone which plays a central role in metabolic functions and tumorogenesis, as well as reproductive and immune system [1]. It is mainly produced in the pituitary gland and in extrapituitary tissues such as human endometrium, decidua, brain, breast, and adipose tissue [2]. Previous studies have shown a complex relationship between PRL and adipose tissue and PRL is implicated in the regulation of adipogenesis and function of adipocytes [2] .

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Polycystic ovary syndrome (PCOS) is a common hormonal disorder in women of reproductive age characterized by irregular menstruation, chronic anovulation, and clinic and/or biochemical hyperandrogenism with morphologically characteristic feature of polycystic ovaries [3]. Metabolic disorders are more common in these patients, compared to healthy individuals, due to insulin resistance and abdominal obesity [4].

Although the regulation of PRL release is altered in PCOS patients, the relationship between PRL and adiposity is still unclear [5]. Therefore, the relationship between PRL and increased adipose tissue and metabolic disorders still remains to be elucidated in this patient population.

In the present study, we aimed to investigate the possible relationship between PRL and adipose tissue and metabolic parameters in patients with PCOS compared to healthy controls.

METHODS

Study population

This cross-sectional study was carried out at Department of Obstetrics and Gynecology of Bursa Yüksek İhtisas Training and Research Hospital between September 2018 and March 2019. A total of 58 patients with PCOS and 34 body mass index (BMI)-matched healthy controls were included. In the patient group, the diagnosis of PCOS was made according to the Rotterdam criteria including at least two of the following three features: i) oligo/anovulation, ii) clinical and/or biochemical hyperandrogenism, and iii) polycystic ovaries on ultrasonography (USG) (6). The control group consisted of healthy individuals in whom no clinical, laboratory, or USG signs of PCOS were present. Exclusion criteria were as follows: history of diabetes, hyperprolactinemia, Cushing syndrome, congenital adrenal hyperplasia, thyroid disorders, and hypertension. Patients who received oral contraceptives, anti-androgens, aspirin, statin, and insulin-sensitizing agents within the past six months were also excluded. A written informed consent was obtained from each participant. The study protocol was approved by the Ethics Committee of Bursa Yüksek İhtisas Training and Research Hospital. (2011-KAEK-25 2018/06-31). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Biochemical analyses and hormone assays

Blood specimens were collected for biochemical and hormone analyses in the early follicular phase (between Day 2 and Day 5 of the menstrual cycle) with at least 12-h overnight fasting between 8.00 and 10.00 AM.Follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), total testosterone, dehydroepiandrosterone sulfate (DHEA), insulin, and 17-hydroxyprogesterone (17-OHP) were analyzed using the Abbott ARCHITECT® assay (Abbott Laboratories, Singapore). In addition, fasting blood glucose, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) were evaluated using the Synchron LX20, Beckman Coulter Diagnostics, USA. Insulin resistance was calculated using the Homeostatic Model Assessment Insulin Resistance (HOMA-IR) formula (fasting glucose (mg/dL) x fasting insulin (μU/mL)/405). The model of adipose distribution (MOAD) for women was calculated using the following formula: waist circumference/[36.58+(1.89xBMI)]. The visceral adiposity index (VAI) was calculated as MOADx(TG/0.81)x(1.52/HDL).

Anthropometric measurements

The body weight, height, and BMI were calculated for each participant. The waist circumference was measured at the narrowest part between the lower border of the rib cage and the iliac crest, while the hip circumference was measured at the greater trochanter while standing erect. The BMI was calculated as the weight in kilogram divided by height in meters squared.

Carotid intima-media thickness measurement

The carotid intima-media thickness (CIMT) was defined as the average of the three thickness measurements between the intimal and medial-adventitial interfaces and was measured in the supine position with head flexion. The CIMT measurements were performed by an experienced radiologist.

Adipose tissue measurement

The thickness of subcutaneous, preperitoneal,

intraperitoneal, and perirenal adipose tissues was measured using USG. The measurement was performed in the supine position in a fasting state by having the patient hold his/her breath to avoid any possible effect of respiration and abdominal wall tension. To avoid fat compression errors, the USG probe was placed above a given site without any pressure. Minimum subcutaneous and preperitoneal fat thickness were measured by longitudinal scanning with the use of (Toshiba Aplio 500, Japenese) 7 Mhz transducer from the xyphoid process, while maximum subcutaneous fat thickness was measured using the same transducer at the level of the umbilicus. Intraperitoneal fat thickness was measured by transverse scanning with the use of 5 Mhz probe in the midline of the abdomen, 2-cm above the umbilicus. Three measures were obtained based on the intraperitoneal fat thickness measurement: the distance from the fascia of rectus abdominis muscle to the vertebral column, the distance from the peritoneum to the vertebral column, and the distance from the linea alba to the vertebral column. Perirenal fat thickness was measured from the perirenal fascia to the renal surface on a long-axis view of the right kidney.

Statistical Analysis

Statistical analysis was performed using the SPSS version 23. Descriptive data were expressed in mean ± standard deviation (SD), median (min-max) values, and number and frequency. The Kolmogorov-Smirnov test was used to check the normality assumption. The Mann-Whitney U test was performed to compare variables between the patient and control groups. The Spearman's rank correlation analysis was used to investigate any relationship between serum PRL and other variables. After necessary adjustments for age, BMI, hip circumference, and waist-to-hip ratio were made, the partial Spearman's rank correlation analysis was performed. A multiple linear regression model was used to analyze the impact of potential variables on serum PRL levels. A p value of < 0.05 was considered statistically significant.

RESULTS

A total of 92 participants were included in this study. The study group consisted of 58 patients with PCOS and the control group consisted of 34 healthy individuals. However, as not every participant underwent all tests, the number of participants in both patient group and control group varied. The mean age was 27.07 ± 4.88 years in the patient group and 28.58 ± 4.78 in the control group, indicating no significant difference between the groups. Baseline demographic and clinical characteristics of the study population are shown in Table 1.

The median body weight ($p = 0.004$), BMI ($p = 0.001$), Ferriman-Gallwey Hirsutism (FGH) scores ($p = 0.001$), waist circumference ($p = 0.002$), hip circumference ($p = 0.003$), waist-to-hip ratio ($p = 0.013$), LH ($p = 0.012$), total testosterone ($p = 0.004$), DHEA-S ($p = 0.049$), 17-OHP ($p = 0.001$), insulin ($p = 0.001$), CIMT, minimum preperitoneal fat thickness ($p = 0.001$), maximum preperitoneal fat thickness ($p = 0.048$), and intraperitoneal fat thickness ($p = 0.018$) were significantly higher in the PCOS group compared to the control group. There was no significant difference in other variables between the groups.

Table 2 shows monotonic relationship between serum PRL and other variables. There was no significant correlation between serum PRL and any of the variables in the control group, while serum PRL significantly decreased with increasing age in the PCOS group ($p = 0.001$). However, there was no significant linear correlation between serum PRL and other variables in the PCOS group.

After necessary adjustments for age, BMI, hip circumference, and waist-to-hip ratio were made, correlation analysis was repeated. A positive and significant correlation was found between serum PRL levels and FGH scores ($p = 0.025$), TG levels ($p = 0.020$), and mid CIMT ($p = 0.039$) in the control group, while no significant correlation was found in the PCOS group (Table 3).

A multiple linear regression model was used to analyze the impact of potential variables on serum PRL levels. In the PCOS group, there was a significant correlation between age ($p = 0.020$), waist-to-hip ratio ($p = 0.044$), and HDL-C ($p = 0.049$) (Table 4).

DISCUSSION

Polycystic ovary syndrome is a reproductive endocrinopathy, predominantly accompanied by

Table 1. Demographic and clinical characteristics of the study population

Variable	Control group (n = 34)						PCOS group (n = 58)						*p* value
	N	Mean	SD	Percentiles			N	Mean	SD	Percentiles			
				1st	Median	3rd				1st	Median	3rd	
Age (years)	33	28.58	4.78	24.50	27.00	33.50	58	27.07	4.88	23.00	27.00	30.25	0.193
Height (cm)	33	160.30	17.13	157.00	164.00	169.00	57	162.72	5.77	160.00	162.00	167.00	0.890
Weight (kg)	33	70.94	18.21	56.00	64.00	79.50	57	81.34	16.93	70.00	78.00	94.50	**0.004**
BMI (kg/m^2)	33	31.26	29.61	21.87	25.80	30.22	57	30.61	5.46	26.98	30.85	34.43	**0.001**
FGH score	32	9.03	5.59	4.00	9.00	13.00	57	16.16	7.97	10.00	16.00	23.00	**0.001**
Waist circumference (cm)	33	86.79	15.26	73.50	83.00	100.50	57	96.39	12.17	88.50	97.00	105.00	**0.002**
Hip circumference (cm)	33	106.79	12.81	96.50	102.00	114.00	57	114.09	11,17	106.00	113.00	121.00	**0.003**
Waist-to-hip ratio	33	0.81	0.06	0.76	0.80	0.86	57	0.84	0.05	0.81	0.85	0.87	**0.013**
SBP (mmHg)	33	111.21	10.83	110.00	110.00	120.00	57	115,19	10.25	110.00	110.00	120.00	0.198
DBP (mmHg)	33	66.67	11.09	60.00	70.00	75.00	57	70,42	9.46	60.00	70.00	80.00	0.134
TSH (µU/ml)	34	2.38	1.55	1.44	1.79	2.94	58	1.97	1.00	1.23	1.77	2.55	0.528
PRL (ng/mL)	34	16.81	7.90	11.20	14.75	23.40	57	15.95	7.88	10.35	14.12	19.75	0.504
FSH (mIU/mL)	34	5.92	1.60	5.09	5.67	6.19	57	4.93	1.47	3.84	4.88	5.75	**0.003**
LH (mIU/mL)	34	4.01	1.46	3.05	3.61	4.98	57	5.63	3.37	3.53	4.68	6.50	**0.012**
Estradiol (pg/mL)	34	44.51	22.57	27.85	42.50	52.25	57	37.18	13.78	28.00	35.00	47.50	0.208
Total testosterone	33	1.16	0.45	0.88	1.09	1.30	56	1.39	0.44	1.05	1.34	1.70	**0.004**
DHEA-S (µg/dL)	33	198.36	95.97	130.50	172.00	235.00	55	227.44	86.04	155.00	239.00	292.00	**0.049**
17-OHP (ng/mL)	33	0.54	0.63	0.20	0.29	0.58	52	1.12	0.88	0.35	0.90	1.88	**0.001**
Insulin (µIU/mL)	33	11.05	13.85	5.00	7.00	11.50	55	12.14	6.66	7.30	10.10	16.00	**0.010**
FBG (mg/dL)	33	90.03	11.50	83.00	87.00	96.00	57	91.91	10.45	83.50	91.00	99.00	0.357
HDL-C (mg/dL)	32	50.22	10.81	42.25	47.00	58.00	57	50.81	16.54	41.00	46.00	58.50	0.666
LDL-C (mg/dL)	32	96.21	28.37	74.50	102.00	114.75	54	106.89	33.81	85.50	109.50	132.25	0.145
Total cholesterol (mg/dL)	33	173.18	30.95	162.00	176.00	191.50	56	180.02	35.48	157.25	177.50	204.75	0.405
TG (mg/dL)	33	107.00	65.44	60.00	87.00	130.00	56	120.45	57.84	78.25	112.50	157.50	0.136
CIMT (right) (mm)	26	0.50	0.12	0.43	0.46	0.56	38	0.56	0.11	0.46	0.58	0.63	**0.029**
CIMT (left) (mm)	26	0.50	0.12	0.43	0.46	0.60	38	0.56	0.10	0.46	0.58	0.63	**0.023**
CIMT (mid) (mm)	26	0.50	0.11	0.43	0.47	0.57	38	0.56	0.10	0.46	0.58	0.63	**0.036**
Min SAT thickness (mm)	26	17.16	6.78	12.43	17.50	21.55	38	19.28	6.20	13.45	19.20	23.70	0.180
Max SAT thickness (mm)	26	26.30	12.16	16.45	26.30	32.53	38	27.42	9.32	19.18	27.25	34.25	0.672
Min preperitoneal thickness (mm)	26	11.09	6.62	6.15	8.50	16.48	38	16.16	5.07	12.85	16.00	20.00	**0.001**
Max preperitoneal thickness (mm)	26	19.25	10.05	12,70	19.00	23.25	38	25.35	13.71	16.60	21.60	30.25	**0.048**
Intraperitoneal thickness (mm)	26	53.71	20.74	33.50	52.00	65.38	38	67.24	21.46	46.35	64.00	87.00	**0.018**
Perirenal thickness (mm)	26	5.66	4.73	1.95	5.35	7.60	38	7.48	5.08	4.38	5.50	9.55	0.124
VAI	31	4.47	3.49	2.17	3.21	5.58	55	5.24	3.24	2.77	4.74	7.12	0.141

PCOS = polycystic ovary syndrome, BMI = body mass index, FGH = Ferriman-Gallwey Hirsutism, SBP = systolic blood pressure, DBP = diastolic blood pressure, TSH = thyroid-stimulating hormone, PRL = prolactin, LH = luteinizing hormone, FSH = follicle-stimulating hormone, 17-OHP = 17-hydroxyprogesterone, FBG = fasting blood glucose, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, TG = triglyceride, CIMT = carotid intima-media thickness, DHEA-S = dehydroepiandrosterone s ulfate, Min = minimum, max = maximum, SAT = subcutaneous adipose tissue, VAI = Visceral Adiposity Index

Table 2. Monotonic relationship between serum prolactin and other variables

Variable	Serum PRL level					
	Control group (n = 34)			PCOS group (n = 58)		
	r	*p value*	N	*r*	*p value*	N
Age (years)	0.016	0.928	33	-0.525	0.001	57
Height (cm)	-0.159	0.376	33	0.121	0.375	56
Weight (kg)	-0.325	0.065	33	-0.090	0.512	56
BMI (kg/m^2)	-0.272	0.126	33	-0.108	0.429	56
FGH score	0.231	0.203	32	0.111	0.414	56
Waist circumference (cm)	-0.206	0.249	33	-0.047	0.730	56
Hip circumference (cm)	-0.221	0.216	33	-0.053	0.696	56
Waist-to-hip ratio	-0.046	0.798	33	-0.008	0.951	56
SBP (mmHg)	0.055	0.762	33	-0.050	0.715	56
DBP (mmHg)	-0.001	0.993	33	0.027	0.842	56
TSH (µU/ml)	-0.039	0.825	34	0.191	0.154	57
FSH (mIU/mL)	-0.123	0.487	34	-0.010	0.940	57
LH (mIU/mL)	0.152	0.390	34	-0.108	0.425	57
Estradiol (pg/mL)	0.180	0.308	34	-0.061	0.655	57
Total testosterone	-0.061	0.735	33	0.019	0.890	56
DHEA-S (µg/dL)	-0.167	0.352	33	0.166	0.229	54
17-OHP (ng/mL)	0.242	0.174	33	0.216	0.124	52
Insulin (µIU/mL)	0.055	0.763	33	-0.070	0.614	55
FBG (mg/dL)	-0.209	0.244	33	-0.152	0.258	57
HDL-C (mg/dL)	0.196	0.284	32	0.134	0.319	57
LDL-C (mg/dL)	-0.166	0.364	32	-0.022	0.873	54
Total cholesterol (mg/dL)	0.060	0.742	33	-0.073	0.93	56
TG (mg/dL)	0.239	0.181	33	-0.153	0.261	56
CIMT (right) (mm)	0.246	0.246	24	0.022	0.97	36
CIMT (left) (mm)	-0.085	0.693	24	-0.011	0.951	36
CIMT (mid) (mm)	0.079	0.715	24	0.007	0.966	36
Min SAT thickness (mm)	-0.042	0.844	24	0.252	0.37	36
Max SAT thickness (mm)	-0.211	0.322	24	0.042	0.809	36
Min preperitoneal thickness (mm)	-0.044	0.837	24	0.228	0.182	36
Max preperitoneal thickness (mm)	-0.226	0.288	24	0.058	0.735	36
Intraperitoneal thickness (mm)	-0.156	0.468	24	0.205	0.230	36
Perirenal thickness (mm)	-0.237	0.264	24	0.050	0.772	36
VAI	0.106	0.569	31	-0.077	0.578	55

PCOS = polycystic ovary syndrome, BMI = body mass index, FGH = Ferriman-Gallwey Hirsutism, SBP = systolic blood pressure, DBP = diastolic blood pressure, TSH = thyroid-stimulating hormone, PRL = prolactin, LH = luteinizing hormone, FSH = follicle-stimulating hormone, 17-OHP = 17-hydroxyprogesterone, FBG = fasting blood glucose, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, TG = triglyceride, CIMT = carotid intima-media thickness, DHEA-S = dehydroepiandrosterone sulfate, Min = minimum, max = maximum, SAT = subcutaneous adipose tissue, VAI = Visceral Adiposity Index

Table 3. Corrected correlation analysis with adjusted variables

Variable		Control group (n = 34)		PCOS group (n = 58)	
		Serum PRL level		Serum PRL level	
		r	*p value*	*r*	*p value*
BMI & waist-to-hip ratio	FGH score	0.499	**0.025**	0.210	0.302
	SBP (mmHg)	0.144	0.545	0.076	0.711
	DBP (mmHg)	-0.114	0.631	0.099	0.631
	TSH (µU/ml)	0.186	0.433	-0.218	0.285
	FSH (mIU/mL)	-0.136	0.569	-0.011	0.958
	LH (mIU/mL)	-0.020	0.933	-0.148	0.471
	Estradiol (pg/mL)	0.328	0.158	-0.026	0.901
	Total testosterone	0.268	0.253	-0.023	0.911
	DHEA-S (µg/dL)	0.219	0.353	-0.124	0.545
	17-OHP (ng/mL)	0.174	0.464	-0.014	0.947
	Insulin (µIU/mL)	0.297	0.204	0.099	0.629
	FBG (mg/dL)	-0.098	0.682	-0.193	0.344
	HDL-C (mg/dL)	0.354	0.126	0.203	0.319
	LDL-C (mg/dL)	-0.001	0.997	-0.078	0.706
	Total cholesterol (mg/dL)	0.182	0.442	-0.041	0.841
	TG (mg/dL)	0.516	**0.020**	-0.164	0.423
	CIMT_SAG	0.599	**0.005**	-0.181	0.377
	CIMT_SOL	0.296	0.205	-0.245	0.228
	CIMT_ORT	0.465	**0.039**	-0.224	0.271
	Min SAT thickness (mm)	0.108	0.651	0.190	0.351
	Max SAT thickness (mm)	-0.085	0.723	0.005	0.981
	Min preperitoneal thickness (mm)	0.188	0.427	0.087	0.671
	Max preperitoneal thickness (mm)	-0.071	0.767	-0.001	0.995
	Intraperitoneal thickness (mm)	0.002	0.994	0.245	0.228
	Perirenal thickness (mm)	-0.132	0.580	0.035	0.865
	VAI	0.387	0.092	-0.175	0.392

PCOS = polycystic ovary syndrome, BMI = body mass index, FGH = Ferriman-Gallwey Hirsutism, SBP = systolic blood pressure, DBP = diastolic blood pressure, TSH = thyroid-stimulating hormone, PRL = prolactin, LH = luteinizing hormone, FSH = follicle-stimulating hormone, 17-OHP = 17-hydroxyprogesterone, FBG = fasting blood glucose, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, TG = triglyceride, CIMT = carotid intima-media thickness, DHEA-S = dehydroepiandrosterone sulfate, Min = minimum, max = maximum, SAT = subcutaneous adipose tissue, VAI = Visceral Adiposity Index

Table 4. Multiple linear regression analysis

Group		Unstandardized Coefficients		Standardized Coefficients	*t*	*p value*
		β	SE	β		
PCOS	Age (years)	-1.362	0.529	-0.885	-2.572	**0.020**
	BMI (kg/m^2)	0.384	0.807	0.282	0.476	0.640
	FGH score	-0.231	0.275	-0.239	-0.839	0.413
	Waist-to-hip ratio	107.688	49.518	0.812	2.175	**0.044**
	SBP (mmHg)	-0.003	0.236	-0.004	-0.014	0.989
	DBP (mmHg)	0.037	0.238	0.048	0.156	0.878
	TSH (µU/ml)	0.268	1.821	0.045	0.147	0.885
	FSH (mIU/mL)	-0.443	1.668	-0.087	-0.266	0.794
	LH (mIU/mL)	-0.894	0.720	-0.322	-1,243	0.231
	Estradiol (pg/mL)	0.155	0.110	0.383	1.411	0.176
	Total testosterone	-3.806	5.265	-0.182	-0.723	0.480
	DHEA-S (µg/dL)	-0.012	0.026	-0.121	-0.467	0.647
	17-OHP (ng/mL)	-0.322	4.116	-0.030	-0,078	0.939
	Insulin (µIU/mL)	0.125	0.197	0.190	0.635	0.534
	HDL-C (mg/dL)	0.488	0.233	0.731	2.000	**0.049**
	TG (mg/dL)	-0.059	0.139	-0.384	-0423	0.677
	CIMT (mid) (mm)	6.249	25.749	0.083	0.243	0.811
	Min SAT thickness (mm)	0.462	0.605	0.399	0.764	0.455
	Max SAT thickness (mm)	-0.260	0.377	-0.333	-0.689	0.500
	Max preperitoneal thickness (mm)	-0.181	0.193	-0.330	-0.938	0.362
	Intraperitoneal thickness (mm)	-0.112	0.172	-0.321	-0.654	0.522
	Perirenal thickness (mm)	0.246	0.631	0.166	0.390	0.701

PCOS = polycystic ovary syndrome, BMI = body mass index, FGH = Ferriman-Gallwey Hirsutism, SBP = systolic blood pressure, DBP = diastolic blood pressure, TSH = thyroid-stimulating hormone, LH = luteinizing hormone, FSH = follicle-stimulating hormone, 17-OHP = 17-hydroxyprogesterone, HDL-C = high-density lipoprotein cholesterol, TG = triglyceride, CIMT = carotid intima-media thickness, DHEA-S = dehydroepiandrosterone sulfate, Min = minimu, max = maximum, SAT = subcutaneous adipose tissue

insulin resistance, obesity, and metabolic disorder [3]. Nearly 30 to 50% of lean patients with PCOS and those with obesity have insulin resistance and lipid metabolism disorders,suggesting that obesity is not the sole driver of metabolic alterations [7]. Several studies have demonstrated that metabolic disorders are more frequently associated with the distribution of adipose tissue rather than absolute amount of the body fat [8]. In the literature, some authors have reported no significant difference in the body composition and fat distribution between lean patients with PCOS and healthy controls [9], while some others have shown that PCOS patients have more visceral fat ratio proportionally to total body fat, suggesting a relationship between PCOS and glucose intolerance, type 2 diabetes, hypertension, and hyperlipidemia [10, 11]. Furthermore, although there are studies showing an increase in the subcutaneous adipose tissue in PCOS patients [12], some authors have not demonstrated such an increase [13]. Similarly,there are some studies showing an increase in the visceral adipose tissue [10, 14], whle some others have found no increase [13]. In

a study, Jena *et al*. [15] found increased subcutaneous adipose tissue in patients with PCOS and obesity, compared to BMI-matched healthy controls. However, in the aforementioned study, there was no significant difference in the subcutaneous adipose tissue between lean PCOS patients and controls. In addition, the authors reported increased visceral adipose tissue in PCOS patients with and without obesity compared to healthy controls [15]. In another study evaluating subcutaneous, preperitoneal, intraperitoneal, mesenteric, epicardial, and perirenal adipose tissue through USG, a significant increase in the visceral adipose tissue, particularly mesenteric and intraperitoneal, was observed in patients with PCOS with and without obesity, compared to the control group [16]. In our study, although we found no significant difference in the subcutaneous fat thickness between the groups, we observed a significant increase in the preperitoneal and intraperitoneal fat thickness in the patients with PCOS.

Visceral adipose tissue is a more active driver of metabolic alterations than subcutaneous adipose tissue and is more resistant to anti-lipolytic effects of insulin, thereby, increasing abnormal lipid production and insulin resistance [17]. Although preperitoneal adipose tissue is not a part of visceral adipose tissue, it has similar properties to visceral adipose tissue, as it is anatomically located close to peritoneal, omental, and retroperitoneal adipose tissues [18].

It has been well-established that PRL plays a central role in the reproductive system. In recent years, there are also several reports suggesting that PRL can be used as a useful biomarker for metabolic syndrome, diabetes mellitus, cardiovascular and all-cause mortality [19, 20]. It has been proposed that PRL exerts its effects on adipose tissue development and functions and pancreatic β cells [21]. There is a complex relationship between PRL and adipose tissue: PRL has not only an effect on adipogenesis and adipocyte functions, but also is produced in adipose tissues [2]. However, the effect of PRL on systemic circulation has not been clearly understood, yet. Some authors have suggested that PRL released by the adipose tissue shows an autocrine/paracrine effect [22].

In the literature, there are several reports showing a positive or negative or no correlation between the amount of adipose tissues and PRL levels [23-25]. The discrepancy among the studies can be attributed to the type of adipose tissue examined. In a study, Kok *et al*. [26] found a higher rate of basal and pulsatile PRL release in premenopausal women with visceral obesity, compared to lean controls. In another study, patients with obesity had a lower PRL release from subcutaneous adipose tissue, compared to visceral adipose tissue, indicating an inverse relationship between PRL release from subcutaneous adipose tissue and BMI [2] . On the other hand, the rate of PRL released from subcutaneous and visceral adipose tissues was similar in patients without obesity. Unlike this study, we found no significant relationship between PRL levels and subcutaneous and visceral adipose tissue in our study population. Similarly, in their study, Ernst *et al*. [25] found no significant difference in the serum basal PRL levels one year after gastric bypass in patients with obesity, compared to baseline, despite severe weight loss.

Adipose tissue dysfunction is considered an important contributor to obesity-related metabolic disorders. In patients with obesity, excessive fat deposition leads to insulin resistance, impaired adipogenesis, altered adipokine secretion, increased inflammation and fibrosis, and reduced angiogenesis [27]. Therefore, adipose tissue modeling is critical to prevent insulin resistance and associated metabolic disorders [28]. A healthy expansion of the adipose tissue is of utmost importance to maintain insulin sensitivity, while PRL is involved in the healthy expansion of the adipose tissue and maintenance of insulin sensitivity [19, 29].

On the other hand, PRL may have adverse metabolic effects in patients with high serum PRL levels due to prolactinoma or the use of antipsychotics, leading to type 2 diabetes(30). Bromocriptine, a dopamine agonist, inhibits PRL levels and increase insulin sensitivity and has been used in the treatment of type 2 diabetes in recent years [31]. However, there are several studies showing no direct correlation between corrected BMI and metabolic parameters and reduced PRL levels [32].

Review of the literature reveals controversial results regarding the relationship between serum PRL levels and metabolic parameters. Some authors reported an inverse correlation between PRL levels and diabetes, metabolic syndrome, HOMA-IR, and impaired lipid metabolism [19, 33], while some others found a positive correlation between PRL levels and

hypertension, insulin resistance, and aortic stiffness [20, 34, 35]. In a study, high physiological concentrations of PRL increased adiponectin release, showing a protective effect against metabolic dysfunction [28]. In another study, Albu *et al*. [5] found a positive correlation between adiponectin and PRL levels in patients with PCOS. In this study, VAI, but not adiponectin, was found to be a useful marker for predicting serum PRL levels. In our study, we found no significant correlation between serum PRL levels and metabolic parameters. Of note, we were unable to examine adipokine levels for adipose tissue dysfunction, although we used VAI. However, we found no significant correlation between serum PRL levels and VAI.

Limitations

Nonetheless, there are some limitations to this study. First, the study has a relatively small sample size. Second, only PCOS patients with obesity and BMI-matched healthy controls were included in this study and lean PCOS patients were unable to be evaluated. Third, thickness measurements of adipose tissues were made using USG. Finally, computed tomography and magnetic resonance imaging are more useful in the evaluation of subcutaneous and visceral adipose tissues(36), the outcomes of both methods are similar to USG [37, 38]. In our study, we used USG as it is an inexpensive, non-invasive imaging method in our study.

CONCLUSION

In conclusion, although there was an increase in the preperitoneal and intraperitoneal fat thickness in the PCOS group compared to the control group, no significant correlation was observed between PRL levels and visceral and subcutaneous adipose tissues. In addition, we found no significant correlation between serum PRL and metabolic parameters. We, therefore, recommend further large-scale studies to establish a definite conclusion on this topic.

Authors' contribution

GAA = designed the study, searched the literature, planned the concept, made statistical analysis, prepared and edited the manuscript, reviewed the manuscript and copy-edited the text and made contributions to improve the quality of the study. HGTÖ = designed the study, searched the literature, planned the concept, made statistical analysis, prepared and edited the manuscript.

Conflict of interest

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

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Biphasic anaphylaxis after use of foam polidocanol sclerotherapy

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ABSTRACT

Polidocanol is a sclerogenic agent commonly used for the treatment of varicose veins. Common complications such as hyperpigmentation, telangiectasia matting on skin may be observed in relation with foam sclerotherapy. Complications such as deep vein thrombosis, stroke and tissue necrosis are rarely seen. Fatal complications such as anaphylaxis are seen very uncommonly. A 50-year-old female patient underwent polidocanol foam sclerotherapy for left leg reticular varicosity (CEAP 2-3). Our patient has experienced biphasic anaphylaxis, which was observed as two attacks following the foam sclerotherapy. Biphasic anaphylaxis complication after the foam sclerotherapy is not known, this presentation may even be the first condition experienced ever.

Keywords: Varicose vein, polidocanol, biphasic anaphylaxis

Polidocanol foam sclerotherapy (PFS) is currently used in the treatment of reticular and telangiectasic veins. Patients may commonly experience complications such as cutaneous hyperpigmentation, telangiectatic matting and migraines following the foam sclerotherapy. They may experience deep vein thrombosis, stroke, tissue necrosis rarely and anaphylaxis reactions uncommonly [1, 2].

It is notified in the literature that anaphylactic reactions emerging following the sclerotherapy are generally seen in the first 15 minutes [3]. The fact rending this case as interesting is the manifestation of anaphylactic reaction in late hours in two attack phases. We could not find the literature relevant in the post-PFS biphasic anaphylactic reaction cases. We have reported this case that was seen uncommonly and progressing different than common anaphylactic reaction.

CASE PRESENTATION

A 50-year-old female patient underwent PFS treatment for the reticular varicose veins (CEAP 2-3) [4] in her left leg . It was determined from the medical background of patient that she had no allergic condition before and that PFS to be performed for the first time. Policocanol 0.5% (Aethoxysklerol® Hameln, Germany.) and ¼ air mixture is prepared by using Tessari method for the patient [5]. Approximately 8 cc foam has been injected slowly in 10 minutes to 5-6 different reticular veins sections with 25 G winged needle (Braun Venofix A Winged Infusion Set, Germany). Following the procedure, extremity was wrapped with elastic compression plaster. Patient has experienced dizziness and mild shortness of breath in 10 minutes following the PFS. Arterial tension was 80/60 mmHg. Patient had left the our outpatients'

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clinic with good clinical conditions after half an hour.

Approximately 3 hours after sclerotherapy, an anesthesiologist of another hospital reported on the phone that the patient was taken to intensive care unit because of tachycardia, severe shortness of breath, severe hypotension and shock. Patient was employed as operation room personnel in other hospital and the incident was occurred in the hospital. Therefore, first medical intervention was performed immediately in intensive care unit. During the consultation held with anesthesiologist, information was shared that the PFS was performed on the patient. It was understood that the patient had biphasic anaphylactic reaction in late period during her care in another medical center. Single dose 0.5 mg epinephrine injection, isotonic serum and i.v. 8 mg corticosteroid (dexamethasone) treatment was applied for the treatment of the patient. Patient was transferred from intensive care unit after one day treatment to service. Then the patient was discharged from the service at second day of the anaphylactic reaction.

DISCUSSION

Chronic venous disease (CVD) of the lower extremities is defined as a dysfunction in the venous system due to venous hypertension caused by valve insufficiency and / or obstruction in venous flow. CVD is a disease with high prevalence morbidity and chronicity. Surgery, medical therapy and sclerotherapy are some treatment options [11].

Polidocanol is commonly used for the treatment of reticular and telangiectasic varicose veins sclerotherapy. PFS commonly used for the treatment of varicosity with high efficiency and low side effect incidence. Severe anaphylaxis (1/10000) due to the use of polidocanol used in liquid or foam form can be seen. Anaphylaxis reaction in patients may cause symptoms as advanced as respiratory distress, cardiac arrhythmia or cardiac arrest [3, 6]. Anaphylaxis is a hypersensitivity reaction that rapidly develops and may result in death. An anaphylaxis reaction model may occur in the form of non-phase (monophasic) and biphasic (also known as delayed or late phase) [7].

Exposure to anaphylaxis reaction antigen occurs in 5-30 minutes through parenteral means and in first 2 hours through oral means. In some cases, a new attach may be developed in 2-24 hours following the complete recovery of initial attack. This condition is known as biphasic anaphylaxis. Biphasic anaphylaxis is seen on cases, where epinephrine treatment is delayed. Some studies have found a relationship between delayed epinephrine treatment and biphasic anaphylaxis [7, 8].

In our case, patient has experienced mild shortness of breath and hypotension in 10 minutes following the polidocanol injection. We have not encountered with any other finding in the examination. We have performed and monitored the patient for half an hour. In this period, shortness of breath has regressed and tension has reached normal levels. We have not considered the risk of anaphylaxis since there was no dramatic change in the condition of patient and symptoms have progressed mildly. We didn't do any treatment. However, approximately 3 hours later, via call doctor of another hospital in the same city has informed that the patient was admitted due to severe hypotension and dyspnea and tachycardia. It was understood from the information obtained from the patient that she did not come into contact with allergens in this period. It was found that the patient had a biphasic anaphylaxis reaction with the symptoms and findings.

Frequency of allergic reactions notified is around 0.6% following the sclerotherapy. Anaphylaxis reactions are uncommonly notified. In general, anaphylaxis reactions may occur due to hypersensitivity for any allergen. Currently, there is no method to identify the predisposed individuals and hence such adverse reactions are not preventable. However, patients implemented with multiple sclerosing agent may contain more risk of anaphylaxis. It is vitally important to implement epinephrine injection, liquid replacement and respiratory support for the emerging anaphylaxis [9].

Lee *et al*. [10] have notified that biphasic anaphylaxis are seen in the rate of 1/20 in meta-analysis, where they have assessed anaphylaxis patients comprised of 4,114 patients. It is notified in this meta-analysis that it is not possible to predetermine biphasic anaphylaxis in practical means and that hypotension developed in patients may be related with biphasic anaphylaxis. They have not specified any

concrete finding apart from that [10].

We believe that reason of failure to determine anaphylaxis reaction in the first phase is mild progression of symptoms and pain due to PFS procedure may be the reason for this condition. We have not performed epinephrine injection since we did not considered anaphylaxis in the first phase. We also believe that failure to implement epinephrine to the patient in the first phase has caused severe phase of biphasic anaphylaxis reaction. It must be considered that mild progression of first phase in biphasic anaphylaxis reaction may be related with possible anaphylaxis reaction even in rare conditions as occurred in our case. We believe that it may be beneficial to observe such patients for longer period. In the literature search we have conducted (MEDLINE, Google Scholar) we have not encountered with any literature study relating to content of ''biphasic anaphylaxis, polidocanol''. Post PFS anaphylaxis cases are rarely notified in the literature for the varicosity treatment [3, 9]. However, biphasic anaphylaxis cases are very rare as in our case and this report may be the first one in the literature. We believe that the our case is significant in this aspect.

CONCLUSION

This case presented here may be a very rare clinical condition in literature. In our opinion, nonspecific symptoms in the first moments after PFS may rarely be the first phase of the biphasic anaphylactic reaction. A slightly longer observation of such patients may prevent later biphasic anaphylaxis.

Informed consent

Written informed consent was obtained from the patient for publication of this case and any accompanying images.

Conflict of interest

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

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A compelling case of postpartum symmetrical peripheral gangrene

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ABSTRACT

Symmetrical peripheral gangrene is one of the very rare problems that is usually secondary to intense inotropic therapy in critically ill patients, but also due to use of ergot alkaloids or sepsis in the postpartum period. We present a case of symmetrical peripheral gangrene developed in lower extremities of a woman in postpartum period ten days after discharge from hospital to which she was admitted and transfused 2 units of erythrocyte suspension due to significant bleeding following delivery conducted at home by an unskilled midwife in Mogadishu, Somalia. After pregnancy, whatever the cause, if developing symmetrical peripheral gangrene is not diagnosed in time and not treated as early as possible can cause limb loss and disability. Detection and investigation of this condition will also be enlightening for the mechanisms for ischemia and subsequent recuperation of circulation.

Keywords: Ergotalkaloids, postpartum, symmetrical peripheral gangrene

Distal symmetrical peripheral gangrene, which is not a classical peripheral vascular disease, is one of the rare cases seen after delivery. Whether it is because of physiological changes in the peripheral vascular system during pregnancy (increased plasma volume, decreased plasma osmolality and decreased peripheral resistance) that actually obscure a severe vasospasm is not fully understood. However, ergot alkaloids used during childbirth, sepsis during or after delivery, and inotropic agents used in the treatment of septic shock are thought to be associated with distal symmetric peripheral gangrene [1, 2].

CASE PRESENTATION

A 34-year-old P2L2 female patient underwent 2 intermittent blood transfusions at the health center, where she was admitted for significant bleeding following delivery by an unskilled midwife at home in Somalia, where traditional methods are often preferred.The patient was discharged after controlling the bleeding and was admitted to our hospital 10 days later due to coldness in the feet, bruising on the skin, pain and progressive gangrene. The patient was hospitalized for examination and treatment. The general condition of the patient was good; vital signs were recorded as stable. All peripheral pulses were palpable. Black, wet-looking dried skin images were present on both dorsal and plantar sides of the feet (Fig. 1a and 1b). On abdominal examination a well contracted uterus was felt. There was no prior history of intermittent claudication, cold or heat intolerance, tobacco smoking, collagen vascular disease or similar family history.

Laboratory data at the time of admission showed

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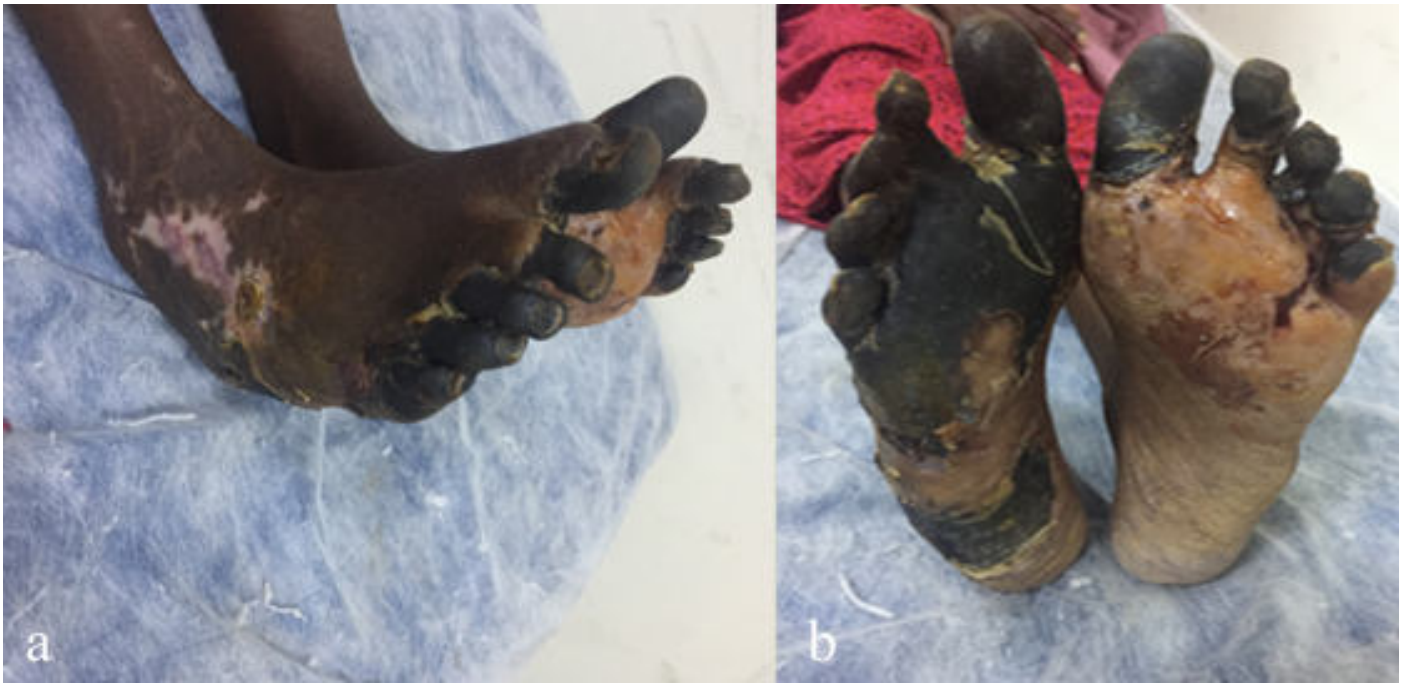

Fig. 1. Postpartum 10th day extremity appearance (a, b).

that hemoglobin (Hb) was 9.9 g/dL; hematocrit (Hct) 32.8%; white blood cell (WBC) count 12.470/mm3; platelet count 674.000/mm3; liver function tests: total serum bilirubin 8 mg/dL, alanine aminotransferase (ALT) 30 U/L, aspartate aminotransferase (AST) 37 U/L; and kidney function tests: serum creatinin 0.65 mg/dL and blood urea 27 mg/dL. Total protein was 6.9 g/dL; albumin 3.3 g/dL, and CRP 41.6 mg/dL. On sepsis work-up, her blood and urine cultures were sterile. On clotting screen prothrombin time (PT) was 12 sec-

Fig. 1. Postpartum 40th day right (a) and left (b) extremity appearance.

onds and partial thromboplastin time test kaolin (PTTK) 32 seconds. Lipid profile was as follows: Serum cholesterol 153 mg/dL, serum triglyceride 173 mg/dL, high-density lipoprotein cholesterol (HDL) 40 mg/dL, low-density lipoprotein cholesterol (LDL) 113 mg/dL. Amilase was 37 U/L, lipase 25 U/L, total IgE 357.5 IU/ml, alkaline phosphatase (ALP) 224 U/L, gamma glutamyltransferase (GGT) 132 U/L, lactate dehydrogenase (LDH) 436 U/L, antistreptolysin O (ASO) 326 IU/ml, rheumatoid factor (RF) 21 IU/ml. Color doppler ultrasound of the lower extremity vessels revealed three-phasic character of normal circulation. The biopsy of the gangrene tissue was consistent with epidermal necrosis. Medical treatment consisting of broad spectrum antibiotics, low molecular weight heparin and pentoxifylline was initiated. The necrotic appearance of the feet limited itself with treatment (Fig. 2a and 2b). The patient underwent regular follow-up after discharge and underwent amputation distaltothe the right ankle. The lesion on the left toes limited itself by auto amputation.

DISCUSSION

The development of postpartum gangrene during pregnancy has been reported, sporadically. However, in most of these, it has been emphasized that the existing peripheral vascular disorder may be caused by the use of ergot alkaloids used in relieving pain symptoms [1]. In another study, they found a relationship between the symmetrical peripheral gangrene after sepsis [3]. In addition, it has been mentioned that high doses of dopamine and noradrenaline, which can be used for inotropic support, may lead to decreased ischemia, and ultimately leading to gangrene, together with vasoconstriction [4, 5]. However, the extent of the effect of the use of these drugs has not been specified. It has not been denied that they may be accompanied by the contribution of common intravascular coagulation and development of disseminated intravascular coagulation (DIC) [6].

In our patient's history, she had no previous signs and symptoms of peripheral vascular disease, and she did not experience any similar complaints or discomfort after her previous delivery. In the postpartum period, symmetrical gangrene occurred in the distal part of both lower extremities 10 days after blood transfusion, which was not expected. It is stated that sporadic cases of distal gangrene can be seen after ergot alkaloids used for painless delivery and postpartum sepsis due to various reasons. In our case, the presence of blood transfusion historymay suggest the effect of transfusion. Although a variety of conditions have been observed from simple redness to renal failure and more severe conditions due to transfusion reaction, such cases of symmetrical gangrene have not been reported. A herbal or chemical agent that may have been used in traditional births may also play a role in the development of gangrene in our case.

CONCLUSION

In cases of peripheral gangrene developing postpartum due to various reasons, especially if the cause cannot be determined as in our case, having high index of suspicion during the onset of symptoms would prevent the necrosis to reach more proximal segments of the limb, and thus limb would be saved with early and proper institution of medical treatment. In addition, the detection and examination of this condition will be enlightening for the pathophysiological mechanisms of ischemia and the subsequent restoration of circulation.

Informed consent

Written informed consent was obtained from the patient for publication of this case and any accompanying images.

Conflict of interest

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

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