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Instructions to Authors

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2. Abstract
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ii. Walker M, Hull A. Preterm labor and birth. In: Taeusch HW, Ballard RA, eds. Avery's Diseases of the Newborn. Philadelphia: WB Saunders, 1998: 144,153.

iii. Hagström H, Nasr P, Ekstedt M, et al. Fibrosis stage but not NASH predicts mortality and time to development of severe liver disease in biopsy-proven NAFLD. J Hepatol 2017; 67: 1265-73. doi: 10.1016/j.jhep.2017.07.027.

iv. WONCA Ad Hoc Task Force on Tobacco Cessation.

http://globalfamilydoctor.com/publications/new/november/09.htm
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Original Article

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Bitter melon *(Momordica charantia)* fruit extract ameliorates methotrexate-induced reproductive toxicity in male rats

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ABSTRACT

Objective: Methotrexate (MTX) is a drug commonly used for the treatment of malign neoplastic and inflammatory diseases. Anti-oxidant and anti-inflammatory effects of bitter melon (BM) were reported. The aim of this study was to examine the effects of BM fruit extract on MTX-induced testicular and epididymal damage.

Materials and Methods: Sprague Dawley male rats were divided into three groups (n=8) as control, MTX and MTX+BM. A single dose of MTX (20 mg/kg) was injected intraperitoneally to the MTX and MTX+BM groups. BM fruit extract (600 mg/kg) was applied to the MTX+BM group orally for 5 days. Testes were examined for general histopathology, proliferating and apoptotic cells. The epididymis samples were used for the evaluation of sperm morphology. Oxidative and inflammatory markers were analysed biochemically.

Results: Increased abnormal spermatozoa, degenerated seminiferous tubules with increased apoptotic cells and decreased proliferative cells were observed in the MTX group. TNF-α, IL-1β, 8-hydroxy-2-deoxyguanosine and caspase-3 levels increased, superoxide dismutase and catalase levels decreased in both testis and epididymis samples. All these histological and biochemical parameters were ameliorated in the MTX+BM group.

Conclusion: Methotrexate causes testis damage by decreasing spermatogenic cells and increasing apoptosis through oxidative stress and inflammation. BM extract improves testis and epididymis damage with its possible anti-oxidant and anti-inflammatory effects.

Keywords: Methotrexate, Bitter melon, Testis, Epididymis, Oxidative stress

1. INTRODUCTION

Methotrexate (MTX), a folic acid anti-metabolite drug, is commonly used in the treatment of many clinical diseases such as romatoid arthritis, systemic lupus erythematosus, psoriasis and some malign neoplastic diseases [1]. Its use is limited due to its side effects including pansitopenia, infections, hepatotoxicity and neuropathy [2]. In addition, the male genital system is also adversely affected by MTX. An increase in degenerated seminiferous tubules with apoptotic cells and a decrease in proliferative cells in the germinal epithelium have been reported [1, 3-8]. Various mechanisms have been proposed to explain its side effects, but not yet fully elucidated. MTX induces apoptosis by causing DNA damage [1, 3-5, 7, 8], disrupting the oxidant and anti-oxidant balance [3-9] and increasing inflammation [5, 6, 10] thereby leading to toxicity in male reproductive systems.

Anti-oxidants and anti-inflammatory compounds such as folic and folinic acid [1], propolis [3], zinc [4], chrysin [5], protocateuchuic acid [6], vitamin B17 [7], apocynin [8] and agomelatine [10] have been indicated to prevent spermatogenic cells from MTX-induced male reproductive system damage. These studies have shown that anti-oxidants are thought to help protect testicular tissue against oxidative stress.

Momordica charantia, known as bitter melon (BM), is a vegetable belonging to Cucurbitaceae family. BM is grown in tropical and subtropical regions of many countries. This plant, rich in carbohydrates, proteins, fiber, minerals and vitamins, has been used for traditional treatment. Many parts of this plant (fruit, seed, root) are used in traditional treatments as well as being food. BM contains essential oils, flavonoids, phenolic acids, fatty acids, amino acids, lectins, etc. and they are responsible for its biological activity [11]. Many usefull effects of BM was reported such as anti-oxidant activity [12-14], anti-inflammatory effect [15, 16],

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anti-diabetic property [17, 18], wound healing effect [19, 20], antiviral activity [21] and anticancer effect [22-26]. Protective effects of BM have been reported on damaged tissues such as liver [27], brain [12, 16] and testis [17] in previous studies.

Yet, effects of BM against testis and epididymis damage induced by MTX have not been documented. So, the purpose of this study was to search the possible anti-oxidant and anti-inflammatory effects of BM fruit extract on testicular damage in MTX-induced rats.

2. MATERIAL and METHODS

Experimental animals

Twenty-four Sprague Dawley male rats (twelve weeks old) were purchased from the Marmara University Experimental Animals Research and Implementation Centre. They were transferred and kept in polycarbonate cages with soft rice husk bedding in a room controlled for ventilation (air exchange 18 time/hour), 12-h light-dark cycle, relative humidity (50-60%) and temperature (23-25ºC) during the study. The animals were given balanced food and water ad libitum. This study was approved by Marmara University, School of Medicine, Animal Care and Use Committee (08.2020.mar).

Preparation of the Bitter Melon Fruit Extracts

Bitter melon fruits were collected from the rural area of Umurbey, Gemlik district of Bursa, Turkey in August 2019. The botanical determination of the plant was made by Dr. Ismail Senkardes. The voucher specimens have been stored in the Herbarium of School of Pharmacy, Marmara University (MARE No: 22446). BM fruit extract was prepared in the Marmara University Pharmacognosy Department. Briefly, fresh fruits with seeds were smashed by blender, and then, were macerated by ethanol 95% (1000 mL) for 48 h. The extraction process was repeated until the solvent becomes colourless. The filtrate was evaporated and concentrated at 40 °C using a rotary evaporator. The dried ethanol extract, obtained with a yield of 5.35%, was stored at +4 °C until the analysis.

Experimental groups

The rats were randomly divided into three groups (n=8) as control, MTX and MTX+BM. A single dose of MTX (20 mg/kg) was applied to MTX and MTX+BM groups by intaperitoneal injection. MTX+BM group was given BM fruit extract dissolved in saline (600 mg/kg) by oral gavage for 5 days. BM fruit extract administration dose was decided according to a previous study of Subramani and Krishnamurthy (2019), that reported the therapeutic efficacy at a dose of 600 mg/kg [28]. The control and MTX groups were given saline for 5 days. The animals were decapitated and testis and epididymis samples were obtained at the end of the experiment.

Measurement of superoxide dismutase, catalase, caspase-3, 8-hydroxy-deoxyguanosine, interleukin-1β, and tumor necrosis factor – α levels

The superoxide dismutase (SOD), catalase (CAT), caspase-3, 8-hydroxy-deoxyguanosine (8-OhDG), interleukin-1β (IL-1β) and tumor necrosis factor (TNF)-α levels were measured in epididymis and testis homogenates by using commercial Enzyme Linked Immunosorbent Assay (ELISA) kits (Abbkine Inc. Wuhan, China). The results were given as ng/mg for CAT, 8-OhDG and IL-1β; U/mg for SOD; nmol pNA/mg for caspase-3 and pg/mg for TNF-α levels.

Morphological evaluation of epididymal sperm

The left caudal epididymis was cut into small pieces and a routine density gradient method was used to examine the sperm. For sedimentation, the supernatant was removed using 5 ml Earle's Balanced Salt Solution (Sigma, USA), then the pellet was diluted with sperm washing medium (SAGE, UK) and centrifuged. The pellet was then diluted with sperm preparation medium (SAGE, UK). Smears were stained with Diff-Quick kit (Medion Diagnostics, Grafelfing, Germany) for morphological evaluation. One hundred spermatozoa were evaluated under a photomicroscope at 400× magnification for tail, neck and head morphology [29].

Light microscopic preparation

The right testes were collected and fixed with 10% neutral buffered formalin and processed for routine paraffin embedding technique. Paraffin sections (5 µm) were stained with hematoxylin and eosin (H&E) for general morphological evaluation, periodic acid Schiff (PAS) reaction for evaluation of basement membrane and evaluated under a photomicroscope (Olympus BX51, Tokyo, Japan). In each section, 20 seminiferous tubules were measured for germinal epithelium thickness and evaluated with histopathological Johnsen's score [30] at 200x magnification. Each tubule was scaled from 1 (absence of germinal epithelium) to 10 (complete spermatogenesis) according to the epithelial maturation.

Proliferating cell nuclear antigen immunohistochemistry analysis

Paraffin sections (4 µm) were placed in a 57 °C oven for 1 hour, then deparaffinized with xylene. After decreasing series of alcohol, they were put into 3% hydrogen peroxide solution. They were boiled in citrate buffer (10 mM; pH 6.0; 20 min) for antigen retrivial. After washing in phosphate buffered saline (PBS), sections were incubated with blocking solution (SensiTek HRP Anti-Polyvalent Lab Pack, AEM080). Sections were incubated overnight (4 °C) with rabbit anti-proliferation cell nuclear antigen (PCNA) primary polyclonal antibody (ab152112, Abcam, Cambridge, UK) in a humidified chamber. Slides were washed with PBS and incubated with biotinylated secondary antibody (20 min). After washing with PBS, the sections were placed in streptavidin peroxidase (20 min), washed with PBS, and applied 3, 3-diaminobenzidine tetrahydrochloride dihydrate (DAB) chromogen and counterstained with hematoxylin. In each section, PCNA-positive and negative cells were counted in 20 seminiferous tubules. The proliferation index was calculated by the percentage of PCNA-positive spermatogonia (PCNA-positive spermatogonia / total number of spermatogonia) in each seminiferous tubule.

Terminal deoxynucleotidyl transferase dUTP nick end labelling analysis

The terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) method was used according to the instructions of kit (Apoptag Kit Plus Peroxidase, In Situ Apoptosis Detection Kit, Millipore, S7101, Temecula, CA, USA). Paraffin sections were counterstained with hematoxylin. In each section, TUNEL-positive cells were counted in 20 seminiferous tubules. Apoptotic index was calculated according to tubules which is containing three or more TUNEL-positive cells were reported as the percentage of twenty tubules.

Statistical Analysis

One-way ANOVA analysis of variance was used for data analysis and Tukey's multiple comparisons test was used for determination of differences between groups. The results were represented as mean ± standard deviation (SD) using Graph Pad Prism Version 8.0 (Graph Pad Software, San Diego, USA). Significant difference was considered at the level of $p<0.05$.

3. RESULTS

Results of SOD, CAT, caspase-3, 8-OHdG, IL – 1 β, and TNF – α levels

SOD ($p<0.001$) and CAT ($p<0.001$) levels reduced, 8-OHdG ($p<0.001$), caspase-3 ($p<0.01$), TNF-α ($p<0.001$) and IL-1β ($p<0.01$) levels increased in epididymis samples of the MTX group compared to the control group. However, SOD ($p<0.05$) and CAT ($p<0.05$) levels were higher, 8-OHdG ($p<0.01$), caspase-3 ($p<0.01$), TNF-α ($p<0.05$) and IL-1β ($p<0.01$) levels were lower in the MTX+BM group than the MTX group (Figure 1).

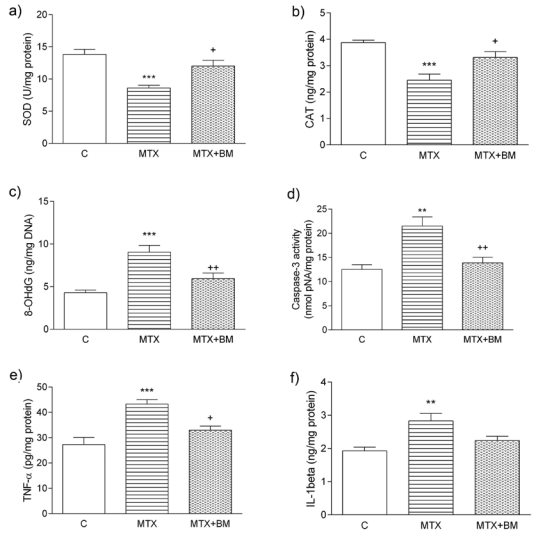

Figure 1. SOD (a), CAT (b), 8-OHdG (c), caspase-3 (d), TNF-α (e) and IL-1b (f) levels of epididymal tissues are seen in the experimental groups. *** p<0.01 and *** p<0.001 compared to control (C) group, + p<0.05 and ++ p<0.01 compared to MTX+BM group.*

SOD ($p<0.001$) and CAT ($p<0.01$) levels decreased, 8-OHdG ($p<0.001$), caspase-3 ($p<0.001$), TNF-α ($p<0.001$) and IL-1β ($p<0.01$) levels increased in the testis of the MTX group compared to the control group. But, SOD ($p<0.05$) and CAT ($p<0.05$) levels increased, 8-OHdG ($p<0.01$), caspase-3 ($p<0.01$), TNF-α ($p<0.01$) and IL-1β ($p<0.01$) levels decreased in the MTX+BM group compared to the MTX group (Figure 2).

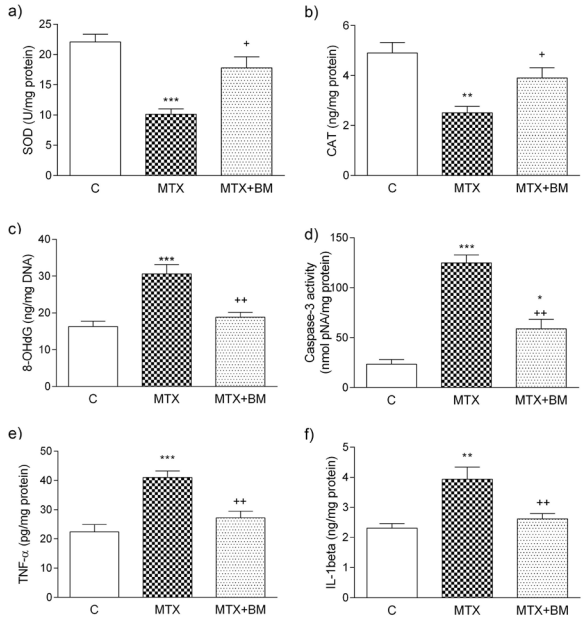

Figure 2 SOD (a), CAT (b), 8-OHdG (c), caspase-3 (d), TNF-α (e) and IL-1b (f) levels of testis tissues are seen in the experimental groups. ** p<0.05, ** p<0.01, *** p<0.001 compared to control (C) group. + p<0.05, ++ p<0.01 compared to MTX group.*

Histopathological results

According to our Diff Quick stained epididymal sperm morphological analysis, normal and abnormal sperms with tail, neck and head defects were seen in each of the experimental groups (Figure 3 A_1-C_1). Spermatozoa with head ($p<0.001$), neck ($p<0.05$) and tail ($p<0.05$) abnormalities increased and normal spermaotozoa decreased ($p<0.001$) in the MTX group compared to the control group. However, spermatozoa with head ($p<0.01$) and neck abnormalities decreased and spermatozoa with tail abnormalities ($p<0.05$) increased in the MTX+BM group compared to the MTX group (Figure 3 D_1) .

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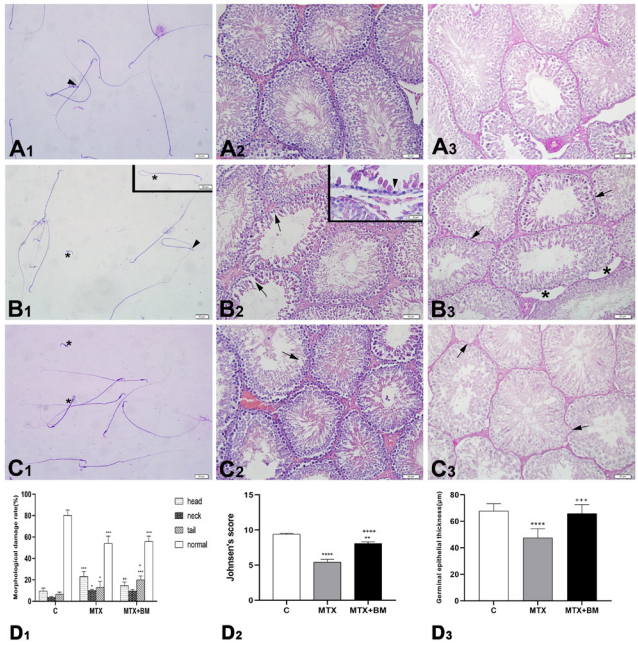

Figure 3. Representative light micrographs of spermatozoa (A_1-C_1), testis (A_2-C_3), sperm morphologic damage ratio (D_1), germinal epithelium thickness (D_2) and Johnsen's histopathological scores (D_3) are seen in the experimental groups. *Normal spermatozoa and spermatozoa with head (arrow head) and tail (*) anomalies are seen in the control (A_1), MTX (B_1) and MTX+BM (C_1) groups. Normal seminiferous tubules with germinal epithelium (A_2) and regular PAS positive basement membrane (A_3) are seen in the control group. Degenerated seminiferous tubules with dilatation of the germinal epithelial cells (arrow), decreased number of germinal epithelial cells (arrowhead) are seen in MTX group (B_2). PAS positive stained irregular basement membrane (arrow) and large vacuol formation in between the seminiferous tubules (asterisk) are seen in this group (B_3). Normal seminiferous tubules and some degenerated tubules with dilatation of germinal epithelium (arrow) are seen in MTX+BM group. PAS positive irregular basement membrane in some area (arrow) are seen in this group (C_3). A_1-C_1: Diff-Quick staining. Scale bar: 20 µm. A_2-C_2: H&E staining. A_3-C_3: PAS staining. Scale bar: 50 µm, inset B_2: 20 µm. * p<0.05 , ** p<0.01, *** p<0.001 and **** p<0.0001 compared to control (C) group. + p<0.05 , ++ p<0.01, +++ p<0.001 and ++++ p<0.0001 compared to MTX group.*

Regular seminiferous tubules with basement membrane, spermatogenic and Sertoli cells and sperms in lumen were seen in the control group (Figure 3 A_2, A_3). Degenerated seminiferous tubules with dilatations of the germinal epithelium, reduction of the germinal cell line, irregular basement membrane and large dilatations between the seminiferous tubules were observed in the MTX group (Figure 3 B_2, B_3). Although, some dilatations were found in seminiferous tubules, quite normal appearing seminiferous tubules were observed in the MTX+BM group (Figure 3 C_2, C_3). Johnsen's score (p<0.001) and germinal epithelium thickness (p<0.001) decreased in the MTX group compared to the control group. However, Johnsen's score (p<0.001) and germinal epithelium thickness (p<0.0001) increased in the MTX+BM group compared to the MTX group (Figure 3 D_2, D_3).

PCNA immunohistochemistry results

PCNA-positive cells were observed as dark brown colour in seminiferous tubules of the experimental groups (Figure 4 A_1-C_1). Proliferation index was lower (p<0.0001) in the MTX and MTX+BM groups compared to the control group (Figure 4 D_1).

TUNEL results

TUNEL-positive cells were seen as dark brown colour in all of the experimental groups (Figure 4A_2-C_2). However, the number of TUNEL positive cells was the highest in the MTX group (Figure 4 B_2). Apoptotic index was higher in the MTX group (p<0.0001) compared to the control group and this apoptotic index value was lower in the MTX+BM group (p<0.0001) compared to the MTX group (Figure 4 D_2).

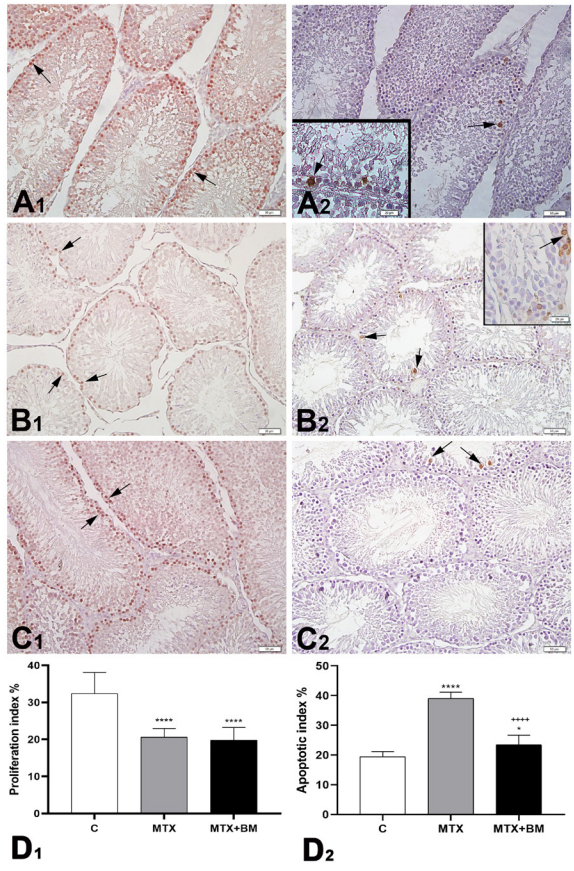

Figure 4. Representative light micrographs of PCNA-immunostained (A_1-C_1) and TUNEL-stained (A_2-C_2) testis samples, proliferation index (D_1) and apoptotic index (D_2) are seen in the experimental groups. *Normal seminiferous tubules with numerous PCNA-positive (arrow, A_1), a few number of TUNEL-positive (arrow, A_2) spermatogenic cells are seen in the control group. A few number of PCNA-positive (arrow, B_1), increased number of TUNEL-positive (arrow, B_2) spermatogenic cell are seen in the MTX group. Normal seminiferous tubules with many PCNA-positive cells (arrow, C_1), but decrease number of TUNEL-positive (arrow, C_2) spermatogenic cells are seen in the MTX+BM group. Scale bar: 50 µm; insets in A_2 and B_2: 20 µm. * p<0.05 and **** p<0.0001 compared to control group, ++++ p<0.0001 compared to MTX group.*

4. DISCUSSION

In the present study, the ameliorating effects of BM on MTX induced testicular and epididymal damage were demonstrated by histological, immunohistochemical and biochemical methods. The results of this study showed that MTX induction caused changes in testicular morphology with decreasing proliferative cells and increasing apoptotic cells, increase of abnormal spermatozoa, testicular and epididymal oxidative stress and inflammation. BM fruit extract treatment ameliorated these histopathological and biochemical alterations in MTX-induced testis and epididymis damage by its anti-oxidant and anti-inflammatory properties.

Chemotherapeutic agents are used for treatment but they might cause excessive reactive oxigen species (ROS) accumulation. ROS increase causes infertility by disrupting the process of the male genital system. Therefore, anti-oxidant compounds are needed to protect the body from oxidative stress damage [29]. It was shown that the oxygen radical level of testis, epididymis and semen fluid are important and critical to preserve sperm vitality and function. Increase of ROS level in these tissues causes DNA damage or lipid peroxidation in spermatozoa; thus sperm capacitation, maturation and even vitality are negatively affected and fertility is reduced [31]. MTX is also a chemotherapeutic agent which is used in monotherapy or combined with other medicines for treatments of inflammatory and some malign neoplastic diseases [1, 2]. A decrease in the activities of antioxidant endogenous enzymes SOD and CAT has been shown in MTX-induced testicular damage [2, 5, 7, 11]. Benefical effects of BM such as anti-oxidant [12, 14], anti-inflammatory [15, 16] and anti-apoptotic activity, have been reported [17]. It was observed that BM aqueous extract reduced mitochondrial ROS in mice with restriction stress and prevented liver damage through its anti-inflammatory effect by reducing inducible nitric oxide synthase and the most protective effect of BM extact was found in 750 mg/kg dose [27]. In an another study, oral administration of BM fruit ethanolic extract in 250 and 500 mg/kg doses to diabetes induced testis damage showed anti-oxidant and anti-apoptotic effects, but BM extract was more protective in high doses [17]. In parallel with previous studies, it was found that oral administration of BM extract at a dose of 600 mg/kg has been found to have anti-oxidant, anti-apoptotic and anti-inflammatory effects in MTX-induced testicular and epididymis damage.

Methotrexate treatment increases ROS and lowers anti-oxidant defense, which results in increase in inflammation-related markers. Persistent inflammation has been shown to produce more ROS, which worsens oxidative damage [1, 2, 4-8]. TNF-α and IL-1β are cytokines that cause inflammatory reaction and control the inflammatory process. Some studies, have shown that MTX increases the levels of TNF-α and IL-1β in MTX-induced testis and epididymis damage in rats [7, 8]. BM has been shown to prevent depressive-like behaviors in mice by reducing proinflammatory cytokines such as TNF-α and IL-1β levels in hippocampus [19]. In another study, BM was shown to reduce mitochondrial ROS in mice with restriction stress and prevented liver damage through its anti-inflammatory effect by reducing inducible nitric oxide synthase [30]. In this study, TNF-α and IL-1β levels rised in both testis and epididymis samples in MTX group, so our study showed that MTX-induced damage in those tissues was associated with inflammation. BM treatment decreased these inflammatory markers to the control levels in both testis and epididymis samples.

8-OHdG which is formed by ROS in damaged tissue, is a marker of DNA damage. Previous studies have shown that MTX treatment increases 8-OHdG in testis tissue [5]. Caspase-3 is the main executor caspase in the apoptotic pathway, its increase suggests apoptosis activation. A study has been shown to increase caspase-3 activity in the testis of animals treated with MTX [8]. Moreover, it has been shown that there is an increase in the number of apoptotic cells in the testis in experimental animals administered MTX [1, 4, 5]. In diabetic rats, BM has been shown to increase testicular anti-oxidant enzymes, reduce caspase-3 activity and inhibit apoptosis, resulting in histopathological improvement [20]. BM has been shown to inhibit the activation of JNK3/c-Jun/Fas-L and JNK3/cytochrome C/caspase-3 signaling cascades and prevent apoptosis due to its anti-oxidant properties during cerebral ischemia/reperfusion injury [15]. In an in vitro study, it has been shown that MTX causes apoptosis and autophagy in spermatocyte cell line via formation of ROS [10]. Parallel to these findings, an increase in 8-OHdG level and caspase-3 activity in the epididymis and testis samples and an increase in apoptotic cells in the testis in MTX group has been observed in our study. However, BM treatment in MTX applied rats reduced caspase-3 activity and 8-OHdG level in both the testis and epididymis samples and apoptotic index in the testis samples.

It was reported that chemotherapeutic agents cause male genital system toxicity such as damage to the seminiferous tubules of the testis and epididymis, reducing sperm count and leading to genetic mutations in sperms [1, 3-5]. It has been shown in many studies that these harmful effects are caused by oxidant damage [3-6, 8-10], inflammation [5, 6, 10], DNA damage and apoptosis [1, 3-7] and decreased FSH, LH and testosteron hormone levels in the serum [4, 7, 8, 10, 17]. Previous studies have shown that MTX causes a decrease in proliferation [3, 4], an increase in apoptosis and DNA damage in the testis, also it decreases the number of spermatozoa and increases the number of abnormal spermatozoa [1, 3-5]. Parallel to the previous studies, we have observed decrease in endogeneous anti-oxidant SOD and CAT activities, as well as an increase in caspase-3 activity and 8-OHdG level in both the testis and epididymis samples in the MTX group. Histological findings showed that increased degenerated seminiferous tubules with a decrease in proliferative cells and an increase in apoptotic cells and increased abnormal spermatozoa were present in the MTX group. BM extract amelioriated MTX-induced testicular and epididymal damage via increase of endogenous anti-oxidant level and inhibition of apoptotic activity.

As a result, MTX is used for the treatment of many diseases, but it has side effects on male genital system toxicity. MTX leads to a decrease in anti-oxidant defence, an increase in inflammation and DNA damage in both testis and epididymis. Moreover, MTX

causes damage in the seminiferous tubules of the testes with a decrease in the germinal epithelium thickness and proliferation. It also increases apoptosis and abnormal spermatozoa. Our study showed that aqueous fruit extract of BM protects MTX-induced testicular and epididymal damage in rats by its anti-oxidant, anti-apoptotic and anti-inflammatory response. The use of aqueous fruit extract of bitter melon may be an effective way to ameliorate potential male fertility in the MTX-induced damage. The limitation of this study is a need for further studies with different dose and time adjustments to obtain more accurate results.

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Compliance with Ethical Standards

Ethical Approval: This study was approved by Marmara University, School of Medicine, Animal Care and Use Committee (08.2020. mar).

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

Author Contributions: F.K., D.O., G.S. and F.E. contributed to the conception and design. F.K., D.O., A.S., O.C., G.S. and F.E. performed experiments and did data collection, F.K., G.S. and F.E. analyzed data. F.K., G.S. and F.E. contributed to the writing and F.E. did the critical revision of the article. All authors approved the final version of the article.

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The natural course of gastric intestinal metaplasia in Turkish patients: A single-center observational cohort study

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ABSTRACT

Objective: Gastric intestinal metaplasia (GIM) is considered as a predisposing lesion for the development of gastric cancer and is recommended to be kept under surveillance in designated intervals. We aimed to assess the natural course of GIM in a large Turkish cohort.

Materials and Methods: We retrospectively reviewed findings from pathology reports of gastric biopsies conducted between 2011 to 2018 to reveal patients diagnosed with solitary GIM in their index pathology report. Progression of GIM was pre-defined as; low-grade dysplasia (LGD), high-grade dysplasia (HGD), or gastric malignancy.

Results: The median follow-up period of the study population was 34 (12-128) months. Out of 109 patients with GIM at the entry, 54 (49.6%) patients had stable GIM, whereas 53 (48.6%) cases had no signs of GIM at their final endoscopy. Only two (1.8%) patients progressed to LGD, but no HGD or malignancy was detected in the follow-up.

Conclusion: Although, considered as a premalignant lesion and offered surveillance globally, progression of GIM was very low in a large Turkish cohort. Further prospective studies in larger cohorts are required to enlighten the obscure strategies in the surveillance of gastric malignancy.

Keywords: Gastric intestinal metaplasia, Gastric cancer, Dysplasia, Surveillance

1. INTRODUCTION

Gastric intestinal metaplasia (GIM), defined as the replacement of the gastric mucosa by the intestinal mucosa, is a well-established precursor lesion for gastric cancer development [1]. The major risk factor for GIM development was shown to be *Helicobacter pylori (H. pylori)* infection with a 3 to 8 fold increased risk compared to the uninfected population [2]. The remaining potential risk factors are known as older age, male gender, low socioeconomic status, and smoking status [3,4]. Since, GIM is usually asymptomatic and found incidentally in patients undergoing upper gastrointestinal (GI) endoscopy, the exact incidence of GIM remains skeptical. The incidence of GIM was suggested to be about 25% for patients undergoing upper GI endoscopy, whereas it differed from 9% to 29.3% in previous reports from East Asia [5-7]. In 2015, the prevalence of GIM in Turkey was reported as 13.8% with the predominance of incomplete subtype [8].

Patients with GIM demonstrate a 6 to 9 fold higher risk of gastric cancer compared with the general population [9,10]. The development of gastric cancer is generally considered as a multistep process including sequential changes of the gastric mucosa from non-atrophic gastritis to atrophic gastritis, GIM, dysplasia, and finally cancer. *H. pylori* is generally thought to be responsible for pulling the trigger of this carcinogenic process [11]. However, it is still an undetermined issue as to whether all patients with GIM require a strict endoscopic surveillance program despite the fact that gastric cancer usually arises with

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concomitant GIM [12]. In 2019, the updated international guideline (Management of precancerous conditions and lesions of the stomach-MAPS-II) recommended endoscopic surveillance for those with extensive GIM located in antrum and corpus as well, or single location but with a family history or incomplete subtype with persistent *H. pylori* gastritis [13]. However, even in those suggested subgroups, the majority remains stable or show regression that may be either true regression mainly related to *H. pylori* eradication or pseudo-regression due to sampling and interobserver variation in histologic examination [14].

The present study aimed to investigate the natural course of GIMs in Turkish patients for the first time and expose the proportion of patients with progression to dysplasia or invasive carcinoma. Moreover, the histological changes in the characteristics of GIM throughout the follow-up period are investigated as well.

2. MATERIALS and METHODS

Patient selection and data collection

We retrospectively reviewed findings from 22.465 pathology reports of gastric biopsies conducted between 2011 to 2018 to reveal patients diagnosed with GIM in their index pathology report (n=372). All upper GI procedures and histopathologic evaluation were performed at a tertiary center with a busy endoscopic practice. Patients lost to follow-up (n=203), having inconsistent surveillance intervals lower than one year (n=25), lack of data (n=15), low-grade dysplasia (LGD) (n=8), high-grade dysplasia (HGD) (n=1) or gastric malignancy (n=6) at the initial screening or gastrectomy operation at the entry (n=2) were excluded. As a result, all patients over the age of 18 with GIM in their index upper GI endoscopy pathology report who underwent at least one surveillance upper GI endoscopy after the index endoscopy (n=109) were included for the analysis. The flow diagram of the study is presented in Figure 1.

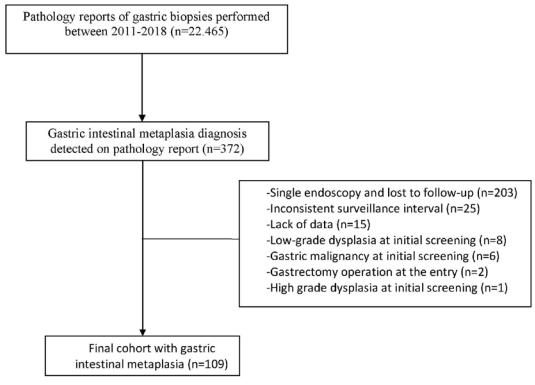

Figure 1. *Flow diagram of the study*

The demographics, details of initial upper GI endoscopy, number of biopsies taken, and histopathologic characteristics were provided from the electronic hospital database. The symptoms and/or findings leading to index upper GI endoscopy were mainly dyspepsia, epigastric pain, diarrhea, loss of weight, iron deficiency anemia, and a family history of GI malignancy.

Progression of GIM was defined as; LGD, HGD, or gastric malignancy. The follow-up time was calculated from the date of index endoscopy to the date of final endoscopy. Patients with the progression of GIM were censored for follow-up at the time of detection of progression, otherwise, patients were censored at the time of final upper GI endoscopy obtained from the hospital database.

Upper GI endoscopy procedure and histopathologic evaluation

All endoscopies were performed using a standard forward-viewing video-gastroscope (PENTAX Medical, New Jersey, USA). Both index endoscopies and surveillance endoscopies were performed by the experienced endoscopists in the same tertiary center using the local protocol with at least two biopsied samples from the antrum (including incisura angularis) and corpus. The number of biopsies may have increased based on the visible lesions observed in the upper GI endoscopy or the physician's preference.

Biopsy samples were fixed with formalin in paraffin blocks and stained with hematoxylin & eosin. All biopsy samples were evaluated by an experienced gastrointestinal pathologist who has experience for more than 10 years in this field.

The study followed the tenets of the Helsinki Declaration and it was approved by the local Ethics Committee (Protocol No: 09.2019.808) of Marmara University, School of Medicine. Owing to the retrospective nature of the study, the need for informed consent was waived.

Statistical analysis

The analysis was primarily descriptive. Data are reported as number (%) of patients unless indicated otherwise. All statistical analyses were conducted using the SPSS software version 20.0 (IBM, Armonk, NY, USA).

3. RESULTS

Through a careful investigation of 22.465 gastric pathology reports and exclusion consistent with the aforementioned criteria, 109 patients had GIM at their index endoscopy and were followed up for a period of 34 (12-128) months. Among them, 62 (56.9) were female and the mean age of the study population at the entry was 61.3 ± 11.6 years. The features of index endoscopies are presented in Table I. Diagnosis of index upper GI endoscopy was antral gastritis in the majority (n=74, 67.9%), followed by atrophic gastritis (n=17, 15.6%), pangastritis (n=5, 4.6%), erosive gastritis (n=5, 4.6%), antral ulcer (n=3, 2.8) and duodenal ulcer (n=3, 2.8). Only one (0.9%) patient was reported as normal upper GI endoscopy and one other (0.9%) had gastric polyp located in the antrum. The median number of biopsies taken in the index endoscopy was 2 [2-8]. The localization of GIM in the index endoscopy was dominantly

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antrum (n=43, 39.4%), followed by corpus (n=13, 11.9%) and both of each (n=9, 8. 3%). The type of GIM was incomplete in 37 (33.9%), complete in 26 (23.9%), and the combination of both in 46 (42.2%) at the index endoscopy. The involvement pattern of GIM was focal in 72 (66.1%) patients and diffuse in 37 (33.9%) cases. Out of 109 analyzed patients, 29 (26.6%) had *H. pylori* at their index endoscopy.

Table I. *Endoscopic and histologic findings of patients at the entry and final*

	Initial Findings	Final Findings
Endoscopic diagnosis, *n (%)*		
Normal	1 (0.9)	-
Antral gastritis	74 (67.9)	73 (67)
Pangastritis	5 (4.6)	13 (11.9)
Atrophic gastritis	17 (15.6)	9 (8.3)
Erosive gastritis	5 (4.6)	7 (6.4)
Antral ulcer	3 (2.8)	1 (0.9)
Duodenal ulcer	3 (2.8)	2 (1.8)
Antral + Duodenal ulcer	-	1 (0.9)
Gastric polyp	1 (0.9)	3 (2.8)
Number of biopsy specimens, med (min-max)	2 (2-8)	2 (2-7)
Intestinal metaplasia localization, *n (%)*		
Antrum	43 (39.4)	29 (51.8)
Corpus	13 (11.9)	14 (25)
Antrum-corpus	9 (8.3)	4 (7.1)
Unspecified	44 (40.4)	9 (16.1)
Histologic duodenitis, n (%)	2 (1.8)	2 (1.8)
Histologic atrophy, n (%)	36 (33)	41 (37.6)
Histologic gastritis, n (%)	97 (89)	97 (89)
Helicobacter pylori, n (%)	29 (26.6)	9 (8.3)
Metaplasia type, n (%)		
None	-	53 (48.6)
Incomplete	37 (33.9)	12 (11)
Complete	26 (23.9)	22 (20.2)
Incomplete + Complete	46 (42.2)	22 (20.2)
Metaplasia involvement, n (%)		
Focal	72 (66.1)	42 (75)
Diffuse	37 (33.9)	14 (25)
Follicular hyperplasia, n (%)	26 (23.9)	25 (22.9)
Lymphoid aggregate, n (%)	14 (12.8)	16 (14.7)
Neuroendocrine cell hyperplasia, n (%)	6 (5.5)	3 (2.8)

Characteristics of the final endoscopies are presented in Table I as well. In the final endoscopies, the distribution of endoscopic diagnoses was quite similar with dominance of antral gastritis (n=73, 67%) followed by pangastritis (n=13, 11.9%), atrophic gastritis (n=9, 8.3%), erosive gastritis (n=7, 6.4%), antral and/or duodenal ulcer (n=4, 3.6%). Two more patients were found to have gastric polyps at their final endoscopies. The median number of biopsies taken in the final endoscopy was 2 [2-7]. The localization of GIM in the final endoscopy was dominantly antrum (n=29, 51.8%), followed by corpus (n=14, 25%) and both of each (n=4, 7.1%). Approximately, half of the study population (n=53, 48.6%) were found to have no GIM in their final endoscopy. The type of GIM in the remaining was as follows; complete in 22 (20.2%), incomplete in 12 (11%), and combination of both in 22 (20.2). The involvement pattern of GIM at the final endoscopy was focal in 42 (75%) patients and diffuse in 14 (25%) cases. The number of detected patients with *H. pylori* decreased to 9 (8.3%) in the final endoscopy, mainly due to treatment.

Fifty-four (49.6%) patients had stable GIM in a median follow-up period of 30 (12-97) months, whereas 53 (48.6%) cases had no signs of GIM at their final endoscopy in a median follow-up period of 39 (13-87) months (Figure 2). Out of 109 reviewed patients with solitary GIM at the entry, only two (1.8%) patients were progressed to LGD. Case-1 with detected LGD in her final endoscopy recruited to a repeat endoscopy in the following 6 months and 1 year and no signs of LGD were observed in both. On the other hand, Case-2 with detected LGD underwent endoscopic ultrasonography due to suspicious antral ulcer 3 months later and was biopsied again under endoscopic ultrasound guidance. The evaluation of the biopsy sample obtained under endoscopic ultrasound guidance showed that the LGD was regressed as well, and no signs of LGD was observed in the subsequent endoscopies. The details of the two cases with detected LGD is exhibited in Table II.

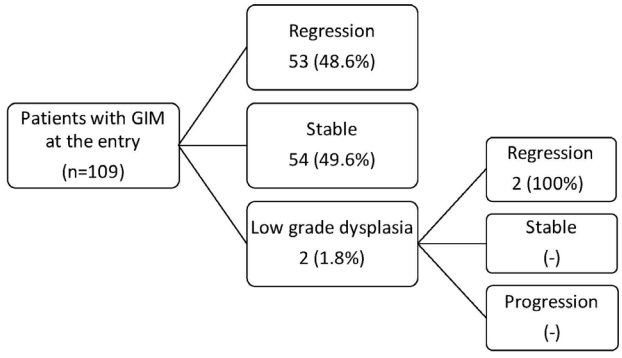

Figure 2. *Natural course of gastric intestinal metaplasia in Turkish patients*

GIM: Gastric intestinal metaplasia

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Table II. *Details of 2 cases with detected low-grade dysplasia*

	Age	Gender	Initial Endoscopic Findings	Initial IM localization	Initial IM type /involvement	*H. Pylori* status	Time to progression(months)
Case-1	72	Male	Antral gastritis	Antrum	Incomplete/focal	(-)	54
Case-2	60	Female	Antral gastritis	Antrum+Corpus	Incomplete+Complete/ diffuse	(-)	129

H. pylori: Helicobacter pylori

4. DISCUSSION

The present study has demonstrated the general non-progressive disease course of GIM in the majority of patients during an average 3 years follow-up period. To the best of our knowledge, this is the first study to evaluate the natural course of GIMs in the Turkish population. In total, only two patients showed progression to LGD, but none of them progressed to HGD or invasive carcinoma. The progression rate was comparable with a recent large European multicenter prospective cohort study conducted in low incidence regions [15]. Out of 279 patients with GIM, only 4 (1.4%) progressed to HGD or gastric cancer. The neoplastic progression ratio was 0.3% in that study which may be accepted as comparable considering the longer follow-up period of approximately 4.7 years and the larger size of their cohort. About two-thirds of their patients remained stable, while the remaining one-third were found to be regressed in the follow-up. In our study, nearly half of our patients remained stable, and the remaining half showed no signs of GIM in the follow-up endoscopy.

The lower rate of regressed GIM patients detected in our cohort may be caused by the unmeasurable pseudo-regression rates mainly due to sampling and histological examination differences. One other contributor to this issue may be the success of *H. pylori* eradication, which decreased the initial rate of 26% to a final rate of 8%. Another interesting finding of our study is that the rate of diffuse involvement pattern of GIM decreased from 34% to 25% throughout the study, in line with the reduction in *H. pylori* rates. Therewithal, an increase in the number of GIMs limited to antrum from 39.4% to 51.8% was also observed, which may be related to the decrease in *H. pylori* and the diffuse involvement pattern. In 2018, the reversibility of GIM and its association with *H. pylori* eradication has been shown in a large Korean cohort [16]. Out of 598 prospectively enrolled patients, significant improvement of GIM was only shown in the *H. pylori* eradicated group compared to *H. pylori*-negative and *H. pylori* non-eradicated group. Still, our observational findings require validation and explanation with further prospective studies with a larger number of patients and translational investigations.

Gastric cancer screening is recommended to a subset of GIMs and the intensity of the surveillance program is decided based on the criteria such as extension, complete/incomplete subtype, etc. Patients with extensive GIM both in the antrum and corpus are recommended to undergo gastric cancer screening every 3 years, while a stricter surveillance program is only recommended to those with a family history of gastric cancer or advanced stages of atrophic gastritis [13]. The majority of our patients underwent a screening endoscopy within 1 or 2 years, but none exceeded 3 years as recommended. Besides, we offered screening endoscopy to all patients with GIMs in our center and did not apply a selection criterion to enter the surveillance program. In our initial cohort, 39.4% would not have been candidates for gastric cancer surveillance according to the aforementioned guideline recommendations, as they were restricted to antrum only. The lack of selection criteria implementation at the entry may be another explanation for the very benign behavior of GIMs in our study. For instance, a retrospective study conducted in Thailand with 91 GIM patients and followed-up for 5 years, showed that none of the GIMs with complete subtype has progressed, whereas a progression rate of 50% was detected in incomplete GIM subtype [17]. In our cohort, 24% did not show the characteristics of the more aggressive incomplete subtype and had complete GIM at the entry.

There are several limitations to our study. First, this was a retrospective observational study conducted in a single tertiary center. The biopsy taking in our center was implemented by experienced endoscopists and generally in line with the Sydney protocol [18] throughout the study, and all specimens were evaluated by an experienced gastrointestinal pathologist. Nevertheless, the retrospective nature of the study prevented us from homogenizing the biopsy taking and histological evaluation process. Besides, not all screening endoscopies were implemented within the same intervals, but none has exceeded the 3-year time interval suggested by the MAPS-II guideline.

In conclusion, although, considered as a preneoplastic lesion and offered surveillance globally, progression to dysplasia or invasive carcinoma was very low in a large unselected Turkish GIM cohort. Further prospective studies in larger cohorts are required to enlighten the obscure strategies in the surveillance of gastric malignancy.

Compliance with Ethical Standards

Ethical Approval: The study followed the tenets of the Helsinki Declaration and it was approved by the local Ethics Committee (Protocol No: 09.2019.808) of Marmara University, School of

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Medicine. Owing to the retrospective nature of the study, the need for informed consent was waived.

Financial Support: No special funding was obtained.

Conflict of Interest Statement: There is no conflict of interest.

Authors' Contributions: C.O.D. : Drafting of the work. C.O.D., F.G. : Concept and design of the study. M.K., M.Y., M.Z.S., M.T.S., C.A.C. : Data acquisition. C.O.D. : Statistical analysis. C.A.C.: Reviewing pathologic specimens and interpretation of the results. All authors critically revised the manuscript, approved the final version to be published, and agreed to be accountable for all aspects of the work.

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The effects of melatonin on the striatum

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ABSTRACT

Objective: Some of the neurological diseases cause morphologic changes in the striatal neurons. Medial forebrain bundle (MFB) lesion is a commonly used method to produce a Parkinsonian model rat. Melatonin is a hormone which exerts a neuroprotective effect on the neurons. The aim of this study is to investigate the effect of melatonin on the dendritic morphology of striatal medium spiny neurons (MSNs) in rats with MFB lesion.

Materials and Methods: Twelve male Wistar albino rats were given saline injections into the MFB and divided into sedentary and treatment groups. The treatment group was administered a 10 mg/kg dose of melatonin intraperitoneally for 30 days. The lesion was confirmed histologically by Nissl staining. Golgi staining technique was applied to observe neuronal morphology. Neuronal structures were analysed from three-dimensional images by Neurolucida (MBF Bioscience) software.

Results: The MFB lesion caused a reduction in the total dendritic length and in the number of dendritic endings. The melatonin enhanced the number of dendritic endings compared to the sedentary group. The melatonin led to an increase in the total spine density, spine densities of thin and mushroom types.

Conclusion: Melatonin improved the dendritic degeneration due to MFB lesion.

Keywords: Dendritic spine, Melatonin, Golgi, Neurolucida

1. INTRODUCTION

The dendritic spines are small fingerlike protrusions that form excitatory synaptic connections. The spine morphology adjusts the stability and strength of the synapse [1]. The dynamic structural property of spine has a crucial role for synaptic plasticity [2]. Alterations in dendrite and spine morphology have been correlated with several neurological diseases [3,4].

The spine morphology has a wide diversity with different head and neck sizes. The spine types are classified according to the ratio of head and neck diameter such as; thin (ratio<2), mushroom (ratio>2), stubby (ratio<1) and also branched (two heads and one neck) types [1,5,6]. The mushroom type is a stable and least dynamic spine. The mushroom spine is able to have strong synaptic connections and also essential for memory storage [7]. The thin type is a more dynamic spine that represents a small synapse and is significant for learning [8]. The stubby type is a dynamic and immature spine. The stubby type may be a general precursor of more mature spine form of mushroom and thin types [9]. The branched type has bifurcated head with a neck which is rarely seen [10]. The morphological alterations of the spines reflect significant knowledge about the neuronal plasticity.

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone that is mainly synthesized in the pineal gland. Melatonin controls various physiological functions [11]. It also has anti-apoptotic, anti-tumor, and anti-oxidative properties [12,13]. Melatonin is highly lipophilic and passes across all morphological barriers (blood-brain barrier-BBB) and diffuses into all body fluids [14]. The disrupted BBB leads to the inflammatory reaction and neuronal cell damage that demonstrates the development or progression of central nervous system (CNS) diseases [15,16]. Melatonin reveales its beneficial effects in acute and chronic inflammatory processes [17,18].

The recent techniques enable to observe a more detailed neuronal morphology. Three-dimensional computer-based microscopical systems provide fundamental opportunity to attain quantitative

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information of neuron morphology and pathological changes. The Neurolucida is a commonly used software that facilitates different laboratories to standardize the parameters of somato-dendritic morphological data [19].

The striatum is a part of the basal ganglia. It contains medium spiny neurons (MSNs) and a few interneurons [20]. Neurodegenerative diseases like Parkinson's disease lead to degeneration of the striatal neurons. In experimental models, medial forebrain bundle (MFB) is a common target to induce 6-hydroxydopamine (6-OHDA) lesion to produce a Parkinsonian model rat. Many electrophysiological studies showed that the 6-OHDA lesion in the nigrostriatal pathway led to functional loss of striatal neurons and interneurons. After the formation of the lesion, the inhibitory effect of GABAergic interneurons against the MSNs increased [21]. The impairment of striatal neurons showed motor, cognitive and behavioral symptoms that were correlated with the functional and morphological deterioration [22].

In view of high technology, spine morphology alterations reflect different functions. In the current study, we aimed to investigate the effects of melatonin on the dendrite and spine morphology of striatal neurons after minimal MFB lesion.

2. MATERIALS and METHODS

Animals and Groups

Twelve male Wistar albino rats (weighing 250-300 g, 9 weeks of age) were used in this study. The rats were obtained from Marmara University Experimental Animal Research Center. All experimental procedures were approved by the Ethics Committee for Animal Experimentation of Marmara University (42.2021mar, 3.15.2021).

All animals were maintained in cages (2 rats per cage) in a room with controlled temperature (20°C±2°C) under a 12-h light/dark cycle with standard rat feed and water supply. The rats were randomly divided into 2 groups (6 in each group); MFB injection + sedentary and MFB injection + melatonin.

Animal Model

Stereotaxic surgery was performed to inject saline into the MFB. A mixture of ketamine (100 mg/kg) and xylazine (50 mg/kg) were administered intraperitoneally to anaesthetize the rats. The coordinates of right MFB were the rostral AP (anterior posterior): – 2.1, L: 2.0, V: – 7.8 (in mm) and caudal AP: – 4.3, L: 1.5 V: – 7.8 [23]. A total of 8 µl 0.9% saline were injected at a rate of 1 µl/min using a Hamilton syringe (Stoelting/10µl) and an infusion pump (KdScientific, MA, USA). At the end of the injection, the syringe was left in the MFB for 5 minutes. Then, it was pulled back slowly. After the procedure, 5 ml of saline (0.9% NaCl) was injected subcutaneously.

Melatonin Preparation

Melatonin (Sigma, St Louis, MO, USA, M5250) was dissolved in ethanol, and diluted in saline solution (5:95). The final concentration of the melatonin was 10 mg/ml. The melatonin was administered intraperitoneally at a dose of 10 mg/kg at the same day time (for 30 days). The drug was prepared freshly before the injection.

Tissue Collection and Golgi Staining

The rats were killed by transcardial perfusion with 0.9% saline followed by 4% paraformaldehyde (PFA) (Merck, Darmstadt, Germany), (0.1 M phosphate buffer, pH 7.4) after deeply anesthetized with ketamine (100 mg/kg, intraperitoneally). The brains were removed and sectioned for staining. In order to determine the accuracy of the MFB injection site, Nissl staining was performed and examined through a light microscope (Figure 1).

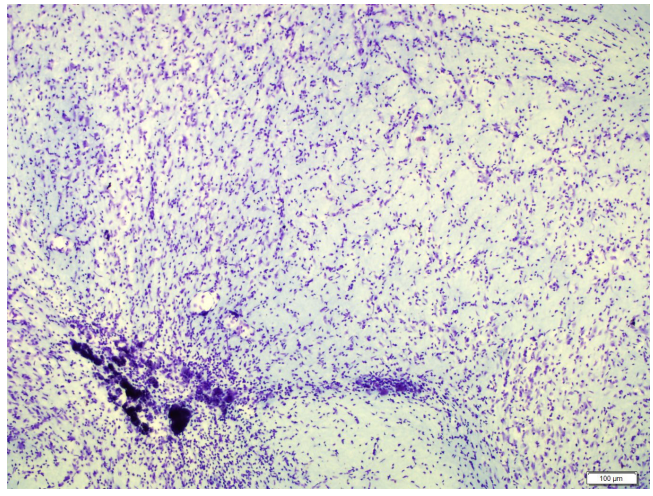

Figure 1. *Examination of the MFB injection site by Nissl staining using a 10 X objective under a light microscope.*

Golgi-cox staining technique was performed to observe the morphological features of the dendrite and the spine in detail [1]. FD Rapid GolgiStain Kit™ (FD NeuroTechnologies Inc. Elliot City, MD, USA) which is a commercially available Golgi staining kit was used according to the instructions. The kit contains standard A, B, C, D, E solutions. After perfusion, the brain tissues were rinsed with double distilled water then they were immersed in the impregnation solution, made by mixing equal volumes of solutions A and B (mercuric chloride, potassium dichromate and potassium chromate). Then, it was stored at room temperature for 3 weeks in the dark. The brain tissues were replaced in the impregnation solution (A and B again) after 24 hours then tissues were transferred into solution C and stored at room temperature in the dark for one week before slicing. The brain sections were cut in 100 µm on a cryostat (Leica Biosystems, CM 1950, USA) at –20°C. Each section was transferred with a glass specimen and mounted on gelatin-coated microscope slides with solution C and stored at room temperature in the dark until they were dried out. The slices were rinsed in double distilled water and then placed in a mixture consisting of 1 fraction solution D,

1 fraction solution E and 2 fractions of double distilled water for 10 minutes. After sections were rinsed in double distilled water, slices were dehydrated in series of 50%, 75%, 95% and 100% ethanol respectively and lastly cleared in xylene and cover-slipped by the aid of permount.

Light Microscopic Analysis

Golgi-stained sections were investigated using an Olympus BX51 microscope and Q Imaging Retiga-2000R camera. Neuronal structures were examined from three-dimensional images using Neurolucida software (MBF Bioscience, Williston, WT, USA). MSN selection and classification of neuronal features were made intently by an observer blinded to the animal groups. For each group, 2 neurons on the lesion and contralateral sides were analysed giving a total of 48 neurons and 96 dendrites. For quantitative analysis, neuron body and dendrites were drawn using a 60 X objective. After, the dendritic spines of the first 10 µm length from the branching area on the secondary dendrites (nearest branch point) were marked. Then, the spines were classified according to the dendritic spine head and neck ratio by a 100 X objective. Sholl analysis was performed for each neuron to maintain morphologic parameters per 10 µm in diameter concentric circles (Figure 2). The dendritic morphometric analysis was performed by Neurolucida Explorer. Total dendritic length (total length of primary and secondary dendritic branches), number of dendritic branches (total number of primary and secondary dendritic segments), nodes (total dendritic bifurcation points), endings (total dendritic termination points), total spine density (spines/10 µm dendrite), morphologic classification of spines (thin, mushroom, stubby, branched) and densities were analized.

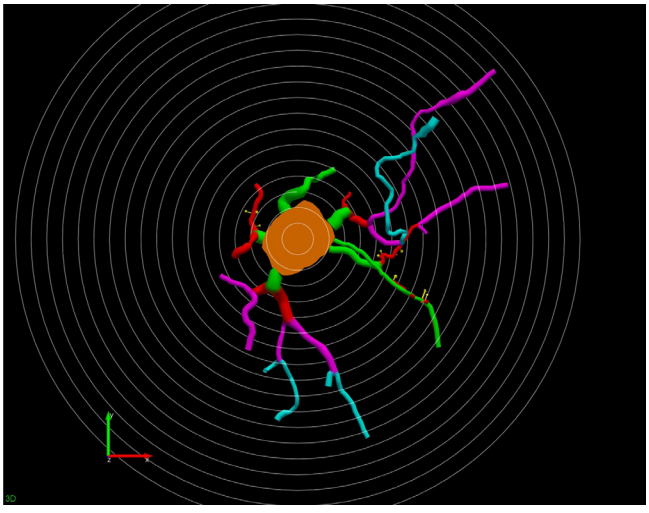

Figure 2. *3D reconstruction of the Sholl analysis of a MSN in the melatonin group. (Scale bar 10 µm). The neuron body is shown in orange. The dendritic branches of neuron are represented with different colors (green-primary, red-secondary, pink-tertiary, blue – quaternary). The spines are shown in yellow arrows on secondary branches of dendrites.*

Statistical Analysis

The GraphPad Prism 6 software was used for the analysis of the data. The normal distribution of data was evaluated by Kolmogorov-Smirnov test. Based on this, whenever the data pass the normality test, parametric Sample t-test was performed. When the data did not pass the normality test, non-parametric Mann-Whitney *U* test was performed. Data in the text and figures were shown as mean ± standard error of mean (SEM). The data was represented as "p value". A value of $p<0.05$ was considered statistically significant.

3. RESULTS

Morphological Analyses of MSNs

The dendritic branching pattern and spine morphology were observed by using Neurolucida software on MSNs in the striatum. The MFB lesion resulted in the reduction of the total dendritic length (181.1 ± 28.1µm, n=6) compared to the contralateral side (366.3 ± 58.7µm, n=6) of the sedentary group (t=1.40, df=10; p=0.0174; Figure 3). The MFB lesion led to a significant reduction in the total dendritic endings (6 ± 0.5, n=6) compared to the contralateral side (9 ± 0.8, n=6) of the sedentary group (t=2.98, df=10; p=0.0137, Figure 4). The total dendritic length on the lesion side did not show any significant difference between the melatonin (244 ± 34.9µm, n=6) and sedentary (181.1 ± 28.1µm, n=6) groups (p=0.19). The melatonin (8.3 ± 0.8, n=6) enhanced the number of dendritic endings compared to the sedentary group (2.4 ± 0.6, n=6), (t=5.81, df=10; p=0.0002, Figure 5). Moreover, the number of dendritic branches and nodes on the lesion side did not show statistically significant difference either between the contralateral sides of each group or melatonin and sedentary groups (branches; p=0.93, nodes; p=0.99). The dendritic complexity on the lesion side did not reveal a significant difference either between the melatonin and sedentary groups (p=0.88) or contralateral sides of each group (p=0.97).

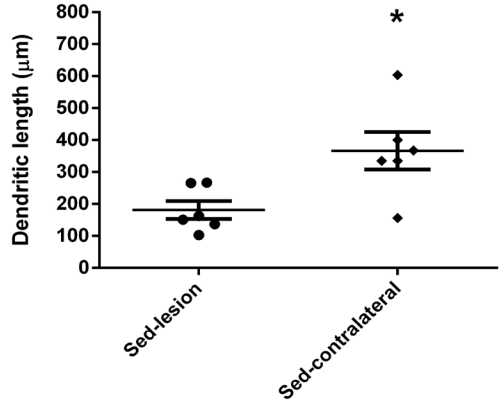

Figure 3. *The effects of MFB lesion on total dendritic length (µm). The lesion side of the number of the total dendritic length was significantly lower compared to the contralateral side of sedentary group (p=0.0174, *p<0.05).*

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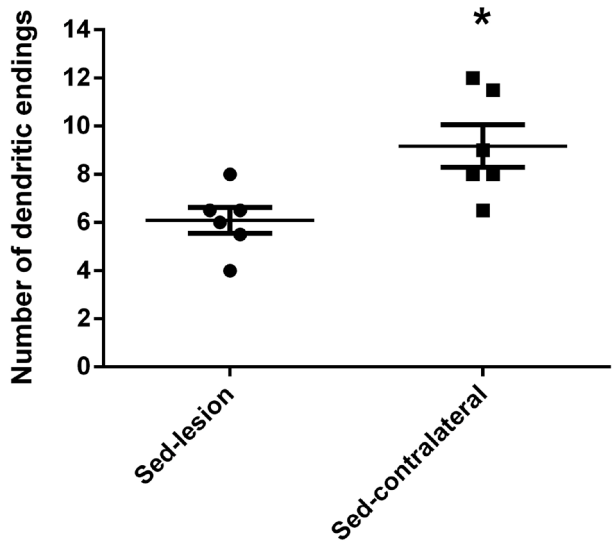

Figure 4. *The effects of MFB lesion on number of dendritic endings. The lesion side of the number of the dendritic endings was significantly lower compared to the contralateral side of sedentary group (p=0.0137, *p<0.05)*

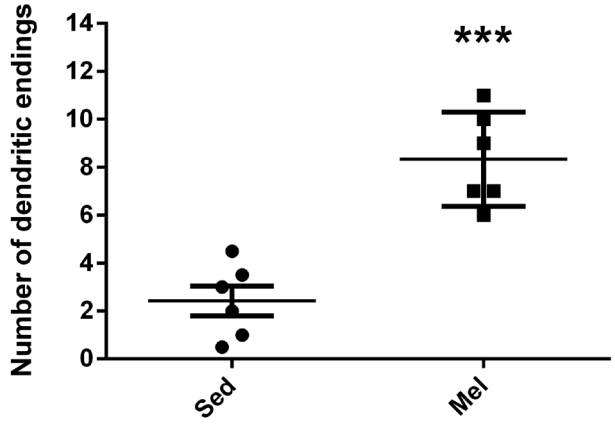

Figure 5. *The effect of melatonin on number of dendritic endings. The melatonin increased the number of dendritic endings compared to the sedentary group (p=0.0002, ***p<0.001).*

The effect of melatonin (7.4 ± 1.1 spines/10μm, n=6) increased the total spine density compared to the sedentary group (3 ± 1.2 spines/10μm, n=6) (t=2.70, df=10; p=0.0223; Figure 6). The spine density of thin type was increased by the melatonin (3.1 ± 0.6 thin spines/10μm) compared to the sedentary group (1.1±0.1 thin spines/10μm, n=6) (t=3.2, df=10; p=0.0092; Figure 7). The spine density of mushroom type was enhanced in the melatonin (3.2 ± 0.6 mushroom spines/10μm, n=6) group compared to the sedentary group (1.1 ± 0.5 mushroom spines/10μm), (t=2.25, df=10; p=0.0303; Figure 8). The spine densities of stubby and branched types did not show statistically significant difference either between melatonin and sedentary groups or contralateral sides of each group (stubby; p=0.07, branched; p=0.35).

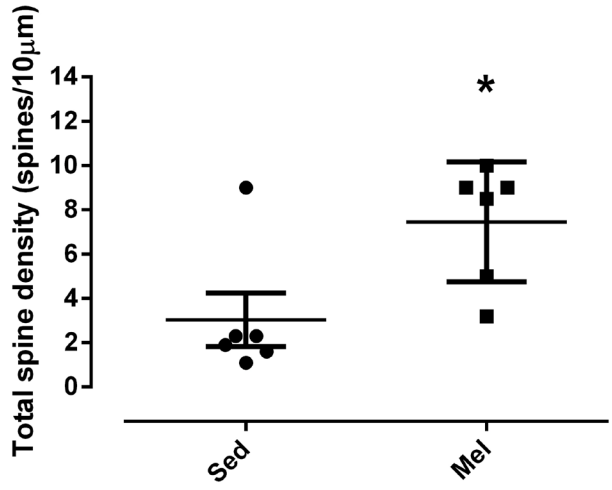

Figure 6. *The effects of MFB lesion and melatonin on total spine density (spines/10 μm). The melatonin group increased the total spine density compared to the sedentary groups (p=0.0223, *p<0.05).*

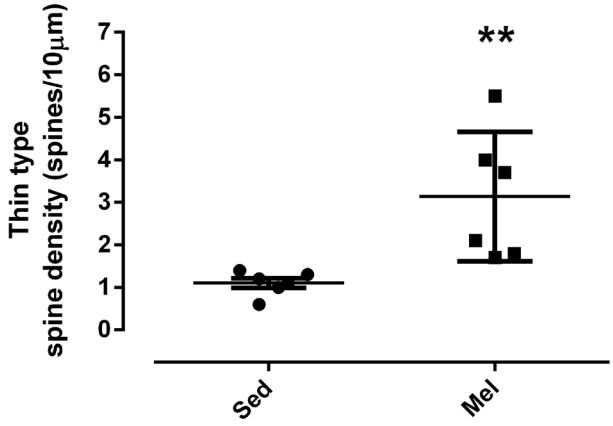

Figure 7. *The effects of MFB lesion and melatonin on spine density of thin type (thin spines/10μm). The thin type spine density was higher in the melatonin group compared to the sedentary group (p=0.0092, **p<0.01).*

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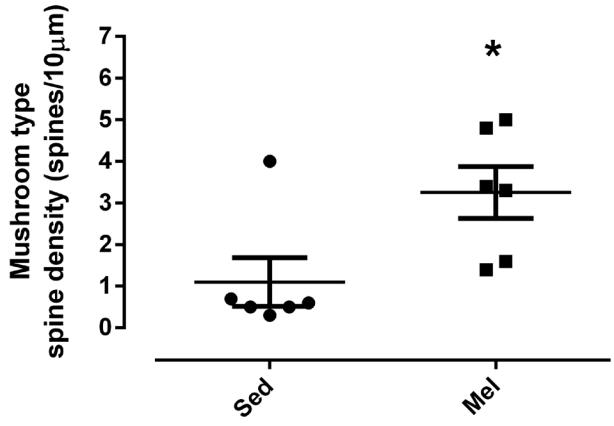

Figure 8. *The effects of MFB lesion and melatonin on mushroom type spine density (mushroom spines/10μm). The mushroom type spine density was higher in the melatonin group compared to the sedentary group (p=0.0303, *p<0.05*

4. DISCUSSION

The potential effects of melatonin on the lesioned striatal neurons were analysed in this study. The MFB lesion led to a reduction of the total dendritic length and the number of dendritic endings compared to the contralateral side of the sedentary group. The melatonin improved the number of dendritic endings compared to the sedentary group. The number of dendritic branches and the nodes were not affected by MFB lesion and also melatonin did not cause any significant effect on these structures. Therefore, the dendritic complexity did not show any significant difference between the melatonin and sedentary groups. The administration of melatonin increased the total spine density and the spine densities of thin and mushroom types.

The dendritic branching pattern of MSNs was affected by many abnormal conditions [24,25]. The cerebral hypoxic ischemia revealed the impaired dendritic arbors such as; branches, nodes, endings and spines in an animal model of MSNs [26]. Some psychostimulant drugs have been shown to increase the dendritic branching in animal models of drug abuse [27]. In contrast, the 6-OHDA and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) injected Parkinsonian models, there was a decrease in the dendritic length and spine density that was correlated with the loss of dopamine [28,29]. The recent literature showed that dopamine secretion regulates the formation and the density of dendritic spines in the striatum [30]. A previous study demonstrated the decrease of the spine densities of thin and mushroom types and an increase of the spine density of stubby type in MSNs following 6-OHDA lesion [8]. This study showed that a minimal trauma to the MFB may cause a limited degree of damage by affecting the dendrite. This information should be kept in mind during planning the Parkinson's disease model studies.

Exogenous melatonin is an important endocrine hormone due to its BBB penetration and easy tolerability without any side effects. Melatonin regulates the expression of neurotrophins and also ameliorates the motor and behavioral functions [31].

Melatonin has antioxidative and neuroprotective effects. It has been suggested as a beneficial antioxidant in the treatment of central nervous system disorders such as brain injuries, Parkinson's disease, Huntington and Alzheimer's disease [32,33]. The studies reported that melatonin had an effect on oxidative stress by increasing antioxidant enzymes and mitochondrial complex-I [34,35]. A study reported that melatonin improved the reduction in the spine density of MSN after MPTP injection [36]. The literature suggested that several types of spines reflect different synaptic functions [9,37]. In the present study, melatonin improved the dendritic endings, total spine density and spine densities of thin and mushroom types. The mushroom type has more synaptic ability because of containing excess glutamatergic receptors compared to the other types [7,38]. Thin spines are more dynamic form for synaptic plasticity that are also associated with the cognitive function [8,39]. The neuronal plasticity occurs in two ways which are functional and structural. In addition, the neuronal plasticity involves alterations in the dendritic spines rather than dendrites in the mature brain [40]. In our study, the structural changes observed in melatonin group were considered as plasticity. The increases of thin and mushroom spine types in the melatonin group represented a more stable morphological form of the spine. This is probably correlated with its synaptic ability. The total dendritic length in the striatum was positively affected by melatonin. Moreover, there was no statistically significant difference in the spine densities of stubby and branched types between the groups. We put forth that the melatonin was more effective on the spine density compared to the dendritic arborization.

Our study showed the effects of MFB lesion and the effects of melatonin on the striatal neurons. A minimal MFB lesion influenced the dendritic parameters rather than the spines. The melatonin ameliorated the morphologic features of dendrites and spines. Melatonin has beneficial effect on the spine density. There is conflicting data in the literature regarding the functions of different spine types [41,42]. The data provided from our study will also contribute to the understanding of the morphology and function of dendrites and spines in the striatum.

Compliance with Ethical Standards

Ethical Approval: The study protocol was approved by the Animal Experimentation Ethical Committee of Marmara University (approval number: 42.2021.mar).

Financial Support: No special funding was obtained.

Conflict of Interest Statement: There is no conflict of interest.

Author Contributions: : S.G., U.S.S. Finding the topic, performing the experiment, analysis and writing the manuscript. O.K. Performed the Golgi staining and statistical analysis. H.B.Performed the Golgi staining. S.D.Y. Contributed to the

analysis at Neurolucida software. All authors have read and approved the final version of the article.

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SREBP1c silencing reduces endoplasmic reticulum stress and related apoptosis in oleic acid induced lipid accumulation

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ABSTRACT

Objective: Sterol regulatory element binding protein 1c (SREBP1c) is one of the major transcription factors that is involved in non-alcoholic fatty liver disease (NAFLD) development by increasing hepatic fatty acid and triglyceride synthesis. Our study aimed to investigate the interaction of SREBP1c with endoplasmic reticulum (ER) stress in oleic acid (OA) induced lipid accumulation.

Material and Methods: Optimum lipid droplet (LD) formation and SREBP-1c induction were determined in alpha mouse liver 12 (AML12) hepatocytes following the incubation with different OA concentrations. To determine the effect of SREBP-1c, cells were transfected with siRNA specific for SREBP-1c. LD formation and SREBP-1c induction were determined via Oil Red O and immunblotting, respectively. Phospho IRE1, GRP78, CHOP, ATF6 and JNK levels were determined with immunofluorescence staining.

Results: Optimum LD formation and SREBP-1c induction were achieved at 0.5 mM oleat concentration. While SREBP-1c silencing decreased LD formation in non-OA treated cells, no significant effect of silencing was determined following OA administration. On the other hand, SREBP-1c silencing in OA treated cells reduced phospho IRE1, ATF6, JNK and CHOP expressions.

Conclusion: Our results suggest that the novel function of SREBP-1c can regulate ER stress response in OA induced lipid accumulation.

Keywords: Lipid accumulation, SREBP1c, ER stress, Apoptosis

1. INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD), one of the essential components of metabolic syndrome, is one of the major health problems today and is currently recognized as a disease entity by the World Health Organization and numerous professional societies. NAFLD is characterized by the formation of lipids in more than 5% of liver weight. Studies have shown that 25% of the population is suffering from fatty liver disease and 90% of these patients are obese individuals [1]. Therefore, understanding the mechanisms regulating lipid accumulation in the pathogenesis of NAFLD is extremely important, however, there are not published studies [2]. It has been shown that the abnormalities in the mechanisms regulating lipogenesis are major cause of triglyceride deposition in hepatocytes [3]. Sterol regulatory element binding proteins (SREBPs) are transcription factors which modulate lipogenesis. SREBPs consist of three isoforms, termed SREBP1a, SREBP1c and SREBP2, and are encoded by two genes: SREBF1 and SREBF2. They are synthesized as inactive precursor proteins attached to the ER membrane, and each isoform has a unique effect on lipid homeostasis. Proteolytic processing of precursors generates transcriptionally active forms that control the expression of a range of genes involved in cholesterol, fatty acid, phospholipid and triacylglycerol synthesis [4]. SREBP1c meditates the transcription of genes involved in fatty acid and triglycerides synthesis, including ATP citrate lyase, acetyl-CoA synthetase (ACS), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), steroyl-CoA desaturase-1 (SCD-1) and glycerol-3-phosphate acyltransferase (GPAT), while SREBP2 is specifically responsible for cholesterol synthesis and transport genes as well as LDL receptor gene transcription [5]. SREBP1a targets the genes of both transcription factors. SREBP1a targets the genes of both pathways.

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Another important issue in NAFLD pathogenesis is endoplasmic reticulum (ER) stress which is characterized by abnormalities in lipid synthesis and chaperones. ER is a crucial organelle responsible in the proper synthesis/folding of proteins, calcium storage, biosynthesis of sterols and phospholipids, and detoxification of toxic substances. Under basal conditions, glucose regulated protein 78 (GRP78) is attached to three major ER stress sensor proteins; inositol requiring kinase 1 (IRE1), double-stranded RNA-activated protein kinase (PKR)-like endoplasmic reticulum kinase (PERK) and activating transcription factor 6 (ATF6). In stress conditions that exceed the folding capacity, GRP78 binds to incorrectly folded or unfolded proteins, and dissociates from IRE1, PERK and ATF6 are activated to promote a physiological response called ER stress [6]. In addition to protein misfolding, irregularities in lipid homeostasis activate ER stress and the excessive ER stress response plays a role in the development and progression of NAFLD by enhancing lipid accumulation, inflammation, insulin resistance, autophagy and apoptosis [7].

Although, SREBP1c is a well-identified transcription factor that has been shown to play a role in disease progression by increasing hepatic fatty acid and triglyceride synthesis, its interaction with ER stress in oleic acid (OA) induced lipid accumulation has not been fully understood yet. In this study we aimed to contribute to literature by clarifying the association between ER stress and SREBP1c and their role in OA induced lipid accumulation.

2. MATERIALS and METHODS

Cell culture and treatments

Alpha mouse liver 12 (AML12) hepatocytes were maintained in Dulbeco's Modified Eagle's Medium (DMEM) (Merck KGaA, Darmstadt, Germany) supplemented with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific, Massachusetts, USA), 100 U/mL penicillin and 100 mg/mL streptomycin (Thermo Fisher Scientific, Massachusetts, USA) at 37 °C with 5% CO_2. To stimulate intracellular lipid accumulation, cells were exposed to different concentrations of oleic acid:bovine serum albumin (BSA) complex (Sigma Aldrich, St. Louis, USA) for 24 h. Cells were seeded in 6/24-well plates for 48 h before treatment with vehicle (DMEM) or OA:BSA complex at the indicated concentrations and time periods. All experiments were applied according to protocols approved by Marmara University, School of Medicine Ethics Committee (protocol number 09.2019.188).

SREBP1c siRNA transfection

Alpha mouse liver 12 cells were seeded at a density of 8×10^5 in 6 well plate for 24 h and then transfected with siRNA specific for SREBP1c (Thermo Fisher Scientific, siRNA ID: 151861) by using Lipofectamine RNAiMax Reagent (Thermo Fisher Scientific, Massachusetts, USA, Catalog No: 13778075) according to manufacturer's instructions. Briefly, 80 pmol SREBP1c siRNA in 1:3 and 1:6 ratio of siRNA: Lipofectamine RNAiMax Reagent was prepared in OptiMEM (Thermo Fisher Scientific, Massachusetts, USA) and incubated for 5 min at room temperature. The mixture was gently added dropwise to the cells in OptiMEM and then incubated at 37 °C with 5% CO_2. 24 and 48 hours after the transfection, cells were harvested for immunoblot experiment to confirm that the SREBP1c was silenced.

Following the optimization of siRNA transfection, cells were seeded in 6/24-well plates for 48 h before pretreatment with SREBP1c siRNA and then treated with vehicle (DMEM) or OA:BSA to divide into four groups totally; i) Control, ii) SREBP1c siRNA, iii) OA, iv) SRBEP1c siRNA + OA.

Oil Red O staining

Following the siRNA and/or OA administrations, cells were fixed with 10% neutral-buffered formalin for 10 min, washed twice with PBS, and stained with 0.2% Oil Red O (Sigma Aldrich, St. Louis, USA) in isopropanol for 15 min. Cells were then washed in PBS, and visualized and photographed using light microscope (Zeiss, Amsterdam, Netherlands). Lipid accumulation was quantified by counting the number of lipid droplets in at least thirty cells for each group.

Immunoblot analysis

Cells were collected and lysed in radioimmunoprecipitation assay (RIPA) buffer (Cell Signalling Technology, Massachusetts, USA) in accordance with the manufacturer's instructions. Protein concentrations were measured with BCA assay (Thermo Fisher Scientific, Massachusetts, USA). Total 20 µg of protein samples were separated by 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membrane. Following the blocking with 5% BSA in Tris-buffered saline with Tween TBST solution for 1 h, membranes were probed with primary antibodies against SREBP1c (Abcam, Cambridge, UK, Catalog No: ab3259) and β-actin (Cell Signaling Technology, Massachusetts, USA, Catalog No: 4967) overnight. After washing of unbound primary antibodies with TBST and use of HRP-conjugated secondary antibodies, blots were visualized with chemiluminescence kit (Cell Signaling Technology, Massachusetts, USA). The density of bands was quantified and normalized with β-actin using Image J software.

Immunofluoresence analysis

AML12 cells were seeded on twelve-well glass chamber slides and treated with OA for 24 h with or without SREBP1c silencing. After incubations, cells were fixed in 4% formaldehyde for 15 min, blocked in 10% goat serum, and incubated for overnight at 4°C with indicated antibodies; GRP78 (Cell Signaling, Massachusetts, USA, Catalog No: 3177), phospho IRE1 (Abcam, Massachusetts, USA, Catalog No: ab104157), CHOP (Aviva Systems Biology, California, USA, Catalog No: ARP31591_P050), JNK (Novus Biologicals, Colorado, USA, Catalog No: NBP2-25115) and ATF6 (Novus Biologicals, Colorado, USA, Catalog No: NBP2-76329). Following use of Alexa Fluor 488 or Alexa Fluor 594 secondary antibodies and DAPI, images were captured using a Zeiss LSM700 confocal microscope (Amsterdam, Netherlands) and analyzed using Image J software.

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The average number of fluorescence intensities per cell was recorded in at least thirty cells for each group.

Statistical analysis

Statistical analysis was performed using Prism 4 (GraphPad) software. For determination of statistical significances of differences, one-way ANOVA was performed followed by multiple comparisons using the Student-Newman-Keuls test. P-value less than 0.05 has been accepted to be statistically significant.

3. RESULTS

Inducing lipid accumulation in AML12 cells

Increased lipid droplet formation in hepatocytes is a typical finding of hepatic steatosis. The water-soluble OA:BSA complex has earlier been shown to efficiently stimulate lipid accumulation and used in the literature (8-10). Here in our study, we first confirmed this finding via Oil Red O staining and established an *in vitro* hepatic steatosis model in AML12 cells. As shown in Fig. 1A, AML12 cells were treated with concentrations of 0.06 mM, 0.5 Mm and 1 mM OA to stimulate lipid droplet formation. Analysis of the lipid droplet number per cell revealed a significant increase in all OA treated groups compared to control (Fig. 1B). In addition to Oil Red O data, SREBP1c protein expression was also observed. We determined that exposure of AML12 cells to 0.5 mM OA stimulated the expression of SREBP1c (Fig. 1C) as well as the formation of lipid droplets. In light of these findings, we decided to apply 0.5 mM OA for 24 h in our study, since the major effect to SREBP1c expression was detected in that concentration.

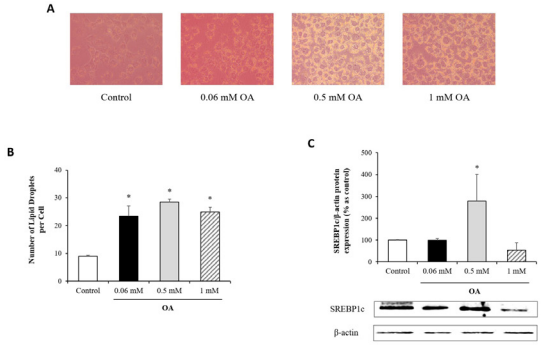

Figure 1. *Inducing lipid accumulation in AML12 cells*
AML12 cells were treated with different concentrations of OA:BSA complex (0.06-0.5-1 mM) for 24 hours. (A) Represantative light microscopic images show lipid droplet formation following Oil Red O staining (400X magnification). (B) Quantification of the numbers of lipid droplet formation per cell. (C) SREBP1c protein expression was analyzed by western blotting followed by densitometric analysis of protein bands and relative ratios were quantified and normalized relative to β-actin.
Data are expressed as mean ± S.D.
**p < 0.05 vs. control, (n=3).*

SREBP1c inhibition and its effect on lipid accumulation

To assess the role of SREBP1c in our *in vitro* hepatic steatosis model, we aimed to reduce SREBP1c levels via siRNA transfection. Therefore, we established different time and siRNA:transfection reagent ratio conditions to determine the most effective inhibition. Protein expression of SREBP1c was effectively reduced in 1:3 siRNA:transfection reagent ratio for 24 h compared to other conditions (Fig. 2A). To confirm the functional impact of SREBP1c knockdown in oleat induced lipid droplet generation, cells were also treated with OA at 0.5 mM concentration for 24 h and stained via Oil Red O (Fig. 2B). Analysis of the images revealed a decrease in lipid droplet formation in SREBP1c siRNA group compared to the control. Differently from non-OA treated ones, siRNA transfection followed by OA administration had no significant effect on lipid droplets compared to OA group (Fig. 2C).

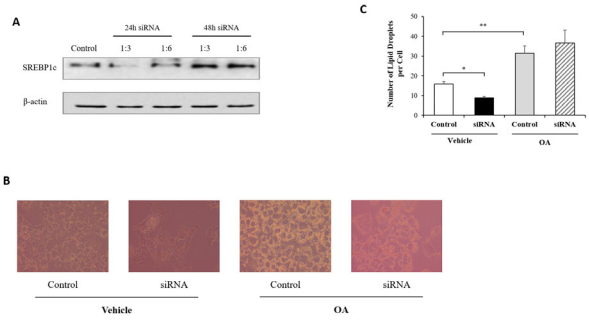

Figure 2. *SREBP1c inhibition and its effect on lipid accumulation*
(A) AML12 cells were transfected with 1:3 and 1:6 siRNA:transfection reagent ratios for either 24 h or 48 h. Protein bands of SREBP-1c and β-actin were visualized with chemiluminescence kit following the use of HRP-conjugated secondary antibodies. After SREBP1c silencing, cells were treated with vehicle (DMEM) or OA:BSA (0.5 mM) to divide into following groups; i) control, ii) SREBP1c siRNA, iii) OA, iv) SREBP1c siRNA + OA. (B) Represantative light microscopic images show lipid droplet formation following Oil Red O staining (400X magnification). (C) Quantification of the numbers of lipid droplet formation per cell.
Data are expressed as mean ± S.D.
***p < 0.01, and *p < 0.05, (n=3).*

Effect of SREBP1c silencing on ER stress and related signaling status following OA administration

After the verification of our steatosis model and SREBP1c silencing, we next tried to gain insight into the importance of SREBP1c signaling in ER stress and related apoptosis. We monitored well-identified parameters of ER stress and apoptosis, including phospho IRE1, GRP78, CHOP, ATF6 and JNK, using immunoflouresence. Microscopic images for selected parameters are shown in Fig. 3A. Anaylsis of images revealed that neither OA application nor siRNA tranfection had any effect on GRP78 expression (Fig. 3B). Activation status of ATF6 is presented as the ratio between nucleus and cytoplasmic levels

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[11]. To calculate ATF6 activation in each group, we measured the ratio between nuclear-and cytoplasmic fluorescence intensities to quantify nuclear localization (N/C). As shown in Fig. 3B, the ratio of nuclear-to-cytoplasmic ATF6 fluorescence intensity was increased in siRNA transfected cells under normal conditions, which was reduced in siRNA transfected cells after OA application. However, the expression of ER stress-related proteins, phospho IRE1 and JNK was up-regulated following OA application, while transfection with SREBP1c siRNA reversed OA induced activation of phosho IRE1 and JNK. CHOP levels was also reduced in SREBP1c silenced cells following OA in spite of no change in OA treated control cells (Fig. 3B).

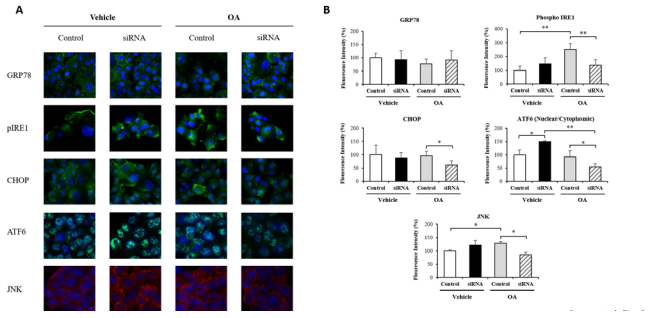

Figure 3. *Effect of SREBP1c silencing on ER stress and related signaling status following OA administration*

AML12 cells were first transfected with SREBP1c siRNA at 1:3 siRNA:transfected reagent ratio for 24 h and then with vehicle (DMEM) or OA:BSA (0.5 mM) to divide into following groups; i) control, ii) SREBP1c siRNA, iii) OA, iv) SREBP1c siRNA + OA. Cells were fixed, and stained for GRP78, phospho IRE1, ATF6, CHOP (green), JNK (red) and nuclei by DAPI (blue). (A) Representative confocal microscopic images of cells from each group (400x magnification). (B) Levels of GRP78, phospho IRE1, nuclear (N) and cytoplasmic (C) forms of ATF6, JNK and CHOP were examined via quantification of relative fluorescence intensities.

Data are expressed as mean ± S.D.

***p < 0.01, and *p < 0.05, (n=3).*

4. DISCUSSION

Non-alcoholic fatty liver disease is one the most common diseases of the metabolic disorder, which is defined by the presence of lipids in more than 5% of liver weight. As a result of increase in obesity and type 2 diabetes mellitus (T2DM) worldwide, the prevalence of NAFLD is rising continuously and is progressing from steatosis to non-alcoholic steatohepatitis (NASH) and liver cirrhosis [12, 13]. According to the literature, in vitro models of steatosis in NAFLD studies can be categorized into four groups; i) primary cell cultures (Hepatocytes, Kupffer cells, stellate cells), ii) immortalized cell lines (AML12, HepG2), iii) co-culture models, and iv) three-dimensional cell cultures [14]. Ethical issues and isolation problems of the primary cell cultures make the immortal cell lines more suitable [14]. In the present study we used AML12 cells in establishing *in vitro* steatosis model due to their extended replicative activity and stable phenotype. Palmitic acid (PA) and OA are two most abundant long-chain free fatty acids in the normal and fatty liver specimens. It has been identified that different combinations of PA and OA induce the steatosis in a manner of dose – and time-dependent [15]. Accordingly, induction of steatosis in human hepatocellular carcinoma (HepG2) cells was established after OA administration [8]. In an another *in vitro* study using rat primary hepatocyte cells, administration of PA and OA combination induced lipid accumulation and cytotoxicity [16]. In our study, we established an *in vitro* hepatic steatosis model by treating AML12 cells with different OA concentrations for 24 hours. We also checked the SREBP1c protein expression to determine the most effective OA dose in inducing SREBP1c activation. Despite the finding of lipid accumulation in all OA administrated cells, only 0.5 mM OA was significantly induced the SREBP1c expression. Therefore, we decided to apply 0.5 mM OA in our further experiments in determining the effect of SREBP1c silencing in a manner of lipid accumulation and ER stress.

Sterol regulatory element binding proteins are important players of lipid metabolism, involved in pathological and physiological conditions, including nutrition, cell growth, energy stress, inflammation. SREBP1c is a transcription factor that is highly expressed in a variety of tissues, including liver, adipose, and skeletal muscle. Its ability to increase the transcription of genes involved in fatty acid and triacylglycerol synthesis has been well identified. SREBP1c overexpression has been shown to induce lipid accumulation insulin resistance, diabetes and NAFLD, in both *in vitro* and *in vivo* studies [17-19]. Conversely, SREBP1c inhibition is reduced the risk of metabolic disorders, such as obesity, atherosclerosis, and fatty liver [20]. In our study, SREBP1c silencing (without OA administration) in AML12 cells resulted in a decrease in lipid droplet formation. Although, inhibition of SREBP1c had no effect against OA induced lipid droplet accumulaiton.

A wide range of cellular disturbances, including high fat diet, impair the efficacy of protein folding in the ER leading to accumulation of misfolded and unfolded proteins [21, 22]. Liver cells cope with ER stress by an adaptive response mechanism, known as unfolded protein response (UPR) [23]. UPR has both cytoprotective and cytotoxic effects. The activity of UPR maintains homeostasis during transient ER stress in normal circumstances. However, under prolonged or excessive ER stress conditions in hepatocytes, UPR fails to recover the normal function of the ER and leads to apoptosis via the upregulation of the pro-apoptotic CHOP and JNK [24-26]. Studies have reported that CHOP is essential for the secretion of proinflammatory factors and hepatocyte apoptosis. In a study, CHOP deletion has been shown to provide resistance in HepG2 cells following palmitate administration [27]. The contribution of ER stress in NAFLD progression was determined either in rats fed a high fat diet [22] or in the livers of patients with NASH [28, 29]. Both IRE1 and ATF6 are well-identified and sensitive ER stress indicators found in ER membrane bound to chaperones [30]. Accumulation of unfolded proteins results in the dissociation of GRP78 from luminal domain of IRE1, thus IRE1 is oligomerized and auto-phosphorylated [31]. Beside its endonuclease activity

that mediates the activation of XBP-1 via splicing the inactive transcript, IRE1 activates apoptosis signal-regulating kinase 1 (ASK1) and phosphorylates JNK by its kinase domain [25, 32]. Apart from IRE1, ATF6 itself acts as a transcription factor that mediates a number of target genes, including GRP78, GRP94 and CHOP [33]. Following the dissociation from GRP78, ATF6 translocates to golgi, undergoes a cleavage, and transports to nucleus [34]. In this regard, increase in nucleus to cytoplasm ratio of ATF6 is accepted as an indicative for ER stress associated ATF6 activation [35]. In our study, we observed the status of ER stress activation via forming OA induced steatosis in AML12 cell line. In this context, OA mediated lipid accumulation induced ER stress in a manner of IRE1 phosphorylation and its downstream JNK without affecting the ratio of nuclear-to-cytoplasmic ATF6 and GRP78 levels, indicating the role of OA in UPR.

Steatosis can be either a cause or consequence in the context of ER stress. Lipid induced ER stress has been shown in several studies [16, 36, 37]. Conversely, it has been demonstrated that chronic ER stress has an important role in lipostasis and transition from steatosis to steatohepatitis [38, 39]. There are a number of well-identified mechanisms which ends up lipogenesis through ER stress, including IRE1 mediated XBP1 activation [40, 41]. There are also many evidences that ER stress induced lipogenesis drives with SREBPs which are main transcription factors of de novo lipogenesis [42, 43]. It has been shown that ER stress initate SCAP independent SREBP1/2 activation in the ER via caspase 2 mediative S1P activation that drives the cell to lipogenesis [44]. Recent studies using *in vitro* and *in vivo* fatty liver models have identified the effect of ER stress in inducing lipid accumulation and SREBP1c activation [45]. Specifically, Damiano et al. [46], observed that tunicamycin-mediated ER stress induction in HepG2 cells was related with SREBP1c activation. In another study using HepG2 and L02 cells, it was found that thapsigargin – induced ER stress increased SREBP1c, FASN, ACC protein expressions along with triglyceride levels, resulting in hepatic steatosis [47]. Despite these studies underline the association between ER stress related de novo lipogenesis and SREBP activation in steatosis progression, the effect of SREBP1c silencing in ER stress activation during OA induced steatosis has not been investigated yet. We now demonstrated that SREBP1c has effect on ER stress in a positive fashion in OA induced steatosis. According to our results, SREBP1c silencing in OA induced lipid steatosis lead to a significant decrease in ER stress (phosho IRE1, ATF6) and ER stress triggered apoptosis (JNK, CHOP) parameters.

In conclusion, SREBP1c might have an individual effect that changes state of affairs in OA-induced lipid accumulation via altered ER stress response. Interestingly, our ATF6 finding suggests that a complex and potential relationship exists between ATF6 and SREBP1c. The discrepancy in the ATF6 intensity following SREBP1c silencing among vehicle and OA groups can be a consequence of response diversity of cells in normal and OA-induced conditions. There are some studies which claimed that ATF6 reduce hepatic steatosis by antagonizing SREBPs [48]. Our results can be an evidence for presumptive bidirectional involvement of SREPB1c which should be investigated with further investigations. Decrease in CHOP and JNK levels in SREBP1c siRNA+OA group also indicated the crucial role of SREBP1c silencing in reducing ER stress mediated apoptosis, the ultimate response to UPR. It is also highlighted that other ER stress sensors, including PERK-ATF4 signaling, might involve in CHOP activation. Therefore, further investigations are needed to clarify whether oleat induced CHOP activation is related to ATF6 or not. Our data collected so far in OA-induced steatosis model gain insight for therapeutic potential of SREBP1c to inhibit the impact of fatty liver by means of ER stress and related apoptosis.

Compliance with Ethical Standards

Ethical Approval

The study protocol was approved by the Marmara University, School of Medicine Ethics Committee (February 01, 2019, protocol number 09.2019.188).

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Conflict of Information

The authors declare no conflict of interest to declare.

Author Contributions

E.S., T.D. and N.K.O. generated the initial idea and conducted experimental design. E.S., T.D., D.D.D. and B.O. performed experiments and analyzed data. T.D. conducted cell culture experiments. E.S. conducted confocal microscopy experiments. All authors performed critical revision of the manuscript and gave final approval of the submitted version.

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Impact of ankyloglossia on the language development of children

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ABSTRACT

Objective: Ankyloglossia is a benign anomaly of the tongue which may cause functional limitation. Evidence regarding the impact of ankyloglossia on children's language development is limited. We aimed to evaluate the language development of children born with ankyloglossia.

Patients and Methods: Children diagnosed with ankyloglossia were followed up prospectively. Demographic characteristics, degree of ankyloglossia assessed by Hazelbaker score in infancy and its effects on breastfeeding were evaluated. Language development was tested by the Turkish version of the Test of Early Language Development-Third Edition and the Denver II Test at 3-5 years of age.

Results: Out of 53 children diagnosed with ankyloglossia, 38 (71.7%) children had language development testing and were included into the study. Significant ankyloglossia was detected in infancy in 10 of these children (26.3%). Median time of exclusively breastfeeding was not different according to the severity of ankyloglossia. All children evaluated with Denver II Test were developmentally normal in all domains. Scores of Test of Early Language Development-Third Edition were not different between children with and without significant ankyloglossia.

Conclusion: Long term language development of children with ankyloglossia was not adversely affected. Parents should be appropriately informed and efforts must be paid to prevent unnecessary surgical interventions concerning language delay.

Keywords: Ankyloglossia, Children, Language development

1. INTRODUCTION

Ankyloglossia, or tongue-tie, is a congenital anomaly of the tongue characterized by short, tight and thick frenulum [1]. The prevalence of ankyloglossia ranges from 0.1% to 10.7% in different populations with a male predominance. This range, in the reported prevalence is large because of the lack of a uniform definition and diagnostic criteria [2].

Ankyloglossia is usually a benign condition, which generally needs no intervention. However, if the mobility of the tongue is severely affected, it may result in functional limitations. These include breastfeeding difficulties in infancy causing significant nipple pain and nipple trauma in mothers. Traumatized nipples can cause the baby to remove milk less efficiently than baby without tongue-tie, therefore, the mother may have severe nipple pain due to incomplete breast drainage. In rare instances, interference with breastfeeding may be so severe that it may be the only cause of failure to thrive [3]. The historical practice of treating ankyloglossia in all infants has already been dropped but there is still no consensus on how to approach ankyloglossia among medical specialists, such as pediatricians, plastic surgeons, otolaryngologists, lactation consultants and speech language pathologists [4]. Available studies about the effects of ankyloglossia on breastfeeding and its surgical management are heterogenous in terms of study design and definition of ankyloglossia [2,5,6]. It is not possible to establish a practice guideline for the management of ankyloglossia due to the low strength of available studies and the lack of knowledge on the natural course of ankyloglossia.

Even though, most of the studies on ankyloglossia are about the effects of breastfeeding, another concern related to ankyloglossia is speech outcomes in children. Available

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studies have conflicting results and the outcome measures are different [7-10]. Therefore, there is no universal consensus on how to approach ankyloglossia regarding future speech problems. Besides, studies on the speech outcomes of ankyloglossia focus on articulation disorders and social consequences but many parents also worry about their child's language development. In this study, our aim was to evaluate the language development of children born with ankyloglossia using objective tests and comparing children's language development according to the severity of ankyloglossia assessed in infancy.

2. PATIENTS and METHODS

Study design and participants

Children born with ankyloglossia between the years 2012 and 2015 and followed up at a well – child clinic at a university hospital were recruited into the study. Between the years 2012 and 2015, the clinic provided care for 2714 children and among them 53 children with ankyloglossia were identified during infancy and noted on personized patient files. In the outpatient clinic, babies were regularly assessed according to the recommendations of the American Academy of Pediatrics. Physical examination, growth and developmental surveillance, immunization and anticipatory guidance were provided during the visits. For the aims of this study, patients' data regarding demographic characteristics, duration of breastfeeding and surgery for ankyloglossia were subtracted from each patient's personalized file. The degree of ankyloglossia was assessed in infancy by the Hazelbaker Assessment Tool for Lingual Frenulum Function (HATLFF) in well – child visits by the pediatricians. HATLFF has five appearance items and seven function items [11]. Maximum score of appearance items is 10 and of function items is 14. Greater scores mean less severe ankyloglossia. Significant ankyloglossia was defined when the function score was less than 11 or the function score was 11 but appearance score was less than 10 or appearance item score was less than 8. Tongue-tie of 10 children (26.3%) was classified as significant during infancy. All children with significant ankyloglossia were referred to the plastic surgeon for further evaluation. The primary outcome of the study was the language development of the children. Duration of exclusive breastfeeding and total duration of breastfeeding were secondary outcomes. The study was approved by the Marmara University, School of Medicine Ethics Committee (Date: 05.10.2018, Number: 09.2018.674). This study was conducted in accordance with the Declaration of Helsinki.

Evaluation of global development and language

Families were called back at 3-5 years of age to assess the children's language development. Denver II Test was performed to examine the global development of children [12]. Language development was further evaluated specifically with the Turkish version of the Test of Early Language Development-3 (TELD-3) [13,14]. A child developmental specialist who had certificates for Denver II and TELD-3 performed both tests. Written and verbal informed consent was obtained from parents. Since, the evaluation of articulation was beyond the scope of this study, no articulation tests were performed.

Denver II Test

All children were tested by Denver II for assessment of global development. Denver II is a screening test for the development of children aged 0-6 years and covers four developmental domains for testing: Fine motor, gross motor, personal-social and language skills. The Turkish version was validated in 1995 by Anlar and Yalaz [12]. Performance of children in Denver II was evaluated according to the children's age. Results were recorded in each domain according to child's performance as normal, suspicious or abnormal.

Test of Early Language Development-3

The Turkish version of the TELD-3 which was originally created by Hresko et al., was adapted by Topbaş and Güven in 2011 [13,14]. Both expressive and receptive language are assessed by TELD-3 and can be used for children between 2-7 months old [11]. The test consists of 2 forms; form A and form B, each with a 37-item receptive language subtest and a 39-item expressive language subtest. Scores were calculated first as raw scores and then converted into standard scores. In addition, we calculated the children's scores as age in months.

Statistical Analysis

Data analysis was performed by IBM SPSS statistical software (Version 20.0. Armonk, NY: IBM Corp.). Values for continuous variables were expressed as medians, interquartile range (IQR), means, standard deviations, minimum and maximum and compared by Mann-Whitney *U* test, after testing normal distribution by Shapiro-Wilk test. Descriptive variables were reported as numbers and percentages. Comparisons between categorical variables were performed by Chi-Square testing. P values of <0.05 were described as significant.

3. RESULTS

Fifty-three children were born with ankyloglossia between the years 2012-2015 and were followed up in our well – child outpatient clinic. In our cohort, the prevalence of ankyloglossia was identified as 2.0%. Out of 53 children, the families of 40 children accepted their child to be evaluated by Denver II and Turkish TELD-3. One boy in a bilingual family and another boy with a diagnosis of epilepsy were excluded from the study. 38 (71.7%) children had language development testing and were included into the study. All of the analyses described below were performed only on 38 children who were assessed for language development. Degree of ankyloglossia was assessed by HATLFF in infancy at a

median age of 2 (IQR:1-5) months. Significant ankyloglossia was detected in 10 (26.3%) children.

Most children were of male gender (n:25, 65.8%). The median age at the evaluation of language development was 56 months (IQR:51-60) and was not different between children with significant and non-significant ankyloglossia. Four children (10.5%) were the product of consanguineous marriage and 10 (26.3%) children had a family history positive for ankyloglossia **(Table I)**.

Table I. *Comparison of children with significant and non-significant tongue-tie*

	Significant	Non-significant	P value	Total
Number of children, n (%)	10 (26.3)	28 (73.7)	NA	38 (100)
Age, months, median (IQR)	58.5 (53.2-62.5)	56.0 (49.5-59.0)	NS	56 (51-60)
Gender, male, n (%)	6 (24.0)	19 (76.0)	NS	25 (100)
Consanguinity, n (%)	2 (50.0)	2 (50.0)	NS	4 (100.0)
Family history of ankyloglossia, n (%)	4 (40.0)	6 (60.0)	NS	10 (100.0)
Exclusive breastfeeding, months, median (IQR)	6.0 (0.7-6.0)	6.0 (5.0-6.0)	NS	6.0 (5.0-6.0)
Total breastfeeding, months, median (IQR)	21.0 (10.7-25.5)	24.0 (11.7-24.0)	NS	24.0 (12.0-24.0)
Denver-II, Normal, n (%)	10 (26.3)	28 (73.7)	NS	38 (100)

IQR: interquartile range, NA: Not applicable, NS: Non-significant

Most of the children (n:28, 71.8%) were exclusively breastfed in the first 6 months. Median duration of exclusive breastfeeding was 6 (IQR:5-6) months and total breastfeeding was 24 (IQR:12-24) months. Duration of exclusive breastfeeding and total breastfeeding were not different between children with significant and non-significant ankyloglossia **(Table I)**. None of the infants required further breastfeeding counseling other than that which was provided during regular well-child visits.

All children with significant ankyloglossia according to HATLFF were also examined by a plastic surgeon. Eight children (21.1%) were operated at a median age of 7 (IQR; 3.2-21) months. Surgery was offered to all children with significant ankyloglossia according to the consensus decision of the pediatricians and the plastic surgeon and was performed in three children with significant ankyloglossia while the families of seven children with significant ankyloglossia opted not to be operated. Additionally, the families of five children with non-significant ankyloglossia had their children's tongue-tie operated elsewhere, even though, surgery was not recommended by our team. A history of the operation was gained through interviewing the parents and was confirmed by physical check. There were no significant differences in HATLFF appearance and function scores between the children who were operated and those who were not. Duration of exclusive breastfeeding and total breastfeeding did not show any differences between children who had undergone tongue-tie division and those who had not.

All children were developmentally normal. The results of Denver II and TELD-3 expressed as age in months of all children were shown in Tables I and II. All children were evaluated as normal in all domains of Denver II. Accordingly, none of the families described any speech delay. The results of TELD-3 were not statistically different in children with and without significant ankyloglossia according to HATLFF score. There were also no differences of TELD-3 scores in children who had frenotomy (n=8) and those who did not (n=30).

Table II. *TELD-3 results of children with significant and non-significant ankyloglossia. No significant differences were detected between the two groups in TELD-3 receptive language scores (P=0.33) and expressive language scores (P=0.25).*

	Age		TELD-3 Receptive Language		TELD-3 Expressive Language	
	Significant	Nonsignificant	Significant	Nonsignificant	Significant	Nonsignificant
	n=10	n=28	n=10	n=28	n=10	n=28
Mean ±SD	57.2±7.4	54.6±7.6	60.1±9.4	58.4±8.8	59.9±9.3	58.4±9.2
Median (IQR)	58.5 (53.2-62.5)	56 (49.5-59)	62 (58.7-64)	61 (52-63)	62.5 (58.5-63.2)	61 (49.5-63)
Minimum	40	40	38	40	38	40
Maximum	66	77	74	85	73	85

IQR: interquartile range, SD: standard deviation, TELD-3:Test of Early Language Development-3.

All values are represented in months

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4. DISCUSSION

Most of the studies regarding ankyloglossia focused on breastfeeding issues. However, in daily clinical practice, parents often share with physicians their concerns about the late outcomes of ankyloglossia. Speech problems are one of the major concerns of parents but there is inadequate evidence to objectively inform parents about the effects of ankyloglossia on the language development. In our study, we performed specific language development tests in addition to evaluation of global development and showed that children with ankyloglossia were able to present age – appropriate language development skills regardless of the severity of their tongue-tie assessed during infancy, whether operated or not.

Clinical significance of ankyloglossia has been a subject of debate among medical professionals. In a survey conducted among otolaryngologists, pediatricians, lactation consultants and speech pathologists, it has been shown that there is no consensus about the significance of ankyloglossia regarding speech, breastfeeding and surgery outcomes [4]. Some of the authors propose that neonatal frenulum is naturally short and with age and repeated use, the short frenulum may elongate and will not necessarily result in functional problems [15-17]. The lower incidence in adults may support this idea [1,2]. However, no study prospectively evaluated the natural course of ankyloglossia to confirm this assumption. On the contrary, there is a body of evidence that it may cause infant feeding difficulties, speech and articulation disorders and social distress [1,18,19]. Some parents may also think that ankyloglossia may be a reason for speech delay, but children with ankyloglossia are expected to follow regular developmental milestones in language, as we showed in our study, although, to our knowledge no prior study has investigated the language development in children with ankyloglossia.

Speech problems are the second most prevalent outcome described in the ankyloglossia literature [7]. Concerns related to speech is one of the main reasons why parents seek for frenotomy [20,21]. However, studies are insufficient to prove a causative association of ankyloglossia with speech disorders [9]. There is some literature reporting an improvement in speech through treatment, but results commonly rely on parent reporting without standardized measurement tools for speech outcomes [8,21]. One of the distinguishing features of our study from other reports in the literature is that we performed standardized tests to assess the language development of the children. In the study of Klockars et al., the most common indication for tongue-tie division was speech and articulation problems and 84% of parents reported a benefit from the surgery, but in this study, it was not clear how speech problems were evaluated and diagnosed. In addition, the benefit of the operation was reported only by parents without any objective measurements [21]. Walls et al., questioned parents regarding the speech intelligibility of their children three years after neonatal frenotomy and compared results with a control group and with a non-surgical intervention group. Parental subjective evaluation of speech regarding vocabulary, articulation and sentence structure were graded higher by a Likert Scale survey in the group of surgically treated children [8]. In contrast, Daggumati et al., found no parenteral perceived difference in speech quality between surgically treated and conservatively managed children [22]. In four studies, speech was evaluated by speech-language pathologists rather than objective parental assessment. Heller et al., compared two surgical methods in 16 patients with persistent articulation problems and presented that 4-flap Z-frenuloplasty provided better improvement in articulation judged by speech-language pathologists [23]. The test used by speech language pathologists was not identified in this study. Similarly, in the study of Messner et al., 82% patients with ankyloglossia were reported to have improved speech postoperatively judged by nonblind speech-language pathologists without a standardized test [18]. In two studies, standardized articulation tests were used. Ito et al., from Japan evaluated changes in speech by a standardized articulation test in five patients. They concluded that articulation errors can be improved after tongue-tie division, even though their study was limited by a low number of patients, nonblind assessment of speech and lack of a control group [24]. In the retrospective cohort of Dollberg et al., speech intelligibility of children with untreated and treated ankyloglossia with an age matched control group was assessed by a standardized articulation test. Articulation errors were more common in children with untreated ankyloglossia but there was no statistical difference in word, sentence and fluent-speech intelligibility [25]. Recently, Salt et al., revealed that objective measures of speech outcomes did not differ between treated-, untreated-, and non-tongue tie groups [26]. These studies with conflicting results cause confusion and discrepancy among medical specialists who encounter ankyloglossia. Moreover, studies investigating the effects of ankyloglossia on speech are focused on articulation rather than language development which is often a point of discussion with parents of children with ankyloglossia in well child visits. At this point, we believe that our study adds to the limited evidence about language development in children with ankyloglossia since we used language development tools in the assessment. It has been shown that parents are the main decision makers for frenotomy following lactation consultants [20]. Therefore, adequate and reliable information must be given to families by health care professionals caring for newborns and infants. We believe that our study provides evidence to reassure parents that children with ankyloglossia exhibit normal language development same as their peers. Therefore, delayed language development in children with ankyloglossia should never be solely attributed to ankyloglossia and a usual evaluation including neurodevelopmental and audiologic assessment should not be deferred while waiting for the benefits of surgery.

Ankyloglossia has been blamed for interfering with successful breastfeeding. As part of efforts to increase breastfeeding rates, there is a substantial increase in the diagnosis and surgical treatment of ankyloglossia [27-29]. However, studies are heterogeneously designed and not enough to recommend frenotomy to improve breastfeeding [6]. In the recent Cochrane review, it has been emphasized that frenotomy reduces maternal nipple pain in the short term evaluation but does not have a consistent positive effect on breastfeeding [30]. Furthermore, no study investigated the effects of ankyloglossia on long-term breastfeeding and resolution of maternal pain whether frenotomy is performed or not. Available studies did not study whether

infants were breastfed for longer after frenotomy [6,30]. In our study, we were able to show that the mean durations of exclusive and total breastfeeding were not different between infants who had frenotomy and who those had not. Besides, breastfeeding duration also did not change according to the severity of ankyloglossia assessed by HATLFF. In our well-child clinic, mothers are informed about the importance of breastfeeding and counselled on successful breastfeeding. Breastfeeding issues are discussed in every visit from the first visit on and lactation specialists observe mother-infant dyad for breastfeeding at least once and every time the mothers have a concern. According to World Health Organization (WHO), infants should be exclusively breastfed for the first six months and continue breastfeeding up to the age of two years or beyond [31]. In our study group, infants were exclusively breastfed for a median of 6 months and median duration of total breastfeeding was 24 months consistent with the WHO recommendations. Breastfeeding counselling efforts in our clinic may have resulted in the success of mothers with tongue-tied infants and may have prevented unnecessary interventions. Therefore, intensive lactation support should be implemented before tongue-tie is blamed for the failure of breastfeeding. In line with this, Dixon at al., noticed that breastfeeding rates remained stable despite an increase in frenotomy rates in the last years and showed in their study that a multidisciplinary program including objective assessment of tongue function and anatomy along with lactation support is effective in reducing frenotomy rates while improving breastfeeding [32]. Muldoon et al., revealed that frenotomy reduced self-reported maternal nipple pain and provided an overall improvement in breastfeeding but rates of exclusive breastfeeding remained unchanged following frenotomy compared with pre-frenotomy [20]. These results show that frenotomy does not always resolve breastfeeding difficulties and highlight the importance of breastfeeding counselling for mother-infant dyads by experienced health care professionals.

Our study has several limitations. Our sample size may seem too small to generalize our findings for all patients with ankyloglossia but we prospectively followed all patients with ankyloglossia in our cohort. Besides, the prevalence of ankyloglossia in our cohort (2%) was within the range of the reported prevalence in the literature [2]. Another limitation is that the natural course in patients who had undergone frenotomy according to the family request is unknown. Even though, articulation was also evaluated while performing language development tests to inform the parents, a standardized articulation test was not used, since it was not the scope of this study. Other limitations are that there was no control group to compare the language development of children with ankyloglossia and the groups which were compared were uneven in number. Despite these limitations, we believe that our study adds to the limited literature comparing speech outcomes of ankyloglossia between treatment and nontreatment groups, as most of the studies compared surgical modalities or changes before and after surgical intervention. Due to prospective design, we were also able to watch the natural course of ankyloglossia in patients who had no frenotomy. Besides, to our knowledge, this is the first study evaluating objectively the language development of children with ankyloglossia. Furthermore, even though there is no universally accepted method of scoring for ankyloglossia, our study is one of the rare studies in which patients were assessed objectively by a validated tool in infancy. The decision on surgery was not only based on scoring but also on consensus reached between pediatric and plastic surgery teams, except for the five patients who were operated upon family request.

In conclusion, language development of children born with ankyloglossia are expected to follow regular steps as normal children. Families with a concern of speech delay should be informed comprehensively to avoid unnecessary surgical interventions which may prevent timely evaluation and management of language delay. Evidence-based guidance on treatment of ankyloglossia is still lacking. Communication between pediatric teams and surgeons is essential in determining the need for surgery until evidence-based guidelines are constituted. Future prospective studies with a larger sample size and long term follow up in which tongue – tie severity is assessed relying on objective tests, would be ideal for a comparison between patients who were surgically treated and those who were not.

Compliance with Ethical Standards

Ethical Approval: The study was approved by the Marmara University, School of Medicine Ethics Committee (Date: 05.10.2018, Number: 09.2018.674). This study was conducted in accordance with the Declaration of Helsinki. Written and verbal informed consent was obtained from parents.

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Contributors' contribution: H.E.B.: participated in data collection, data analysis, data interpretation, literature search, generation of figures and writing first draft of the manuscript. H.G.: participated in data collection, data interpretation and literature search and drafting the article. S.K.: participated in performing developmental tests of the subjects, data collection and interpretation and drafting the manuscript. F.N.D.K.: had participated in the study design, patient follow up, interpretation of the data and critical revision of the manuscript. P.B.: had primary responsibility for concept and design of the study, patient enrollment, patient follow up, outcome assessment, data analysis, writing manuscript and critical revision of the manuscript. All authors read and approved the final version of the manuscript.

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Differential diagnosis of classical Bartter syndrome and Gitelman syndrome: Do we need genetic analysis?

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ABSTRACT

Objective: Classical Bartter syndrome (cBS) and Gitelman syndrome (GS) are genotypically distinct, but there is a phenotypic overlap among these two diseases, which can complicate the accurate diagnosis without genetic analysis. This study aimed to evaluate the correlation between clinical and genetic diagnoses among patients who have genetically defined cBS and GS.

Patients and Methods: The study included 18 patients with homozygous/compound heterozygous *CLCNKB (NM_000085)* (n:10/18) and *SLC12A3 (NM_000339)* (n:8/18) mutations. Biochemical, clinical and radiological data were collected at presentation and at the last visit.

Results: In cBS group age at diagnosis, median plasma potassium and chloride concentrations were significantly lower and median plasma HCO3 and blood pH values were significantly higher. Patients with GS had significantly lower median plasma magnesium concentrations and urinary calcium/creatinine ratio. One child with GS had normocalciuria, two children with cBS had hypocalciuria and hypomagnesemia. Low estimated glomerular filtration rate (eGFR) (ml/dk/1.73m2) and growth failure were more evident in cBS group. In patients with cBS, nine different CLCNKB gene mutations were detected, five of them were novel. Novel mutations were: one nonsense (c.66G>A, p.Trp22*), one missense (c.499G>A, p.Gly167Ser) and three splice-site (c.867-2delA; c.499-2insG; c.1930-2A>C) mutations. In patients with GS, six different *SLC12A3* gene mutations were found.

Conclusions: It may not always be possible to clinically distinguish cBS from GS. We suggest to perform a genotypic classification if genetic analysis is possible.

Keywords: Bartter syndrome, Gitelman syndrome, Kidney tubuler disease, Hypokalemic metabolic alkalosis

1. INTRODUCTION

Bartter (BS) and Gitelman syndromes (GS) are autosomal recessive inherited salt-losing tubulopathies, characterized by hypokalemic hypochloremic metabolic alkalosis and normal blood pressure in the context of elevated renin and aldosterone levels [1]. Clinically, these tubulopathies are often categorized into 3 major subgroups: antenatal BS (aBS), classical BS (cBS) and GS. BS type-3 also known as cBS is caused by loss of function mutations in *CLCNKB* gene. *CLCNKB* gene encodes a chloride channel protein CIC-Kb, expressed in the thick ascending limp (TAL) of Henle's loop, the distal convoluted tubule (DCT), the connecting tubule and the collecting duct [1-3]. CIC-Kb has a role in the reabsorption of sodium chloride in the TAL and the loss of function mutations in *CLCNKB* gene cause electrolyte abnormalities called 'loop phenotype'. The use of loop diuretics results in the same electrolyte abnormalities [2,3]. GS was initially thought to be a subtype of BS but advances in molecular genetics showed that it was a separate entity. GS is caused by loss of function mutations in the gene *SLC12A3,* encoding thiazide-senstitive NaCl cotransporter called NCCT of the DCT [3]. The NCCT is responsible for the sodium reabsorption in the DCT and like with loop dysfunction, impaired sodium reabsorption in the DCT causes characteristic electrolyte abnormalities, called 'DCT' phenotype [3].

Classical BS and GS share some metabolic abnormalities such as hypochloremic hypokalemic metabolic alkalosis. Clinical and metabolic findings like hypercalciuria, normomagnesemia,

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early onset at younger age or severity of the disease are used to distinguish cBS from GS. In most cases, the correct diagnosis can be made without genetic analysis. However, it may not always allow distinction between them. Because hypercalciuria is not present always in all cases of cBS and some patients with cBS have hypomagnesemia, which is a typical feature of GS. Also, neither hypocalciuria nor hypomagnesemia are consistent findings in GS [4,5]. This study aimed to clarify clinical and laboratory findings among patients with genetically defined cBS and GS and evaluate the correlation between clinical diagnoses and genetic diagnoses.

2. PATIENTS and METHODS

Patients and biochemical analysis

The study included eighteen patients with homozygous or compound heterozygous *CLCNKB* (n:10/18) and *SLC12A3* (n:8/18) mutations presenting to the pediatric nephrology division of a university hospital. Biochemical, clinical and radiological data were collected at presentation and at the last clinic follow-up visit. Hypomagnesemia was defined as a serum magnesium concentration less than 1.7 mg/dl, hypokalaemia was defined as a serum concentration less than 3.5 mEq/L, hypochloremia was defined as a serum chloride concentration less than 98 mg/dl. Normocalciuria was defined with the normal ranges for the patient's age. Estimated glomerular filtration rate (eGFR) was calculated using the modified Schwartz formula, with a k-value of 0.413. Body height percentiles of patients were recorded at the presentation and at the last follow-up visit. Growth failure was defined as a height persentile less than 3. Kidney ultrasound imaging was performed at presentation and in the follow-up of all children.

Molecular Genetics studies

After detailed pedigree analyses and written informed consents were obtained, all patients' and their parents' total genomic DNA was extracted from peripheral blood using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). For all patients sequencing of *CLCNKB (NM_000085)* and *SLC12A3* genes *(NM_000339)* were performed using two different methods: Sanger sequencing and Next-Generation Sequencing (NGS). All samples were first analyzed by NGS and after detection of sequence changes, confirmation of them were performed using Sanger sequencing with targeted sequencing for those already detected mutations/variations. The Nephropathies Solution (NES) kit by Sophia Genetics (Switzerland) was used for NGS (Illumina Nextseq 500) and single nucleotide polymorphisms (SNPs) and copy number variations (CNVs) were analyzed through Sophia-DDM-v4 platform [ ]. For both of these genes, specific primers were designed for all exons and exon-intron junctions and Sanger sequencing was performed via ABI PRISM 3130xl Genetic Analyzer (AppliedBiosystems, Foster City, CA, USA). SNPs were analyzed with Chromas software version 2.4.1. Variants with minor allele frequency (MAF) <1% according to population studies [ESP, ExAC, 1000 Genome (1000G), and Genome aggregation database (gnomAD)] were filtered and retained variants were searched in Human Gene Mutation Database (HGMD), Clinvar and Varsome Databases. Pathogenicity scores were predicted using Mutation taster, Provean, Polyphen, Human Splicing Finder (HSF) and Sorting Intolerant From Tolerant (SIFT) in – silico tools.

Statistical analysis

All data were analyzed using the Statistical Packages for the Social Sciences (SPSS Inc., Chicago, IL, USA) 21.0 package. A One Sample Kolmogorov-Smirnov test was used to determine the normality of data. Results were expressed as mean with standard deviation (mean ± SD) in case of normal distribution and median (range) in case of non-normal distribution. Differences between categorical data were evaluated using t-test and Mann-Whitney non-parametric U test in normal distribution and non-normal distribution, respectively. A p-value of less than 0.05 was considered statistically significant.

3. RESULTS

Biochemical features and clinical features at presentation and at the last follow-up visit are summarized in Table I. Ten patients presented with cBS and eight patients with GS. Five patients (50%) with cBS and five patients (62.5%) with GS were male. Patients with cBS had a significantly lower median age at diagnosis than the patients with GS (11.5 vs. 128 months, respectively). The median follow-up time was 33 months in patients with cBS and 47 months in patients with GS.

Table I. *Clinical, demographic and biochemical characteristics of the patients*

	Classical Bartter Syndrome (n=10)	Gitelman Syndrome (n=8)	p
Age, months	11,5 (1-76)	128 (44-209)	**<0.001**
Female/male, n (%)	5/5 (50%)	3/5 (60%)	0.664
Follow-up, months	33 (3-120)	47 (19-174)	0,200
Prematurity, n (%)	3 (30%)	0 (0%)	0,090
Blood pH	7.57 (7.40-7.70)	7.44 (7.40-7.59)	**0.003**
Blood HCO3 (mEq/L)	41.1 (21.8-51.5)	30.6 (27.4-32.4)	**0.010**
Plasma potassium (mEq/L)	2.2 (1.9-3.1)	2.8 (2.2-3.7)	**0.002**
Plasma chloride (mg/L)	85 (67-96)	95 (88-101)	**<0.001**
Plasma magnessium (mg/dl)	2.15 (1.6-2.7)	1.4 (1.30-1.50)	**0.001**
Urinary calcium/creatinine ratio (mg/mg)	0.6 (0.03-1.9)	0,01 (0.01-0.67)	**<0.001**
eGFR (ml/dk/1.73 m^2) (at presentation)	103 (49-172)	137 (99-172)	**0.043**
eGFR (ml/dk/1.73 m^2) (at the last visit)	103 (76-192)	112 (89-271)	0.410
Height <3.persentile (at presentation) , n(%)	5 (50%)	2 (25%)	0.280
Height <3.persentile (at the last visit), n(%)	3 (30%)	1 (12.5%)	0.370

Values are represented as median (min – max), eGFR, estimated glomerular filtration rate

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Median plasma potassium and chloride concentrations were significantly lower in cBS group (2.2 vs. 2.8 mEq/L; 85 vs. 95 mg/L, respectively). Median plasma HCO3 concentrations and blood pH values were significantly higher in cBS group (41.1 vs. 30.6 mEq/L; 7.57 vs. 7.44 respectively). Patients with GS had significantly lower median plasma magnesium concentrations (1.40 vs.2.15 mg/dl respectively) and urinary calcium/creatinine ratio (0,01 vs. 0,6 respectively). One child with GS had normocalciuria and two children with cBS had both hypocalciuria and hypomagnesemia at presentation. Median eGFR (ml/dk/1.73m^2) at presentation were significantly lower in cBS patients than patients with GS (103 vs. 137 ml/dk/1.73m^2 respectively). At the last follow-up, patients with cBS had still lower eGFR (103 vs. 112 ml/dk/1.73m^2 respectively). Growth failure was the most frequent complaint of patients with cBS and it was more evident in patients with cBS (50% at presentation; 30% at last follow-up visit). Ultrasonography performed at presentation and in the follow-up showed nephrocalcinosis in only one child with cBS.

Molecular analyses, revealed mutations that are shown in Tables II and III. In ten patients with cBS, nine different *CLCNKB(NM_000085)* gene mutations were detected (Table II). Five of them were novel mutations. Novel mutations were: one nonsense (c.66G>A, p.Trp22*), one missense (c.499G>A, p.Gly167Ser) and three splice-site (c.867-2delA; c.499-2insG; c.1930-2A>C) mutations. In eight patients with GS, six different *SLC12A3 (NM_000339)* gene mutations were detected (Table III). Only one patient had compound heterozygous mutation and the majority of mutations caused a frameshift c.237_238dupCC (p.Arg80Profs*35) mutation.

Table II. *Nine variants with five novel variants (showed in bold) in classical Bartter syndrome-related gene*

Patients	Gene	Status	Mutation	Position	Type of mutation	Reference
B1	*CLCNKB*	Homozygous	c.371C>T (p.Prol24Leu)	Exon 5	Missense	Simon et al.,1997 [6]
B2	*CLCNKB*	Homozygous	c.371C>T (p.Prol24Leu)	Exon 5	Missense	Simon et al., 1997 [6]
B3	*CLCNKB*	Homozygous	**c.867-2delA**	Intron 8	Splice-site	This study
B4	*CLCNKB*	Homozygous	Exon 2-20 deletion	Exon 2-20	Gross deletion	Simon et al.,1997[6]
B5	*CLCNKB*	Homozygous	**c.499-2insG**	Intron 4	Splice-site	This study
B6	*CLCNKB*	Homozygous	c.910C>T (p.Arg304*)	Exon 10	Nonsense	Messa et al., 2020[7]
B7	*CLCNKB*	Homozygous	c.910C>T (p.Arg304*)	Exon 10	Nonsense	Messa et al., 2020[7]
B8	*CLCNKB*	Homozygous	**c.1930-2A>C**	Intron 18	Splice-site	This study
B9	*CLCNKB*	Homozygous	**c.499G>A (p.Gly167Ser)**	Exon 5	Missense	This study
B10	*CLCNKB*	Compound heterozygous	**c.66G>A (p.Trp22*)** c.865G>C (p.Gly289Arg)	Exon 2 Exon 9	Nonsense Missense	This study and Sahbani et al.,2020[8]

Table III. *Six variants in Gitelman syndrome-related genes*

Patients	Gene	Status	Mutation	Location	Type of mutation	Reference
G1	*SLC12A3*	Homozygous	c.1180+1G>T	IVS 9	Splice-site	Coto et al., 2004[9]
G2	*SLC12A3*	Compound heterozygous	c.237_238dupCC (p.Arg80Profs*35) c.514T>C (p.Trp172Arg)	Exon 1 Exon 4	Frameshift Missense	Mastroianni et al., 1996[10] Syren et al., 2002[11]
G3	*SLC12A3*	Homozygous	c.625C>T (p.Arg209Trp)	Exon 5	Missense	Simon et al., 1996[3]
G4	*SLC12A3*	Homozygous	c.1175C>T (p.Thr392Ile)	Exon 9	Missense	Colussi et al., 2007[12]
G5	*SLC12A3*	Homozygous	c.237_238dupCC (p.Arg80Profs*35)	Exon 4	Frameshift	Mastroianni et al., 1996[10]
G6	*SLC12A3*	Homozygous	c.237_238dupCC (p.Arg80Profs*35)	Exon 4	Frameshift	Mastroianni et al., 1996[10]
G7	*SLC12A3*	Homozygous	c.237_238dupCC (p.Arg80Profs*35)	Exon 4	Frameshift	Mastroianni et al., 1996[10]
G8	*SLC12A3*	Homozygous	c.1964G>A (p.Arg655His)	Exon 16	Missense	Simon et al., 1996[3]

4. DISCUSSION

The present study describes the initial clinical and biochemical characteristics and the genetic findings of the patients with cBS and GS. According to our findings, age at diagnosis, the urinary calcium/creatinine ratio, plasma chlorid, magnesium, bicarbonate levels may be useful markers for differentiating cBS and GS. Although, cBS and GS are genotypically distinct, there is considerable overlap in clinical presentation. cBS may sometimes mimic GS and some patients with cBS appear to have a phenotypic switch [13]. cBS is generally considered to be a disorder of TAL, whereas GS is a disorder of DCT. Initially, some cBS patients present with loop (or BS) phenotype but later they revert to a DCT (or Gitelman like) phenotype [14]. The expression of *CLCNKB* also in DCT may explain the phenotypic similarity of cBS and GS.

It is known that cBS often presents clinically before the age of two [15]. In this study, the age at diagnosis differed significantly between two diseases. Only one patient with cBS was diagnosed after the age of two years, whereas the youngest patient in GS group was 44 months old at the time of diagnosis.

Hypochloremia, hypokalemia and metabolic alkolosis are hallmarks of cBS. In our study, serum chloride and potassium levels were significantly lower and bicarbonate levels were higher in patients with cBS than GS as expected.

Hypercalciuria and normomagnesemia are used to distinguish cBS from GS. Hypercalciuria and nephrocalcinosis which are very important risk factors for chronic kidney disease progression, are less commonly seen in patients with cBS than aBS and not an expected finding in GS. Impaired salt reabsorption in the TAL leads to an impaired paracellular cation uptake, mostly manifesting as hypercalciuria as with *CLCNKB* mutations [1]. In contrast to loop dysfunction, impaired salt reabsorption in the DCT paracellular calcium reabsorption in the TAL is unaffected and patients typically have hypocalciuria due to a compensatory increase in salt and calcium reabsorption in the proximal tubule as with *SLC12A3* mutations [1]. Hypomagnesemia is present in 20-52% of patients with BS and this is caused by magnesium wasting [16]. Among our patients, hypercalciuria was found in six of ten patients with cBS. However, some patients with cBS may have hypomagnesemia and hypocalciuria [14,17-19]. Zelikovic et al., had reported that four patients in the 12 family members with *CLCKNB* mutations had concominant hypomagnesemia and hypocalciuria [20]. In our study, two children with cBS (B4, B7) had both hypocalciuria and hypomagnesemia at presentation. Patient B4 had a gross deletion (Exon 2-20 deletion) and patient B7 had a homozygous nonsense mutation (p.Arg304*) in exon 10 of *CLCNKB* gene. But we did not observe hypomagnesemia and hypocalciuria in another patient (B6) with the same mutation as patient B7. Recently, Ring et al. and Kamel et al., also reported that patients with GS may have normocalciuria [4,5].

In the present study, patients with GS had a significantly lower median plasma magnesium concentrations and urinary calcium/creatinine ratio. But similar to the above mentioned studies, one child with GS (G5) had normocalciuria. Patient G5 had a homozygous frameshift mutation (p.Arg80Profs*35) in exon 4 of *SLC12A3* gene. However, our other patient (G6) with the same mutation had hypocalciuria. It is still unclear why the same genetic defect causes different clinical outcomes but Zelikovic et al., hypothesized that a modifying genetic effect on any one of channels participating in Cl^- transport in the TAL and the DCT may influence the disease phenotype [20]. Genetic analysis is pivotal for an accurate diagnosis in such patients.

Impaired GFR is observed more commonly in patents with BS than GS. It has been reported that 25% of patients with cBS suffer from CKD [15,21-24]. In our study, four patients with cBS had impaired GFR at admission, whereas only two patients had CKD stage-II at the end of follow-up. However, all patients with GS had an eGFR greater than 90 ml/dk/1.73 m^2 at admission and at the end of follow-up period. The mechanism of CKD development is probably multifactorial. Nephrocalcinosis, chronic hypokalaemia, prematurity, long-term treatment with NSAIDs and damaging effect of elevated aldosterone levels on podocytes are some of the possibilities for CKD development in patients with BS and GS.

One recent study covering 30 patients with cBS detected no genotype-phenotype association [25], whereas Seys et al., reported an association between complete loss of function (CL/CL) mutations of *CLCNKB* mutations and severe phenotypes [21]. Although, we did not demonstrate by functional analysis, there was only one large deletion (Exon 2-20 deletion, patient B4) in our cohort that could be predicted to cause complete loss of function. This patient had the lowest eGFR at presentation. Although, previous studies from different countries [18,21,26] reported that whole gene deletion was the most common mutation in *CLCNKB* gene, in our cohort only one patient had this mutation. When our patients were evaluated in terms of genotype-phenotype correlation, no significant correlation was found even among patients harboring the same mutation.

The major limitations of our study were the low number of our patients and the relatively short period of follow up. Long term follow up of a larger number of patients could allow us to identify a significant difference in growth between these two study groups. Nonethless, the strength of our study is that the diagnosis of our patients were confirmed by very detailed genetic analyses. Prospective studies with larger number of patients, longer follow-up time and genetic confirmation will allow better demonstration of the genotype-phenotype correlation.

Conclusions

According to our findings, age at diagnosis, plasma chlorid, magnesium, bicarbonate levels and the urinary calcium/creatinine ratio may be useful markers for differentiating cBS and GS. But, in some cases, cBS cannot be distinguished clinically from GS, especially in patients with overlapping features. For this reason we suggest to perform a gene-based classification especially in countries where genetic analysis is possible.

Compliance with the Ethical Standards The study was approved by the Ethics Committee of Marmara University, School of Medicine (Protocol number: 09.2021.157). The

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study protocol was described to all patients and their parents and written informed consent was obtained from all individual participants older than 18 years of age and from the parents of all children included in the study.

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Comparison of postoperative pain and pain control techniques in uniportal and biportal VATS and open surgery patients

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ABSTRACT

Objectives: Thoracotomy causes intense postoperative pain which may become chronic. Video-assisted thoracic surgery (VATS) leads to less postoperative pain compared with thoracotomy. In this study, we analyzed pain scores in patients who underwent lung resections with VATS or thoracotomy.

Patients and Methods: Patients who underwent lung resections with uniportal, biportal VATS or thoracotomy between May 2015 – May 2017 were included in the study. Visual Analogue Scale (VAS) pain scores were recorded on postoperative day 1, 5 (or at discharge), 2nd week, 1st and 3rd months. Patients were classified in 3 groups, uniportal VATS (n=178), biportal VATS (n=15), thoracotomy (n=60). Demographics, resection type, mortality, morbidity and epidural catheter use were recorded.

Results: Two hundred and fifty-three patients (average age was 57.3 ± 12.7, 94 females) were included in the study. Median hospital stay was 5 days. Uniportal and biportal groups had significantly lower pain scores in all intervals compared with thoracotomy. No chronic pain was seen in VATS groups. Uniportal and biportal groups had similar pain scores at all times. Epidural use or size of specimen did not affect pain in VATS patients (p=0.18 vs p=0.68).

Conclusion: Video-assisted thoracic surgery decreases the need for epidural patient control analgesia. Specimen size does not affect postoperative pain and chronic pain is rare.

Keywords: Thoracoscopy, Uniportal, Biportal, Postoperative analgesia

1. INTRODUCTION

Pain control after thoracic operations is of critical importance. Thoracotomy causes intense postoperative pain, which may require epidural analgesia and/or opioid medications (morphine, oxycontine, tramadol etc.) [1]. Postoperative intensive pain control approaches may hinder the patient's compliance to pulmonary rehabilitation, inadequate ambulation may lead to atelectasis and pneumonia. Pain may still persist after discharge, affecting patient's toleration for adjuvant treatments, cause poor quality of life and also chronic pain syndromes may occur in 5-10% of patients who underwent thoracotomy [2].

Video-assisted thoracic surgery (VATS) has become the standard of care in the last 15 years for early stage non-small cell lung cancer (NSCLC). The reason of this popularity is mainly due to decreased postoperative pain with VATS compared to thoracotomy. This has been reported in comparative and randomized studies which mainly use visual analogue scale (VAS) for pain quantification [3,4]. Improvements in VAS scores have also been correlated with functional gains after thoracic surgery [5].

Video-assisted thoracic surgery approach can be used for various procedures ranging from anatomical resections to pleural

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biopsies. For anatomical resections, standard VATS approach uses 3 ports, but as the technique gained popularity and surgeons completed their learning curve, biportal and uniportal anatomic resections have been reported in large case series. Comparative studies for those procedures are being published and the functional gain of these patients (if any) is not clear [6, 7]. There is also no currently established guideline delineating the use of basic (medication) or advanced (epidural, paravertebral) pain control modalities in various VATS techniques.

The aim of this study is to compare three different thoracic surgical techniques (uniportal VATS, biportal VATS, thoracotomy) and the size of lung specimens (wedge resection vs anatomical resection) in terms of pain scores, necessity of epidural analgesia and presence of chronic pain.

2. PATIENTS and METHODS

Patient selection

All patients undergoing anatomical or limited lung resection between May 2015-May 2017 were prospectively enrolled in this study. Patients were evaluated in 3 groups; Uniportal VATS (n=178), biportal VATS (n=15) and thoracotomy (open surgery) (n=60). All patients received standard preoperative work up according to their condition (lung cancer, solitary pulmonary nodule, bronchiectasis etc.) and anesthetic needs (routine laboratory studies, chest X-ray, electrocardiogram, pulmonary function tests, required consults according to present comorbidities). Patients with pre-diagnosed chronic pain syndromes or on chronic opioids and patients who did not consent to participate or could not cooperate for the application of the VAS were excluded. Demographic data, medical history (comorbidities, previous surgery, malignancy, etc), operative data (type of surgery, presence of epidural catheter, etc), perioperative data (length of hospital stay, opioid use during hospital and after discharge) and VAS for pain were prospectively recorded in a database.

The study was approved by the Marmara University, School of Medicine Clinical Research Ethics Committee on 02.12.2016 with the protocol code of 09.2016.589. Per regulations of the review board and the hospital, written consent was taken from each patient.

Surgical technique

Uniportal VATS incision was made on the anterior axillary line, at the 5th intercostal space. 3-5 cm incision was made and a wound protecting retractor was used in every patient. A 28-32 F drain was placed through the same incision at the end of the operation.

Biportal VATS approach included a second incision in addition to the incision described in uniportal technique, which was usually at the 7th or 8th intercostal space, on the anterior axillary line. A 28-32 F drain was placed through the lower port at the end of the operation.

Open surgery was performed through a classical posterolateral or anterolateral thoracotomy through 5th intercostal space. Rib retractor was used in all cases. 2 chest drains were placed from separate incisions.

Analgesic technique and follow up

All patients enrolled in the study received either intravenous (pethidine) or epidural (bupivacaine) patient controlled analgesia (PCA). Pethidine PCA settings were loading dose of 0.6 mg/kg, and a request dose of 0.2 mg/kg with basal infusion of 0.1 mg/kh/hour. An analgesic solution of bupivacaine (0.125%) was used for epidural PCA. In both groups, locked out time was set as 30 minutes. Thoracic epidural catheters were placed preoperatively in the operating theater, just before the operation. Anesthesiology postoperative pain team followed up the PCAs for 3 days every 12 hours and per needed basis in between. After postoperative day 3, analgesic maintenance was through oral and IV medications (tramadol, acetaminophen, ibuprofen). PCA was terminated on the day of discharge if the hospital stay was shorter than 3 days.

Patients were provided with a single script for 20 tramadol tablets, 50 mg, taken up to thrice daily as needed at discharge. They were instructed to rely on diclofenac 75 mg (twice a day) and acetaminophen 500 mg tablets (thrice a day) first, before using tramadol.

Visual analogue scale (VAS) pain scores

Visual Analogue Scale is an established method used for pain quantification. In our study the type of VAS that was provided had a 1 to 10 scale with 1 as pain free and 10 as the worst pain imaginable. The scale also included graphical references, pictured as representative faces at that corresponding pain level [8]. The measurements were taken on postoperative 1st, 5th days and on 2nd week, 1st month and 3rd month.

Statistical Analysis

Data were analyzed using SPSS 22.0. $p<0.05$ was determined as statistically significant. Mean, standard deviation and median values were calculated accordingly.

Distribution of variables were determined by using Kolmogorov – Smirnov test. Qualitative independent variables were evaluated by using Kruskal-Wallis and Mann-Whitney-U tests. Wilcoxon test was used for dependent qualitative parameters. For quantitative assessment, Chi-Square was used when conditions were met, otherwise Fisher's exact test was performed.

3. RESULTS

Patient characteristics

Among the patients included in the study, 178 patients underwent uniportal, 15 patients biportal VATS and the remaining 60 patients underwent thoracotomy. Operation breakdown is shown in Table I.

Mean age was 57.3 ± 12.7. There was no statistically significant age difference between groups (Table I).

Epidural cathater PCA was applied to 183 patients (114 uniportal, 12 biportal, 57 open), while in 70 patients analgesia was provided (64 uniportal, 3 biportal, 3 open) via IV PCA (Table I).

Uniportal and biportal groups had significantly lower pain scores on all intervals when compared to open group, however on the postoperative day 14, although the difference was evident, it was not statistically significant between biportal and open surgery groups (p:0.087). The difference in pain scores between these 3 groups was most pronounced in postoperative day (POD)1 (3.3±1.6, 3.3±1.3 vs 4.7±1.1 p<0.001). (Table II, Figure1).

On postoperative day 5, significantly lower percentage of patients in uniportal group scored higher than 4 when compared to open group (18/160 [11.3%] vs 17/60 [28.3%], p=0.01).

In chronic phase (3rd month) 14 uniportal, 3 biportal and 27 open surgery patients had very low pain intensity. While none of the uniportal and biportal patients described significant pain at 3rd month, 4 open surgery patients still had medium pain intensity. (0.1±0.4, 0.3±0.6 vs 0.8±1.1 p<0.001).

The subgroup analysis in regards to operation type (anatomic resection vs non anatomic resection) did not yield to any statistically significant different results in terms of VAS score composition (Table III). Also, in uniportal cases epidural and IV PCA groups were comparable in all intervals regardless of the operation type (Table IV).

Length of hospital stay

Uniportal surgery group had statistically a shorter length of hospital stay when compared to open surgery group (4.7±3.3, 5.5±2.2 vs 7.3±4.1 p<0.001). There was no significant difference in regards to the length of hospital stay in either uniportal versus biportal or biportal versus open surgery comparisons.

Opioid usage after discharge

While a greater percentage of open surgery patients filled in and used their opioid (tramadol 50 mg, 20 tablets, thrice a day) prescsriptions than uniportal patients (27% vs 11%, p<0.04), biportal group's opioid consumption was comparable to uniportal and open surgery groups (p>0.05) .

Table I. *Patient characteristics, operation and analgesic type of subgroups*

	Uniportal (n=178)	Biportal (n=15)	Open surgery (n=60)
Age (mean ±SD, Median)	56.8±12.4, 58.0	63.7±9.1, 63.0	57.0±13.9, 59.5
Sex (n,%) Female	N=72, 40 %	N=3, 20 %	N=19, 31 %
Male	N=106, 59 %	N=12, 80 %	N=41, 68 %
Anatomic resection (n, %)	N=89, 50 %	N=14, 93.3 %	N=50, 90 %
Wedge resection (n,%)	N=89, 50 %	N=1, 6.7 %	N=6, 10 %
Analgesic control Epidural PCA	N=114, 64 %	N=12, 80 %	N=57, 95 %
(n,%) IV PCA	N=64, 36 %	N=3, 20 %	N=3, 5 %

PCA: Patient Controlled Analgesia

Table II. *Distribution of VAS pain scores according to the groups at different intervals*

	Uniportal				Biportal				Open Surgery				p	
	Mean±s.d.			Median	Mean±s.d.			Median	Mean±s.d.			Median		
VAS														
Postop Day 1	3.3	±	1.6	3.0	3.3	±	1.3	4.0	4.7	±	1.1	4.5	**<0.001**	K
Postop Day 5	1.9	±	1.1	2.0	2.2	±	1.0	2.0	3.0	±	0.9	3.0	**<0.001**	K
Postop 2nd week	0.6	±	0.8	0.0	1.1	±	0.9	1.0	1.6	±	0.7	2.0	**<0.001**	K
Postop 1st month	0.2	±	0.6	0.0	0.2	±	0.4	0.0	0.9	±	0.8	1.0	**<0.001**	K
Postop 3rd month	0.1	±	0.4	0.0	0.3	±	0.6	0.0	0.8	±	1.1	0.0	**<0.001**	K

K Kruskal-Wallis, VAS: Visual Analogue Scale

Table III. *Distribution of VAS pain scores in terms of resection types*

	Wedge				Anatomic Resection				p	
	Mean.±s.d.			Median	Mean ±s.d.			Median		
VAS										
Postop Day 1	3.27	±	1.69	3.00	3.37	±	1.42	4.00	0.172	m
Postop Day 5	1.88	±	1.05	2.00	1.94	±	1.16	2.00	0.555	m
Postop 2nd week	0.46	±	0.66	0.00	0.71	±	0.89	0.00	0.091	m
Postop 1st month	0.17	±	0.48	0.00	0.30	±	0.65	0.00	0.143	m
Postop 3rd monthW	0.03	±	0.18	0.00	0.17	±	0.48	0.00	0.052	m

m Mann-Whitney U test, VAS: Visual Analogue Scale

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Table IV. *Distribution of VAS pain scores between epidural and IV PCA in uniportal incision*

	Epidural (-)				Epidural (+)				P	
	Mean ± s.d.			Median	Mean ± s.d.			Median		
VAS										
Postop Day 1	3.17	±	1.45	3.00	3.40	±	1.61	3.50	0.335	m
Postop Day 5	1.70	±	0.90	2.00	2.03	±	1.19	2.00	0.092	m
Postop 2nd week	0.45	±	0.75	0.00	0.66	±	0.81	0.00	0.078	m
Postop 1st month	0.08	±	0.27	0.00	0.32	±	0.67	0.00	0.009	m
Postop 3rd month	0.06	±	0.30	0.00	0.12	±	0.40	0.00	0.242	m

m Mann-Whitney U test

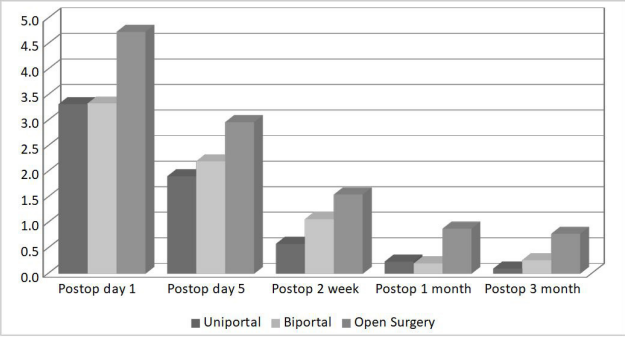

Figure 1. *VAS pain scores of three groups at different intervals*

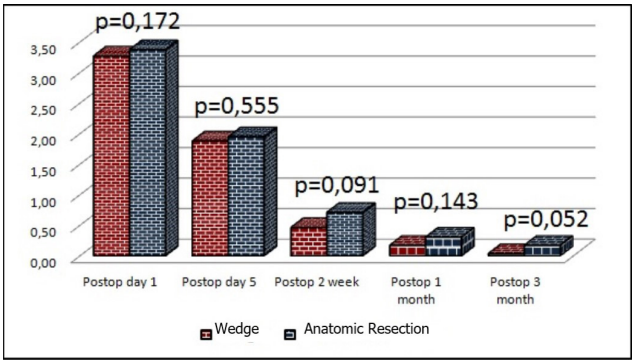

Figure 2. *Distribution of VAS pain scores according to the resection types*

4. DISCUSSION

A total of 253 patients who have undergone uniportal and biportal VATS and thoracotomy for various indications were included in this study. No significant difference was found in terms of pain between the uniportal and the biportal groups at all times except from the resection size comparison. Although, there was no significant difference between biportal and open surgery at the postoperative 2nd week, uniportal and biportal groups had lower pain scores compared with the open surgical group at all other times (Figure 1).

The first comparison of postoperative pain in VATS versus thoracotomy approaches was done by Landreneau et al., in 1993, which concluded in favor of VATS [9]. After that, various articles elaborated on postoperative pain and other aspects like length of hospital stay, length of chest drains, complications, quality of life, survival etc. 39 of those studies were reviewed in a meta-analysis by Whitson et al., among 6370 patients. VATS was found to be superior in terms of postoperative complications, length of chest drainage and hospital stay [3]. Since, the nature of the problem makes it very difficult to design a double blind randomized study and recruit adequate number of patients, the National Comprehensive Cancer Network guidelines now accept VATS as a first line approach for early state NSCLC, citing meta-analysis and well designed propensity score match studies show both superiority and non-inferiority to thoracotomy in regards to perioperative and oncological outcomes [10].

Enhanced recovery after surgery (ERAS) pathways were initially developed in colorectal surgery. In a meta-analysis of 38 studies, ERAS pathways were seen to be effective in reducing hospital length of stay and postoperative complication rates [11]. Guidelines for enhanced ERAS have recently been published for lung surgery. A standardized multimodal approach to pain relief, including good regional anaesthesia, is recommended with the aim of reducing postoperative opioid use. Acetaminophen and non-steroidal anti-inflammatory drugs should be administered regularly to all patients unless contraindications exist [12]. The use of ERAS after lung surgery has the potential to improve patient outcomes. Early mobilization and VATS lung resection were independently associated with reduced morbidity while early mobilization was the only independent factor influencing a reduced length of hospital stay [13]. Our standard approach was to have patient walk in-room after 6th postoperative hour, right after introduction of a liquid diet. Since, the time of the disposition of the patient (family home, skilled nursing facility, rehabilitation facility, assisted living, etc.) is dependent on the practice environment we were not able to directly compare our

hospital stay statistics but 5-7 days is within the range of the established literature [7, 12,13].

Video-assisted surgery is a versatile approach, allowing application to a wide range of pathologies with different modifications. Uniportal VATS became popular after 2008 and some articles compared uniportal vs multiportal approaches in terms of postoperative pain and opioid consumption. While Jutley et al., found uniportal VATS to be more favourable in pneumothorax cases in acute postoperative period, uniportal and multiportal approaches were found to be equivalent in the chronic phase [14]. McElnay et al., found both approaches comparable when the population of the study was limited to anatomic resections [15]. This report's cohort, regarding the range of pathologies covered with VATS and postoperative pain outcomes, was comparable in both acute and chronic phases regardless of the number of the incisions.

In another study, similar to ours, by Sebastian et al., patients were divided into 3 groups: robotic surgery, VATS and open surgery and pain scores were compared. There was not a significant difference between VATS and robotic-assisted thoracoscopic surgery (RATS) groups in terms of acute and chronic pain and pain scores were less than the open surgery group in the acute period. However, different from our study, in the chronic period, there was not a significant difference in pain scores between 3 groups [16].

Although, the pathogenesis of pain is not clear, the most common opinion is that postthoracotomy pain is caused by intercostal nerve injury. Pain can sometimes exist after 4 to 5 years in 30% of patients [17]. In our study, during the chronic pain evaluation in the third month: no patients in the uniportal (0/178) and biportal (0/15) groups had pain, while in the open surgery group (4/60) 4 patients had moderate pain (Table II, Figure 1). The reason of this pain is thought to be nerve injury caused by intercostal retractions during surgery.

Epidural analgesia, paravertebral blocks, intercostal nerve blocks, PCA are the most common used pain control methods after thoracic surgery. Jie Ae Kim et al., compared the epidural PCA and paravertebral block methods for pain control in 37 patients who underwent VATS during the postoperative 5 days period and there was no significant difference between pain levels and pulmonary functions [18]. Epidural PCA or IV PCA were applied to all patients in our study. Pain scores were evaluated in 2 groups named as epidural PCA and IV PCA in 178 patients who had uniportal resection. There was not a significant difference in pain levels between these groups in our study (Table IV). Our study revealed that lung resections with uniportal incisions result in lower pain scores and pain control was comparable with systemic or epidural pain control methods in accordance with the reported experiences of different clinics in the literature [19, 20]. We compared the patients in 2 groups according to the size of resection: named as wedge resection and anatomical resection. The results showed that the size of resection has no effect on pain. There are no studies in literature comparing the size of the specimen and its relevance with postoperative pain.

Thoracic surgery is associated with high level of pain and an elevated incidence of long term opioid use after surgery [21]. Opioid use is also associated with significant risk for both mobidity and mortality [22].There are no clinical studies which analyze opioid needs after discharge following lung cancer surgery at different types of incision. However, many news and studies derived from especially the USA [23, 24] show that the increase in the number of IV opioid users and the change in their demographic profile in these days are caused by the opioid prescriptions during discharge and their easy access. Due to this reason, it is better for patient safety to choose the methods that do not require opioid consumption in discharge. Our study showed that in the postoperative fifth day (which is usually the day of discharge) patients who had 4 and more in pain scores in uniportal VATS group were significantly low. Since, sociocultural factors affect pain resistance and analgesic requirements, in our study opioid consumption was overall low in all groups. However, if this is not the case in other cultures, uniportal VATS may be a solution to decrease opioid consumption.

This study has several limitations. First of all, this study is not randomized and possibility of selection bias cannot be excluded. Thoracotomy patients may have had more advanced stage tumors. Secondly, the number of patients are not evenly distributed. Biportal patients are usually conversions from uniportal VATS due to difficulty in stapler introduction or lung manipulation. The study also does not have a power analysis, thus some of the significances may have been unnoticed due to the limited number of patients. Additionally, our study is terminated with 3 month follow-up and we could not assess pain scores at 6 months and 1 year postoperatively.

In conclusion, this study shows that VATS technique, independent of the number of port incisions, may decrease or eliminate the need for an epidural PCA for postoperative analgesia, specimen size does not affect postoperative pain and chronic pain is not highly expected in VATS patients.

Compliance with Ethical Standards

Ethical approval: Ethical approval was obtained from the Marmara University Faculty of Medicine Clinical Research Ethics Committee on 02.12.2016 with the protocol code of 09.2016.589. Per regulations of the review committee and the hospital, written consent was taken from each patient.

Funding: The study was not supported by any funds.

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

Author Contributions: C.C. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work, Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Final approval of the version to be published. Z.B. Drafting the work or revising it critically for important intellectual content, Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work, Final approval of the version

to be published. T.L. Drafting the work or revising it critically for important intellectual content, Final approval of the version to be published. K.B. Drafting the work or revising it critically for important intellectual content, Final approval of the version to be published. B.Y. Drafting the work or revising it critically for important intellectual content, Final approval of the version to be published. M.Y. Drafting the work or revising it critically for important intellectual content, Final approval of the version to be published. H.F.B. Drafting the work or revising it critically for important intellectual content, Final approval of the version to be published. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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The palmaris longus muscle: A surface study of the population of North Cyprus

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ABSTRACT

Objective: The aim of this research is to determine the frequency of surface anatomical variations of palmaris longus (PL) muscle in the population of North Cyprus and their association with gender, body side, and hand dominance.

Materials and Methods: The presence of PL was determined in 1280 subjects (660 females and 620 males) using four testing methods; Schaeffer's test, the Thompson's test, Pushpakumar's test and the Bhattacharya's test. Where the presence of the PL could not be determined by any of these tests, palpation was performed as the final confirmatory test.

Results: The overall frequency of absence was 17.4%. Female subjects showed a higher frequency of absence of 10.6% compared to males (6.8%). The absence was more likely to occur in the non-dominant hand. In those that had the PL, there were 28 (2.6%) cases of a split tendon and 3 cases (0.2%) of a laterally displaced tendon of the PL.

Conclusion: The overall frequency of absence of PL in North Cyprus was 17.4% and absence is more likely to occur in females, on the left side and in the non-dominant hand. Other variations recorded are the split tendons and laterally displaced tendons.

Keywords: Palmaris longus, Anatomical variation, Agenesis, Forearm, North Cyprus

1. INTRODUCTION

The palmaris longus (PL) muscle is one of the four superficial muscles on the flexor aspect of the forearm. It originates from the common flexor tendon at the medial epicondyle of humerus and converges into a long and slender tendon that passes anterior to the flexor retinaculum before it becomes flattened to blend with the palmar aponeurosis. It flexes the wrist joint and tenses the palmar aponeurosis [1-3].

The PL has received worldwide attention from researchers, despite the fact that functionally, it is a negligible muscle. It is believed to be functionally more active in mammals that use their upper limbs for mobilization [4, 5]. It has lost its function in the course of human evolution and is gradually becoming extinct [6-8]. This evolution-induced morphometric change has made the PL one of the most variable muscles in the body [4, 5, 9]. It may be absent on one or both sides [3, 10] or may be reversed, duplicated or digastric [3].

Surgeons consider the tendon of PL as the tendon of choice in reconstructive surgeries, because it is of the right length and diameter, it is easily accessible and its absence does not produce any functional deformity [4, 11]. It is used by plastic surgeons in treatment of facial paralysis, repairing ptosis, lip augmentation, and in the restoration of lip and chin defects [12-14]. The tendon of PL is also harvested to repair oncologic defects of head and neck and arthritis of the thumb [15].

The variations of PL can cause many clinical syndromes as reported by various researchers. A study reported a clinical case of a bitendinous PL causing median nerve compression during a standard carpal tunnel release [16].

Many textbooks of anatomy show a worldwide prevalence of absence of PL as 10-15% [3, 17] although, prevalence as low as 0.6% has been recorded in the Korean population [18] and as high as 63.9% in the Turkish population [19].

However, despite the several studies to determine the prevalence of absence of the PL in various ethnic groups worldwide, there is a dearth of information on the North Cyprus population. To the best of the authors' knowledge, the prevalence of absence of

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the PL in the North Cyprus population is unknown. Therefore, the aim of this research is to determine the frequency of surface anatomical variations of PL muscle in North Cyprus and their association with gender, body side and hand dominance. The findings will be useful to surgeons working in North Cyprus, especially in the fields of plastic surgery and orthopedics. It will also increase the available data on the PL, thus contributing to the research community.

2. MATERIALS and METHODS

The study was done in the Faculty of Medicine, Department of Anatomy, Near East University after approval of the Ethical Board of the Institute of Health Sciences, Near East University (Project Number: 807, Meeting Number: 2019/68, Date of the Ethical Board Meeting: 02/05/2019) and the Ministry of Education, North Cyprus. A written informed consent was sought from each participant before the study was carried out.

The study was conducted in 3 schools within the Nicosia district of North Cyprus; the Turkish High School of Nicosia, the Turkish Education College, and the Near East University. The study was planned as a cross sectional study and the sampling was done sequentially until the target population is reached. The sample size was determined using the population of 300,000 North Cypriots using a confidence interval of 95% and a margin of error of 3% and was calculated to be 1,064.

A total of 1280 participants were assessed for surface variations of the PL. Four testing methods were used, which included the Schaeffer's test, the Thompson's test, Pushpakumar's test and the Bhattacharya's test [20]. Where the PL was not visualized by the 4 methods, palpation was done as the final confirmatory for absence. Presence of other variations of the PL tendon was also assessed. Subjects with hand and wrist deformities, previous injuries or surgeries in the forearm and wrist, less than 10 years age or above 60 years, were excluded from the study.

Statistical Analysis

The data collected was entered into a Microsoft Excel spreadsheet then exported to SPSS version 20. All statistical analyses were carried out using SPSS. These include frequencies, percentages and chi-square tests. A value of $p < 0.05$ was considered to indicate a statistical significance between variables and the degree of freedom was taken as 1. The Yates correction was applied in 2 data sets.

3. RESULTS

A total of 1,280 subjects were examined out of which 660 were females and 620 were males. PL agenesis was found in 223 cases (17.4%). 28 cases (2.6%) showed a split (bifid) tendon and 3 cases (0.2%) presented with a laterally shifted tendon (Figure 1).

Out of the total, unilateral absence was observed to be 158 (12.3%), of which right unilateral absence was 40 (3.1%), and left unilateral absence was 118 (9.3%). The frequency of bilateral absence of PL was observed to be 65 (5.1%). The distribution of absence based on gender is shown in Figure 2.

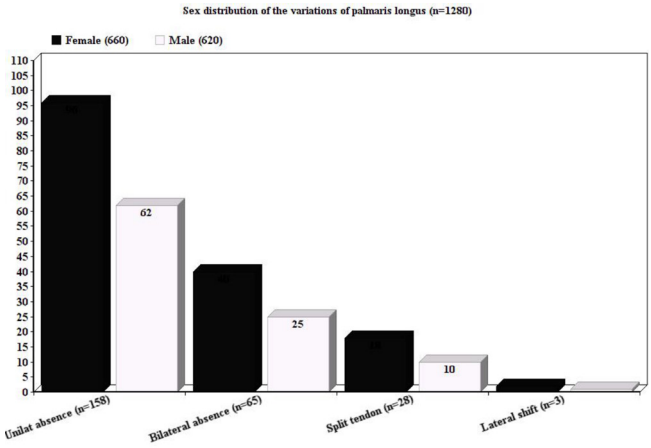

Figure 1. *Distribution of variations based on sex*

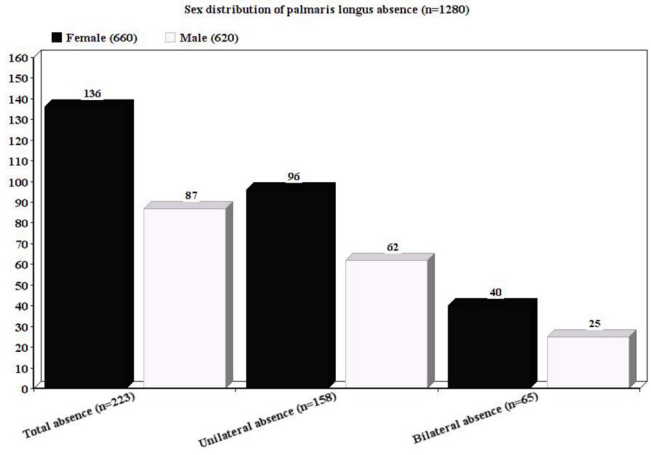

Figure 2. *Distribution of PL absence based on sex*

The distribution of PL agenesis among the different nationalities and/or regions was found to be 96 (7.5%) for Cypriots, 86 (6.7%) for Turks, 30 (2.3%) for Arabs, 4 (0.3%) for Africans and 7 (0.5%) for others (Table I).

Table I. *Distribution of palmaris longus absence based on nationalities and/or regions (North Cyprus, Nicosia, 2019) (N=1280)*

	Right Absence		Left Absence		Bilateral Absence		Total	
	n	%	n	%	n	%	n	%
Cypriots (n=633)	20	20.8	43	44.8	26	27.1	96	100
Turks (n=518)	16	18.6	45	52.3	32	33.3	86	100
Arabs (n=66)	2	6.7	25	83.3	3	10	30	100
Africans (n=45)	1	25.0	1	25.0	2	50	4	100
Others (n=18)	1	14.2	4	57.1	2	28.6	7	100
Total	40		118		65		223	

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Unilateral and bilateral absence of PL is shown by Figures 3A-B. The presence of a split tendon was observed in 28 subjects (2.6%); 24 cases occurred on the right, 2 on the left, and 2 bilaterally (Figures 4 A-B).

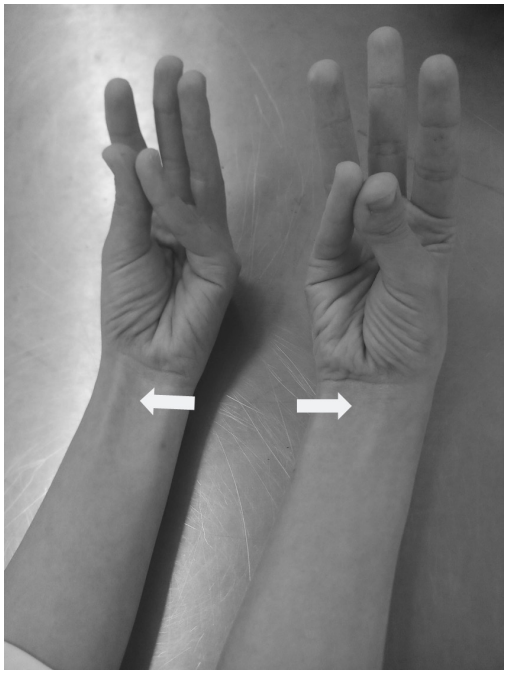

Figure 3A. *Bilateral absence of palmaris longus tendon in a 22y old Persian female demonstrated by the Shaeffer's test*

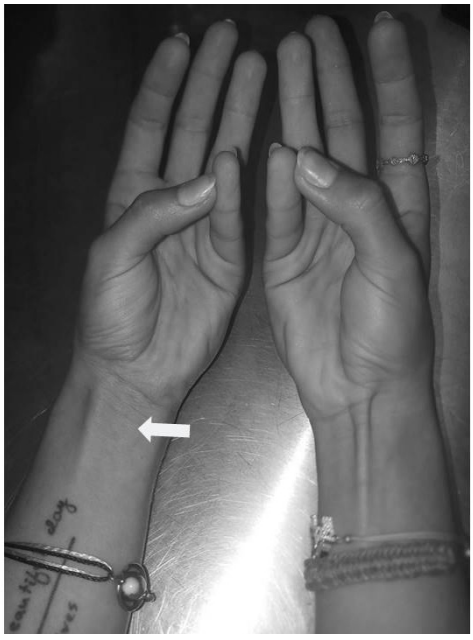

Figure 3B. *Unilateral left absence of palmaris longus in a 19y old Cypriot female. The arrow heads point to the tendon of flexor carpi ulnaris which has become more prominent*

Out of the total number of cases that presented with PL tendon (n=1057), 3 cases (0.2%) were observed to have a laterally displaced tendon; 2 cases were females (an African and a Turk) and the other was a Turkish male (Figures 5A-B).

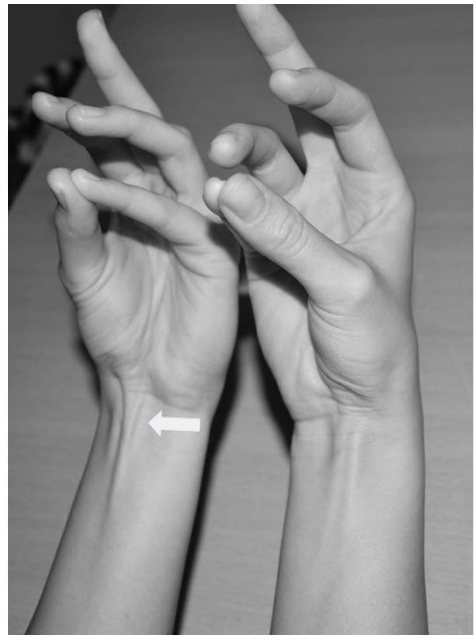

Figure 4A. *Left split tendon (shown by arrow head) in a 22y old Russian female visualized by Shaeffer's test*

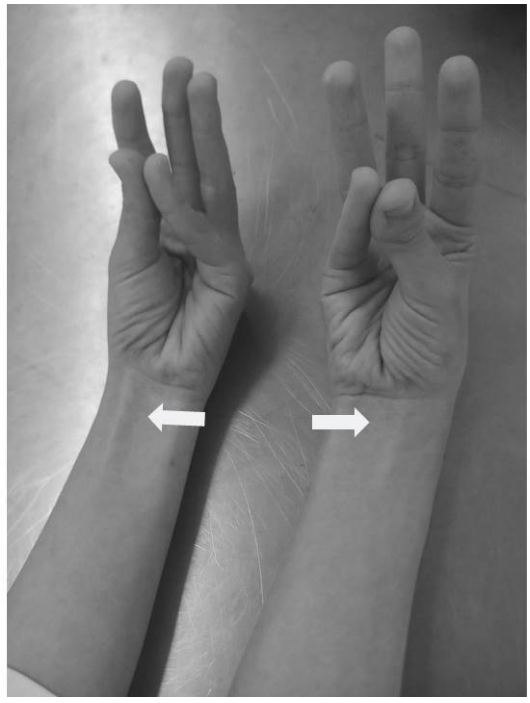

Figure 4B. *Right split tendon in a 16y old African female*

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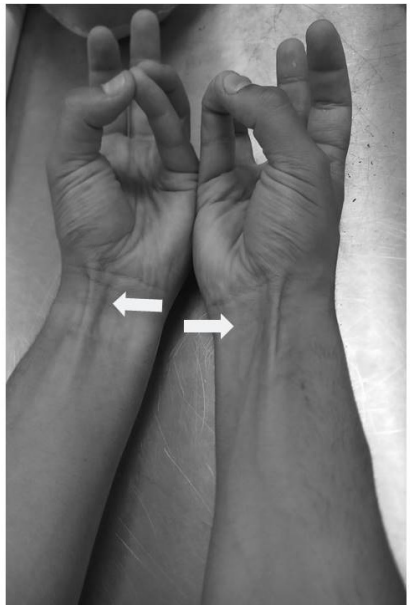

Figure 5A. *Laterally displaced tendon of palmaris longus in the right distal forearm of a 24y old Turkish male and split tendon of palmaris longus on the right*

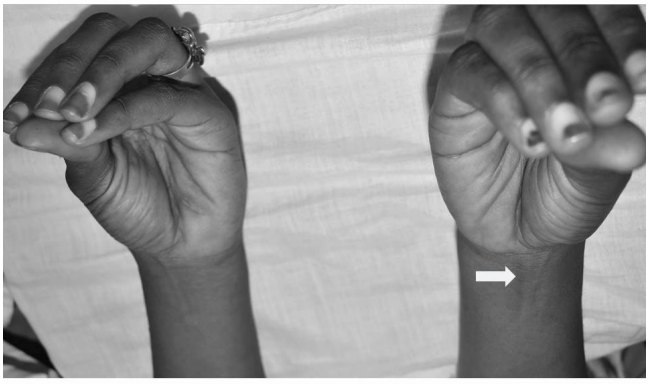

Figure 5B. *Laterally displaced left palmaris longus and right split tendon in an African female*

The overall difference in PL agenesis between males and females was statistically significant ($p < .05$), which implies that females have a higher frequency of absence of PL muscle (Table II).

Table II. *Relationship between absence of palmaris longus and gender (North Cyprus, Nicosia, 2019) (N=1280)*

Gender	Present		Absent		Total	χ^2	p-value
	n	%	n	%			
Female	524	79.4	136	20.6	660	9.61	0.002
Male	533	86.0	87	14.0	620		
Total	1057		223		1280		

While trying to find a correlation between the occurrence of the split tendon and gender, the result was not statistically significant ($p > .05$). Although, the frequency of a split tendon was higher in females, the chi-square test was not statistically significant (Table III).

Table III. *Correlation between split tendon and gender (North Cyprus, Nicosia, 2019) (N=1280)*

Tendon	Normal		Split		χ^{2*}	p-value
	n	%	n	%		
Male	533	98.2	10	1.8	1.39	0.238
Female	524	96.7	18	3.3		
Total	1057		28			

**Yates correction*

Concerning laterality or body side, there was also a significant statistical difference ($p < .05$ for left side) between the right and left sides, which shows that PL agenesis occurs more frequently on the left side (Table IV).

Table IV. *Laterality in palmaris longus muscle absence (North Cyprus, Nicosia, 2019) (N=1280)*

Laterality	Present		Absent		Total	χ^2	p-value
	n	%	n	%			
Left	1162	90.8	118	9.2	1280	41.04	P<0.001
Right	1280	96.9	40	3.1	1280		

The result of the correlation between body side and occurrence of a split tendon was statistically significant at $p < .05$, and shows that the split tendon is more likely to occur on the right side as shown in Table V.

Table V. *Relationship between body side and split tendon of palmaris longus (North Cyprus, Nicosia, 2019) (N=1280)*

	Normal		Split tendon		Total	χ^{2*}	p-value
	n	%	n	%			
Right	1240	98.1	24	1.9	1264	13.40	p<0.001
Left	1162	99.7	4	0.3	1166		

**Yates correction*

Out of the 1280 subjects, 1214 were right handed and 66 were left handed. 40 cases showed absence of PL on the right and 118 on the left. Out of the total 223 cases of absence, bilateral absence occurred in 57 right hand dominant subjects and 8 left hand dominant subjects. This is shown in Table VI.

Table VI. *Relationship between handedness and frequency of absence of palmaris longus (North Cyprus, Nicosia, 1280) (N=1280)*

	Handedness		Absence of PL		χ^2	p-value
	n	%	n	%		
Right	1214	96.8	40	3.2	609.27	P < 0.001
Left	66	35.9	118	64.1		
Total	1280		158			

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Left sided unilateral absence occured in 109 right handed subjects and 9 left handed subjects while right sided unilateral absence occurred in 37 right handed subjects and 3 left handed subjects. The relationship between handedness and frequency of absence of PL was statistically significant ($p < .05$) in that the absence was more likely to occur in the non-dominant hand. The p-value for the left hand was < .001 which meant PL agenesis was more likely to occur on the left side in a right-handed individuals.

All the cases of the split tendon occurred in right handed subjects, so a comparison could not be made. There were only 3 cases of a laterally displaced tendon, so no statistical analysis could be made.

4. DISCUSSION

The tendon of PL muscle, an anterior forearm flexor muscle, is found at the level of the wrist lying between the tendons of the flexor carpi ulnaris and flexor carpi radialis Various studies have reported many different variations of the tendon of PL, especially its absence [3-5, 20-28]. More studies have been conducted to establish a correlation of the absence of PL to body side, gender and hand dominance [6, 29, 30-32].

In this study, the overall frequency of absence of PL among the North Cyprus population was found to be 17.4% (n=223) (Figure 1). This value is comparable to the study recorded with a 17.09% frequency of absence among first year Filipino medical students [15]. A similar study conducted on 500 Indian subjects recorded a similar prevalence of absence of 17.2% [33]. Another study on an Indian population also reported a similar prevalence of 17.2% [27]. The value is also similar to the worldwide prevalence of absence of 15% [17]. A study in Van, Turkey recorded a total prevalence of 15.1% [7]. Higher prevalence of PL agenesis have been given in Chilean subjects as 21% [6] while it was 22.8% in Iran [4], 26.6% in Turkey [8], 26.7% in Nigeria [29], 28% in India [13], and 40.5% in Saudi Arabia [12]. A study conducted in the Gaziantep population of Turkey reported the highest prevalence as 63.9% [19]. Many studies have recorded lower prevalence of absence especially in studies conducted in Africa and Asia. In South Africa it was reported as 11.5% [34]; In Iranian subjects it was reported as 10.2% prevalence of absence [4], while it was 9.3% in Malaysia [14]; Yoruba population of Nigeria showed 6.7% [15]; East Africa as 4.4% [9]; Korea as 4.1% [18]; Ghana as 3.8% [35]; and Zimbabwe as 1.6% [30].

Out of the total prevalence of absence in this study (17.4%), a total of 158 (12.3%) cases exhibited unilateral absence while 65 (5.1%) cases showed bilateral absence. In the unilateral cases, 40 subjects (3.1%) exhibited unilaterality on the right, and 118 (9.3%) showed left unilateral absence (Figure 2). From this study, it was inferred that there is a higher frequency of unilateral absence of the PL tendon. This was supported by a report with the total prevalence of absence of 21%, 11% were unilateral and 9% bilateral [6]. In another study, it is also recorded with a 6.4% unilateral absence and 2.9% bilateral absence, out of the total 8.3% [14]. The study by Yong et al., also reported that left sided absence was more common [32]. Also, the East African study reported that out of the total 4.4% cases of absence, 3.3% were unilateral and 1.1% was bilateral [9]. The results of the current study were in contrast to the results of a study that reported a higher incidence of bilateral absence (6.7% out of the total 10.2%) [4]. Some other studies showed no significant difference between unilateral and bilateral cases; one study reported that of the 26.7% cases of absence, 13% were unilateral and 13.7% were bilateral [29] while another one reported no statistically significant difference in terms of sidedness [15]. Similarly, one of the studies reported that the development and the prevalence of agenesis of the PL in the fetal period had no significant differences based on sidedness, although, bilateral absence was significantly higher (62.2%) when compared to unilateral prevalence (15%) [36]. In the current study, the unilateral cases were 3.1% on the right and 9.3% on the left (Table IV). This contrasted with the study that showed a higher distribution on the right side (10.2%) compared to the 5.9% on the left [4]. In comparison, a study showed equal distribution on both right and left sides (5.6%) [29], same as another study which showed 1.4% prevalence of absence on both sides [35]. One study showed a slightly higher prevalence on the left (6%) compared to the 5% on the right side [6].

Studies have tried to correlate the frequency of absence of the PL with gender. The report of a study showed a higher frequency of absence in females (15.1%) compared to males (11.2); of these cases, 9.0% cases were on the left side [6]. The current study also showed a higher frequency of absence in females (10.6%) compared to the males (6.8%). The relationship between gender and absence of the PL was statistically significant ($p < .05$) (Table II). The total frequency of absence on the right side was 3.1% while on the left, it was 9.2%; 19 of the female subjects (1.9%) had unilateral absence on the right and 77 (6.0%) had unilateral absence on the left; while the male frequency of absence was 1.6% on the right and 3.2% on the left. In this study, the p-value for the left was statistically significant ($p < .05$) (Table IV). This finding was also supported by the study which reported 37.5% absence in females and 27.9% in males, with a p-value for left hand being 0.017 [37]. While, one study also reported a higher frequency of absence in females and on the left [29], another study reported males to have 16% unilateral agenesis and 4% bilateral agenesis while females had 29% unilateral agenesis and 14% bilateral agenesis [18]. In contrast, another study reported a higher frequency of absence in males (4.7%) compared to 3.3% in females, with no statistically significant difference as to laterality. A study conducted in Malaysia reported a higher frequency in females (11.5% compared to 7.1% in males) but there was no statistically significant difference in laterality as the p-value for the left was 0.105 [14]. This finding was in contrast to the findings of studies that showed no statistically significant difference in genders [4, 34]. The study of frequency in Chilean subjects showed that the PL was most frequently absent on the left side and in women, but the statistical test was not significant [6].

The current study recorded a bilateral absence of PL in 57 (4.5%) right hand dominant subjects and 8 (0.6%) in left hand dominant subjects. 109 cases (8.5%) had unilateral absence on the left side

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while 9 cases (0.7%) had unilateral absence on the left side. On the other hand, 37 right-handed subjects (2.9%) had unilateral absence on the right; while 3 (0.2%) left-handed subjects had absence on the right. The difference between right and left hand dominance and frequency of absence of PL was statistically significant (p-value is < .001 at p < .05) for the non-dominant hand, which showed that absence of PL was more likely to occur in the non-dominant hand (Table VI). This finding was supported by a study concluded that right-sided absence was more common in left-handed persons while left-sided absence was more common in the right-handed persons [38]. Kigera and Mukwaya reported similar findings that PL agenesis was more likely to occur in a non-dominant hand [9]. Another study reported no significant difference in terms of gender, body side and handedness, but concluded that the absence of PL tendon was more likely to occur in the non-dominant hand [35]. Another study however, reported a contradicting result that there was a significant relationship between PL agenesis and left hand dominance, that those with PL agenesis were 3.7 times more likely to be left-hand dominant and left handed people were 3.7 times more likely to have PL agenesis [4]. No other study has reported similar findings. Kyung et al., concluded that there was no relationship between hand dominance and PL absence [18].

This study also documented 28 cases (2.5%) of the total number of those that were positive for the PL tendon of a split (bifid) tendon of PL, of which 10 (0.9%) cases occurred in males and 17 (1.6%) in females. Although, the frequency of a split tendon was higher in females, the chi-square test was 1.43 and p-value was .23 which was p > .05 (Table III); this implied that the difference in gender was not statistically significant. In the cases with split tendon, 24 cases occurred on the right, 1 bilaterally and 2 on the left side. The chi-square test was 14.86 and p – value was < 0.001, which was statistically significant at p < .05, which means that the split tendon is more likely to occur on the right side (Table V). However, no similar studies were found to compare and contrast this result. In the literature, there is a case report of 2 cases of an anomalous V-shaped bifid tendon of PL [39].

There were 3 cases (0.2%) of a laterally displaced tendon of PL on the right forearms of a Turkish male (Figure 3A), Turkish female, and the left forearm of an African female (Picture 3b). Another study reported a lateral shift in the tendon of PL in 1.1% of subjects [30].

The frequency of absence in the ethnic groups of North Cyprus could not be compared as there were no similar studies in the region to compare. No other abnormalities have been observed in this study.

In summary, the goals of this research were to establish the frequency of surface anatomical variations in the different races in North Cyprus and to correlate these variations with gender, body side and hand dominance. All these have been established in this research. The frequency of absence was 17.4%, frequency of split tendon was 2.5% and lateral tendon was 0.2%. There was a significant statistical correlation between these variations, gender, body side and hand dominance. From this study, it can be concluded that PL agenesis is more likely to occur in females and on the left side, and in the non-dominant hand.

Compliance with Ethical Standards

Ethical Approval: This study was approved by the Ethics Committee of the Institute of Health Sciences, Near East University (Project Number: 807, Meeting Number: 2019/68, Date of the Ethical Board Meeting: 02/05/2019) and the Ministry of Education, North Cyprus. A written informed consent was sought from each participant before the study was carried out.

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Conflict of interest: The authors declare no conflict of interest.

Authors' Contrubition: M.N.A., M.T.: Idea/ Concept. M.N.A.:: Design. M.T.:. Control/Supervision. N.N.A.:Data Collection and processing. M.N.A., M.T.: Analysis. M.N.A., M.T.: Literature review. M.T.: Critical Review. Both authors approved the final version of the article.

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Genetic alterations and pathways in patients with Hereditary Angioedema of Unknown Cause (U-HAE)

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ABSTRACT

Objective: Hereditary angioedema (HAE) with normal C1 inhibitor (HAE-nC1-INH), is a genetically complex, rare disease and mutations in *F12, ANGPT1, PLG, MYOF* genes are found in some families with HAE-nC1-INH. However, often a specific mutation cannot be identified and this type is called as hereditary angioedema of unknown cause (U-HAE). Our aim was to identify putative causative genetic alterations and/or pathways by whole exome sequencing in patients with U-HAE.

Patients and Methods: Nine patients from 8 families between the ages of 3 to 63 years with U-HAE and 6 controls were enrolled for the study and whole exome sequencing were performed.

Results: No significant difference was found between the case and control group for the *a priori* suspected set of genes. Variants in the genes; *RAMP2, IL6, GP1BA, C1QBP* were significantly different between U-HAE and control group. Downstream functional analysis found that blood coagulation pathways were enriched in these genes.

Conclusion: Proteins that are not involved in contact pathways may also play a role in U-HAE. These variants need to be replicated in larger cohorts and studied at the functional level to verify our findings.

Keywords: Hereditary angioedema of unknown cause (U-HAE), Whole exome sequencing (WES), Genetic

1. INTRODUCTION

Hereditary angioedema (HAE) is a rare autosomal dominant disease that results in recurrent attacks of swelling and has a highly variable clinical course [1]. There are two well defined types of HAE: HAE due to C1 inhibitor deficiency (C1-INH) and HAE with normal C1-INH deficiency (HAE-nlC1-INH). HAE due to C1-INH deficiency is further subdivided into type 1 (low plasma c1INH antigen) and type 2 (normal, but dysfunctional C1INH). Both HAE types are caused by mutations in *SERPING1* gene [2,3].

Hereditary angioedema with nlC1-INH is a genetically complex, rare disease first described in 2000. Prevalence is estimated at 1:100.000. Mutations in *F12* gene are seen in approximately 30% of the patients and recently mutations in *ANGPT1, PLG* and *MYOF* were shown in some families with HAE-nC1-INH [4] .However, most of the time a specific mutation cannot be shown. There is a need for methods that can identify affected individuals with HAE-nC1-INH with unknown genetic cause (U-HAE) [5,1] .

Functional genomics approaches attempt to discover, define and describe function of genes and interactions thereof, making use of the vast data generated by genomic and transcriptomic projects [6]. One initial step into this endeavor, is the discovery of causative genes, in particular for a disease or trait of interest

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via observational studies, where features (e.g. structural variants in the genome) are associated with the traits.

2. PATIENTS and METHODS

After ethics approval (Ankara University Ethics Committee, date: 08/27/2012 number: 13-419-12) and consent forms were taken, 9 patients between the ages of 3 to 63 years with U-HAE were enrolled in the study (Table I). 8 patients were from a Turkish decent and 1 was Irish-American. 2 patients were father and his adult daughter, and the rest belonged to unrelated families. 3 of the index cases were younger than 11 years of age. 6 controls were taken randomly from a sample of patients with congenital heart disease. All index cases presented with recurrent angioedema symptoms and met the criteria for HAE-nlC1-INH as described by Cicardi et al [1].

WES analysis, Identification of variants and statistical procedures, bioinformatics analyses.

All patients (controls and cases) underwent to whole-exome sequencing (WES) analysis. The raw sequence files were quality filtered (average Phred score >30, minimum 50X depth of coverage) using FastQC. The resulting sequence files were mapped to human genome (hg19) using Bowtie2 [7] or Burrows-Wheeler Alignment (BWA) tool [8] and downstream analyses were performed using Sam tools [9]. The mapped reads were further used for variant detection using standard bioinformatics workflows of samtools and/or GATK [10]. All detected variants were listed and inspected for their PolyPhen [11] and SIFT [12] scores and further checked in COSMIC database [13]. The variations identified as "benign" were discarded and variations with outcomes as "possibly damaging" and "unknown" were kept.

Construction of Variant profiles per gene

To assess potential association between the U-HAE and structural variants, variant profile for each gene is constructed as follows: for each gene and each sample considered, a binary variable that represents the presence or absence of a structural variation (with suitable PolyPhen outcome) is generated. In doing so, all variations in the selected gene are aggregated. In case there are multiple variations in a gene, the binary variable is still taken as one. This yields variant profiles per gene for case and control groups. The resulting contingency table, taking cases and controls as well as number of variations and non-variations is tested using Fisher's exact test [14] for the null hypothesis that there is no difference in variation profile between the case and control group. $\alpha = 0.05$ is taken as the significance threshold.

Identification of potential candidate genes

Initial gene set, for which a variation profile is generated and tested for significance among groups is constructed based on reported genes for HAE variants and literature search. To expand and identify further potential genes, we used the initial gene set consisting of *F12*, *ACE*, *ADM* and *SERPINA1* as a starting point, and considered genes interacting at protein level (protein-protein interactions) to potentially associate with U-HAE case. The protein-protein interactions are retrieved from STRING database [15] .

Downstream Bioinformatics analyses

The final gene list containing the genes with statistically significant difference in their variation profile between the case and control is further functionally analyzed for their function and GO-terms using The Database for Annotation, Visualization and Integrated Discovery (DAVID) database [16,17] and amiGO tool [18].

3. RESULTS

Characteristics of the Patients

In this study, 9 patients from 8 families and 6 controls with different clinical profile were analyzed. Characteristics of the patients are shown in Table I.

Table I. *Characteristics of patients with U-HAE*

Patient	Sex	Age at diagnosis	Presenting Symptoms
1	M	33	Lip swelling
2	F	9	Lip and hands swelling
3	M	18	Lip and scrotum swelling
4	F	22	Tongue and lip swelling
5	F	34	Tongue swelling
6	F	7	Lip swelling
7	M	50	Tongue swelling, abdominal pain
8	F	19	Acute abdominal pain with free fluid collection
9	F	28	Hands swelling

Analysis of Variant profiles for F12, ACE, SERPINA1 and ADM

In the first round the initial seed gene list consisting of *F12, ACE, SERPINA1* and *ADM* are inspected for their variant profiles, different from case to control.

In an attempt to characterize correctly the HAE and making sure that these patients are U-HAE, an additional list of genes was also inspected for their variant profile. This list is composed of genes that are used to characterize the HAE in earlier works. The results, including the seed gene list as well as candidate genes, are given in Figure 1. Only high-quality variants were included in the analysis with possibly damaging and unknown variants based on their polyphen scores. The analysis resulted in no gene with a differential variant profile among case and control groups. Interestingly, no variation was observed for SERPINA1 and ADM genes in the cohort and almost all individuals including control had variation for F12 ($p = 0.476$) and ACE ($p = 0.999$). The lowest among those was the variant profile of SERPING1 gene which was only marginally significant ($p = 0.044$) (Figure 1).

Expanding the search space using interactome information

In searching for causative genes in this dataset, we expanded the initial gene set by including the genes whose protein products

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were reported to be interacting with the initial set, based on STRING database and selected only the high-confidence pairs (minimum required interaction score > 0.9, Figure 2). The new gene list contained 29 additional genes, for which variation profiles were constructed, tested for significant difference between the case and control groups and found that indeed significant difference was found for this set (p = 0.027).

After seeing that not all genes in the expanded list were different in their variant profile, we focused on genes in the new list that would best differentiate (lowest p-value) the case and control groups, based on the variant profiles. To achieve this, each gene in the expanded list was ranked for their p-value and the final list of genes is given in Table II. Using these 4 genes, p-value was estimated to be 0.023.

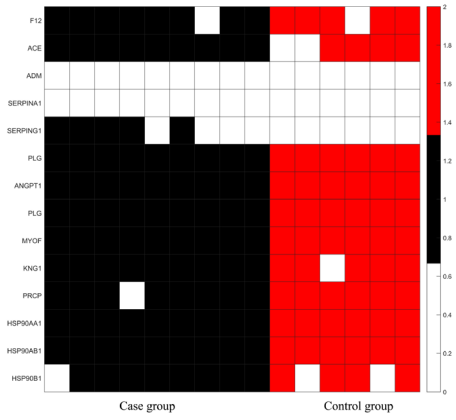


Figure 1. *Variant profiles for possibly damaging and unknown variants for case (black) and control (red) groups. Colored spots present the presence of variant in a selected gene in the respective sample and white represents the absence. In this cohort, there are no possibly damaging and unknown variants for ADM and SERPINA1 genes.*

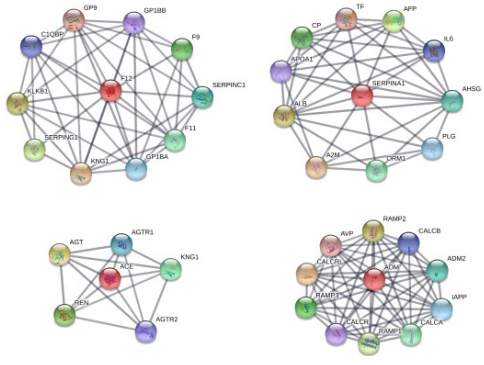
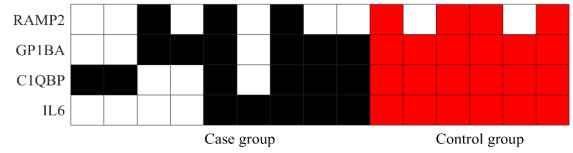


Figure 2. *Searching for causative genes U-HAE. The expanded list of genes constructed by considering highest confidence interactions according to STRING database with the initial set of genes. Each network in the figure is centered around one of the genes in the initial set: F12, SERPINA1, ACE, ADM from left to right, top to bottom. The thickness of the lines connecting each protein couple indicates the strength of data support as provided by STRING database.*

Table II. *Final list of genes proposed in this study, based on statistical association of the variant profile with U-HAE diagnosis state and their variant profiles for case and control groups.*

Gene symbol	Gene Name
RAMP2	Receptor Activity Modifying Protein 2
IL6	Interleukin 6
GP1BA	Glycoprotein Ib Platelet Subunit Alpha
C1QBP	Complement C1q Binding Protein



Downstream analysis of the proposed genes

The obtained set of genes were downstream analyzed to be inspected for their (common) function/pathway using GO Enrichment Analysis via amiGO tool. The GO terms overrepresented for the gene list in Table II is given in Table III.

Table III. *GO terms overrepresented for the candidate genes in Table I.*

GO biological process	FDR corrected p value
blood coagulation, intrinsic pathway (GO:0007597)	3.76E-02
blood coagulation, fibrin clot formation (GO:0072378)	5.54E-02
protein activation cascade (GO:0072376)	4.15E-02
blood coagulation (GO:0007596)	2.95E-02
coagulation (GO:0050817)	2.58E-02
hemostasis (GO:0007599)	2.33E-02
negative regulation of response to external stimulus (GO:0032102)	8.75E-03
regulation of response to external stimulus (GO:0032101)	3.48E-02

FDR: False Discovery Rate, GO: Gene Ontology

4. DISCUSSION

We propose a data-mined set of genes that statistically show variation among U-HAE cases and control groups. Prominent among this set of genes are *RAMP2, IL6, GP1BA* and *C1QBP*. RAMP2 can function as an adrenomedullin receptor (ADM) which is a vasoactive peptide and it has been shown that its level increases during C1-INH-HAE attacks together with other vasoactive peptides arginine vasopressin (AVP) and endothelin-1 (ET-1) to counter-balance the actions of excess bradykinin (BK) and terminate the attacks [19]. HAE-nl-C1INH may also be BK mediated as they show a favorable response to BK pathway-targeted medications [20] .The possible effects of *RAMP2* in vasoactive peptides may reveal a novel pathophysiological aspect.

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Concentrations of interleukin (IL)-1, IL-6, and transforming growth factor (TGF-β) were found to be significantly higher in HAE patients in remission compared with healthy controls. [21]. IL-6 is a soluble mediator with a pleiotropic effect on inflammation, immune response, and hematopoiesis. IL-6 induces excess production of vascular endothelial growth factor (VEGF), leading to enhanced angiogenesis and increased vascular permeability which causes joint swelling and edema. VEGF also interacts with BK to mediate plasma leakage.

Glycoprotein Ibalpha (*GP1BA*) is a platelet surface membrane glycoprotein that functions as a receptor for von Willebrand factor. The binding of VWF to the GP Ib complex initiates signaling events within the platelet that lead to enhanced platelet activation, thrombosis, and hemostasis [22]. Mutations in this gene is associated with Bernard-Soulier syndrome and platelet type Von-Willebrand Disease. This glycoprotein may play a role in U-HAE by platelet activation, but this is another research topic.

Complement component 1q (*C1QBP)* subcomponent binding protein is a multifunctional protein involved in immune response, energy homeostasis of cells as a plasma membrane receptor, and a nuclear, cytoplasmic or mitochondrial protein. It has a role in the mediation of the actions of pro-inflammatory agents, such as high molecular weight kininogen to produce further pro-inflammatory agents [23]. It can play as a mediator in U-HAE since it acts as a bridge between the complement and contact activation system.

.U-HAE is usually misdiagnosed or underdiagnosed due to rarity of the disease and lack of specific biomarkers. This results in mismanagement of this potentially life threatening disease.

Detection of causative genes will help in timely diagnosis of patients and identifying affected family members. It will also allow for understanding the pathophysiology of this disorder, which in turn will lead to better therapeutic options. In our study genes that statistically show variation among U-HAE cases and control group are *RAMP2, IL6, GP1BA* and *C1QBP*.

Our study contributes to understanding the potential genetic pathways in U-HAE. The next step would be to increase our sample size, do family segregation studies, to extend the work to GWAS or RNA-Seq studies to further our understanding via network analyses and functional (e.g., transcriptomic or low throughput) analyses to understand and confirm the disease modifying effects of these genetic variations.

Compliance with Ethical Standards

Ethical Approval: The study was approved by the Ankara University Ethics Committee (date: 08/27/2012 number: 13-419-12) and consent forms were taken.

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

Conflict of interest: The authors declare that they have no conflict of interest to declare

Authors' contribution: H.K.: conceived and designed the analysis, collected the data of the patients, did the literature search and wrote the article. H.A.: conceived and designed the analysis, collected the data of the patients, did the literature search. A.O.C.: performed the genetic analyses. O.G.: collected the data of the patients, did the literature search, contributed to the conception of the research. E.N.G.: collected the data of the patients, contributed to the conception of the research. E.N.: did the statistical analysis and literature search, contributed to the conception of the research, wrote the article. All authors discussed the results and approved the final version of the article.

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The role of serum lactate levels in predicting abdominal surgery in geriatric patients who had computed tomography

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ABSTRACT

Objective: We investigated the importance of serum lactate levels in making decision regarding abdominal surgery in elderly patients who underwent computed tomography.

Patients and Methods: Patients over 65 years of age who presented to the emergency department of a tertiary teaching hospital in 2019 were scanned within the hospital data processing system according to the criteria of tomography of the abdomen. Patients' age, gender, lactate levels, the medical reason for their visit, pathological condition of abdominal computed tomography (aCT), a recommendation of emergent surgery, mortality status, and length of stay in the hospital were collected. The patients were divided into four groups according to surgery recommendation and pathologic status in aCT.

Results: Of the 356 patients who were included in the study, 194 (54.5%) were male. The mean age of the participants was 77.1±7.82 years, and their mean lactate level was 2.59±2.41 mmol/liter. Lactate values were statistically significant according to ROC analysis that predict the state of surgery recommendation (AUC:0.796, p<0.001). There was a statistically significant difference in lactate values between the groups (1.78±1.46, 3.19±2.71, 4.44±3.22, I-II p<0.001, I-III p<0.001, II-III p=0.002).

Conclusion: In geriatric patients, the aCT results and lactate levels were found to be reliable in deciding to eliminate abdominal surgery.

Keywords: Geriatrics, Surgery, Lactates, Computed tomography, Emergency Department

1. INTRODUCTION

Abdominal pain is a common complaint in elderly patients [1]. Due to the increase in the geriatric population, about 25% of patients visiting the emergency department are over the age of 50 [2]. Elderly patients apply to the emergency department at older ages and with more non-specific symptoms [3]. Therefore, the clinical picture of an elderly patient presenting with abdominal pain may differ from that of a young patient [4]. Elderly patients need surgical intervention more than younger patients do [5]. It is important to make the emergency surgery decisions about elderly patients visiting the emergency department promptly, as a late diagnosis leads to both an increase in mortality and morbidity, and is more of a risk of misdiagnosis in elderly patients compared to young patients [4].

Computed tomography (CT), as a diagnostic method, is one of the imaging procedures used in patients with abdominal pain. A routine CT is more reliable in making the surgical treatment decisions of patients with acute abdominal pain [6].

Lactate level is an early marker of tissue hypoxia. Lactate levels have been reported to increase in inflammatory and ischemic conditions, such as acute appendicitis and mesenteric ischemia [7,8]. In addition to the studies indicating that the lactate level increases in patients who undergo surgery due to the acute abdomen [9], there are also studies indicating that these levels are not sufficient for surgical decisions in these patients [10]. Since, elderly patients with acute abdominal pain may visit the hospital much later than younger patients, ischemia is thought to be a likely preexisting pathology, and serum lactate level is expected to increase.

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We investigated the importance of serum lactate levels in making decision regarding abdominal surgery in elderly patients who underwent abdominal computed tomography (aCT).

2. PATIENTS and METHODS

Ethics committee approval for this single-center, retrospective study was obtained from the Necmettin Erbakan University, Meram Medical Faculty, Pharmaceutical and Non-Medical Device Studies Ethical Committee (decision number of 2020/2569).

Patients over 65 years of age who applied to the tertiary teaching hospital emergency department in 2019 were scanned within the hospital data processing system according to the findings of tomography of the abdomen. This procedure was performed by listing the patients aged over 65 years for whom the service codes of aCT and aCT aortography were entered (in the emergency department). A total of 1011 patients' records were examined. Of these, 144 were excluded from the study due to indicated traumatic conditions, 375 were excluded due to lacking blood lactate level measurements, six were excluded because they arrived in the emergency department with a direct cardiopulmonary arrest, and 130 were excluded for having a lung or cardiac-based diagnosis. Eventually, 356 patients who had aCT with no non-abdominal diagnoses and for whom lactate level measurements available, were included in the statistical analysis (Figure 1).

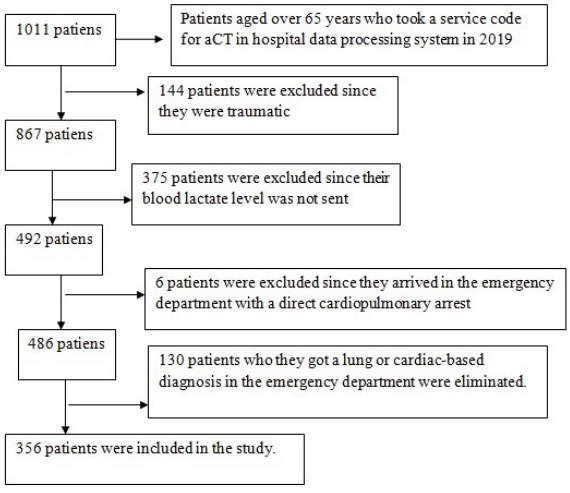

Figure 1. *Study plan*

The following data for 356 patients were saved: age, gender, serum lactate level, the medical reason for their visit to the emergency department, whether he/she had a pathologic diagnosis by aCT, pathological diagnosis type, abdominal surgery recommendation status, hospital mortality status, emergency outcome status (discharge, stay in the service, stay in the intensive care unit, exitus, voluntary discharge), and length of stay in the hospital.

The patients' aCTs were interpreted as "there is a pathological diagnosis" or "no pathological diagnosis" according to the official reports made by the radiology department through the hospital system. Any aCT interpretation, which may be a surgical indication, was considered pathological, regardless of the patient clinical condition. This interpretation was made by an independent physician who was not included in the study and blinded to the lactate levels and the status of surgery recommendations. The recommendation for abdominal surgery for patients was acquired by monitoring the clinical course of the patients.

The patients were grouped based on the status of obtaining a pathological diagnosis in aCT and undergoing an emergent surgery.

Group 1: No pathological diagnosis in aCT; no abdominal surgery was recommended

Group 2: Pathological diagnosis in aCT; no abdominal surgery was recommended

Group 3: Pathological diagnosis in aCT; abdominal surgery was recommended

Group 4: No pathological diagnosis in aCT; abdominal surgery was recommended.

The primary outcome of this study is a comparison of the lactate levels between these four groups and lactate levels between patients who were recommended for surgery and those who were not. Our secondary aim was to compare patients' age, gender, length of stay in the hospital, and mortality by intergroup and surgery recommendation status.

Statistical Analysis

Statistical analyses of the data were made by using SPSS 20.0 (SPSS Inc., Chicago, IL) packaged software. Analyses of normality of the data were made using histograms and Kolmogorov–Smirnov test. Quantitative data were stated as mean±standard deviation, while categorical variables were stated as frequency (percentage). The differences between the groups were investigated using the Mann–Whitney U test in non-normally distributed quantitative variables, while the Student t-test was used for normally distributed quantitative variables. Categorical variables were compared between groups using chi-square test. ROC analysis was performed to predict the status of mortality and the recommendation of emergent surgery by the lactate level. $p< 0.05$ value was accepted as statistically significant.

3. RESULTS

Of the 356 patients who were included in the study, 194 (54.5%) were male, their mean age was 77.1±7.82 years, and their mean lactate level was 2.59±2.41 mmol/liter, their mean length of stay in the hospital was 7.06±9.27 days. Twenty seven pathologies were detected on CT in 144 (40.4%) of 356 patients. Twelve patients had two pathologies, while the others had only one pathology. The three most common pathologies were ileus (48 (33.3%)), acute cholecystitis (14 (9.7%)), and perforation (13 (9%)) (Table I). The patients presented to the emergency

department with a total of 46 different complaints. Of the patients, 156 (43.8%) had one complaint, 133 (37.4%) had two complaints, 58 (16.3%) had three complaints, 8 (2.2%) had four complaints, and 1 (0.3%) had five complaints. The three most common complaints by patients were abdominal pain (193 (54.2%)), nausea (74 (20.8%)), and vomiting (72 (20.2%)). Table II shows the characteristics of the participants.

Table I. *Emergency pathology status in aCT*

Patalogy in aCT	144	100
Ileus	48	33.3%
Acute cholecystitis	14	9.7%
Perforation	13	9%
Herniation	12	8.3%
Mesenteric ischemia	11	7.6%
Cholangitis	8	5.6%
Abscess	7	4.9%
Acute appendicitis	7	4.9%
Pancreatitis	5	3.5%
Diverticulitis	4	2.8%
Abdominal aortic aneurysm	3	2.1%
Aortic dissection	3	2.1%
Anastomosis leakage	2	1.4%
Hematoma	2	1.4%
Necrotizing pancreatitis	2	1.4%
Intra-abdominal bleeding	2	1.4%
Necrotizing fasciitis	2	1.4%
Sigmoid volvulus	2	1.4%
Peritoneal carcinomatosis	1	0.7%
Renal artery thrombosis-renal infarction	1	0.7%
Superior mesenteric vein thrombosis	1	0.7%
Severe stenosis in the abdominal aorta	1	0.7%
Spleen infarction	1	0.7%
Abdominal aortic thrombus	1	0.7%
Rupture of the abdominal aortic aneurysm	1	0.7%
Rectovaginal fistula	1	0.7%

Table II. *The characteristics of patients*

Number of patients		356	100%
Gender	Male	194	54.5%
	Female	162	45.5%
Age		77.1±7.82	
Lactate levels (mEq/L)		2.59±2.41	
Length of stay in hospital (day)		7.06±9.27	
Emergency Outcome Status	Discharge	84	23.6%
	Stay in Service	156	43.8%
	Stay in Intensive Care Unit	96	27%
	Ex	3	0.8%
	Voluntary Discharge	17	4.8%
3 most common complaints	Abdominal pain	193	54.2%
	Nausea	74	20.8%
	Vomiting	72	20.2%
In-Hospital Mortality	Survivor	301	84.6%
	Non-survivor	54	15.4%
Surgery Recommendation	Recommended	65	18.3%
	Not – recommended	291	81.7%
Group 1*		208	58.4%
Group 2**		83	23.3%
Group 3***		61	17.1%
Group 4****		4	1.1%

*: No pathologic diagnosis in aCT, no abdominal surgery was recommended
**: Pathologic diagnosis in aCT, no abdominal surgery was recommended
***: Pathologic diagnosis in aCT, abdominal surgery was recommended
****: No pathologic diagnosis in aCT, abdominal surgery was recommended

Surgery was not recommended for 291 patients (81.7%), while it was recommended for 65 (18.3%) patients. Surgery was performed in 54 of 65 patients for whom surgery was recommended while 11 could not be operated for certain reasons. Surgical operation could not be performed because one of these 11 patients was considered inoperable, 2 of them died before the surgery, 3 of them rejected the operation, and 5 of them had invasive procedure by interventional radiology. Further, no pathology was found during operation in 3 of 54 patients who underwent surgery. The lactate levels of these 3 patients were 1.4, 1.5 and 2.7 mmol/liter and they were categorized in group 3, group 4 and group 3, respectively. The lactate level of patients in the surgery recommended group was statistically significantly higher than those of the group for whom surgery was not recommended (2.18±2, 4.42±3.15, p<0.001) (Table III). Lactate values were statistically significant according to the ROC analysis performed to predict the state of surgery recommendation (AUC:0.796, p<0.001). At 2.55 mmol/liter cut-off lactate level, the rates of 75.38% sensitivity, 79.38% specificity, 45% positive predictive value, and 93.5% negative predictive value were reached (Figure 2). When patients who had no pathology in aCT and who were below 2.55 mmol/liter of lactate were categorized, rates of 98.5% sensitivity, 63.2% specificity, 37.4% positive predictive value, and 99.5% negative predictive value were reached in those who had surgery recommendation.

Table III. *Evaluation of the participants by their surgical status*

		Surgery not recommended (291)	Surgery recommended (65)*	p value
Gender	Male	155(53.3%)	39(60%)	p=0.324
	Female	136(46.7%)	26(40%)	
Number or mortality		33(11.3%)	22(33.8%)	**p<0.001**
Lactate levels (mEq/L)		2.18±2	4.42±3.15	**p<0.001**
Age		77.05±7.92	77.32±7.39	p=0.801
Emergent pathology in CT		83(28.5%)	61(93.8%)	**p<0.001**
Length of stay in hospital (day)		6.42±8.64	9.91±11.32	**p=0.006**

*Those who were considered as inop, who could not undergo a surgery because they died, who rejected surgery although it was recommended, and for whom invasive surgical intervention was performed were also included in this group.

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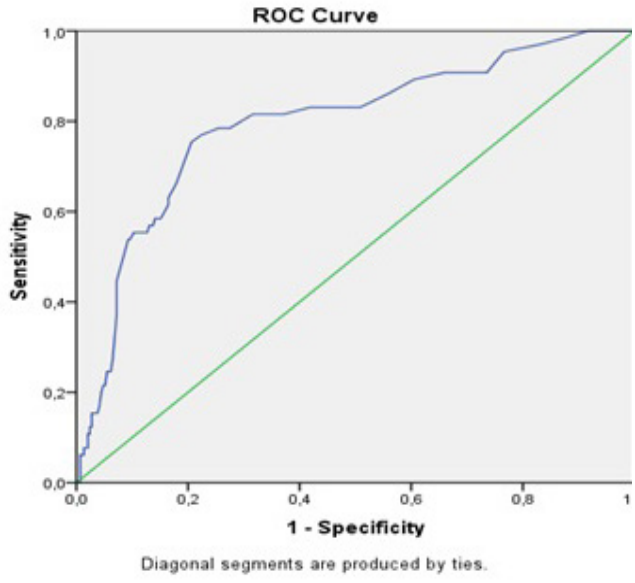

Figure 2. *ROC analysis for the recommendation of surgery with lactate level*

The participants were divided into four groups according to surgery recommendation and pathologic status on aCT. Four patients were found in Group 4, who had no pathology in aCT, but surgery was recommended. In the absence of pathology in CT, surgery might have been recommended based on the more dominant physical examination findings of the patients. These four patients were excluded because the between-groups comparison was not statistically possible. Statistical comparisons were made between the remaining three groups. These four patients in Group 4 were male, their mean age was 67.75±3.59 years, and their mean lactate level was 4.17±2.07 mmol/liter. There was a statistically significant difference in lactate values between the groups (Group 1: 1.78±1.46, Group 2: 3.19±2.71, Group 3: 4.44±3.22, I-II p<0.001, I-III p<0.001, II-III P=0.002). There was a statistically significant difference between Group 1 and Group 3 in terms of the length of stay (days) in the hospital (Group 1: 5.98±8.86, Group 3: 9.89±11.68, P=0.011). There was a statistically significant difference between hospital mortality rates between groups (Group 1: 15(%7.2), Group 2: 18(%21.7), Group 3: 22(%36.1), p<0.001). Table 4 shows the comparisons between groups.

A total of 55 (15.4%) patients died at the hospital. There was a statistically significant difference between the lactate level of patients who survived and non-survived (non-survivor 5.70±4.23, survivor 2.02±1.25, p<0.001). According to the ROC analysis, lactate values were found to be statistically significant in predicting mortality status (AUC: 0.855, p<0.001). At a cut-off of 2.45 mmol/liter lactate level, the rates of 76.36% sensitivity, 75.75% specificity, 36.5% positive predictive value, and 94.65% negative predictive value were reached (Figure 3). Tables IV and V show the comparison of mortality status and other parameters.

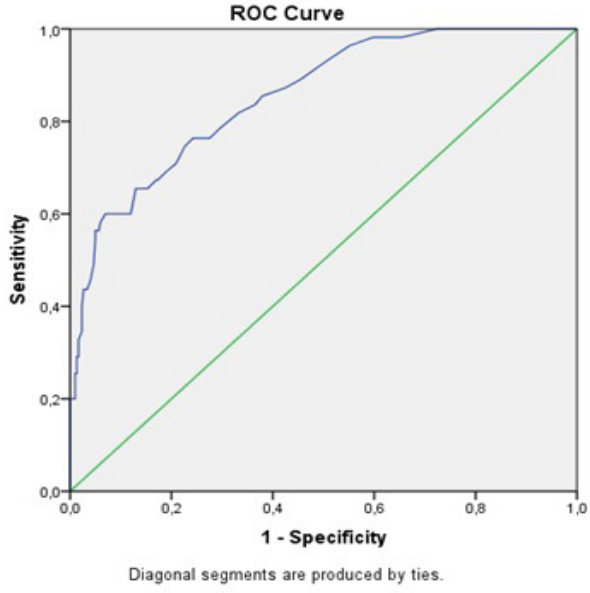

Figure 3. *ROC analysis of lactate level and mortality status*

Table IV. *Comparison of patient groups with other parameters*

		Group1 (n=208)	Group2 (n=83)	Group3 (n=61)	P value	I-II p value	I-III p value	II-III p value
Gender	Male	106(51%)	49(59%)	35(57.4%)	p=0.387			
	Female	102(49%)	34(41%)	26(42.6%)				
Lactate Levels (mEq/L)		1.78±1.46	3.19±2.71	4.44±3.22		**p<0.001**	**p<0.001**	**p=0.002**
Age		76.89±7.91	77.45±8	77.95±7.15		p=0.85	p=0.622	p=0.922
Length of Stay in Hospital (day)		5.98±8.86	7.52±8.02	9.89±11.68		p=0.406	**p=0.011**	p=0.282
Mortality status		15(7.2%)	18(21.7%)	22(36.1%)	**p<0.001**			

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Table V. Comparison of mortality status and other parameters

		Non-Survivor(55)	Survivor(301)	P value
Age		78.96±7.23	76.76±7.89	P=0.055
Gender	Male	35(63.6%)	159(52.8%)	P=0.139
	Female	20(36.4%)	142(47.2%)	
Lactate Levels (mEq/L)		5.70±4.23	2.02±1.25	**p<0.001**
Length of stay in hospital (day)		9.36±9.81	6.63±9.12	P=0.059
Emergent pathology in CT		40(72.7%)	104(34.6%)	**p<0.001**

4. DISCUSSION

Geriatric patients' management in the emergency department is difficult. It is important to detect the pathology that will require abdominal surgery in geriatric patients presenting to the emergency department. In this study, we assessed the potential of making decision regarding abdominal surgery in geriatric patients with the use of aCT results along with the blood lactate level. In this study population, the mean lactate level was significantly higher in those for whom surgery was recommended than in those for whom surgery was not recommended. There was a statistically significant difference and increasing mean blood lactate levels in patients who had no pathology in aCT and for whom surgery was not recommended, who had pathology in aCT and for whom surgery was not recommended, and who had pathology in aCT and for whom surgery was recommended. This is the first time observation of grouping of this kind in the literature.

In a study conducted by Verma et al., examining patients of all age groups over 15 years with acute abdominal pain, 30 of whom underwent an emergent surgery and 20 who did not, peritoneal lactate levels between the groups were found as 14.65±1.19 and 5.92±0.97, respectively, which was a statistically significant difference [9]. The blood lactate level was also significantly higher in the surgery recommended group in this study. The reason for the high lactate levels in the surgery recommended group may be due to the ischemic and inflammatory origin of pathologies that may cause acute surgical indications. Since, geriatric patients have more comorbidity than younger patients, they have a higher risk for the operation. This may have led physicians to take a sounder step in making decisions regarding surgery for geriatric patients. This sound choice may have reduced the likelihood of diagnosing surgical pathologies at the onset of the ischemic and inflammatory conditions with no increased lactate levels.

In this study, there was a statistically significantly higher hospital mortality rate among those for whom surgery was recommended than among those for whom surgery was not recommended. Further, the hospital mortality rate was significantly different between the patients who had no pathology in aCT and for whom the surgery was not recommended, who had pathology in aCT and for whom the surgery was not recommended, and who had pathology in aCT and for whom the surgery was recommended. In their study of 195 patients who had elective major abdominal surgery, Veličković et al., found that perioperative high lactate levels were associated with postoperative comorbidity and mortality [11]. Ravishankaran et al., determined that high lactate levels were associated with prognosis in patients with acute abdominal pain who underwent a surgery [12]. In the current study, a 33.8% mortality rate was observed in the surgery recommended group. In their study of 710 patients over 70 years of age who underwent emergent abdominal surgery, Arenal et al., found a mortality rate as 22% [13]. In another study, Rangel et al., found a mortality rate of 32.5% [14], while Brandt et al., found the 90-day mortality rate of 42.7% in 150 patients who underwent geriatric abdominal surgery [15]. Thus, the findings from our study are consistent with those of previous studies.

In this study, the lactate levels in the non-survivor group was statistically significantly higher than the survivor group. In their study of 455 patients who stayed in the intensive care unit following their application to the emergency department, Dündar et al., found that high lactate levels were associated with in-hospital mortality [16]. In a study conducted with 1278 emergency patients with signs of infection, Shapiro et al., found that serum lactate is reliable in predicting mortality [17]. Portal et al., found that a high lactate level was associated with mortality in patients over 65 years of age who visited to the emergency department, and had no infection [18]. Due to the high mortality relationship of lactate in both sepsis and geriatric patients, the high level of lactate in the non-survivor group in this study was compatible with the literature. In this study, no statistically significant difference was found in the mortality rates between the genders. In their study of 710 patients who were over 70 years of age and underwent emergent abdominal surgery, Arenal et al., did not find any statistical difference between male and female genders [13].

The mean age of the patients in this study was 77.1±7.82 years. In their study carried out with 220 patients who were over 65 years of age and underwent abdominal surgery, Joseph et al., found the mean age as 75.5±7.7 years [19]. The most common complaint in this study was abdominal pain with 54.2%. In the study carried out by Grundmann et al., that examined the epidemiologic review of patients presenting with acute abdomen, patients presenting with acute abdomen applied mostly with non-specific abdominal pain with 24-44.3% [20]. In this study, the most common pathology seen on aCT was ileus (33.3%). This finding is consistent with those of previous studies. For example, Arenal et al., evaluated 710 patients who underwent emergent abdominal surgery and found that the most common etiological reason was intestinal obstruction in the age group between 70-79 with 37% and in the age group over 80 years with 45% [13]. Bugliosi et al's report on 127 patients over 65 years of age who visited the emergency department with non-traumatic abdominal pain indicated that the most common etiological cause in those who underwent an operation was small bowel obstruction and biliary tract diseases [21].

Limitation

This study, in which we suppose that serum lactate with aCT would support the evaluation of surgery for elderly patients,

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had some limitations, given its retrospective nature. The clinical decision of patients excluded due to lack of blood lactate levels were not known; thus, we could not assess how the result would have been affected should their lactate levels available and included in our analysis. The fact that the research was a retrospective study causes the surgical decision-making physician not to be blind to the lactate level. However, we excluded this limitation because only three of the patients who underwent surgery had no pathology at the time of operation (intraoperative) and their lactate levels were not high. Another limitation is that aCT imaging is non standard in terms of contrast. Considering that the physician evaluates the patient as a whole under aCT, physical examination, consultation, and laboratory examination, and eliminates the surgical pathology, we ignored this limitation. Further, the fact that the study was single-centered increased the subjectivity of the pathology and surgical decisions. Another limitation is that patients who underwent emergency surgery with ultrasonography were not evaluated.

5. CONCLUSION

We investigated whether serum lactate levels together with aCT findings would support emergent surgery decisions in elderly patients. The results of aCT and lactate levels in geriatric patients were found to be reliable physician's decisions to eliminate acute abdominal surgery. Our findings reveal that geriatric patients are less likely to be diagnosed with surgical pathologies at the beginning of the ischemic and inflammatory disorders with no increased lactate levels. Yet, there is a need for prospective multicenter studies to further validate these findings.

Compliance with Ethical Standards

Ethical approval: Ethics committee approval for this single-center, retrospective study was obtained from the Necmettin Erbakan University, Meram Medical Faculty, Pharmaceutical and Non-Medical Device Studies Ethical Committee (decision number of 2020/2569).

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

Authors' Contributions: KK: Idea/Concept, KK: Design, KK: Data Collection/Processing, KK and MKA: Analysis/Interpretation: KK and MKA, Literature Review: KK and MKA, Drafting/Writing: KK and MKA, Critical Review: KK and MKA. Both authors read and approved the final version of this article.

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The effects of low bone mineral density on pain, quality of life and fatigue in patients with epilepsy

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ABSTRACT

Objectives: To investigate the effects of low bone mineral density (BMD) on pain, quality of life (QoL), and fatigue in epileptic patients who use anticonvulsants.

Patients and Methods: Epileptic patients aged 18 years or older who use anticonvulsant drugs were recruited into the study. Demographic and clinical features were recorded, including the duration of epilepsy, number of anticonvulsants used, previous fracture history and BMD scores. The functional parameters included back pain measured with the visual analogue scale (VAS) and brief pain inventory (BPI) scale, QoL assessed with the Qualeffo-41 questionnaire, and fatigue assessed with the fatigue severity scale (FSS).

Results: Of the 100 patients screened for inclusion in the study, 63 epileptic patients met the inclusion criteria. The mean age and mean disease duration of all participants was 39.5 (±11.2) and 19.3 (±11.6) years, respectively. The median scores for VAS back pain, VAS low back pain, Qualeffo-41, FSS, pain severity, and pain interference (BPI) were significantly higher in patients with secondary osteoporosis compared to patients with normal BMD. There were significant correlations between lumbar spinal BMD and VAS back pain (rho = – 0.58, $p < 0.0005$), BPI pain severity (rho = – 0.56, $p < 0.0005$), BPI pain interference (rho = – 0.52, $p < 0.0005$), Qualeffo-41 (rho = – 0.56, $p < 0.0005$), and FSS (rho = – 0.41, $p = 0.001$).

Conclusion: Epileptic patients suffering from low BMD showed increased pain, fatigue and impaired QoL. Therefore, BMD measurement should be recommended for the evaluation and management of epileptic patients.

Keywords: Epilepsy, Quality of life, Bone mineral density, Fatigue, Pain

1. INTRODUCTION

Epilepsy is a chronic disorder with several social and psychological impacts. Patients with epilepsy often report concerns about having a seizure attack, social isolation, adverse effects of anticonvulsants, and transportation/driving restrictions [1]. In general, epileptic patients perform significantly less physical activity and have higher rates of obesity [2]. The reason for reduced physical activity in many patients is that they incorrectly believe that physical activity can induce seizures or increase their frequency, and they may also fear prejudice and perceived social and cultural stigma [3]. However, regular physical activity is important as it increases muscle and bone strength. In addition, anticonvulsant therapy can negatively impact bone health in several important ways, ranging from asymptomatic high-turnover bone disease to decreased bone mineral density (BMD) and an increased fracture risk [4]. Both children and adults under anticonvulsant treatment have been found to have decreased BMD, often more than two standard deviations below that of healthy young controls [5]. Thus, patients with a history of long-term therapy should be evaluated for anticonvulsant-induced bone disease. Pain, muscle weakness, and skeletal deformities lead to a decrease in QoL in these patients [6,7]. In addition, patients with osteoporosis may have concerns about

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falls, fractures and an eventual loss of independence, which could negatively influence their lives [7,8]. The use of anticonvulsants that cause low BMD can lead to fragility fractures [8,9]. As far as we know, over 60% of fractures occur in younger women, with T-scores ranging from – 1 to – 2.5 [10]. Furthermore, all types of fractures are associated with higher mortality rates [11]. Chronic pain, disability, postural changes, and impaired quality of life (QoL) secondary to fractures are commonly observed in osteoporosis; therefore, the assessment and management of pain should be considered in detail [12].

Fatigue related to osteoporosis has not been extensively examined in the literature. In a previous study, osteopenic and osteoporotic patients experienced greater fatigue compared to healthy controls. In patients with a low BMD, there is known to be an interaction between pain, QoL, and fatigue, where a problem in one of these factors can trigger a disturbance in the others [7]. It is important to assess BMD in patients under anticonvulsant treatment. In the literature, there is limited information on the consequences of low BMD in epileptic patients. Furthermore, the effects of low BMD on pain, QoL, and fatigue in patients with epilepsy have not yet been analysed. The aim of this study was to investigate the effect of low BMD on QoL, pain, and fatigue in these patients.

2. PATIENTS and METHODS

Participants

Of the 100 patients screened for inclusion in the study, 63 epileptic patients were included due to the strict exclusion criteria. Seventy-five percent of our patients had generalized epilepsy, the rest had focal seizure. Participants were recruited from the outpatient clinic of Marmara University, School of Medicine Departments of Neurology and Physical Medicine and Rehabilitation. The inclusion criteria were an age of 18 years or older, a diagnosis of epilepsy, and anticonvulsant treatment. The exclusion criteria included Alzheimer's disease, severe psychiatric disorders (bipolar disease, schizophrenia), mental retardation, rheumatologic disorders (rheumatoid arthritis, fibromyalgia, etc.), endocrine disorders associated with osteoporosis (diabetes mellitus, hyper – and hypothyroidism, hyperparathyroidism, pituitary gland disorders, hypogonadism), other medications that can cause osteoporosis (corticosteroids, warfarin, heparin, etc.), malignancy, pregnancy, lactation, and chronic renal/hepatic failure. Ethical approval was obtained from the Ethical Committee of Marmara University, School of Medicine with a registry number of 09.2016.367. Both verbal and written consent were obtained from each participant.

Bone mineral density measurements in the anterior-posterior position were performed at the level of the lumbar vertebra using a dual-energy X-ray absorptiometry (DXA) device (Lunar DPX Prodigy-Tech; General Electric, Madison, WI, USA). Based on the average value for age-matched adult individuals, a T – or Z-score up to one standard deviation (SD) was considered normal. A T – or Z-score greater than one SD or the presence of a pathologic fracture (vertebral, hip, non-vertebral) was considered secondary osteoporosis [13]. In men under 50 years of age and premenopausal women, Z-scores were used [14].

Demographic data (age, gender, body mass index [BMI], education level), number of births, duration of lactation, presence of menopause, age of menopause onset, regularity of menstrual cycle, alcohol consumption, smoking, and physical activity level were noted. Clinical parameters (disease duration, number of anticonvulsants, type of anticonvulsants) were recorded. The drugs that induce or not cytochrome p450 were classified as inducers, non-inducers, respectively. The drugs that affect bone with other mechanism of action such as valproate classified as others. The participants were requested to complete the following scales: visual analogue scale (VAS) for back pain, brief pain inventory (BPI), quality of life questionnaire of the European Foundation for Osteoporosis (Qualeffo-41), and fatigue severity scale (FSS).

Instruments

The VAS is a unidimensional measure of pain intensity for the assessment of back pain (VAS back pain). It consists of a 10-cm horizontal line on which the patient's pain intensity is represented by a point between the extremes of "no pain" (score of 0) and "worst imaginable pain" (score of 10) [15].

The BPI is one of the most commonly used outcome measures for evaluating pain. The BPI assesses pain severity as "worst", "least", "average", and "now". The pain severity dimension of the BPI consists of four items that are scored from 0 (no pain) to 10 (worst possible pain), whereas the pain interference dimension consists of seven items that are scored from 0 (no interference) to 10 (complete interference). A pain severity score is calculated from the mean of the four pain intensity items, and a pain interference score is calculated from the mean of the seven pain interference items [16]. It has been validated in a Turkish population in surgery patients [17].

The Qualeffo-41 is a disease-specific questionnaire to measure QoL in osteopenia and osteoporosis. It has five domains which are pain (5 items), physical function (17 items), social function (7 items), general health perception (3 items), and mental function (9 items). The score for each domain is calculated as an average value of all the answered items linearly converted on a 0–100 scale. The total Qualeffo-41 score is calculated as the sum of all answers to items and then converted to the 0–100 scale. The QoL worsens as the total Qualeffo-41 score increases [18]. The Turkish version of Qualeffo-41 has been validated and is commonly used in studies [19].

The FSS contains nine items that evaluate the severity of patients' fatigue symptoms. Each item is scored on a 7-point scale where 1 represents "strongly disagree" and 7 represents "strongly agree". The total score ranges between 9 and 63. The severity of fatigue symptom increases as the total score increases [20].

Statistical Analysis

Descriptive statistical analyses were performed. The Mann–Whitney U test was used to assess the mean differences between groups. Spearman's correlation coefficient was used to assess

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the relationship between continuous parameters. Correlation coefficients >0.50, 0.35–0.50, and <0.35 were considered strong, moderate, and weak, respectively [21]. We were unable to calculate the sample size as there was no previous reference study. Therefore, a post hoc power analysis was performed. Cohen set out standardised measures of effect size, proposing a simple categorisation of small, moderate, and large effect sizes [22]. A formula based on G power version 3.0 was used to determine post hoc power [23]. We determined the power of the study by computing an effect size of 0.80 (large effect) using a significance level of 0.05. The post hoc power was found to be 0.93, which is considered good. Hierarchical multiple regression analysis was used to examine the differential contributions of variables to the lumbar BMD score. The variables that were correlated significantly with BMD scores were entered into three models including different categories of variables. Scatterplots of distribution of residuals to the models were found acceptable. SPSS version 20 was used for all statistical analyses, and p-values less than 0.05 were considered statistically significant.

3. RESULTS

In total, 63 epileptic patients who were under anticonvulsant treatment (35 females, 28 males) were enrolled in the study. The mean age of participants was 39.5 (±11.2) years, ranging between 20 and 66 years. The demographic and clinical features of the patients are shown in Table I.

Table I. *Demographic and clinical features of the participants (n=63)*

Gender	**N (%)**
Female	35 (55.6)
Male	28 (44.4)
Education	
Primary-secondary school	40 (63.5)
High school	19 (30.2)
University	4 (6.3)
Presence of fracture	
Present	9 (14.3)
Not present	54 (85.7)
	Mean (SD), min-max
Age (mean)	39.6 (11.2), 20-66
BMI	26.5 (5.1),15.9-37.2
Disease duration (years)	19.3 (12.6), 3-47
Number of anticonvulsants	2.3 (1.2), 1-5

N: Number; BMI: Body Mass Index; SD: Standard deviation; Min-max: Minimum-maximum

Sixteen female patients were nulliparous. Among the remaining 19 female patients, 5 had one previous pregnancy (14.3 %), 10 had two pregnancies (28.6 %), 3 had three pregnancies (8.6 %), and 1 patient had four pregnancies (2.9 %). The mean duration of lactation in female patients who gave birth was 12.4 ± 17.3 months. The menstrual irregularity was present in 6 patients (9.5%). Eleven female patients (31.4%) were in menopause. None of the patients were using alcohol, only 5 (7.9%) of them were active smokers. Regarding anticonvulsant use, 26 (41.3%) patients were using one anticonvulsant and the rest were using two or more. The patients' scores were compared according to their anticonvulsant types. Inducers, non-inducers and the drugs that effect bone with other mechanisms such as valproate did not differ significantly when scores of BMD, pain, QoL and fatigue compared (p>0.05). All of the patients were sedentary. The patients with secondary osteoporosis had higher scores of VAS-back pain, BPI-pain severity, BPI-pain interference, Qualeffo-41, FSS compared to the patients with normal BMD which was depicted in Table II.

Table II. *Comparison of patients with secondary osteoporosis and normal BMD values**

	Patients with secondary osteoporosis (n=31)	Patients with normal BMD values (n=32)	P value
VAS-back pain	4.9±3.6	1.2±1.6	<0.0005**
BPI-pain severity	4.2±3.1	1.2±1.6	<0.0005**
BPI-pain interference	4.4±3.3	1.1±1.6	<0.0005**
Qualeffo-41 pain	30.6±26.2	9.8±15.1	0.002**
Qualeffo-41 physical function	25.5±22.2	10.7±16.3	0.001**
Qualeffo-41 social function	30.6±26.2	9.8±15.1	0.001**
Qualeffo-41 GHP	49.9±20.1	29.9±22	<0.0005**
Qualeffo-41 mental function	50.5±19.4	26.7±18.3	<0.0005**
Qualeffo-41 total score	40.7±18	19.3±14.9	<0.0005**
FSS	40.9±18.5	19±13.5	<0.0005**

**:Mann-Whitney U test; **all values were significant; N: Number; BMD: Bone Mineral Density; VAS: Visual Analogue Scale; BPI: Brief Pain Interference; Qualeffo-41: Quality of Life Questionnaire of the European Foundation for Osteoporosis-41; GHP: General Health Perception; FSS: Fatigue Severity Scale*

There were significant moderate correlations between lumbar BMD scores and BMI, number of anticonvulsants, VAS back pain score, BPI score, Qualeffo-41 score, and FSS (Table III). The mean BMD score (g/cm^2) did not differ between genders (p = 0.65).

A hierarchical regression analysis was used to examine the differential contributions of different independent variables. The overall model accounted for 45% of variance to the lumbar BMD scores. In hierarchical multiple regression, three models were used. Model 1, demographic and clinical variables (BMI, number of anticonvulsants) predicted the BMD scores since its contribution to the models was 21% of the variance [F (2, 60) = 8.3, (p = 0.001)]. Controlling the BMI and the number of anticonvulsants, the self-reported measures assessing QoL and fatigue contributed significantly, accounting for an additional 12% of the variance [F (2, 58) = 5.4, p=0.007] (Model 2). The addition of scales that assessed pain (VAS-back pain, BPI-pain

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severity, BPI-pain interference) to the prediction of BMI (Model 3) also led to a statistically significant increase in R^2 of 0.11, F(3, 55) = 3.8, p=0.015. Table IV showed the full details on each regression model.

Table III. *Relation of the Lumbar BMD (g/cm2) scores with demographic and clinical parameters (n=63)*

	Spearman's correlation coefficient (rho)	P significance value
Age	-0.12	0.34
BMI	0.42	**0.005***
Disease duration (years)	-0.16	0.22
Number of anticonvulsants	-0.40	**0.001***
VAS-back pain	-0.58	**<0.0005***
BPI-pain severity	-0.56	**<0.0005***
BPI-pain interference	-0.52	**<0.0005***
Qualeffo-41	-0.56	**<0.0005***
FSS	-0.41	**0.001***

**Significant values; N:number; BMD: Bone Mineral Density; BMI: Body Mass Index; VAS: Visual Analogue Scale; BPI: Brief Pain Index; Qualeffo-41: Quality of Life Questionnaire of the European Foundation for Osteoporosis-41; FSS: Fatigue Severity Scale*

Table IV. *Final model for the associations between the lumbar BMD score and the other variables entered in three models*

Variables	*R^2 change*	*F change*	*P value*
Model 1: Demographic and clinical variables BMI Number of anticonvulsants	0.22	8.2	0.001*
Model 2: Scales assessing QoL and fatigue Qualeffo-41 FSS	0.12	5.4	0.007*
Model 3: Scales assessing pain VAS-back pain BPI-pain severity BPI-pain interference	0.11	3.8	0.015*

*BMD: Bone Mineral Density; BMI: Body Mass Index; QoL: Quality of Life; Qualeffo-41: Quality of Life Questionnaire of the European Foundation for Osteoporosis-41; FSS: Fatigue Severity Scale; VAS: Visual Analogue Scale; BPI: Brief Pain Index; *p<0.05 accepted as significant*

4. DISCUSSION

This study aimed to assess the relationship of BMD with pain, QoL, and fatigue in epileptic patients under medical treatment. Anticonvulsants decrease BMD and increase the risk of fractures by two-fold due to high bone turnover, and secondary hyperparathyroidism [24]. Epilepsy and its treatment are associated with a decrease in BMD in patients of both genders, independent of vitamin D levels [25].

Hierarchical multiple regression analysis performed to predict the differential contributions of different independent variables. The overall model accounted for 45% of variance to the lumbar BMD scores. All of the three models contributed significantly to the variance. Chin et al., describes R^2 values of 0.67, 0.33, and 0.19 in multiple regression models as substantial, moderate, and weak, respectively [26].

The QoL of individuals with epilepsy is related to their perception of the impact of the disease itself and its treatment. Thus, the QoL of epileptic patients could be negatively affected by the adverse effects of anticonvulsants [27]. Indeed, uncontrolled epilepsy, adverse effects of anticonvulsants, and psychological comorbidities (i.e., depression, anxiety) have been found to negatively affect the QoL of patients with epilepsy [28]. In these patients, QoL is determined by the balance between seizure control by anticonvulsants and their adverse effects. The pain, muscle weakness, skeletal deformities, and psychological disorders associated with osteoporosis may have negative effects on QoL [7]. Furthermore, the pain, anxiety, and depression associated with osteoporosis may cause sleep disturbances in epileptic patients [28]. In accordance to this, the patients with secondary osteoporosis had a decreased mental and social function compared to the patients with normal BMD in our study. Furthermore, we showed that the epileptic patients with secondary osteoporosis had poor QoL.

Chronic pain is one of the most consequential symptoms of age-related and secondary osteoporosis. The most common symptom reported by patients with a diagnosis of osteoporosis is back pain resulting from osteoporosis-related fractures, skeletal deformities, joint imbalance, and tension in muscular structures [12]. Accordingly, epileptic patients with osteoporosis had greater VAS back pain scores compared to those without osteoporosis. Also, there were significant moderate correlations between the BMD scores of epileptic patients and pain severity (rho = – 0.56, p < 0.0005) and pain interference (rho = – 0.52, p < 0.0005), assessed using the BPI.

Fatigue is commonly seen in epilepsy and osteoporosis, both of which are chronic conditions [7, 29]. The frequency of fatigue was 47.1% in epileptic patients. Fatigue is known to be significantly related to sleep and depression [29]. However, the relationship of epilepsy-related fatigue with BMD and osteoporosis has not yet been investigated. In our study, epileptic patients with secondary osteoporosis had higher fatigue scores compared to the patients with normal BMD. Furthermore, BMD scores were significantly negatively correlated with FSS scores (rho = – 0.41, p = 0.001). As the BMD scores of epileptic patients decreased, the patients complained of greater fatigue (higher FSS score).

In our study, we found that as the number of anticonvulsants increased, lumbar BMD scores decreased (rho = – 0.40, p = 0.001). In accordance with the findings of our study, Farhat et al., found that the multiplicity of anticonvulsant therapies was a significant negative determinant of BMD [25]. We found no correlation between lumbar BMD scores and disease duration. However, Farhat et al., showed that the duration of anticonvulsant therapy was significantly negatively correlated with BMD. Nevertheless, two other studies failed to show a relationship

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between disease duration and BMD. In those studies, the mean duration of disease was longer than that reported by Farhat et al., but similar to our results, so most of the deleterious skeletal effects may have already occurred [25, 30, 31]. Cytochrome P450 enzyme inducers phenytoin (PHT), phenobarbital (PB), primidone (DRE), carbamazepine, oxcarbazepine (OXC) are associated with a decrease in BMD. However, valproate, which inhibits cytochrome P450 is also associated with decreased BMD suggesting different mechanism of effect on bone metabolism. Previous studies suggested that valproate affects directly the functions of bone cells. New generation anticonvulsants are thought to be less harmful to bone metabolism, however new researches should be carried out to prove it definitely. In our study, we compared the anticonvulsants by mechanism of their action on bone, however no significant difference was found. There was no relationship between age and lumbar BMD scores in our study. This could be attributed to the fact that the participants enrolled in our study were relatively younger, with an age range of 20 to 66 years. As far as we know, osteoporosis is associated with a low BMI [32, 33]. In accordance with this, we found a positive, moderate correlation between BMI and lumbar BMD scores.

A strength of this study is that several instruments were used to evaluate the deleterious effects of anticonvulsants on BMD, pain intensity, QoL, and fatigue in epileptic patients, which have not been investigated until now. However, the sample size was limited, as most of the epileptic patients treated at our clinic were unable to fill out the questionnaires due to mental problems and various other comorbidities.

Compliance with Ethical Standards

Ethical approval: This study was approved by the Ethical Committee of Marmara University, School of Medicine with a registry number of 09.2016.367. Both verbal and written consent were obtained from each participant.

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Conflict of interest: All authors declare that there is no conflict of interest.

Authors' contributions: CUU, and GYS gathered and performed the measurements, IM and GDA were involved in planning and supervised the work, CUU processed the experimental data, performed the analysis, drafted the manuscript and designed the figures. All authors aided in interpreting the results and worked on the article. All authors discussed the results and commented on the article. All authors approved the final version of the article.

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Topical and systemic analgesia versus caudal epidural and dorsal penile nerve block in relieving pain after pediatric circumcision

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ABSTRACT

Objective: There is no consensus on the use of optimal analgesic method after pediatric circumcision, although, caudal block (CB), dorsal penile nerve block (DPNB), topical local anesthetic application and systemic analgesic administration are frequently used methods. In this study, we aimed to compare the postoperative analgesic effects and side effects, as well as parental satisfaction concerning these methods.

Patients and Methods: Eighty children, aged 3-12 years, undergoing circumcision were randomized into four groups (n=20). Group Caudal Anesthesia (CA), Group Dorsal Penile Blok (DP), Group Topical Analgesia (TA), Group Systemic Analgesics (SA). The severity of pain was measured with NRS or CHEOPS scores. Time to awakening, first analgesic requirement, hospital discharge, side effects and parental satisfaction were recorded.

Results: In groups CA and DP, pain scores during the first postoperative hour were lower than the pain scores of other groups and in group CA, the first analgesic requirement time was significantly longer compared to other groups ($p<0.05$). In the early postoperative hour, parental satisfaction was higher in group CA compared to other groups.

Conclusion: In the early postoperative period, CB and DPNB reduce pain more effectively and provide more parental satisfaction than other applications, (in children who underwent) circumcision.

Keywords: Circumcision, Pain, Postoperative, Dorsal penile nerve block Caudal epidural block, EMLA

1. INTRODUCTION

Providing an effective postoperative analgesia is essential following pediatric day case surgery in children [1]. Circumcision is a minor and day case surgery, usually represented as the most undertaken procedure on a day case basis, and it usually ends up with significant postoperative pain and distress [2, 3]. Pain leading to involuntary movements in the early postoperative period may result in bleeding at the operative site.

Ideal analgesia method after circumcision should provide an immediate, long lasting and effective pain relief with minimal to no side effects. Nonsteroidal anti-inflammatory agents (NSAIDs) and opioids are the most widely used systemic analgesics after circumcision, however, their use may be limited because of the inadequate analgesic effects of NSAIDs, when used alone and the potential adverse effects of opioids [1]. Although, local anesthetic techniques such as Caudal Block (CB) and Dorsal Penile Nerve Block (DPNB) have been shown to be more effective than the administration of systemic opioids after circumcision, CB may be complicated with motor block or delayed micturition [4]. Studies on the effectivity of DPNB have also conflicting results with a reported overall failure rate being 4-6% [3, 5]. The topical administration of local anesthetic (LA) agents seems to be promising for its noninvasiveness [6], although, some authors claim that topical analgesia is not effective [2]. All in all, the best and minimal invasive method in relieving post circumcision pain has not yet been determined and a Cochrane review on this subject claims that trials comparing all the methods are still lacking [4].

Our aim was to compare the early postoperative analgesic effects, side effects and parental satisfaction regarding four analgesic methods; CB, DPNB, topical and systemic analgesic administration in children undergoing elective circumcision.

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2. PATIENTS and METHOD

After obtaining ethical committee approval (Ethics Committee Approval: Decision No. 82 dated 24.09.2010 from the Turkish Ministry of Health, General Directorate of Pharmaceuticals and Pharmacy, Pharmaceutical Clinical Research Ethics Advisory Board-I) and written informed consent of the parents, eighty boys aged between 3-12 years, scheduled for circumcision were enrolled into the study. The study design was prospective, randomized, controlled and comparative. Randomization was done by a sealed envelope method. Exclusion criteria were parental refusal, history of coagulopathy, atopic dermatitis or allergy to local anesthetics, history of methemoglobinemia and contraindication to NSAIDs or CB, history of developmental delay or mental retardation which might disturb the pain intensity assessment, neurological diseases, and analgesic use in the previous week of the surgery.

Patients were allocated into four groups:

Group CA (n=20) received caudal block with 0.25% bupivacaine in 1ml/kg, after anesthesia induction;

Group DP (n=20) received DPNB with 0.25% bupivacaine in 0.3ml/kg, after anesthesia induction;

Group TA (n=20) received topical analgesia, one hour before anesthesia induction.

Group SA (Control group) (n=20) received systemic analgesics (rectal paracetamol after anesthesia induction, rectal or oral paracetamol postoperatively). General anesthesia was induced with thiopental 5-7 mg/kg IV or inhalation of 8% sevoflurane with 70% N_2O in oxygen in children who refuse intravenous line access. Muscle relaxation was maintained with 0.5 mg/kg atracurium bromide IV. An appropriate-sized laryngeal mask airway was inserted and anesthesia was maintained with 1 MAC sevoflurane and 70% N_2O in oxygen.

Topical analgesia with 3 g 2.5% lidocaine and 2.5% prilocaine cream, eutectic mixture of local anesthetics (EMLA) was applied to the distal half of the penis under an occlusive dressing, one hour before the surgery, in the preoperative room before the assistance of the parents (Group TA). Application did not include the mucosal surface. CB was performed by the same experienced anesthesiologist with 0.25% bupivacaine hydrochloride, 1 $mLkg^{-1}$ (maximum 20 mL) (Group CA). DPNB was performed by the same experienced pediatric surgeon, with infiltration of 0.3 $mLkg^{-1}$ 0.25% bupivacaine hydrochloride to both sides of the pubic arch via a 21 G needle (Group DP). In group SA, 40 $mgkg^{-1}$ paracetamol were given rectally after the induction of anesthesia, 20 $mgkg^{-1}$ paracetamol rectally or orally four times a day were administered postoperatively.

Heart rate, non-invasive blood pressure, peripheral oxygen saturation (SpO_2), end-tidal carbon dioxide pressure ($ETCO_2$) and sevoflurane concentrations were monitorized and recorded with 15 minute intervals peroperatively.

Postoperative pain intensity was assessed with a 10-point numeric rating scale (NRS) (0=none, 1-3=mild, 4-6=moderate, 7-10=severe) in children older than 6 years (school-aged) or with Children's Hospital of Eastern Ontario Pain Scale (CHEOPS: minimum score 4 points and maximum score 13 points) in younger (preschool) children [7].

Pain scores were recorded at 0, 2nd, and 4th h postoperatively. In the postanesthesia care unit or in the ward, 1 $mgkg^{-1}$ meperidine hydrochloride intravenously was administered whenever NRS scores were higher than 3 or CHEOPS scores were higher than 5. All data collection was done by an anesthesiologist who was blinded to the group assignment. At the end of anesthesia, awakening times (time to follow verbal commands after the discontinuation of sevoflurane), the first analgesic requirement time, the need for rescue analgesic, the first urination, mobilization and hospital discharge time, adverse effects and parental satisfaction via a 4-point scale, edited by researchers (4=very satisfied, 3=satisfied, 2= dissatisfied, 1=very dissatisfied) were recorded.

Statistical analysis

Statistical evaluation of the collected data was performed using GraphPad Instat Version 3.00 for Windows 95. The primary outcome was pain scores as measured by NRS or CHEOPS. Secondary endpoints included the first analgesic requirement time, the need for rescue analgesic, the first urination, mobilization and hospital discharge time, adverse effects and parental satisfaction. Sample size was calculated based on previous studies [8] to detect a difference of 2 in CHEOPS pain scores at early postoperative hours, between groups with a standard deviation of 2.75. At the end, we found that at least 20 patients in each group required to demonstrate a significant difference for a type I error of 0.05 and a power of 0.8. Repeated measures of ANOVA were used to analyze the variations of parameters in different times. Tukey's test was considered as post-hoc analysis. Non parametric data were evaluated with Kruskal-Wallis and Mann-Whitney U tests. Chi-square or Fisher's exact test were used to evaluate the proportional variables. Data were expressed as mean ± standard deviation (SD). A p value less than 0.05 was considered as statistically significant.

3. RESULTS

Descriptive analysis of the patients' demographic and clinical characteristics is shown in Table I. Demographic datas, duration of surgery and anesthesia were similar.

While the heart rate did not differ between the groups in the first 15 minutes peroperatively ($p > 0.05$), the 30th minute value was significantly lower in Group CA compared to other groups ($p < 0.05$). Table II shows peroperative heart rates and mean arterial pressures.

Table III shows awakening, first mobilization, first urination and hospital discharge times.

Figure 1 shows the pain scores evaluated with CHEOPS. At the first postoperative measurement time (0.s), CHEOPS values were significantly lower in Group CA and Group DP than in Group TA and Group SA ($p < 0.05$). There was no significant difference between the groups at other measurement times ($p > 0.05$).

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There was no significant difference between the groups in terms of weaning time, first analgesic requirement time, first mobilization time, first urination time (p> 0.05). The proportion of patients who required additional analgesics during the study was significantly lower than Group TA and Group SA in Group CA and DP (p <0.05).

Figure 2 shows the NRS scores evaluated with NRS. In Group CA, NRS scores were significanty lower than the scores in Group TA and Group SA.

Figure 3 shows additional analgesic requirement. In Group CA, analgesic requirement was significantly lower than the analgesic requirement for Group TA and Group SA.

Figure 4 shows the parental satisfaction scores. Parental satisfaction with analgesia for Group CA and Group DP was significantly higher than the parental satisfaction for Group TA and Group SA.

Table I. *Descriptive analysis of the patients' demographic and clinical characteristics.*

	Group CA (n=20) Mean (±SD)	Group DP (n=20) Mean (±SD)	Group TA (n=20) Mean (±SD)	Group SA (n=20) Mean (±SD)	p value
Age (years)	6.70 (± 2.60)	5.93 (± 2.98)	5.68 (± 3.20)	5.55 (± 2.88)	0,59
Weight (kg)	24.40 (± 7.88)	24.50 (± 11.87)	21.20 (± 6.93)	21.15 (± 5.87)	0,39
Duration of anesthesia (min)	42.60 (± 11.21)	37.35 (± 5.12)	37.35 (± 13.95)	39.30 (± 10.58)	0,35
Duration of surgery (min)	28.40 (± 9.77)	29.70 (± 5.47)	27.50 (± 11.77)	30.40 (± 8.59)	0,75

All data are presented as mean (± Standard Deviation). ANOVA test

Table II. *Peroperative heart rate and mean arterial pressure values*

	HR (Beats/min)			MAP (mmHg)		
	0 min	15. min	30. min	0 min	15. min	30. min
Group CA	123.4 (±19.2)*	112.1 (±19.7)	100.6 (±18.4) *⊥	80.5(±11.4)*	67.7 (±9.5)*	65.8 (±8.4)*
Group DP	118.9 (±20.6)	117.7 (±18.3)	115.4 (±18.0)⊥	81.1(±14.1)	71.8 (±13.7)	71.7 (±12.0)
Group TA	123.8 (±19.0)	122.9 (±18.6)	115.6 (±14.5)⊥	75.0 (±11.0)	73.9 (±9.1)	73.7 (±9.9)
Group SA	120.4 (±21.9)	125.2 (±16.4)	117.7 (±15.3)⊥	73.3 (±13.5)	75.2 (±11.7)	71.9 (±8.9)

*All data are presented as mean (± Standard Deviation), * p<0.01, within group comparison, ⊥ p<0.05, group CA compared to other groups, HR: heart rate, MAP: mean arterial pressure, ANOVA test (with posthoc Tukey test)*

Table III. *Awakening time, first analgesic requirement, first mobilization, first urination and hospital discharge times*

	Group CA (n=20)	Group DP (n=20)	Group TA (n=20)	Group SA (n=20)
Awakening time (min)	6.03±4.46	7.65±4.22	7.95±4.94	7.03±3.69
First analgesic requirement time (min)	25.33±16.48*	6.30±4.27*	5.16±5.04*	3.95±3.35*
First mobilization time (hr)	2.45±1.28	1.75±1.16	1.45±0.71	1.98±1.49
First urination time (hr)	2.68±1.23**	1.98±1.21	1.30±0.75**	2.23±1.61
Discharge time (hr)	4.35±0.99	3.93±1.08	3.70±1.19 †	4.73±1.21 †

*Data are expressed as mean ± SD, * p<0.0001, Group CA compared to other groups, ** p<0.01, Group CA, compared to group TA, † p<0.05, Group TA compared to group SA, ANOVA test (with posthoc Tukey test)*

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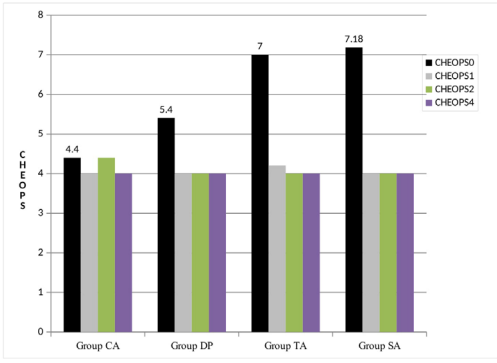

Figure I. *Postoperative CHEOPS pain scores*
**, p<0.05 Group CA compared to groups TA and SA*
***, p<0.05 Group DP compared to groups TA and SA*
Kruskal Wallis/Mann-Whitney U tests

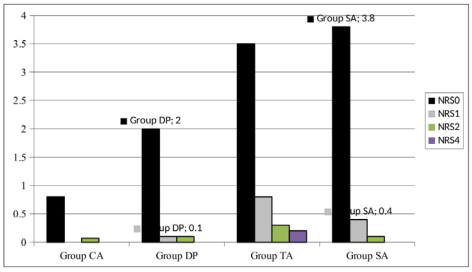

Figure 2. *Postoperative NRS scores*

**, p<0.05 Group CA compared to groups TA and SA*
***, p<0.05 Group CA compared to group TA*
Kruskal Wallis/Mann-Whitney U tests

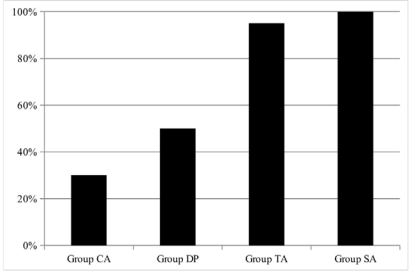

Figure 3. *Percent of patients requiring additional analgesic(%)*

**, p<0.05 Group CA compared to groups TA and SA*
†, p<0.05 Group DP compared to groups TA and SA
Chi-square test

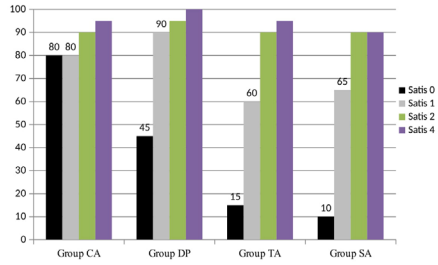

Figure 4. *Prental satisfaction (score of 4)*
**, p<0.05 Group CA compared to groups DP, TA and SA*
***, p<0.05 Group DP compared to groups TA and SA*
†, p<0.05 Group CA compared to groups TA and SA
¶p<0.05 Group DP compared to groups TA and SA
Kruskal Wallis/Mann-Whitney U tests

4. DISCUSSION

The main result of this study is that the first term postoperative analgesic effects of CB and DPNB are more prominent compared to noninvasive methods, in children undergoing circumcision. In regard of the parental satisfaction, they also offered advantageous findings. In other words, noninvasive methods such as topical LA application and less invasive method such as systemic analgesic administration appeared to be less effective than invasive methods in reducing pain during the early postoperative period.

Circumcision is a minor but painful surgery and children deserve to receive adequate analgesia during the immediate post-circumcision period. Early postoperative pain results in crying and agitation with excessive body movements leading to an increased risk for bleeding at the surgical site. So, the ideal analgesia method after circumcision would provide effective pain relief with minimal side effects.

Systemic analgesics such as opioids (pethidine hydrochloride, morphine sulphate and codeine) or NSAIDs are routinely administered in relieving postoperative pain following circumcision in children. Even though, these drugs are effective as painkillers, their unwanted side effects limit their use: opioids are usually administered in subtherapeutic doses as a result of the fear of opioid induced sedation, respiratory depression, nausea or urinary retention. NSAIDs are generally used as adjuvants to opioids in order to reduce their doses, as well as the incidence of side effects [9]. Nevertheless, nephrotoxicity, gastrointestinal bleeding and thrombocyte dysfunction stand as limiting factors in the use of NSAIDs [9]. To overcome these disadvantages, the application of local anesthetic techniques such as CB and DPNB in relieving post circumcision pain, became popular [10,11]. The main limiting factor of these methods is their invasiveness. Topical analgesia for pain relief seems promising but repeated application is usually required. Up to date, most of the studies concerning the effectivity of the methods compared only two

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methods, so we aimed to compare the most commonly used four methods and find the optimal one to provide post circumcision analgesia.

Caudal Block is the most commonly performed pediatric regional analgesia technique in children undergoing perineal surgery. Major disadvantages of CB are urinary retention and lower extremity motor block, leading to the denial of its routine use as an ideal method in day case surgeries [10]. Local anesthetic agent concentration is the major determinant of the degree of motor block. Wolf et al., determined that in caudal anesthesia, the use of bupivacaine at a concentration of 0.125% instead of 0.25% resulted in a lower incidence of motor block, whereas a further reduction in bupivacaine concentration to as low as 0.0625% resulted in a lower incidence of motor block with less analgesic effect [13]. We used 0.25% bupivacaine and motor block was only observed in two children. This finding does not overlap with the suggestion of the Cochrane review which claims that due to the possible risk of motor weakness, CB should not be performed in children old enough to walk, in day case surgeries [4]. The low incidence of motor block in our study was probably related to the low concentration of LA, and we believe that CB is an advantageous method to reduce pain after circumcision, as long as temporary leg weakness is prevented with reduced LA concentration. It is true that the ideal agent, dose, and concentration for a caudal block has not yet been determined as previously reported [2].

Dorsal Penile Nerve Block has the advantages of providing rapid recovery, early urination and discharge times, after circumcision [11-13]. In this method, there is no need to change the patient's position from supine to the lateral decubitus and a substantial number of studies claim that it provides analgesia as effective as CB [10,13]. In our study, DPNB resulted in lower CHEOPS scores in the immediate postoperative period and in higher parental satisfaction than noninvasive (topical LA administration) and control (systemic analgesic administration) groups. Although, time to first analgesic requirement was significantly shorter than CB, DPNB offered advantageous findings regarding lower number of children requiring additional analgesics than topical and systemic analgesia groups.

The incidence of side effects in DPNB is less compared to CB and only described as bleeding or hematoma formation at the site of injection [15]. The occurrence of penile ischemia is out of question if large volumes of LA agents and epinephrine containing solutions are avoided [16]. We did not observe any side effect with DPNB. Naja Z et al., suggested that DPNB is a blind approach since, its application relies on the subjective estimation of the dorsal nerve location in the Buck's fascia [3].

In terms of CB, volumes and concentrations used in our study were similar to those used in the literature (0.25% bupivacaine, 1mlkg^{-1}); but the concentration of bupivacaine used in DPNB was lower than that used in Weksler's study (0.25% versus 0.5%), volumes being similar [11-13]. In the aforementioned study, although the induction, skin incision and hospital discharge times were shorter than the CB group, the administration of high concentration of bupivacaine in DPNB made no difference in terms of postoperative analgesia. In Beyaz et al., study CB was performed with 0.25% levobupivacaine (0.5mlkg^{-1}) and DPNB with 0.25% levobupivacaine (0.5mlkg^{-1}) and analgesic efficacies, additional analgesic requirements were similar [15]. Margetts et al., as well, used 0.5% bupivacaine (0.25mlkg^{-1}) in DPNB and found no difference in analgesic efficacy when compared to CB performed with 0.25% bupivacaine (0.5mlkg^{-1}) with the addition of ketamine [18]. Regarding the concentrations and volumes used in our study, we also found equipotent analgesic efficacy between CB and DPNB in the immediate and early postoperative hours, with no difference in hospital discharge times. We question the necessity of using high concentrations and high volumes in DPNB. As mentioned earlier, we believe that administering low LA concentrations in CB may prevent motor block and make its use convenient in day case surgeries.

Topical LA application has a single side effect that manifests as a skin reaction, but it is suggested that it does not provide analgesia as effective as DPNB [19]. The reason of this suggestion may lie in the application time of the LA (EMLA) cream. Sufficient time must be allowed for it to become effective. There will not be enough time left to begin its effect if it is applied at the end of the surgery. Choi et al., performed topical analgesia one hour before surgery and determined that analgesia is as effective as DPNB but with a shorter duration of action [19]. We also applied EMLA cream one hour before surgery. Pain scores at the immediate postoperative hour and patient number requiring additional analgesics were higher in group TA than groups CA and DP. The ineffective analgesic profile of the topical LA application may be explained by the removal of LA from the surgical site during the sterilization period and loss of the effectivity of the cream. This explanation is also supported by some authors [19]. This problem may be solved by lengthening the application time to at least two h before surgery. Additionally, we performed DPNB with bupivacaine, a long lasting and more potent LA compared to lidocaine and prilocaine found in EMLA and this explains that the analgesic efficacy of DPNB was not observed in TA group. In this point, topical analgesia only seems to be advantageous due to its noninvasiveness and we recommend that it should not be used as the sole method of analgesia. This recommendation is also supported by Paix et al [3].

In summary, in the immediate postoperative period, CB and DPNB reduce pain more effectively and provide more parental satisfaction than topical and systemic analgesic applications, in children undergoing circumcision. Need for an additional analgesic requires longer time in CB and DPNB. There is a misconception that these blocks are difficult to perform and time-consuming, but our present study demonstrated that they are more effective in post circumcision pain relief compared to noninvasive and systemic methods. TA may only decrease the need for supplementary analgesics.

Compliance with Ethical Standards

Ethical approval: Ethics Committee Approval: Decision No. 82 dated 24.09.2010 from the Turkish Ministry of Health, General Directorate of Pharmaceuticals and Pharmacy (Pharmaceutical Clinical Research Ethics Advisory Board-I)

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A.K.,E.Z.E.,S.U.Z. Analysis. A.K.,E.Z.E.,S.U.Z.,F.Y.G. Literature review. A.K.,E.Z.E.,T.U. A.K./E.Z.E.,T.U.,S.U.Z.,F.Y.G. Critical review. A.K.,E.Z.E.,T.U.,S.U.Z References. All authors read and approved the final version of the article.

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Evaluation of skills of intensive care nurses regarding central venous catheter care: An observational study

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ABSTRACT

Objective: Central venous access is a common procedure performed in many clinical settings for a variety of indications. Yet, there are multiple complications related to central venous catheter use. Significant morbidity and mortality can result from complications related to central venous catheters. Therefore, safety and effectiveness of central venous catheter care by nurses is crucial. The aim of this study was to evaluate the intensive care nursing skills associated with central venous catheter care.

Materials and Methods: This observational study was conducted with 37 volunteer nurses who were employed in adult intensive care units of three hospitals in Istanbul, Turkey. A total of 111 observations were collected by using the Nurses' Descriptive Characteristics Form and the Intensive Care Nurses' Central Venous Catheter Care Skills Observation Checklist.

Results: The average age of the nurses was 33.2 ± 5.9 years. The study showed that 93.7% nurses had performed the hand hygiene before starting the central venous catheter care but seldom after the procedure. Sterile gloves were not used by majority of the nurses during the care. They almost never disinfected the catheter hub, and they did not adequately obey the rules after completing the care procedure.

Conclusions: This study demonstrated that nurses had inadequate skills of central venous catheter care. We recommend that nurses' skills must be enriched with professional theoretical and practical trainings.

Keywords: Central venous catheter, Intensive care, Nurse, Nursing care, Skill.

1. INTRODUCTION

Central venous catheters (CVCs) are invasive tools utilized, especially in intensive care units (ICUs), for infusion treatments, nutritional support, hemodynamic monitoring, blood and blood product transfusion, and hemodialysis. CVCs have significant advantages for medical treatment, but they can also cause various serious complications [1]. Side effects associated with long-term CVC use include catheter-related bloodstream infection (CRBSI), bleeding, and thrombosis [2].

Central venous catheters are reported to cause 250,000 cases of CRBSI every year in the United States alone, and of these infections, 67,500 result in death [3]. A Turkish study that investigated healthcare-related infections in ICUs indicated that CRBSI was the most common type of infection, making up 48% of all cases, and had a mortality rate of 63.3% [4]. These CRBSIs not only negatively impact patients' medical conditions but are also known to increase the length of hospital stay and increase workload and costs. With all this in mind, it is extremely important to prevent infections before they occur. Proper nursing care can prevent the development of both infections and other catheter-related complications [5]. CVCs are most commonly used in intensive care settings. The intensive care nurse should be able to follow the patient's overall condition, know the catheter selection and the purpose of closing the catheter site, be able to follow the best techniques

* This study was produced from master thesis. The study, was presented as an oral presentation on December 19-21, 2019 at the 6th International 17th National Nursing Congress organized by Gazi University in Ankara/ Turkey.

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during catheter insertion, correctly practice catheter care, and properly manage the catheter to avoid infections [6]. Therefore, proper CVC management requires both sufficient theoretical knowledge and practical experience.

Most studies that investigated knowledge of nurses on CVC care indicated that they had inadequate knowledge [7-13] and that this knowledge could be improved by training [10,11] and experience [12,13]. Inadequate knowledge also negatively affects nurses' practical skills, since, nursing is an applied health discipline that requires a combination of theoretical content with practical skills [14,15]. However, there are a limited number of studies that evaluate nurses' CVC care skills [11,16-18] and none that directly investigate the CVC care skills of nurses. The most accurate way to evaluate the compliance of nurses with guidelines is to observe nurses directly during daily work routines and to examine their practices [19,20]. Therefore, this study was planned to evaluate the CVC care skills of ICU nurses via an observational technique.

This study aims to evaluate the intensive care nurses' skills associated with CVC care.

2. MATERIALS and METHODS

Design and sample

The research was conducted with nurses employed in adult ICUs of one public hospital, one university hospital, and one private hospital in Istanbul, Turkey, using the non-participant structured observation technique.

The research population consisted of 156 nurses working in the adult ICUs of the three hospitals as of May 2019. Ten nurses working in the general ICU of the public hospital (i.e., the workplace of the researcher) and 16 nurses who could not be reached due to being on a leave of absence were excluded from the study. In addition, due to the lack of patients on CVCs, 29 nurses working in 3 ICUs of the public hospital were also excluded. Therefore, the size of the research population was determined as n = 101.

There was no sampling. Instead, the sample was made up of 37 nurses who volunteered to participate in the study (n=37). In all three hospitals, the nurses worked in shifts, and the clinics employed CVC care procedures.

Measurement and instruments

During the research duration, each nurse was observed 3 times while performing CVC care. The accuracy of how a nurse behaves cannot be determined with one observation, and when two observations are made, it is uncertain which of the two different behaviors more accurately reflects actuality. For this reason, as per the expert opinion of a statistician, we decided to make 3 observations for each nurse to ensure the reliability of the results. Our review of the literature revealed that other observational studies similarly used at least 3 observations [21,22].

We used two data collection tools in the study. These were the Nurses' Descriptive Characteristics Survey Form (Form 1) and the Intensive Care Nurses' Central Venous Catheter Care Skills Observation Form (Form 2).

Form 1: Nurses' Descriptive Characteristics Survey Form

This 12 item form was prepared by the researchers as per the literature [8,22,23]. The form consists of 5 items that investigate the sociodemographic characteristics of the nurses and 7 items concerning training and available resources related to CVC care.

Form 2: Intensive Care Nurses' Central Venous Catheter Care Skills Observation Checklist

This checklist was prepared by the researchers to determine the ICU nurses' CVC care-related skills as per the relevant literature [24-28]. The checklist consists of the CVC care procedure divided into 41 steps. It was designed so that each step is marked as "performed" or "not performed", and the results of all three observations can be recorded on the same checklist. Six experts were consulted to evaluate the content validity of the data collection tools. The results of this evaluation indicated that all items of both forms were sufficient for content validity (content validity index (CVI) > 0.80).

Data collection

First, a pilot study was conducted among nurses working in ICUs of a separate hospital to test the research tools for usability. The study then moved on to the application phase. The study was conducted in the adult ICUs of three hospitals in May 2019. The nurses were provided with an explanation of the study by the researcher and were informed that they would be observed 3 times while providing CVC care. The nurses agreeing to participate in the study signed informed consent forms and filled out Form 1. The study then moved on to the observational stage. If a sufficient number of patients with CVC were available on the day that Form 1 was filled out, the nurses were observed on the same day. If the number was insufficient, each nurse was asked to make an appointment to avoid affecting their routine work, and each nurse was observed three times at different times. Nurses were evaluated using the non-participant structured observational design. Each observation lasted approximately 20 minutes. The nurses were not provided with feedback until, after all three observations were completed in order to avoid affecting their behavior. A total of 111 observations were made.

The study was granted ethical approval by the Ethics Committee of Ankara Yildirim Beyazit University in Ankara, Turkey (Date: 21/11/2018, Number: 102). Written permission was obtained from the administrations of all hospitals. After being informed about the research, all nurses who agreed to participate in the study signed informed consent forms.

Statistical analysis

Data were analyzed using IBM SPSS Statistics software version 25.0. When nurses performed a step indicated in the CVC assessment form two or three times out of three observations,

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they were considered to have "performed" the step. When nurses performed a step indicated in the CVC assessment form once out of three observations or did not perform it at all, they were considered to have "not performed" the step. The observation results of each step of the CVC procedure are presented as numbers and percentages in Table I.

The demographic characteristics of the nurses are presented as numbers and percentages. The nurses' demographic data were compared with the CVC care process steps. To facilitate the presentation of data, we only included the results of the critical steps of the CVC care process (25 steps), as elaborated in the literature [24-28]. The Z-test was used to determine the correlation of the observation results for two-category variables, and the chi-square test was used to determine the correlation of the observation results with variables with three or more categories (e.g., age, educational status). Fisher's exact test was used for pair wise comparisons. A p-value of less than 0.05 ($p<$ .05) was considered statistically significant.

3. RESULTS

The average age of the nurses included in the study was 33.2 ± 5.9 years (range: 21-41), and most (86.5%) were women. Furthermore, 67.6% of the nurses had undergraduate degrees. Other demographic data are presented in Table I.

Table 1. *Demographic characteristics of the nurses (n = 37)*

Demographic characteristics	n	%
Age		
≤ 25	11	29.8
26-29	16	43.2
≥ 30	10	27.0
$\overline{X}$ *±SD =33.2 ±5.9 min= 21 age max=41 age*		
Gender		
Female	32	86.5
Male	5	13.5
Educational status		
Nursing vocational high school	6	16.2
Bachelor's degrees	25	67.6
Master's degrees	6	16.2
Nursing Experience		
<5 years	20	54.0
≥5 years	17	46.0
$\overline{X}$ *±SD=5.9 ± 4.6 min= 8 months max= 21 years*		
Experience in ICU		
<5 years	20	54.0
≥5 years	17	46.0
$\overline{X}$ *±SD=5.9 ± 3.2 min= 3 months max= 13 years*		

It was found that 75.7% of nurses stated that they had received training on CVC care; most (89.3%) stated that they primarily received "in-service" training, and 85.7% stated that the provided training was "adequate". Only 32.4% of the nurses stated that they had utilized resources on CVC care. Other data concerning training and available resources related to CVC care are presented in Table II.

Table II. *Distribution of the nurses' CVC care characteristics (n=37)*

Characteristics regarding CVC care training and resources	n	%
CVC training status		
Received training	28	75.7
Did not receive training	9	24.3
Type of training (n=28)*		
In-service training	25	89.3
Basic vocational training	17	60.7
Conference/convention	8	28.8
Course	6	21.6
Internet	3	10.8
Seminar	1	3.6
Form of training (n=28)		
Only theoretical	2	7.1
Only practical	2	7.1
Both theoretical and practical	24	85.8
Adequacy of training (n=28)		
Adequate	24	85.7
Partly adequate	4	14.3
Resource availability in the workplace		
Available	12	32.4
Not available	25	67.6
Type of resource referred to (n=12)		
Hospital information system	7	58.3
Nursing textbooks	3	25.0
Internet	2	16.7
Frequency of CVC care application *		
Per shift (n=14)		
1-2 times	12	85.8
3-4 times	2	14.2
Per week (n=19)		
2-4 times	17	89.5
5-7 times	2	10.5
Per month (n=10)		
7-12 times	5	50.0
13-18 times	2	20.0
19-24 times	3	30.0

** n has increased due to multiple responses*

Table III presents the results of a total of 111 observations of 37 nurses that were made to determine their skills in the

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critical steps of the CVC care process (25 steps). We compared the observational results with the nurses' demographic characteristics, and we specify our findings in the text.

Ninty-three point seven percent in all three observation stages, nurses performed hand hygiene before starting the care process, while, only 24.3% used masks (Table III). The mask use rate was significantly higher in the group that did not receive training on CVC care than among those who did (p= .015).

We noted that after touching soiled dressings, few nurses removed their gloves (49.5% of all the three observations), performed hand hygiene (16.2% of all the three observations) and used sterile gloves (29.7% of all the three observations). None of the nurses aged ≤ 25 years wore sterile gloves for catheter dressing, while the nurses aged 26-29 years and those aged ≥ 30 years used sterile gloves significantly more than those aged ≤ 25 years (respectively p= .022; p= .035; p< .05). In addition, nurses who had graduated from nursing vocational high schools never wore sterile gloves, while nurses with master's degrees did so significantly more than nurses with vocational high school and bachelor's degrees (respectively p= .030; p =. 043; p< .05).

Nurses waited for the region wiped with an antiseptic solution to air-dry in 72.1% of the observations (Table III). Only 10.8% of the nurses wiped the catheter hub with an antiseptic solution, and nurses with more than 5 years of ICU experience were found to do so significantly more often (p= .004). Additionally, nurses aged ≥ 30 years were found to perform this step significantly more often than nurses aged ≤ 25 years (p= .035; p< .05).

In our study, none of the nurses performed the step in which the syringe was withdrawn to observe positive blood return in any of the 111 observations (Table III). Only 5.4% of all nurses flushed the catheter hub with sodium chloride solution, and 25.2% placed a new cap on the hub (Table III).

Nurses performed hand hygiene after removing their gloves, after providing care only 40.5% of the time, and this rate was significantly higher in the group aged ≤ 25 years than in those aged 26-29 years (p= .015; p< .05). Other observation assessment findings are presented in Table III.

Table III. *Observation results of critical steps of CVC care process*

Critical steps of the CVC care process	CVC Care Observation Results															
	1. Observation (n=37)				2. Observation (n=37)				3. Observation (n=37)				Total (n=111)			
	Performed		Not Performed		Performed		Not Performed		Performed		Not Performed		Performed		Not Performed	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
1. Hand hygiene is performed.	35	94.6	2	5. 4	35	94.6	2	5.4	34	91.9	3	8.1	104	93.7	7	6.3
2. The required equipment is prepared; a) Masks (1 each for the patient and the nurse)	9	24.3	28	75.7	9	24.3	28	75.7	9	24.3	28	75.7	27	24.3	84	75.7
b) Sterile gloves	11	29.7	26	70.3	11	29.7	26	70.3	11	29.7	26	70.3	33	29.7	78	70.3
c) Sterile gauze	37	100.0	-	-	37	100.0	-	-	37	100.0	-	-	111	100.0	-	-
d) An antiseptic solution in accordance with institutional policy for catheter entrance site care	37	100.0	-	-	37	100.0	-	-	37	100.0	-	-	111	100.0	-	-
e) Sterile transparent or gauze dressing	37	100.0	-	-	37	100.0	-	-	37	100.0	-	-	111	100.0	-	-
f) 2% chlorhexidine gluconate, povidone iodine or alcoholic wipes for cleaning the catheter hub	6	16.2	31	83.8	6	16.2	31	83.8	6	16.2	31	83.8	18	16.2	93	83.8
g) 10 mL syringes (as per the number of lumens)	2	5.4	35	94.6	2	5.4	35	94.6	2	5.4	35	94.6	6	5.4	105	94.6
h) 0.9% sodium chloride solution	2	5.4	35	94.6	2	5.4	35	94.6	2	5.4	35	94.6	6	5.4	105	94.6
i) Positive pressure end caps (as per the number of lumens)	11	29.7	26	70.3	10	27.0	27	73.0	10	27.0	27	73.0	31	27.9	80	72.1

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3. The procedure is explained to the patient, provided that they are conscious.	18	48.6	19	51.4	13	35.1	24	64.9	11	29.7	26	70.3	42	37.8	69	62.2
4. The mask is put on.	9	24.3	28	75.7	9	24.3	28	75.7	9	24.3	28	75.7	27	24.3	84	75.7
5. The patient's head is turned in the opposite direction of the catheter, and if it cannot be turned, a mask is put on his/her face.	5	13.5	32	86.5	4	10.8	33	89.2	4	10.8	33	89.2	13	11.7	98	88.3
6. Disposable gloves are put on.	36	97.3	1	2.7	36	97.3	1	2.7	36	97.3	1	2.7	108	97.3	3	2.7
7. The old dressing is removed.	37	100.0	-	-	37	100.0	-	-	37	100.0	-	-	111	100.0	-	-
8. Entrance site, sutures, and surrounding tissues are evaluated.	37	100.0	-	-	37	100.0	-	-	37	100.0	-	-	111	100.0	-	-
9. The integrity of the catheter and its hub is evaluated.	37	100.0	-	-	37	100.0	-	-	37	100.0	-	-	111	100.0	-	-
10. Gloves are removed.	20	54.0	17	46.0	17	46.0	20	54.0	18	48.7	19	51.3	55	49.5	56	50.5
11. Hand hygiene is performed.	7	18.9	30	81.1	5	13.5	32	86.5	6	16.2	31	83.8	18	16.2	93	83.8
12. Sterile gloves are put on.	11	29.7	26	70.3	11	29.7	26	70.3	11	29.7	26	70.3	33	29.7	78	70.3
13. The region is wiped with outward circular movements starting from the entrance site.	37	100.0	-	-	37	100.0	-	-	37	100.0	-	-	111	100.0	-	-
14. The wiped region is allowed to dry.	28	75.7	9	24.3	26	70.3	11	29.7	26	70.3	11	29.7	80	72.1	31	27.9
15. The region is dressed with sterile transparent or gauze dressing, placing the dressing so that the entrance site remains in the center.	37	100.0	-	-	37	100.0	-	-	37	100.0	-	-	111	100.0	-	-
16. The cap of the lumen is removed.	8	21.6	29	78.4	7	18.9	30	81.1	7	18.9	30	81.1	22	19.8	89	80.2
17. The catheter hub is cleaned with chlorhexidine, povidone iodine or alcoholic wipes.	4	10.8	33	89.2	4	10.8	33	89.2	4	10.8	33	89.2	12	10.8	99	89.2
18. The syringe filled with 0.9% saline solution is placed to the hub and the clamp is opened.	2	5.4	35	94.6	2	5.4	35	94.6	2	5.4	35	94.6	6	5.4	105	94.6
19. The syringe is withdrawn to observe positive blood return.	-	-	37	100.0	-	-	37	100.0	-	-	37	100.0	-	-	111	100.0
20. The hub is flushed with the saline solution in the syringe.	2	5.4	35	94.6	2	5.4	35	94.6	2	5.4	35	94.6	6	5.4	105	94.6
21. A new cap is put on the hub.	10	27.0	27	73.0	9	24.3	28	75.7	9	24.3	28	75.7	28	25.2	83	74.8
22. Gloves are removed.	37	100.0	-	-	33	89.2	4	10.8	34	91.9	3	8.1	104	93.7	7	6.3
23. Hand hygiene is performed.	17	46.0	20	54.0	14	37.8	23	62.2	14	37.8	23	62.2	45	40.5	66	59.5
24. The time and date of the dressing change and the initials of the nurse are written on the dressing.	37	100.0	-	-	37	100.0	-	-	37	100.0	-	-	111	100.0	-	-
25. The procedures and observations are recorded on the nurse's observation form.	5	13.5	32	86.5	6	16.2	31	83.8	7	18.9	30	81.1	18	16.2	93	83.8

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4. DISCUSSION

In our study, we found that participants were mostly young nurses and had limited practical nursing experience. We believe that this may be because, in Turkey, newly graduated nurses are commonly appointed to ICUs to gain experience. Other similar studies have obtained comparable results, where the majority of nurses were in the age group of 26-30 and had 1-5 years of professional experience [7,8,29].

Central venous catheterization care training is routinely given in ICUs in Turkey. The majority of the nurses stated that they had received in-service training on CVC care and that they found this training to be "adequate". Aydogdu and Akgun investigated the knowledge of nurses on CVC care and the influencing factors and found that the majority of nurses received "in-service training" for CVC care [30]. We believe that the nurse attendance rate at national and international congresses and conferences is low because hospitals generally do not provide sufficient financial support for nurses to participate in scientific activities.

We determined that the majority of nurses did not have available resources to refer to in the context of CVC care in their workplace and that they most commonly referred to the hospital information system. Whereas, the availability and accessibility of different resources in ICUs are very important to increase and maintain the quality of nursing care.

It is critical to perform hand hygiene after touching a potentially contaminated area and before putting on sterile gloves. All guidelines for CVC care likewise emphasize performing hand hygiene during catheter care [24,26,27,31]. In our study, almost all nurses started the process by performing hand hygiene in all three observations. However, in the following steps, the vast majority did not perform hand hygiene after removing their contaminated gloves or before putting on sterile gloves. In a similar study, Mutlu and Senturan, observed nurses before and after Hickman catheter care training and noted that nurses had shortcomings in performing hand hygiene before wearing sterile gloves in both observations [11]. The literature indicates that hand hygiene compliance rates are higher among nurses than among other health workers but still not at the expected level [32,33]. The reasons for this behavior were as follows: hand hygiene was perceived as less important than other procedures, nurses had insufficient time due to a heavy workload, sinks were inappropriately located and small, experienced health personnel set bad examples, and nurses had allergic reactions to hand antiseptics [32,33].

We observed that the majority of the nurses performed CVC care with disposable gloves without wearing sterile gloves. Basic nursing textbooks [24,26] recommend using sterile gloves in CVC care, while CVC care guidelines [25,27,28] state that this application can also be done with disposable gloves. In our study, the rate of sterile glove use was very low, and we found that this rate was significantly higher in the age group of ≥26 years and among nurses with master's degree. These results are important as they demonstrate the importance of professional experience and education. Snarski et al., examined nursing practices that aimed to prevent CRBSI in hematopoietic stem cell transplant patients and found the rate of sterile glove use to be 82% [34]. Mutlu and Senturan found this rate to be 77.3% [11]. The results of these studies differ from our findings. We believe that the low rate of sterile glove use in our study stems from habit, availability, high cost and time considerations. The nurses found using disposable gloves and an antiseptic solution to be more practical.

In our study, we observed that all nurses wiped the catheter region with sterile gauze and antiseptic solution. The literature recommends using chlorhexidine gluconate (CHG) for catheterization sites and states that 10% povidone-iodine or 70% alcohol may also be used if CHG is contraindicated [25,27,28,31]. However, in our study, we did not observe any nurse using CHG for skin antisepsis; instead, they mostly used povidone-iodine. The reason for this might be the lower cost and accessibility.

Guidelines indicate that to achieve maximum anti-microbial effect in catheter dressings, the skin needs to be allowed to dry after the application of antiseptic solution [24-28,31]. In our study, we observed that a considerable number of nurses applied dressings without letting the antiseptic solution dry. Similar studies have reported variable results. Lai et al. [35] and Mutlu and Senturan [11] indicated that this rate was high among nurses. In contrast, Gerceker et al., stated that nurses did not perform this step prior to training [18]. Arslan et al., indicated that few nurses were observed to scrub the stopcock entrance with 70% alcohol and subsequently wait for it to dry [16]. Nurses need to be motivated to perform this step, it is simple but is significant in preventing infection.

Cleaning the catheter hub, a microorganism entrance site, is crucial for the prevention of CRBSIs. According to the Joint Commission International (JCI) and the Centers for Disease Control and Prevention (CDC), the catheter's hub should be rubbed with the appropriate antiseptic solution before any intervention. If the hub is not cleaned, organisms from the contaminated hub may migrate to the inner surface of the CVC [36,37]. Wright et al., evaluated the effect of catheter hub cleaning on the development of CRBSIs and concluded that hub cleaning significantly decreased the infection rate [38]. However, in our study, the catheter hub cleaning rates were very low. Other studies reported similar results [16,39,40]. In addition, we found that this rate was higher in nurses with more professional experience. This finding shows that clinical experience is an effective factor in skill improvement.

Guidelines recommended CVC lumens to be washed with at least 10 mL of sodium chloride solution before and after each use [25,27]. In contrast, in the present study, almost none of the nurses practiced flushing. Arslan et al., stated that flushing the CVC line was frequently performed incorrectly by the nurses in all three treatment hours [16]. Gerceker et al., also stated that nurses had shortcomings in this step of the procedure and that the concern of nurses in catching up with treatment needs was a contributing factor [18]. Other factors that may have contributed to our result are patients with continuing infusions, concerns over disrupting the current treatment, as it may affect

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the patient's vital findings, and believing that the procedure would be a waste of time.

We observed that the majority of nurses did not put a new cap on the lumen after cleaning the catheter hub, an application that has been shown to reduce the risk of infection. This shows that nurses have shortcomings in these practices. Nursing staff shortages, nurses' work overload, and inadequate supervision could contribute to such a low practice level. Additionally, it was determined that the recording the process on the nurse's observation form was not done by majority of the nurses. Keeping records ensures that the communication between nurses is understandable and healthy and it also constitutes a legal basis in a possible case.

Limitations of the Study

The researcher was able to observe the nurses only during day shifts of the week, as per the request of the nurses in charge of the ICUs. For this reason, nurses who often worked the night shift refused to participate in the study, stating that 3 observations were not feasible. Additionally, in several ICUs, the nurses refused to participate in the study, claiming that it would disrupt their routines and increase the workload of the day shift, as CVC care was mostly done during the night shift, together with patient care. These factors reduced the number of nurses included in the study and affected the participation rate.

Prior to initiation of this observational study, nurses were informed about the ethical principles. The nurses were aware of the fact that being a participant in an observational study, might affect their skills negatively while performing CVC care. In addition, this study was conducted with ICU nurses of three hospitals only, and hence, the results might not be generalized to all nurses working in ICUs in Turkey.

Conclusion

This study showed that nurses had inadequate skills of CVC care applications. According to the results of this study that majority (93.7%) of the nurses had performed the hand hygiene before starting the CVC care procedure but seldom performed hand hygiene after the procedure. The majority of the nurses (70.3%) did not use sterile gloves during the CVC care applications; they almost never flushed and maintained the catheter hub, and they did not adequately keep records after the CVC care procedure. In line with these results, we recommend that nurses' CVC care skills must be enriched with professional theoretical and practical trainings; preparing the CVC care procedure based on evidence-based practices and ensuring that nurses have easy access to these documents. Further studies with larger samples are needed to confirm our results.

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Ethics Committee Approval

The study was approved by the Ethics Committee of Ankara Yildirim Beyazit University, Ankara, Turkey (Date: 21/11/2018, Number: 102). Written informed concent was obtained from each participant.

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The efficacy of epiduroscopic adhesiolysis in patients with chronic back pain after surgery

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ABSTRACT

Objective:Chronic back pain is a common problem with unwanted effects on the quality of life. The choice of treatment is usually patient-specific, but the use of epiduroscopic minimal invasive procedure is increasing. Epiduroscopy avoids surgical complications and improves patient comfort. This study was conducted to compare the efficacy of epiduroscopy in patients with failed back surgery syndrome (FBSS) and in patients without previous back surgery.

Patients and Methods: Forty-one ASA I-II-III patients aged 18–80 years old, with chronic back pain, radiologically and clinically diagnosed with lumbar spinal stenosis (LSS), and FBSS after laminectomy, hemilaminectomy, lumbar discectomy or lumbar spinal stabilization were included retrospectively. Patients were stratified as Group (O) with FBSS and Group (N) without previous back surgery. Baseline visual analogue scale (VAS) scores were obtained before treatment. During the 1st, 2nd and 3rd follow-ups, VAS scores of patients were measured.

Results: Visual analogue scale scores decreased significantly at 1, 2, and 3 months after epiduroscopic adhesiolysis in both groups. The differences in VAS scores of patients with and without previous back surgery were not statistically significant.

Conclusion: Epiduroscopic adhesiolysis neuroplasty was followed by a significant decrease in chronic back pain in LSS and FBSS patients .

Keywords: Epiduroscopic adhesiololysis, Failed back surgery syndrome, Back pain

1. INTRODUCTION

Back pain is described as pain or muscle strain between the twelfth costal margin and the lower gluteal curve and can occur with or without leg pain. In 90% of patients, it resolves without treatment yet 10% have a chronic course of more than 12 weeks duration [1].The estimated prevalence of lifelong back pain is 60%–85%, and it is the most common functional constraint and cause of lost work time among people younger than 45 years of age. The most important cause of back pain is lumbar spinal stenosis (LSS) resulting from lumbar spondylosis, ligamentum flavum hypertrophy, or spondylolisthesis. Many patients benefit from conservative treatment by epidural, transforaminal, or facet joint injection of drugs, but those are most often temporary solutions. Surgery may be indicated for non-responsive patients, but most are not candidates because of age or other factors. Epidural fibrosis, which may occur 6–12 weeks after back surgery, is another cause of back or leg pain [2,3].Rapid pain relief often occurs after surgical procedures but can relapse because of fibrous adhesions [4]. Epiduroscopy is a novel, minimally invasive treatment of spinal pain. Its advantages include: 1) mechanical adhesiolysis with direct observation and laceration of adhesions and scar tissue; 2) saline infusion to dilute inflammatory mediators such as phospholipase-A2 and tumor necrosis factor; and 3) chemical adhesiolysis by injection

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of target-specific drugs. Epiduroscopy has been shown to reduce back pain and increase functional status [5,6-11].

Epiduroscopy was first used in the 1931s to demonstrate vertebral anatomy in cadavers. Clinical applications were recognized after Shimoji et al., described the use of conscious sedation and Saberski and Kitahata developed a caudal technique. Because patients under conscious sedation feel pain when their affected nerve roots are touched assists in identifying the area in which pain originates. The caudal technique minimizes complications such as dural perforation and headaches [12-14].

This study evaluated epiduroscopic adhesiolysis–neuroplasty in patients who visited an algology polyclinic complaining of back pain and were diagnosed with LSS with or without a history of back surgery.

2. PATIENTS and METHODS

Research Ethics Committee of Bezmialem Foundation University (no. 71306642-050.01.04) approved the study prior to initiation. American Society of Anesthesiologists (ASA) I-II-III patients between 18 and 80 years of age who visited the pain clinic for chronic back pain and consented to be treated by epiduroscopic adhesiolysis–neuroplasty. Written informed consent was obtained from all patients.

Patients with imaging evidence of pathologies that could cause LSS such as degenerative lumbar spondylosis, ligamentum flavum hypertrophy, or spondylolisthesis, or with back pain subsequent to back surgery performed at least 1 year previously and were not responsive to conservative medical therapy, physiotherapy, or 3 months of interventional injection treatment were included. Exclusion criteria were, patients with systemic or local infections at the surgical location, coagulopathy, serious pulmonary disease, renal or liver failure, history of cerebrovascular events, intracranial masses that could increase the intracranial pressure, cauda equina syndrome, failed caudal interventions, experience respiratory or hemodynamic disturbance during the procedure. None of the subjects from both groups experinced any failed intervention or hemodynamic and respiratory problems. Study participants were stratified by their history to groups without (Group N), or with (Group O), previous back surgery. Participants were asked to indicate their pain on a vertical, numbered visual analogue scale (VAS) ruler (0= no pain and 10 = worts pain ever had). Initial pain scores were determined with the VAS scale and recorded.

Patients fasted for at least 8 hours before starting the intervention. Venous access was established via the antecubital fossa with a 22 G angiocath for premedication with 0.03 mg/kg midazolam. Patients were placed on the operating table in the prone position and monitored by electrocardiography, non-invasive blood pressure, and peripheral oxygen saturation. Fentanyl 1–2 mcg/kg and propofol 1–3 mg/kg conscious sedation was administered, and the sterile field was prepared. Local lidocaine anesthesia was applied to the insertion site and an 18 G Tuohy needle was inserted into the epidural space through the sacral hiatus. A floppy-tip guidewire (Tria V Guide, Tria Spine Med., Turkey) was passed into the epidural space and its location was confirmed by X-radiography. The site of guidewire insertion into the epidural space was incised and a 10 F dilatator was inserted by pushing it forward over the guidewire. After passing a 12 F valved introducer into the epidural space over the guidewire, the guidewire was removed and the epiduroscopy connections were made. To obtain a clear camera view and dilute inflammatory mediators, an isotonic NaCl solution was infused at 20–60 mL/min depending on patient tolerability. After visual identification of the nerve roots affected by stenosis and the epidural adhesions, a mixture of 80 mg methylprednisolone plus 40 mg lidocaine was injected to the affected area. At the same time, mechanical adhesiolysis of the epidural adhesions was performed by gentle, controlled pressure using an epidural catheter. After completing these procedures, the operation was terminated by catheter removal and dressing and taping the insertion sites. The patients were routinely monitored for at least 1 hour in the recovery room before transfer to the service room. After discharge, patients were evaluated monthly for the next 3 months, including assessment of their VAS.

Statistical Analysis

Statistical analysis was performed with the Statistical Package for Social Sciences (SPSS). Data was tested for normal distribution by the Shapiro–Wilk test. Descriptive statistics were reported as averages ± standard deviation for normally distributed continuous variables and as medians (minimum–maximum) for the variables that were not normally distributed. Values of categorical variables were reported as frequency (n), and percentage (%). The independent-sample *t*-test was used to determine the significance of differences in normally distributed continuous variables. The Mann–Whitney U test was used to compare differences in independent variables that did not have a normal distribution; the Wilcoxon Signed Rank test was used with dependent variables. Pearson's chi-squared test was used to compare differences in the values of categorical variables. The significance level was designated as $\alpha = 0.05$. p-values <0.05 were considered significant. Significant differences are indicated in bold text.

3. RESULTS

Forty-one patients with LSS and epiduroscopic adhesiolysis–neuroplasty were enrolled between June 1 and August 2, 2016. Twenty-one patients were included in Group N (without back surgery) and 20 in Group O (with back surgery). The patient characteristics are shown in Table I. There were no significant differences in the age and sex ratio of the two groups, which were considered as a homogeneous population.

The initial VAS scores and the scores obtained during follow-ups after the epiduroscopic procedure are shown in Table II. After epiduroscopic adhesiolysis–neuroplasty, there was a significant decrease in the back pain scores reported by the patients in both study groups throughout the follow-up period.

The initial and follow-up VAS scores of both groups are shown and compared in Table II

As noted above, the initial scores in patients with a history of back surgery and in those without previous back surgery were not significantly different. Within each group, there was a significant decline in VAS score following epiduroscopy. However, there were no significant differences between two groups ($p > 0.05$), indicating that the effectiveness of the procedure in each group was similar.

Table I. *Patients Baseline Characteristics*

Demographic Data	Group N (n=21)	Group O (n=20)
Age (average ± SD)	66.05 ± 8.880	61.75 ± 10.622
Female Gender n (%)	12 (57.1%)	8 (40%)
Male Gender n (%)	9 (42.9%)	12 (60%)

Table II. *VAS scores and between-group differences in patients with (Group O) and without (Group N) previous back surgery*

Groups		Initial VAS	1MonthVAS	2MonthVAS	3MonthVAS	Differ. 1	Differ. 2	Differ. 3
1 Non – Operated	n (21)							
	Median	8.00	4.00	3.00	2.00	−3.00	−4.00	−4.00
	Minimum	5	0	0	0	−8	−9	−9
	Maximum	10	8	8	8	0	0	0
	Mean	7.81	4.52	3.48	3.24	−3.29	−4.33	−4.57
	Std. Deviation	1.209	2.228	2.379	2.406	2.348	2.497	2.561
2 Operated	n (20)							
	Median	8.00	4.00	2.50	2.50	−4.00	−4.50	−4.50
	Minimum	5	2	0	0	−7	−8	−9
	Maximum	10	8	8	8	0	0	0
	Mean	7.70	4.20	3.45	3.10	-3.50	−4.25	−4.60
	Std. Deviation	1.302	1.989	2.544	2.654	2.188	2.673	2.854
p		0.751				0.634	0.948	0.937

4. DISCUSSION

Chronic back pain is a significant psychosocial problem, especially in the elderly population. It is also a major socioeconomic problem limiting physical activities of people under 45 years of age. Back surgery may result in the development of severe scarring of dermal, subcutaneous, and connective tissues. The scar tissue can restrict and inhibit movements of the affected region producing generalized pain or fibromyalgia. Fibrous epidural scar tissue present in FBSS patients can cause nerve entrapment, stenosis, and functional movement limitations that cannot be detected by magnetic resonance imaging (MRI) [8]. Many patients who suffer from chronic back pain are elderly and relatively less tolerant of general anesthesia and major surgical interventions. However, surgery is indicated in some conditions such as cauda equina syndrome or sequestered disks [4].

Epiduroscopy is an endoscopic procedure for patients with back pain unresponsive to physical therapy, drugs, or transforaminal injections. It has important advantages in determining the cause of pain, mechanically separating adhesions, and injecting medications to the target regions [14-18]. Bosscher and Heavner confirmed the high sensitivity of epiduroscopy by demonstrating its efficacy in diagnosing epidural fibrosis in a series of FBSS patients, compared to MRI (91% versus 16.1%, respectively) [11]. Similarly, in a prospective study concerning target-specific adhesiolysis and epidural injections for relieving radicular pain, this procedure identified epidural adhesions in 19 of 20 patients [7]. Nevertheless, the adhesions could not be detected by MRI, in 8 of the 19 patients [7]. Manchikanti et al., compared the effectiveness of conventional chemical adhesiolysis using steroids and local anesthetics to detect lesions, with or without epiduroscopy [12]. Without the aid of epiduroscopy, adhesiolysis was repeated six times in one, five times in three, and four times in twelve patients. With epiduroscopy, adhesiolysis was performed three times in only one patient during 12 months of follow-up. Effectiveness was defined as more than 50% decrease in pain, and after the first epiduroscopy procedure, a significant decrease in pain was observed in all patients. The comparison of non epiduroscopic adhesiolysis with the epiduroscopic one, revealed that the rate of decrease in pain was 72% versus 97% in the first month, 25% versus 80% in the third month, 10% versus

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52% in the sixth month, and 7% versus 22% in the twelfth month of the procedure, respectively. The results were also comparable after the second procedure. It was concluded that epiduroscopic adhesiolysis had largely positive effects. [12]. In our study we found positive effects on VAS values at each of the 3 months of patient follow up in both operated and non operated groups.

Pereria et al., evaluated the effects of adhesiolysis under epiduroscopy in patients with back or leg pain, unresponsive to the conservative therapy for six months, after lumbar discectomy [14]. Saline infusion was used for soft adhesions and mechanical pressure was applied to hard fibrous tissues using a catheter. Radiofrequency ablation was applied to septas that remained despite those procedures. After adhesiolysis, betamethazone and bupivacaine were injected. to each patient. Effectiveness was assessed by VAS and Oswestry Disability Index 2.0 scores. Significant decreases in VAS scores were seen in 71% of the patients in the first month, in 63% between 3 and 6 months, and in 38% at 12 months after treatment. The decreases in back and/or leg pain were statistically significant [14]. Rafaelli and Righetti have reported successful lysis of adhesions in 14 patients with a 4 Mhz Res-Ablator and over 90% recovery in eight of the patients (57%) [15].

In FBSS patients, epiduroscopy can be used in the lysis of adhesions caused by fibrous scar tissues (adhesiolysis). In LSS patients it can improve the physiological activity of nerve roots and the spinal cord (neuroplasty) by reducing the pressure on the medulla spinalis and nerve roots that results from stenosis. Jo et al., retrospectively evaluated epiduroscopy and ELND (Epiduroscopic laser neural decompression) in 39 patients with characteristics similar to the those enrolled in this study. Laser ablation was applied to pain-causing lesions visualized by epiduroscopy. The patients had not responded to previous medical treatment, epidural steroid injection, or surgery, or were patients with chronic back pain who relapsed 1 week after previous treatment. Some patients had requested ELND as initial treatment. Patient satisfaction was scored as "good," "acceptable," or "bad." Seventeen patients had previous lumbar surgery and 22 did not. Sixteen (94.1%) surgical patients and 19 nonsurgical patients (86.3%) reported that the study treatment outcome was acceptable or good. The difference was not significant but the improvement of chronic back and leg pain was judged clinically significant in both groups. [16]. The results of this study are in line with those of Jo et al., in that epiduroscopic adhesiolysis–neuroplasty was effective in LSS patients with and without FBSS [16].

Complications of epiduroscopic adhesiolysis include pain at the insertion site, dural-perforation headache, infection, cerebrospinal fluid pressure caused by bolus injection. neurologic sequelae caused by epidural hematoma, and steroid side effects [6,7,20]. Although, case studies have shown epiduroscopic adhesiolysis to be a medically safe procedure, some serious complications have been reported [22,23]. No complications occurred in our study. Even though the patient number is statistically adequate to them, the sample size and relatively short follow-up can be seen as limitations. Unfortunately, the patient portfolio in our country does not include very much data on complications compared with controls beyond that needed for a conclusion of adequate safety.

The study evaluated and compared the effectiveness of epiduroscopic adhesiolysis–neuroplasty in FBSS and LSS patients. FBSS is characterized by post-surgical pain caused by pressure on spinal nerves following development of fibrous epidural scar tissue 6–12 weeks after the procedure. The pressure disturbs the physiological activities of nerves. FBSS can occur even after technically and anatomically proper surgical procedures. Mechano-chemical laceration or disruption of epidural fibrosis reducing the pressure on nerves and thus relieves the back pain. Because of this, we expected this procedure to be more effective in FBSS than in LSS patients.

Conclusion

Epiduroscopic adhesiolysis–neuroplasty was effective in decreasing chronic back pain in both LSS patients and FBSS patients with previous back surgery including laminectomy, hemilaminectomy, lumbar discectomy, or posterior stabilization. Considering FBS, epiduroscopic adhesiolysis may be a good choice in appropriate cases with LSS since, it is an effective and a minimally invasive method.

Compliance with Ethical Standards

Ethical Approval: This study was approved by the Ethics Committee of Bezmialem Foundation University (no. 71306642-050.01.04). Written informed consent was obtained from all patients before the procedure.

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Impact of COVID-19 pandemic on maxillofacial trauma etiology

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ABSTRACT

Objective: Maxillofacial trauma (MFT) is a frequent presentation in the emergency department (ED) which requires a multidisciplinary approach. Although, its etiology and diversity of injuries are almost similar worldwide, the causes may differ among the countries depending on the sociocultural and environmental factors and local traffic regulations. This study aims to evaluate etiologies of maxillofacial traumas (MFTs) in ED and to compare etiologies of MFTs during COVID-19 pandemic with the previous year.

Patients and Methods: Totally 84 (61 males, 23 females) patients admitted to ED of our center with MFT between March 15th – April 30th, 2020 were included. The control group consisted of 148 (112 males, 36 females) MFT patients admitted to ED in the previous year (March 15th-April 30th, 2019). Data including age, sex, cause of trauma, treatment, and COVID-19 infection status within 14 days after ED admission were compared between groups.

Results: Mean age was 31.88±2.53 years in the patient group and 31.40±1.74 years in the control group. Number of patients admitted to ED with MFTs decreased by 43.3% during pandemic compared to the previous year. Majority of MFTs occurred at home, followed by public places, traffic, and workplaces in both time periods. During pandemic, the rate of home accidents increased and MFTs occurred in the public places decreased.

Conclusion: COVID-19 pandemic-mandated social restrictions lead to a decrease in the number of MFTs. However, home accidents are the main cause of MFTs. Spread of COVID-19 infection in the hospital setting can be minimized with necessary precautions.

Keywords: Maxillofacial trauma, COVID-19, Emergency department, Plastic Surgery, Lockdown

1. INTRODUCTION

Maxillofacial trauma (MFT) is a frequent presentation in the emergency department (ED) which requires a multidisciplinary approach. Although, its etiology and diversity of injuries are almost similar worldwide, the causes may differ among the countries depending on the sociocultural and environmental factors and local traffic regulations[1-3]. Review of the literature has shown that traffic accidents [4], assaults [5], and traumas during daily life [6] are the main causes of MFTs. Studies conducted in Turkey have shown that assaults and road traffic accidents are the leading causes of MFTs [7-10].

After the first identification of novel coronavirus-2019 (COVID-19) in the Hubei province of China in December 2019 and rapid spread to the whole world, the World Health Organization (WHO) declared COVID-19 pandemic on March 11th, 2020 [11]. The first case of COVID-19 was identified on March 11th, 2020 in Turkey [12]. The pandemic caused an unprecedented disruption in work life, social life, academic life, and healthcare systems with strict restrictions across the country. These restrictions mainly included closing schools and attending to online learning, social distancing, encouraging remote and/or flexible working, limiting social gatherings, postponing meetings, limiting the number of passengers in public transport vehicles, postponing elective surgeries, and weekend lockdowns.

In the present study, we aimed to evaluate etiologies of MFTs in the ED, to compare the etiologies of MFTs at the time of this study with the previous year, to assess whether COVID-19 pandemic affected the treatment decisions, and to investigate the COVID-19 infection rate within the first 14 days after admission.

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2.MATERIALS and METHODS

Study design and study population

This single-center, retrospective study was conducted at Marmara University, Faculty of Medicine between March 15th, 2020 and April 30th, 2020. A written informed consent was obtained from each participant. The study protocol was approved by the Marmara University, Faculty of Medicine, Ethics Committee with the Approval No. 09.2020.540 in May 20th, 2020. The study was conducted in accordance with the principles of the Declaration of Helsinki.

A total of 84 (61 males, 23 females) patients admitted to the ED of our center with MFT during the study period were included in the study. The control group consisted of 148 (112 males, 36 females) MFT patients admitted to the ED in the previous year (March 15th, 2019 and April 30th, 2019). Data including age, sex, cause of trauma, treatment, and the COVID-19 infection status within 14 days after ED admission were recorded. Demographic and clinical characteristics of the control group were also recorded and compared with the patient group.

Study variables

The causes of MFTs were classified as traffic accidents, falls, assault, sports injuries, industrial accidents, injuries related to sharp objects, and human or animal bites. The settings where MFTs occurred including home, work, traffic, and other were documented. Injuries of the soft tissue, bone, tooth, and mucosa were noted.

Treatments applied, medical treatments and recommendations, interventions in the ED setting, operation in the operating room, referral of the patient to an external center, and refusal of the treatment by the patient were assessed.

All patients admitted to the ED were questioned regarding COVID-19 infection using a screening questionnaire (Table I). Physical examination findings including body temperature, and finger oxygen saturation, and complete blood count and biochemistry test results were documented. The patients suspected of COVID-19 underwent reverse transcriptase polymerase chain reaction (RT-PCR) analysis to confirm the diagnosis. The patients requiring hospitalization were taken to the single-patient wards and caregivers and/or companions were not allowed. The patient rooms were visited by the healthcare workers, when necessary, wearing personal protective equipment (PPE) such as face mask, gloves, goggles, glasses, face shields, gowns. All caregivers and/or companions were asked to wear a mask in the patient room. All patients admitted to ED with MFT were followed for COVID-19 infection symptoms for 14 days.

Statistical analysis

Statistical analysis was performed using the SPSS version for Windows 15.0 software (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed in mean ± standard deviation (SD), median (min-max), while categorical variables were expressed in number and percentage. The independent *t*-test was used to compare continuous random variables between the time periods, while the chi-square test was used to compare discrete random variables. A p value of <0.05 was considered statistically significant.

Table I. *COVID-19 infection screening questionnaire*

Question	Yes	No
Have you had a new onset of fever, cough, or diarrhea within the past two weeks?		
Have you travelled overseas or been in contact with a traveller within the past two weeks?		
Have you been exposed to someone known to have had COVID-19 infection within the past two weeks?		

3. RESULTS

Of a total of 84 patients admitted to the ED with MFT, 61 were males and 23 females with a mean age of 31.88±2.53 (range, 1 to 93) years. Of the control group (n=148), 112 were males and 36 females with a mean age of 31.40±1.74 (range, 2 to 95) years. There was no statistically significant difference in the sex and age of the patient and control groups between the time periods (p=0.721 and p=0.874, respectively).

The number of patients admitted to ED with MFTs decreased by 43.3% during the pandemic compared to the previous year. In addition, the rate of falls was higher and the rate of MFTs caused by assault decreased in the COVID-19 pandemic, although not statistically significant (p=0.416). According to the age groups, the rate of MFTs decreased in the 0-20 years age group and increased in the 21-64 and ≥65 years age groups in the COVID-19 pandemic, compared to the previous year (p=0.612). However, it did not reach statistical significance. Etiological factors of MFTs according to age and time periods are shown in Table II.

According to the setting of MFTs, the majority of MFTs occurred at home, followed by public places, traffic, and workplaces in both time periods. However, during the pandemic, the rate of home accidents increased and MFTs occurred in the public places decreased, although the difference was not statistically significant (p=0.815). On the other hand, the rate of traffic related MFT's lightly increased and workplace-related MFTs remained unchanged. In the home setting, the most common cause of injury was fall and this rate increased from 81.08% in the previous year and up to 88% in the COVID-19 pandemic. The second leading cause of MFTs was injuries related to sharp objects to the face which decreased from 17.57% in the previous year to 8% in the COVID-19 pandemic. The settings where MFTs occurred are presented in Table III.

Table IV shows the injury sites of MFTs. The rate of mucosal injuries significantly increased during the pandemic than the previous year (p=0.020). However, the rates of soft tissue lacerations and defects, bone fractures, and tooth trauma decreased during the pandemic, although it did not reach statistical significance (p=0.256). The most common fracture site was the nasal bone in both time periods (43.9% in 2019, 34.5% in 2020).

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According to the treatments applied, the number of ED interventions and the rate of patient discharge with medical treatment and recommendations decreased, while the rate of inpatients having an operation increased during the pandemic, compared to the previous year; however, no statistically significant difference was found (p=0.080) (Table V). In our study, the rate of refusal of treatment was 5.4% in the previous year, while none of the patients refused treatment during the pandemic.

Table II. *Etiological factors of MFTs according to age and time periods*

		Etiological factors n (%)							
Age	Year	Fall	Assault	Traffic accident	Injury related to sharp objects	Sports injury	Industrial injury	Animal/ human bite	Total
0-20 years	2019	29 (51.8%)	13 (23.2%)	4 (7.1%)	7 (12.5%)	2 (3.6%)	1 (1.8%)	0	56 (37.8%)
	2020	22 (81.5%)	2 (7.4%)	2 (7.4%)	1 (3.7%)	0	0	0	27 (32.1%)
21-64 years	2019	23 (28.8%)	26 (32.5%)	16 (20%)	6 (7.5%)	0	8 (10%)	1 (1.3%)	80 (54.1%)
	2020	17 (35.4%)	13 (27.1%)	10 (20.8%)	5 (10.4%)	0	2 (4.2%)	1 (2.1%)	48 (57.1%)
≥65 years	2019	12 (100%)	0	0	0	0	0	0	12 (8.1%)
	2020	8 (88.9%)	1 (11.1%)	0	0	0	0	0	9 (10.7%)
Total	2019	64 (43.2%)	39 (26.4%)	20 (13.5%)	13 (8.8%)	2 (1.4%)	9 (6.1%)	1 (0.7%)	148 (100%)
	2020	47 (56%)	16 (19%)	12 (14.3%)	6 (7.1%)	2 (2.4%)	2 (2.4%)	1 (1.2%)	84 (100%)
p value	2019	p= 0.001*							
	2020	p= 0.029*							
Total		111 (47.8%)	55 (23.7%)	32 (13.8%)	19 (8.2%)	2 (0.9%)	11 (4.7%)	2 (0.9%)	232

*Data are given in number and percentage, unless otherwise stated. *p<0.05 indicates statistical significance. MFT: maxillofacial trauma.*

Table III. *The settings where MFTs occurred*

March 15th – April 30th	Setting of MFTs n %				
	Home	Traffic	Workplace	Other	Total
2019	79 53.4%	19 12.8%	13 8.8%	37 25%	148 100%
2020	50 59.5%	11 13.1%	7 8.3%	16 19.1%	84 100%
p value	0.815				
Total	129 55.6%	30 12.9%	20 8.6%	53 22.9%	232 100%

Data are given in number and percentage, unless otherwise stated. p<0.05 indicates statistical significance. MFT: maxillofacial trauma.

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Table IV. *Injury sites of MFTs*

	Injury sites n (%)				
Year	Soft tissue laceration	Soft tissue defect	Mucosal injury	Bone fracture	Tooth trauma
2019	60 (40.5%)	2 (1.4%)	18 (12.2%)	107 (72.3%)	5 (3.4%)
2020	27 (32.1%)	1 (1.2%)	21 (25%)	54 (64.3%)	1 (1.2%)
p value	0.259	1.000	0.020*	0.234	0.422
Total	87 (37.5%)	3 (1.3%)	39 (16.8%)	161 (69.4%)	6 (2.6%)

*Data are given in number and percentage, unless otherwise stated. * p<0.05 indicates statistical significance. MFT: maxillofacial trauma.*

Table V. *Treatments of MFTs*

Year	**Treatment** n (%)					
	Medical treatment and recommendations	Intervention in ED setting	Hospitalization and operation	Referral	Refusal of treatment	Total
2019	38 (25.7%)	91 (61.5%)	11 (7.4%)	1 (0.7%)	7 (4.7%)	148 (100%)
2020	32 (38.1%)	44 (52.4%)	8 (9.5%)	0 (0%)	0 (0%)	84 (100%)
p value	0.080					
Total	70 (30.2%)	135 (58.2%)	19 (8.2%)	1 (0.4%)	7 (3%)	232 (100%)

Data are given in number and percentage, unless otherwise stated. p<0.05 indicates statistical significance. MFT: maxillofacial trauma.

In the current study, one patient was suspected of COVID-19 based on the COVID-19 screening questionnaire and physical examination findings and underwent thoracic CT and PCR to confirm the diagnosis. Although the patient tested negative, COVID-19 measures were implemented. Repeated RT-PCR yielded a negative result after surgery and, therefore, the patient was considered negative for COVID-19 and discharged from the hospital. During follow-up, no additional signs and symptoms were observed. Another patient developed high fever postoperatively and thoracic CT once and RT-PCR tests were performed with two days interval. Both test results were negative and the patient was considered negative for COVID-19. During 30-day follow-up after surgery, no additional signs and symptoms were observed. In our clinic, none of specialists were infected with COVID-19 which can be explained by the implementation of strict preventive measures (*i.e.*, the patients requiring hospitalization were taken to the single-patient wards and caregivers and/or companions were not allowed; all healthcare workers complied with the donning/doffing procedures of PPE). However, other staff including anesthesiologists and nurses involved in the treatment process were not analyzed, as it is beyond the scope of this study.

One patient suspected of COVID-19 due to fever underwent thoracic computed tomography (CT) and RT-PCR analysis to confirm the diagnosis and the test result was negative.

4. DISCUSSION

Our study results showed that the number of patients admitted to ED with MFTs decreased by 43.2% during the pandemic compared to the previous year. In the literature, the rate of MFTs decreased by 80% [13-19]. This can be attributed to the higher time spent at home due to restrictions worldwide. According to the Istanbul Metropolitan Municipality data, the use of mass transportation vehicles and passage of vehicles decreased by 86.4% in Istanbul province during the pandemic and the number of elderly aged ≥60 years (94%) and students (93%) using these vehicles significantly reduced [20]. As a result, the probability of accidents in the outside decreased. In our study, the rate of injuries in the public places and traffic was higher (47%) than home accidents (37%). In addition to being a pandemic center, our hospital remained open for all emergency cases during the study period. Many patients had a fear of infected with COVID-19 in the hospital setting and were unwilling to visit hospitals during the pandemic. Therefore, most of MFT cases with mild injuries may have visited private hospitals and clinics than state hospitals. The significantly lower rate of soft tissue injuries (63%) during the pandemic (24 in 2019 *vs.* 9 in 2020, respectively) supports this probability. These patients may have applied self-care and self-remedy at home using cream, ointment, or adhesive bandages during the pandemic. The higher number of MFT cases with oral mucosal injuries can be explained by the fact that these patients may have immediately visited the ED after trauma and patients can not treat themselves easily.

Also, the nasal bone was the most affected bone in MFTs in our study, consistent with the literature [21]. However, some authors found the mandibular fractures to be the most common fractures both before and after pandemic [15].

According to the treatments applied for MFT cases, the number of ED interventions such as suturation and external fixation of the nasal bone decreased according to the rate of patient

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discharge with medical treatment and recommendations. This may have resulted from the fact that many patients and healthcare workers have a fear of infected with COVID-19 in the hospital setting and are unwilling to visit hospitals during the pandemic. Therefore, soft tissue wounds can be closed by tissue adhesives rather than sutures in the ED setting. In addition, some authors have discussed conservative treatment methods for head and neck injuries before pandemic [22]; therefore, conservative methods may have been applied in the ED in selected cases. The negligible decrease in the rate of patients operated in the operating room indicates that patients who are not considered eligible for conservative treatment in the ED setting continue to be operated during the pandemic. Although more complex cases can be treated in the ED setting, operating room setting can be preferred to save time spending in ED room during the pandemic. In case of more than two types of injuries or bone involvement, serious trauma is considered. In our study, the rate of traffic accidents slightly increased. Previous studies have shown that road accidents are the leading causes of serious MFT fractures [23]. In our study, similarly, we observed a mild decline in the rate of operated patients. In addition, as many patients avoided hospital admission and readmission during the pandemic as much as possible, none of the patients refused the treatment given in the study.

The unchanged mean age (~31.5 years) and male sex predominance in both study periods can be explained by the fact that the majority of the individuals in the social circle, work life, and drivers in traffic are young males in our country [24]. According to the General Directorate of Security of Turkey data, the rate of traffic accidents decreased by 52.1% with a decrease in the accidents leading to death by 24.03% during the pandemic; however, the severity of the accidents became more traumatic [25]. According to the Istanbul Metropolitan Municipality, Transportation Management Center data, there was an increase in the average road speed of the moving vehicles in Istanbul province [20]. This finding indicates that, although the rate of road accidents decreased by more than 50% and the associated MFT rates decreased by 40% during the pandemic, the severity of the accidents was more traumatic.

Previous studies have shown that traffic accidents, assaults, falls, and sports injuries are the leading causes of MFTs [4-6]. In three of four studies conducted in Turkey, traffic accidents were the main cause of MFTs [8-10], while assaults were the primary reason in the remaining study [7]. However, three of these four studies included only patients with facial bone fractures related to MFTs. However, MFTs consists of many types of injuries including soft tissue, mucosa, tooth, and bone [26]. In our study, all types of MFTs were included to gain a better understanding of the extent of the trauma and falls were found to be the most common etiological factors.

Furthermore, the most common cause of MFTs was fall in both time periods in our study (51.8% *vs.* 56%, respectively), primarily affecting the 0-20 years age group. This result is not surprising, as this age group includes infants and toddlers, restless young children, and those having attention deficits [27], tendency to stumbling [28], and having a high head-to-body ratio [29]. Unlike our study, previous studies showed that the main causes of MFTs were motor vehicle crashes [29] and assault [30]. Some of the studies, however, found falls to be the main cause of MFTs, similar to our study [16,17,19]. Both during the pandemic and the previous year of pandemic, the only cause of MFTs was fall in patients aged ≥65 years; however, one patient had assault-induced MFT in the COVID-19 pandemic. Previous studies have reported a higher rate of MFTs in advanced age than young population, probably due to daily living activities – and fall-related injuries [6]. In a study, Brucoli et al., reported that falls were the main cause of MFTs in elderly [31]. In our study, the number of all cause-related MFTs decreased during the pandemic (43.3%) and the least decline was seen in the rate of falls-related MFTs (27%).

According to the age groups, MFTs related to violence and/or assaults most frequently occurred in the 21-64 years age group and decreased in this age group during the COVID-19 pandemic, compared to the previous year. The increased social distancing and reduced social gatherings during the pandemic may have played a role in the decreased number of assaults-related MFTs. On the other hand, alcohol consumption is common in most violent acts [32]. During the lockdown, the Turkish government implemented an alcohol sales ban, which may have decreased the violent acts.

The higher rate of MFTs related to home accidents can be explained by the fact that individuals spent much more time at home and less time outside. In addition, those who were living in crowded settings may have experienced much more home accidents, such as falls, as reported in the literature [17]. Increased domestic violence and self-destruction may contribute to the increased rates [33]. Also, a higher perceived risk to COVID-19 infection may aggravate anticipatory fear and anxiety which affects mental health and makes individuals more aggressive and irritable during the crisis [18].

There are some limitations to this study. First, different pandemic restrictions were implemented according to age groups in our country and, therefore, we included broad age groups such as 0-20, 21-64, and ≥65 years. Second, the education, employment or retirement status were unable to be evaluated. On the other hand, the main strength of the present study is that it evaluated etiological factors of MFTs according to the age groups during the COVID-19 pandemic. In addition, the rate of COVID-19 infection was examined in the operated cases in the emergency setting.

In conclusion, COVID-19 pandemic-mandated social restrictions lead to a decrease in the number of MFTs. The home accidents are the main cause of MFTs, while the rate of MFTs occurring in the workplace remain unchanged. Based on these findings, we believe that the spread of COVID-19 infection in the hospital setting can be minimized with necessary precautions. The present study may be a useful guide for ED admissions of MFT cases and management planning in the future pandemics.

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Compliance with the Ethical Standards

Ethical Approval: The study protocol was approved by the Marmara University, Faculty of Medicine, Ethics Committee with the Approval No. 09.2020.540 in May 20th, 2020. A written informed consent was obtained from each participant. The study was conducted in accordance with the principles of the Declaration of Helsinki.

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

Authors' Contributions: M.C.O., Conceptualization and design of the study, methodology, data interpretation, writing the original draft, review and editing. O.S., Data collection, formal analysis, review of the original draft. Both authors read and approved the final manuscript.

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The validity and reliability of the Turkish version of the Family Nutrition and Physical Activity screening tool

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ABSTRACT

Objective: The aim of this study is to evaluate reliability and validity of the Turkish version of the Family Nutrition and Physical Activity (FNPA-TR) screening tool. This study was conducted as a methodological research. The data were collected between May and June 2018. The population of the study consisted of 1126 first and fourth grade students and their families in three primary public schools that represent three socioeconomic statuses (high, medium, low) in Istanbul, Turkey.

Materials and Methods: Data were collected from 727 students and their families with an introductory information form and the FNPA-TR screening tool. The validity of content scale was evaluated by comparing the relationship between FNPA-TR scores and answers given to non-scale questions measuring the eating behaviour. In this study, Cronbach's alpha, Kaiser-Meyer-Olkin, Varimax rotation and ICC coefficients statistical tests were used to measure validity and reliability.

Results: The Cronbach alpha coefficient for the internal consistency of the scale was 0.724. The test-retest reliability coefficient of the scale had a medium to very high level that ranged from 0.422 to 0.925. The Kaiser-Meyer-Olkin test result was found to be appropriate as 0.771.

Conclusion: The study shows that the FNPA-TR scale is a valid and reliable measurement tool for the Turkish population.

Keywords: Family nutrition, Childhood obesity, Physical activity

1. INTRODUCTION

Obesity has turned out to be a multi-component disorder with the interaction of genes and the environment. The risk of obesity can be passed from generation to generation as a result of biological factors and/or behavioral factors. Children are known to inherit some characteristics from their families, such as socioeconomic status, cultural norms and behaviors, and family eating and physical activity behaviors, which are effective in the development of obesity in children [1].

Childhood obesity is a growing health problem for Turkish society as well as it is all over the world. The prevalence of obesity has increased threefold since 1975. The prevalence of overweight and obesity in all over the world, increased from 4% in 1975 to 18% in 2016 among children and adolescents aged between 5 and 19 [2]. There is an increase in childhood obesity in Turkey, as it is in the world. According to the Turkey Monitoring Growth in School-Age Children Survey in 2009 (TOCBI), overweight and obesity were 14.3% and 6.5% respectively in children aged between 6 and 10 [3].

Obesity has short and long-term effects on the health of children and adolescents. Compared to normal-weight children, obese children may suffer from absenteeism due to medical checks, health limitations, and diseases. Overweight and obese children

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are more likely to become obese adults and have a higher risk of morbidity, disability, and premature death in adulthood [4-6].

School-age children have less autonomy in their choices of diet and physical activity. Parents have a direct impact on the home environment that may predispose the child to over-eating and inactivity [7]. The World Health Organization (WHO), World Health Assembly 2016 report of the Commission on Ending Childhood Obesity draws attention to the obesogenic environment, which is defined as an environment that supports excess energy intake and sedentary life. Several factors such as commercial factors, agricultural policies, food systems, access to healthy food, the necessary infrastructure for a healthy environment, and the family environment (parental nutrition knowledge and behavior, family economy, family eating behaviors) affect the obesogenic environment [8].

There is considerable research into obesogenic environmental components. Herbenick et al., evaluated the risk of obesity in children using the Family Nutrition and Physical Activity Screening Tool (FNPA) and anthropometric measurements. In the study, children's scores from the FNPA scale were found to increase as a result of training about the obesogenic environment given to school-age children [7]. In another study employing the FNPA scale, the FNPA was emphasized as a useful measurement tool for clinicians and programs aiming to change family behaviors and home environment in the fight against obesity [9]. In a qualitative study assessing the obesogenic environment, five main themes were attained to prevent obesity. Two of these were "positive parenting practices" and "gardens, parks, sports halls, and school meals". These themes demonstrated the effect of parental attitudes and activity to prevent obesity [10]. In another qualitative study conducted to determine parental perceptions of healthy living (especially nutrition and activation) and the content of interventions to be conducted in the future to prevent obesity in children, the nutrition experiences of families were found to be effective and difficult to change. In the same study, it was recommended that increasing self-efficacy in cases such as physical activity should be included in the strategies of the families involved in focus groups [11].

In the 2016 report of the WHO World Health Assembly, the Commission on Ending Childhood Obesity, five propositions were developed to end childhood obesity. These were listed as improving healthy food intake; improving physical activity; prenatal and pregnancy care; diet and physical activity in early childhood; and health, nutrition, and physical activity for school-age children [8].

Toruner et al., in their study comparing the status of measured body weight in children and the perceptions of parents, found that most parents did not notice obesity risk or obesity status in their children [12]. Since, families affect children's nutritional and physical activity behaviors and create an accessible social and physical environment, they are encouraged to be involved in the center of studies on the prevention of obesity in children [13,14,15]. For this reason, obesity screening strategies should also center on evaluating the family environment and their behaviors.

Assessing the nutritional and physical activity status of the family may be important in determining the causes of obesity in the child. We could not reach a reliable and valid screening tool to evaluate the nutrition and physical activity environment of the family in our country. This study aimed to adapt the FNPA Screening Tool to Turkish and evaluate its reliability and validity.

2. MATERIALS and METHODS

Design

A mix of probability sampling methods was used in this study, which was planned in the methodological type.

Language Adaptation

The items of the scale were translated into Turkish independently by three experts and then these texts were translated into English, the original language of the scale, separately by three different experts. Finally, a single translated text was created by comparing these translations by two researchers who have sufficient English language skills and are familiar with the measured concepts and the best Turkish translation form was determined for use in the study. To ensure the face validity of the translated text, the items were submitted to the opinions of three researchers with a background of the public health field, and the final version of the scale was obtained. The final form of the scale was named as "The Turkish Adaptation of the Family Nutrition and Physical Activity (FNPA-TR) Screening Tool". A pilot study was conducted in an elementary school to see how the items of the FNPA-TR worked in the field. For this application, 15 families with children aged between 6 and 10, who were literate, who had no difficulty filling out the form due to health reasons, and who could speak Turkish were reached, and the actual implementation was started after necessary revisions were made. As a result of the pilot study, it was determined that answering the tool took 5-10 minutes on average and there was no problem in understanding the items.

The content validity of the scale was evaluated by academicians who had expertise on the subject. The explanatory factor analysis was employed for determining the item-factor relationship, and the confirmatory factor analysis was used for calculating the extent to which the items and the sub-dimensions explained the original structure of the scale. The time interval test-retest method was used for reliability analysis. Intra-class correlation coefficients (ICC) were found to range from 0.422 to 0.925, and the scale was determined to vary between medium to high test-retest reliability. Cronbach's alpha coefficient related to the internal consistency of the scale was found to be 0.724.

The permission from Ihmels et al., who developed FNPA screening tool was obtained through e-mail [13]. The institutional permission of Istanbul Provincial Directorate of National Education and the approval of Non-Interventional Research Ethics Committee from a university in Istanbul (issue: 23.03.2018/490) were obtained. The verbal consent of the children and the written consent of the parents were obtained.

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Sample

In the first stage of the sampling, schools were stratified according to their income levels, and among them, schools that were heterogeneous in terms of student profile and family profile were included in the study. The sample size was not calculated and it was aimed to reach all 1126 first and fourth-grade students and their families studying in three different primary schools with low, middle and high socioeconomic status in Uskudar, Istanbul.

There were 566 first-grade and 560 fourth-grade students in three selected public schools. Data forms were given to a total of 727 students and their families by researchers using face to face method, including 366 first–grade and 361 fourth-grade students, who agreed to participate in the study. Of the total students, 64.7% of the first-graders and 64.5% of the fourth-graders were contacted. The study was conducted with 591 students and their families who completed the data form and the questions were answered by the parents. Some of the questionnaire items were not responded by all participants, and therefore, % values were calculated over the participants who responded to the items.

Measures

The data were collected using a questionnaire consisting of two parts:

Part 1: It consisted of 39 questions including the descriptive information of the participants and information on nutrition and physical activity (parent height, weight, age, marital status, educational status, occupation, social security, the longest residence, number of households, family monthly income, presence of chronic disease, etc.).

Part 2: The Turkish adapted Family Nutrition and Physical Activity (FNPA-TR)Screening Tool : ABFA-TR is the Turkish version of the FNPA screening tool. The scale was developed in 2009 by Ihmels et al. [13].

This tool is an easy-to-use self-report scale designed to assess the family environment and behavioral factors that may cause the child to gain excess weight. The first version of the FNPA, which was created by identifying 10 main factors positively associated with overweight and obesity, was made up of a total of 21 questions. Then the number of items was reduced to 20 with the arrangements made in 2017 [16].

The conceptual framework of the scale consists of 10 sub-dimensions. Each item is rated on a four-point Likert type scale with options 1 = never / hardly ever, 2 = sometimes, 3 = often, 4 = usually / always. The scores that can be obtained from the scale vary between 1 and 80 points. The scale includes 10 sub-dimensions and two questions for each sub-dimension. Seven of the items (3, 4, 5, 7, 10, 13) were inversely coded.

The sub-dimensions are made of "Meals in the Family", "Family Eating Habits", "Food Choices", "Beverage Choices", "Limitation/ Rewarding", "Screen Time", "Healthy Environment", "Family Activity", "Child Activity", and "Family Planning / Sleep Pattern". The total score is calculated by summing the scores obtained from each sub-dimension. The total score is then used to interpret the physical activity and nutritional status of the family. A high total score obtained from the scale refers to a high-risk family environment and behaviors, while a low total score means a more positive family environment and behaviors.

The data were collected by the researchers between May and June 2018. A pilot study was conducted. To collect the data, the field team handed out the informed consent and the questionnaire involving the FNPA-TR scale in sealed envelope to the children to be taken to their parents. The responses received in the week following the day the envelopes were delivered were collected back in an envelope. Children's height and weight data were collected according to the statements of the families.

Statistical Analysis

The internal consistency of the scale and the subscales was calculated using Cronbach's alpha coefficient. Explanatory factor analysis was applied to 20 questions on the scale. The Kaiser-Meyer-Olkin test was used to determine whether the factor analysis was appropriate for the data structure. Besides, Bartlett's test was used for correlations between questions. Principal Components method was used to obtain factor loads, and the Varimax rotation method was used for identifying meaningful factor loads. ICC coefficients were calculated to find out the test-retest reliability. p-values less than 0.05 were considered statistically significant.

3. RESULTS

In this study the data from a total of 591 students is used including 294 (49.7%) girls and 297 (50.3%) boys from 3 different schools located in an Anatolian side district of Istanbul city, who were enrolled in the first (n = 321, 54.3%) and fourth (n = 270, 45.7%) grades and who filled out the questionnaire completely. Data were collected from primary schools from three different socioeconomic regions of the district. Of the families participating in the study, 115 (19.5%) stated that their income was less than their expenses, 387 (65.5%) reported their income as equivalent to their expenses, and 83 (14%) said that their income was higher than their expenses. In addition, 506 of those who filled out the questionnaire forms were mothers (85.6%) and 46 (7.8%) were fathers.

The test-retest reliability of each item on the scale was analyzed using the intra-class correlation coefficient (ICC). The findings obtained from the analysis are given in Table I. The ICC coefficients were found to vary between 0.422 and 0.925, which indicated test-retest reliability varying from a medium to a high level (Table I).

Table I. *Test-retest reliability results of scale items and sub-dimensions*

Items and Sub-Dimensions	ICC	95 % Confidence interval for ICC		p
Items		Lower	Upper	
A1-1 and A1-2	0.846	0.731	0.912	**0.001**
A2-1 and A2-2	0.522	0.150	0.731	**0.006**
B3-1 and B3-2	0.777	0.611	0.872	**0.001**
B4-1 and B4-2	0.813	0.674	0.893	**0.001**
C5-1 and C5-2	0.614	0.327	0.779	**0.001**
C6-1 and C6-2	0.534	0.187	0.734	**0.004**
D7-1 and D7-2	0.545	0.206	0.740	**0.003**
D8-1 and D8-2	0.716	0.505	0.838	**0.001**
E9-1 and E9-2	0.556	0.225	0.746	**0.002**
E10-1 and E10-2	0.485	0.101	0.705	**0.010**
F11-1 and F11-2	0.422	-0.009	0.669	**0.027**
F12-1 and F12-2	0.729	0.527	0.845	**0.001**
G13-1 and G13-2	0.623	0.338	0.786	**0.001**
G14-1 and G14-2	0.754	0.570	0.859	**0.001**
H15-1 and H15-2	0.799	0.649	0.885	**0.001**
H16-1 and H16-2	0.814	0.675	0.893	**0.001**
I17-1 and I17-2	0.768	0.595	0.867	**0.001**
I18-1 and I18-2	0.925	0.869	0.957	**0.001**
J19-1 and J19-2	0.778	0.613	0.873	**0.001**
J20-1 and J20-2	0.772	0.603	0.870	**0.001**

p<0.05

As a result of the factor analysis applied to the 20 items on the scale, the Kaiser-Meyer-Olkin test result was found to be 0.771. As this value was greater than 0.50, the scale was determined to be appropriate for the factor analysis. Besides, the Sphericity test showed that the correlation matrix was not spherical (p <0.0001). This result indicated that the correlations between the scale items were significant and appropriate for factor analysis. Furthermore, it was concluded that there was no need to eliminate any items from the scale because all diagonal elements of the anti-image matrix were greater than 0.50. The factor analysis revealed that there were 7 factors with eigenvalues greater than 1. The scale exhibited a 7-factor structure explaining 58.1% of the variance in the sample. An analysis explaining 50-75% of the total variance is considered a valid analysis [17]. After obtaining factor loads, they were rotated using the Varimax rotation method, and 7 significant factors were obtained accordingly (Table II).

The first factor consisted of "Child Activity" with two items, "Family Activity" with two items, and the "How often does your family allocate time for physical activities (walking, running, etc.) item of the "Healthy Environment" sub-scale with two items. Its factor loads varied between 0.674 and 0.802, and it explained 18.657 % of the total variance.

The second factor consisted of "Meals in the Family" with two items and "How often does your child consume fruit and vegetables together with meals and as snacks (except for juice)?" item of the "Food Choices" with two items. Factor loads varied between 0.577 and 0.714, and it explained 9.417 % of the total variance.

"Sleep Pattern" sub-group made up the third factor that consisted of 2 items. Also, it explained 7.922% of the variance.

The "How often is fast-food consumed in your family?" item of the "Family Eating Habits" sub-group, the "How often does your family consume packaged fast-food? (frozen food, food heated in microwave, etc.)" item of the "Food Choices" sub-group, and the "How often does your child consume soda or sweetened beverages? (plain or fruit soda, cold tea, juice, energy drinks, etc.)" of the "Beverage Choices" sub-group made up the fourth factor. Factor loads were 0.645 and 0.768 and 0.555. It explained 6.036 % of the total variance.

The fifth factor consisted of the "How often does your family control your child's consumption of sugar, chips, and cookies?" item of the "Limitation/Rewarding" and the two items of the "Screen Time" sub-group. Factor loads were 0.686-0.638 and 0.571. It explained 5.582% of the total variance.

As for the sixth factor, it was made up of the "How often does your child eat while watching TV (including meals and snacks)" item of the "Family Eating Habits" sub-group and the "How often does your child spend time on his computer, mobile devices, and game systems in his/her bedroom?" item of the "Healthy Environment" sub-group. Factor loads were 0.675 and 0.728. It explained 5.391% of the total variance.

Finally, the seventh factor was found to consist of the "How often does your child consume low-fat milk at meals or with snacks? (1% fat or skimmed milk, flavored milk, soy milk, almond milk, etc.)" item of the "Beverage Choices" sub-group and the "How often does your family use sweets, ice-cream, or other foods as a reward?" item of the "Limitation/Rewarding" sub-group. Factor loads were 0.627 and 0.576 and it explained the 5.066% of the variance. The factor structure of the items of the FNPA-TR, variance levels, and the item-total correlation values are presented in Table II.

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Table II. *Factor structure of FNPA-TR scale, rotated factor loads, variance explanation shares and item total correlation values*

Factors	Levels of variance	1	2	3	4	5	6	7	Item total correlation values
Family Activity	18.657								
How often does your family encourage your child to be physically active?		0.802							0.318
How often does your child engage in physical activity with at least one of the other family members?		0.716							0.275
Children's Activity									
How often does your child engage in physical activities in his spare time?		0.705							0.273
How often does your child participate in regular sports or physical activities with a coach or team leader?		0.692							0.308
Healthy Environment									
How often does your family make time for physical activity (family walking, running, etc.)?		0.674							0.250
Meals in Family	9.417								
How often does your child have breakfast at home or at school?			0.714						0.461
How often does your child eat at least one meal a day with at least one other family member?			0.577						0.372
Food selections									
How often does your child consume fruits and vegetables during meals or snacks? Except fruit juice			0.681						0.480
Family Planning / Sleep	7.922								
How often does your child follow his or her sleep habits or time?				0.855					0.591
How often does your child get enough sleep at night?				0.803					0.527
Eating Habits in Family	6.036								
How often is fastfood consumed in your family?					0.645				0.405
Food selections									
How often does your family consume packaged ready-to-eat food (frozen foods, microwave-heated foods, etc.)?					0.768				0.584
Beverage Selections									
How often does your child consume soda or sweetened drinks (plain or fruity soda, cold teas, juice, energy drink, etc.)?					0.555				0.323
Limitation / Rewarding	5.582								
How often does your family supervise your child's consumption of candy, chips and cookies?						0.686			0.495
Display Time									
How often does your child spend less than 2 hours a day on the screen? (television, computer, gaming systems, any mobile devices with visual screens etc.)						0.638			0.433
How often does your family monitor the time your child spends on the screen?						0.571			0.352
Eating Habits in Family	5.391								
How often does your child eat while watching TV (including meals or snacks)?							0.675		0.567
Healthy Environment									
How often does your child spend time in the bedroom on computer, mobile devices, etc.?							0.728		0.609
Beverage Selections	5.066								
How often does your child consume low-fat milk during meals or snacks? (1% or skimmed milk, flavored milk, soy milk, almond milk etc.)								0.627	0.543
Limitation / Rewarding									
How often does your family use sugar, ice cream or other foods as a reward for good behavior?								0.576	0.462

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In the additional analyses conducted to evaluate the content validity, the relationships between the scale score and the answers given to questions which were not included in the questionnaire and which provided information about the feeding behavior of the family were investigated. When these relationships were evaluated, the mean score of those who had regular breakfast was significantly higher (p = 0.001). The mean scale score was significantly higher in subjects who did not eat snacks and junk food (p = 0.001). The mean scale score was found to be significantly higher in subjects who had regular meal hours (p = 0.001). There was no significant relationship between the number of meals a day and the scale score (r = 0.074, p = 0.075). As the number of meals a week eaten together with the family increased, the scale score increased significantly as well (r = 0.167, p = 0.001) (Table III).

Table III. *Comparison of answers given to various questions in terms of total FNPA-TR scale score*

	n	Mean ± SD	p
Regular Breakfasts			
Yes	468	59.21±6.88	0.001
No	106	53.18±5.54	
Junk Food and Snacks Consumption			
Yes	148	55.61±6.77	0.0001
No	108	61.20±7.76	
Sometimes	321	58.12±6.48	
Do you eat meals at regular times?			
Yes	395	59.72±6.92	0.001
No	40	51.90±6.17	
Sometimes	143	55.19±5.62	
Gender			
Female	294	58.44±6.43	0.230
Male	297	57.74±7.70	
Grade			
1st Grade	321	57.19±7.36	0.001
4th Grade	270	59.15±6.62	
Income			
Income is less than expense	115	56.13±7.37	0.001
Income is equivalent to expense	387	58.51±6.92	
Income is more than expense	83	58.63±6.88	

p<0.05

4. DISCUSSION

This study investigated the validity and reliability of the Turkish adapted version of the "Family Nutrition and Physical Activity Screening Tool" (FNPA) [15], which was developed to evaluate the home environment and family nutrition and physical activity practices and behaviors that may be associated with childhood overweight and obesity. The study supported the original FNPA findings

The time interval test-retest method was used for reliability analyses. The intra-class correlation coefficients (ICC) were found to range between 0.422 and 0.925 and have a medium to very high test-retest reliability. Cronbach's alpha coefficient related to the internal consistency of the scale was found to be 0.724. Cronbach Alpha value between 0.71–0.91 indicates that the scale has a good level of reliability [18].

Explanatory factor analysis was employed for determining the item-factor relationship and confirmatory factor analysis was used for calculating the extent to which the items and the factors in the scale explained the original construct of the scale. As a result of the factor analysis conducted to determine the factor construct of the scale, the scale was determined to consist of 7 factors. The item-factor distribution was as follows. Factor 1: child activity (1,2), family activity (1,2), and healthy environment (2); factor 2: meals in the family (1,2), and food choices (2); factor 3: sleep pattern (1,2); factor 4: family eating habits (2), food choices (1), and beverage choices (1); factor 5: limitation / rewarding (1) and screen time (1,2); factor 6: family eating habits (1) and healthy environment (1); factor 7: beverage choices (2) and restriction / rewarding (1) (Table IV). In factor analyses conducted to examine the psychometric properties of the original FNPA scale, 7 factors met the minimum eigenvalue criterion of 1.0.

A significant correlation was found between the total scale score and the BMI of the child (-0.03), the mother (r = – 0.15), and the father (r = – 0.02). This weak correlation was likely to stem from the large sample size. Similar relationship between the BMI of the child and FNPA scores was observed in the original validity study (r = .10.17, p <0.01) and in another study conducted on different age groups (first-grade students: r = −0.17; tenth-grade students: r=−0.15) [12, 14]. Another study showed a negative correlation between age and gender-adjusted FNPA scores and BMI, BMI at the 95th percent, BF %, and all fat prevention measures, including the waist [9].

In the present study, a significant relationship was found between the BMI of the parents and the BMI of the child. Studies show that the environment shared by individuals and their behaviors as well as genetic factors affect the relationship between the BMI of the parents and the child [19-21]. This finding on the relationship between parent BMI and child BMI emphasizes the need for a family-based approach to the prevention of childhood obesity. There was also a significant relationship between the BMI of the mother and the FNPA total score. Parents' behaviors related to nutrition and physical activity are positively related to their own weight management as well as the obesogenic environment in the family and the nutritional quality and/or weight status of children [9, 14, 22, 23]. Williams et al., also found a relationship between the parent's BMI and the family FNPA score and reported that a large proportion of families with a low or normal weight parent had high FNPA scores, and a smaller proportion of families with overweight or obese parents had high FNPA scores [24].

A large portion of studies on childhood obesity has reported an inverse relationship between socioeconomic status (SES) and childhood obesity [25, 26]. However, there are studies showing that the relationship between SES and obesity is directly proportional. These studies have mentioned that in

addition to SES, factors such as ethnic inequalities, parental education, family income, and changes occurring over time may be effective on obesity [27, 28]. In the present study, the mean BMI of children was observed to be higher in schools with high and middle socioeconomic levels compared to the low ones. In addition, the mean child BMI was higher in families reporting higher income than their expenses.

Evaluations of the mean BMI should include not only SES or family income but also environmental, biological, and socio-cultural factors. As studies on inequalities addressing childhood obesity have focused on individual behaviors, maternal education, SES, family behaviors, sedentary behaviors, snacks between meals, eating habits such as consumption of fast-food and sweetened drinks, physical activity, and breastfeeding, the potential effects of social environments on obesity are still unclear [7, 10]. Although, ethnicity is stated to have a role in this difference [11, 29], this study did not include any elements of ethnicity.

The evaluation of cultural and living conditions suggests that children of families with high income and SES have no financial barriers to access to unhealthy snacks such as fast-food, chips, and sweet foods, as well as access to high-tech technology such as television, computer games, and telephones; consequently, these conditions result in decreased physical activity, and they are thought to be effective in the emergence of these results.

The results of the study showed that the higher the socioeconomic status of the schools was, the higher their FNPA total scores were. Also, the mean scale score was found to be significantly higher in those who had regular breakfast, who did not eat junk food and snacks, and who had regular meals. Regular breakfast and eating regular meals were reported to be factors that decreased the rate of obesity in children.

The obesity epidemic makes it essential to improve our understanding of the effect of food environments on children. As healthy habit formation at an early age is essential in primary prevention of obesity, it is necessary to control nutritional behaviours, familial factors and physical activity levels. This tool can be used to evaluate environmental factors affecting obesity to create programs to prevent obesity, as well as other anthropometric measurements such as waist-to-height ratio, BMI and waist circumference.

Studies are carried out to increase adequate and balanced nutrition and physical activity in the prevention of childhood obesity. Parents have a direct impact on the home environment which may predispose their children to excessive food intake, excess energy intake, and inactivity. It is important to provide a healthy family environment that improves eating behaviors and physical activities of children and to take part in their lives as the right role model. Developing and using measurement tools related to the family environment as an element of the obesogenic environment is among important steps to be taken to fight obesity. In conclusion, the Turkish version of the FNPA is a valid and reliable screening tool and it captures various aspects of the home environment rather than just physical activity or diet. We think FNPA will meet the need for a reliable obesogenic environmental assessment in our country.

Compliance with Ethical Standards

Ethical Approval: The study was approved by Uskudar Universty, Non-Interventional Research Ethics Committee (issue: 23.03.2018/490). The institutional permission was obtained from the Istanbul Provincial Directorate of National Education. The verbal consent of the children and the written consent of the parents were obtained.

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Authors' contribution: E.E and H.I. conceived of the presented idea. E.E., H.I., M.C. and E.H.K. developed the theory and collected research data. H.A. verified the analytical methods. All authors discussed the results and approved the final version of the article.

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How do social and spousal support influence postpartum depression?

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ABSTRACT

Objective: Postpartum psychiatric morbidity is one of the most frequent complications of birth, cause of suicides and maternal death. We aimed to determine the prevalence of postpartum depression (PPD) among mothers who gave birth in the last 6 months and also assess the risk factors, particularly poor social and spousal support.

Patients and Methods: This is a cross-sectional study. We included 303 mothers who gave birth in the last 6 months attending three family health centers in a district of Istanbul. Stratified sampling method was used. Edinburgh Postpartum Depression Scale, Postpartum Support Scale, The Scale of Perceived Spousal Support Among Women in Early Postpartum Period were administered. Univariate and multivariate statistical analyses were used.

Results: Postpartum depression prevalence was 9.9% among the participants. According to multivariate statistical analysis; while a higher income (OR : 0.99) and a higher spousal support score (OR: 0.95) were found as protective factors, actively working (OR :8.63), unplanned pregnancy (OR: 3.21), having a first child compared to having two children (OR: 11.20), having low birth weight infant (OR: 8.33) and unmet social support (OR: 1.02) were risk factors for PPD ($p<0.05$).

Conclusion: Considering the results of this study, increasing family income, social and spousal support, prevention of unplanned pregnancies using effective family planning methods and improving the life prosperity of the women are essential to the decrease of PPD prevalence.

Keywords: Postpartum, Depression, Social support, Spousal support

1. INTRODUCTION

Prevalence of psychiatric disorders increases during pregnancy (10%) and postpartum (13%) periods [1]. Postpartum mood disorders are maternity blues, non-psychotic postpartum depression (PPD) and postpartum psychosis (PPP). Maternity blues is the most common one in postpartum period (26-84%) [2, 3]. Generally, spontaneous regression occurs not requiring treatment [4]. Maternity blues increases PPD by 20% and may be a precursor for PPD [3]. PPP is a psychotic attack that occurs within the first 2 weeks postpartum with rapid clinical onset. Its prevalence is 1 to 2 attacks per 1000 births [5]. Previous bipolar disorder is an important risk factor for PPP [6].

Postpartum depression is a non-psychotic depressive episode that starts in the postpartum period [7, 8] and is most commonly seen in the postpartum 4th-6th week but the risk continues up to one year [9]. Its prevalence in the world is reported as 1.9% – 82.2% [10]. In Turkey, PPD prevalence varies between 5.0%-61.8% [11]. Standardized interview forms (International Classification of Disease-10, The Diagnostic and Statistical Manual of Mental Disorders-V based) for diagnosis [12, 13] and various tools such as Edinburgh Postpartum Depression Scale (EPDS) are used for screening [14]. PPD is a multifactorial complex process in which biological, obstetric, pediatric, psychosocial and cultural factors

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are involved. Postpartum psychiatric morbidity is important for being one of the most frequent complications of labor and the third leading cause of disease among perinatal disorders. PPD is also important since it is one of the leading causes of maternal deaths by suicide and has negative impact on marriage and childbirth. The aim of the study is to determine the prevalence of PPD among the mothers of 0-6 month old infants and evaluate the social, economical, obstetrical and infant-related risk factors particularly the effects of social and spousal support on PPD prevalence.

2. MATERIALS and METHODS

In this institutional based cross-sectional study 3547 mothers with 0-6 months old infants registered to three family health centers (FHCs) in a district of Istanbul were included. Sample size was calculated as 386 when PPD prevalence (for postpartum 6 months) was accepted as 35%, error margin 5%, design effect 1.2, confidence interval 95% using OpenEpi program stratified sampling was used according to socioeconomic level. Three FHCs were selected proportionally from low, medium and high socioeconomic level subdistricts in the study district. Postpartum 6 months period mothers registered in the FHCs were included in the study. The inclusion criteria were: having 0-6 months old infant, understanding and speaking Turkish and acceptance of participating in the study. A total of 303 women who gave informed consent priorly, completed the survey and post-hoc power was calculated as 96 % at 0.05 α-error and 0.20 effect size.

Measures

Postpartum Depression

The Turkish version of the Edinburgh Postpartum Depression Scale (EPDS) was used to assess depression symptoms [14]. It is a 10-item scale with 4 options for questioning the mood in the last week. Each item scored on a 4-point scale (0 to 3) and the total score was calculated. EPDS cut-off score is considered as either 12 or 13. Sensitivity of EPDS in the antenatal and postnatal period is 84% and specificity is 88%. [14]. In this study, the cut-off point was accepted as 13 as it is generally accepted as 13 in Turkish literature [16,17] and for defining case level depressive symptoms [18].

Social Support

Postpartum Support Questionnaire (PSQ) was used [19]. Thirty four Likert-type items associated with financial support, emotional support, information support. Scoring between 0 and 7 for 'importance of need' and ' received support' respectively. The highest score is 238. The Turkish version of the PSQ was validated [20]. In this study, the difference between the total score of 'the importance of the need' and the total score of 'received support' was defined as 'unmet social support'.

Spousal Support

Spousal support was evaluated by 'The Scale of Perceived Spousal Support among Women in Early Postpartum Period' [21] composed of 16 items with 5-point Likert-type scoring. Positive questions are 1,2,3,4,5,6,7,11,13,16th questions and negative questions are 8,9,10,12,14,15th questions. Highest score is 80 and the lowest score is 16.

Ethics committee approval and institution permission were received (Marmara University Faculty of Medicine Clinical Research Ethics Committee, 03.11.2017, protocol code: 09.2017.635). Data collection was carried out by face-to-face method getting written informed consent.

Statistical Analysis

Pearson's Chi-squared test, Fisher's exact test, Mann-Whitney U test, Student's t-test and Binary Logistic Regression Analysis were used for statistics. Also Cronbach's Alpha Reliability test was used for EPDS, PSQ (importance of need), PSQ (received support) and 'Perceived Spousal Support among Women in Early Postpartum Period' scale. P values less than 0.05 were considered statistically significant.

3. RESULTS

Sociodemographic Characteristics

The sociodemographic characteristics of the study group as well as a comparison of sociodemographic characteristics and PPD are presented in Table I. Higher educational level and higher income level were protective factors significantly associated with PPD. Also comparison of some health characteristics of mothers and PPD were summarized in Table II.

Reproductive Health and Infant Characteristics

Forty-six percent of participants had their first child. First gestational median age was 27 (23-30) years. One out of every three mothers had pregnancy loss during reproductive life. Last pregnancy was unplanned for 20.5% of the mothers. Seven participants had multiple pregnancies. Twenty-four percent of participants had health problems during their last pregnancy. Cesarean delivery history was present in 70%. 31.4% had used contraceptive methods. Number of female and male infants was similar. Low birth weight (LBW) infant percent was 6.6%. Breastfeeding percent was 93.7% and 68.6% of the participants were feeding their infant by exclusive breastfeeding. Low first gestational age, unplanned last pregnancy, smoking during pregnancy, having health problems and stressful events during the last pregnancy increased the PPD and were found as risk factors in this study. Use of in vitro fertilization in the last pregnancy, weight gain, use of contraceptive method, birth place, delivery method, use of forceps / vacuum and pregnancy type did not affect PPD significantly ($p > 0.05$) (Table III).

Postpartum depression frequency was significantly higher in mothers who had LBW infants compared to those who did not ($p = 0.03$) (Table III). Infant age, gender, prematurity, need for intensive care at birth, presence of congenital disease, feeding with only breastfeeding did not affect PPD significantly ($p > 0.05$).

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Table I. *Comparision of sociodemographic characteristics and PPD*

		PPD (cut off 13)						p value[a]
		No		Yes		Total		
		n	%	n	%	n	%	
Education level	College and above	134	93.7	9	6.3	143	100	**0.04**
	High school and below	139	86.9	21	13.1	160	100	
Occupation	Housewife	139	88.5	18	11.5	157	100	0.53[b]
	Health workers	21	95.5	1	4.5	22	100	
	Others	113	91.1	11	8.9	124	100	
Actively working status	No	255	91.1	25	8.9	280	100	0.06[b]
	Yes	18	78.3	5	21.7	23	100	
Actively working status of spouse	No	9	100.0	0	0.0	9	100	0.60
	Yes	264	89.8	30	10.2	294	100	
Private health insurance	No	208	88.5	27	11.5	235	100	0.085
	Yes	65	95.6	3	4.4	68	100	
		Median	25-75Percentile	Median	25.-75. Percentile			
Age		31	28-35	31	27-35			0.45[c]
Income per capita (TL)		1000	625-1667	666	417-1250			**0.007**[c]

[a] *Pearson's Chi-squared test,* [b] *Fisher's exact test,* [c] *Mann-Whitney U test*

Table II. *Comparison of participants' general health features and PPD*

		PPD (cut off 13)						p value[a]
		No		Yes		Total		
		n	%	n	%	n	%	
Chronic disease	Yes	46	85.2	8	14.8	54	100	0.18
	No	227	91.2	22	8.8	249	100	
Smoking	Yes	37	82.2	8	17.8	45	100	0.10[b]
	No	236	91.5	22	8.5	258	100	
Alcohol use	Yes	11	100.0	0	0.0	11	100	0.61[b]
	No	262	89.7	30	10.3	292	100	
Physical activity	Inactive	255	90.7	26	9.3	281	100	0.25[b]
	Active	18	81.8	4	18.2	22	100	
Body mass index[c]	underweight-normal	122	90.4	13	9.6	135	100	0.89
	Overweight-obese	151	89.9	17	10.1	168	100	

[a] *Pearson's Chi-squared test,* [b] *Fisher's exact test,* [c] *Personal declaration*

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Table III. *Comparison of reproductive health and infant characteristics and PPD*

		PPD (cut off 13)						p value[a]
		No		Yes		Total		
		n	%	n	%	n	%	
Planned pregnancy	Yes	223	92.5	18	7.5	241	100	**0.005**
	No	50	80.6	12	19.4	62	100	
Wanted pregnancy	Yes	252	91.6	23	8.4	275	100	**0.012**[b]
	No	21	75.0	7	25.0	28	100	
Health problem during pregnancy	Yes	62	83.8	12	16.2	74	100	**0.036**
	No	211	92.1	18	7.9	229	100	
Smoking during pregnancy	Yes	21	75.0	7	25.0	28	100	**0.012**[b]
	No	252	91.6	23	8.4	275	100	
Stressfull event during pregnancy	Yes	37	77.1	11	22.9	48	100	**0.003**[b]
	No	236	92.5	19	7.5	255	100	
Low birth weight infant	Yes	15	75.0	5	25.0	20	100	**0,036**[b]
	No	258	91.2	25	8.8	283	100	
		Median	25.-75. Percentile	Median	25.-75. Percentile			
First gestation age		27	23-30	25	21-27			**0.023**[c]

[a] Pearson's Chi-squared test. [b]Fisher's exact test. [c] Mann-Whitney U test

Tablo IV. *Comparision of PSQ and spousal support scores and PPD*

	PPD (cut off 13)						p value[a]
	No		Yes		Total		
	Median	25.-75. Percentile	Median	25.-75. Percentile	Median	25.-75. Percentile	
PSQ 'importance of need'	169	137-201	167	154-197	169	138-201	0.770
PSQ 'unmet social support'	15	0-37	45	25-102	17	0-39	**0.001>**
Spousal support	70	61-77	60	49-66	70	60-77	**0.001>**
	Mean	sd	Mean	sd	Mean	sd	
PSQ 'recieved support'	146	49	106	46	141.74	49.74	**0.001>**[b]

sd: standard deviation,PSQ: postpartum support questionnairw. [a]Mann-Whitney U test, [b]Student's t-test

Psychosociocultural Characteristics

All three scales administered to mothers had high internal consistency. Cronbach's Alpha Coefficients were 0.83; 0.96; 0.96; 0.91 for EPDS, PSQ (importance of need), PSQ (received support) and 'Perceived Spousal Support among Women in Early Postpartum Period' scale respectively. PPD prevalence was 9.9% with cut off score 13 and 14.9% with cut off score 12. Six participants (2%) answered the question 'suicide intention in the last 7 days' as 'sometimes'. Low 'received social support' and spousal support scores but high 'unmet social support' was found to significantly increase PPD frequency (Table IV).

Postpartum depression frequency was higher in mothers who had previous psychiatric disorders and lost relatives in the last 6 months. Family history of psychiatric disorder and previous PPD did not significantly affect PPD (Table V). First marriage age was found to be lower in mothers with PPD and there was no significant difference in terms of marriage number, intermarriage, marriage style and marriage year. Good communication also was found to be protective for PPD (Table VI).

As a result of multivariate analysis, increase in income per capita was found to reduce the risk of PPD significantly (OR: 0.99). Working actively (OR:8.63) , having the first child (compared to having 2 children) (OR:11.20), unplanned pregnancy (OR:3.21), having LBW infant (OR:8.33), high'unmet social support' score (OR:1.02) increased PPD significantly (Table VII).

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Table V. *Comparison of participants' psychological characteristics with PPD*

		PPD (cut off 13)						p value [a]
		No		Yes		Total		
		n	%	n	%	n	%	
Previous psychiatric disorder	Yes	30	78.9	8	21.1	38	100	**0.036**[b]
	No	243	91.7	22	8.3	265	100	
Family history with psychiatric disorders	Yes	43	84.3	8	15.7	51	100	0.13
	No	230	91.3	22	8.7	252	100	
Relative loss in the previous last 6 months	Yes	17	73.9	6	26.1	23	100	**0.017**[b]
	No	256	91.4	24	8.6	280	100	
Previous PPD (n=159)[c]	Yes	35	83.3	7	16.7	42	100	0.07[b]
	No	109	93.2	8	6.8	117	100	

[a]Pearson's Chi-squared test, [b]Fisher's exact test , [c]Women having more than one child

Table VI. *Comparison of participants' sociocultural characteristics and PPD*

		PPD (cut off 13)						p value[a]
		No		Yes		Total		
		n	%	n	%	n	%	
Number of marriages	One	259	90.6	27	9.4	286	100	0.23
	Two	14	82.4	3	17.6	17	100	
Marriage style	Modern	229	90.5	24	9.5	253	100	0.60[b]
	Traditional	44	88.0	6	12.0	50	100	
Intermarriage	Yes	45	93.8	3	6.3	48	100	0.44[b]
	No	228	89.4	27	10.6	255	100	
Communicationwith spouse	Good	262	92.3	22	7.7	284	100	**0.001>**
	Bad	11	57.9	8	42.1	19	100	
Communicationwith spouse family	Good	251	93.7	17	6.3	268	100	**0.001>**
	Bad	22	62.9	13	37.1	35	100	
Communicationwith own family	Good	265	91.1	26	8.9	291	100	**0.006**
	Bad	8	66.7	4	33.3	12	100	
Maritial satisfaction	Good	263	91.3	25	8.7	288	100	**0.002**
	Bad	10	66.7	5	33.3	15	100	
		Median	25.-75. percentile	Median	25.-75.percentile			
First marriage age		24	21-27	22	19-25			**0.03**[c]
Marriage years		5	2-9	5	3-11			0.81[c]

[a]Pearson's Chi-squared test, [b]Fisher's exact test , [c]Mann-Whitney U test

Table VII. *Comparison of risk factors and PPD*

	OR (%95 CI)	p value [a]
Age	0.98 (0.89-1.07)	0.715
Lower educational level (High school and below)	0.52 (0.16-1.63)	0.264
Income per capita [b]	0.99 (0.98-1.00)	**0.005**
Actively working [c]	8.63 (1.71-43.44)	**0.009**
Child number		**0.006**
1	11.2 (2.51-49.87)	**0.002**
2	1	
3 ≤	2.29 (0.51-10.18)	0.273
Unplanned pregnancy	3.21 (1.08-9.47)	**0.035**
Stressful event during pregnancy	2.74 (0.91-8.22)	0.072
Low birth weight infant	8.33 (1.98-35.11)	**0.004**
PSQ 'unmet social support'	1.02 (1.01-1.03)	**0.000**
Spousal support score	0.95 (0.91-0.99)	**0.019**

PSQ: Postpartum Support Questionnaire, [a] Binary logistic regression test, [b] Personal declaration, [c] Not actively working (never worked, paid/unpaid leave)

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4. DISCUSSION

In the present study, PPD prevalence was 9.9% while cut off score was 13 and 14.9% while cut off score was 12. Increase in income per capita reduced the risk of PPD significantly (OR: 0.99). Actively working (OR:8.63) , having a first child (compared to having 2 children) (OR:11.20), unplanned pregnancy (OR:3.21), having LBW infant (OR:8.33), high'unmet social support' score (OR:1.02) increased PPD significantly.

In a comprehensive meta-analysis (n = 12810) of Hara et al., the mean prevalance of PPD was 13% [1]. Studies in Turkey reported 5% – 61.8% value for PPD prevalance. In a meta-analysis composing different regions an average prevalence of 23.8% for PPD [11] was reported. In the present study, PPD prevalence was 9.9%. It may be lower due to the fact that our study was performed in the FHCs not in hospitals. The data collection period was May-July when depression may be seen less, health care behavior in the presence of depression might be decreased. The postpartum period was accepted as 6 months, it was not restricted to the earlier period. All these reasons might have resulted in lower PPD prevalence.

In some of the studies, maternal age, marital status, educational status and number of children did not significantly affect PPD prevalence [1]. On the other hand, in the review of Norhayati et al., low socioeconomical level, low educational level and low-income were associated with PPD [10]. In the meta-analysis of Özcan et al., income level and spouse working status were prominent among the factors associated with PPD [11]. In the study of Özmen et al., the PPD risk was 1.38 times higher in the first child [22]. In this study, PPD prevalence was higher in the lower education group (13.1%) than in the higher education group (6.3%). PPD risk was 5.9 times higher in the actively working group compared to the non-working mothers (never worked, paid or unpaid leave). Although, not working is considered as a risk factor for PPD generally, it is important to point out that working causes serious stress and tiredness for mothers and may induce PPD. As in many studies, income per capita showed a significant negative relationship with PPD in our study.

The study of Palumbo et al., found no association between number of parity and PPD [23]. But it was reported that having many children in countries with economic difficulties such as Nepal increased PPD and in countries such as the United Arab Emirates where many children are considered advantageous for women, the results were reversed [24]. In our study, having the first child increased PPD 10 times compared to having the second. Lack of experience and higher anxiety levels when having a first child was probably responsible for this predisposition to PPD.

Previously, abortion has been shown to increase the risk of PPD [24]. In the review of Özcan et al., history of abortion and stillbirth were positively associated with PPD in 6 of 15 studies [11]. In our study, PPD frequency was higher in women who had at least one pregnancy loss (curettage, miscarriage, stillbirth) compared to those who did not, but this result was not statistically significant. Therefore, we did not include pregnancy loss. Unwanted or unplanned pregnancy is a risk factor for PPD [23, 25]. In the study of Arslantaş et al., unwanted pregnancy significantly decreased PPD [26]. In our study, PPD was positive for 7.5% for planned and 19.4% for unplanned pregnancies.In a study performed in Canada, having health problems during pregnancy was a factor increasing PPD risk by 1.45 times [27]. In our study, the frequency of PPD was higher (16.2%) for those who had any health problems during the last pregnancy compared to women who did not (7.9%). In addition, experiencing a stressful event in pregnancy increased PPD by 3.65 times. It is known that LBW infant or neonatal complications of the infant also triggers PPD [28]. In our study, having LBW infant increased PPD risk 6.75 fold.

While infant gender was not associated with PPD in the study of Sylven et al. [29], it was shown that having a female infant was a risk factor for PPD in the study of Deng et al. from China [30]. In our study, no relationship was found between the infant gender and PPD. The fact that Istanbul is a cosmopolitan province may have caused not finding any significant relationship between PPD and infant gender.

Previous psychiatric disorder is a risk factor for PPD in many countries [10]. In the review of Özcan et al., previous psychiatric disorder was positively associated with PPD in 24 of 33 studies. Family history of psychiatric disorder was associated with PPD in 7 of 15 studies [11]. In this study, PPD was more frequent in the participants having previous psychiatric disorder (21.1%) compared to those who did not (8.3%), but no significant relationship was found between family history of psychiatric disorder and PPD.

Inandı et al., showed that PPD increased by 1.34 if the first marriage age was '18 and below' [31]. In our study, first marital age was lower in PPD positive mothers compared to PPD negative ones. First marriage age in Turkey is associated with many basic characteristics such as education level, working status for women.

In the study of Deng et al., a bad relationship with mother-in-law and sister-in-law was found to increase PPD risk by 2.34 times [30]. The study of Arslantaş et al., showed that relation with spouse, bad relations with own family and poor relationship with friends were found to be positively associated with PPD [26]. In our study, participants having good communication with spouse, spouse family and own family were found as less depressive compared to those having bad communication. Also, having a good maritial relationship was protective against PPD.

In the study performed in Canada, the lack of social support during postpartum period increases the PPD risk by 5.10 times [27]. In a study in Qatar, the lack of family support increases the risk of PPD by 1.6 times [32]. In our study, 'unmet social support' scores were positively correlated with PPD. The absence of sources of social support or poor relations with the social environment is expected to increase the risk of PPD. However, this relation should be interpreted cautiously as women with depression may undervalue social support.

In a prospective study carried out in Korea, 37 mothers in postpartum 1st week were evaluated and their spouses were

provided to support their mother and baby from the 1st to the 6th postpartum week. The spouses' support lowered mothers' postpartum depression (24.3% and 0% respectively for postpartum 1st and 6th week) and increased self-efficacy [33]. Gross et al., carried out a study evaluating the 150 first-time mothers and fathers from the prenatal period to the 4th postpartum month in the United States. Parenting efficacy experiences were found to be negatively associated with PPD for both mothers and fathers especially in the first postpartum month.[34]. In our study it was found that the mothers who did not exprience PPD had higher spousal support scores. Especially in traditional societies, male partners' perceiving parenting or spousal support as sharing responsibilities and not simply as help will contribute to improving parenting both in mothers and fathers.

There are few studies examining the relation between PPD and social and spousal support in Turkey. Including the FHCs from different socioeconomical regions; effects of factors such as income, educational status, working status were observed more clearly. Response rate (95%) and the rate of reaching the target number of sample size (79%) were high.

As the data were collected in the summer months, less health service use for depressive women and the fact that data were collected during visits to FHCs, may be the causes of lower prevalance of PPD.

Conclusion

In our study, one out of every ten mothers in the postpartum 0-6 month period had PPD. PPD risk was less in cases with high income; higher in those working actively, having a first child, an unplanned pregnancy, an LBW infant and unmet social support.

Evaluation of the psychiatric condition and risk factors of mothers should be done during the visits to gynecology and FHCs providing guidance. Responsibilities such as child-care, housework imposed on the female by the community should be shared by spouses and supported by the social environment, especially for women working in the postpartum period. Unplanned pregnancies should be minimized by effective family planning especially for women having three or more children.

Compliance with the Ethical Standards

Ethical Approval: The study was approved by the Ethics Committee of Marmara University, School of Medicine (03.11.2017, protocol code: 09.2017.635). Women were provided with participant information sheets, had the study explained to them by a researcher and had the opportunity to ask questions prior to providing informed consent. Written informed consent was obtained from all participants.

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

Authors' contributions: N.Y. and D.S. Conceived the study. N.Y.Collected the data. N.Y.and D.S.Performed analyses. N.Y.YN Initially drafted the manuscript, and D.S. critically revised it. N.Y. and D.S. Approved the submitted article.

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Case Report

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Constrictive pericarditis diagnosed following liver transplantation

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ABSTRACT

Constrictive pericarditis is frequently diagnosed during evaluation of the patients for hepatosplenomegaly and easily missed if not considered in differential diagnosis. Herein, we present a patient diagnosed with constrictive pericarditis during his investigation for impaired liver functions and right heart failure one year after liver transplantation.

A thirteen-year-old boy presented with abdominal distention, dyspnea and fatigue. He had undergone liver transplantation due to liver failure in the previous year, and the symptoms had recurred in the last three months. Physical examination revealed normal heart sounds. Abdominal distention and ascites were present. Cardiothoracic index and pulmonary vascular markings were normal on chest X-ray. Echocardiography showed biatrial dilatation. Thickening of the pericardium with calcifications was demonstrated by thorax computerised tomography. High pulmonary wedge pressure and equalization of end-diastolic pressures were found during catheter-angiography. Pericardiectomy was performed, histopathology was compatible with chronic fibrinous pericarditis. The patient improved dramatically after surgery, the right heart failure findings resolved, and the liver graft functions turned to be normal. Constrictive pericarditis must be considered in differential diagnosis of hepatosplenomegaly, liver dysfunction and right heart failure since surgical treatment is possible and lifesaving.

Keywords: Constrictive pericarditis, Echocardiography, Liver transplantation, Pericardiectomy

1. INTRODUCTION

Constrictive pericarditis (CP) is a disease presenting with manifestations of right heart failure and hepatosplenomegaly. Hepatosplenomegaly is thickening, fibrosis, adhesion and rigidity of pericardial membranes and causes impaired diastolic filling of the ventricles [1,2]. Patients with CP are usually diagnosed during the investigation of hepatosplenomegaly and liver disease. Diagnosis may be easily missed if CP is not considered in a differential diagnosis. Right heart failure together with massive ascites after liver transplantation (LT) is a rare condition, the causes of which may include bacterial peritonitis, obstruction of portal or hepatic veins, graft rejection, and renal or cardiac dysfunction. Identification and correction of the underlying cause is crucial [3]. It is important to consider CP in the etiology of liver failure, since it can be easily treated with surgical intervention. To the best of our knowledge, there has been no previous report of right heart failure accompanying massive ascites developing after LT due to CP in children. We report a case diagnosed with CP suggesting liver dysfunction with findings of right heart failure and massive ascites one year after LT due to diagnosis of chronic liver disease in our clinic.

2. CASE REPORT

A thirteen-year-old boy presented to our clinic with abdominal swelling, shortness of breath, and early fatigue. The patient had undergone LT with a diagnosis of chronic liver disease in the previous year and the pre-transplantation symptoms had recurred over in the last three months. He was referred to our clinic for evaluation for re-transplantation. In physical examination arterial blood pressure was 110/70 mmHg in both arms, and heart rate was 86/min; his skin was pale, bilateral pretibial pitting edema was present. Cardiovascular system examination revealed normal rhythmic heart sounds, additional sounds or murmurs were not audible. Abdominal distension and ascite was found. Other system examinations were unremarkable.

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In biochemical evaluation; complete blood count was normal; aspartate transaminase 284 IU/l, alanine aminotransferase 452 IU/l, gamma glutamyl transpeptidase 128 IU/l, total protein 6.2 g/dl, and albumin 3.2 g/dl, and mild bilirubin elevation were found. Acute phase reactants were negative. Cardiothoracic ratio and pulmonary vascular markings were normal on chest X-ray. Electrocardiography showed bi-atrial dilatation. Echocardiography revealed normal left ventricular dimensions and systolic functions; both left and right atria were enlarged (Figure 1). Mitral inflow velocities varied significantly with respiration (mitral E flow velocity at inspiration 0.64 m/sec, mitral E in expiration 1.02 m/sec). Tissue Doppler mitral E' was normal (0.98 m/sec). Constrictive and restrictive cardiomyopathy was considered in differential diagnosis. Thoracic computed tomography (CT) and diagnostic catheter angiography were scheduled. Pericardial thickness had increased 2.8 mm and foci of pericardial calcifications were present on thorax CT (Figure 2). Catheter angiography revealed increased right atrial and pulmonary wedge pressures with equalization of end diastolic pressures. The following measurements were obtained: pulmonary artery wedge 18 mmHg, pulmonary artery pressure 30/20 mmHg, right ventricular pressure 31/17 mmHg, right atrial pressure 16 mmHg, left ventricular pressure 80/20 mmHg, and aortic pressure 79/50 mmHg . Pericardiectomy was performed. Histologic examination of the pericardial tissue was compatible with chronic fibrinous pericarditis. Since, tuberculosis is one of the leading causes of CP, and prevelant in Turkey, Quantiferon test was performed and resulted negative. The patient's clinical state improved dramatically after surgery, and the findings of right heart failure resolved entirely at two-year follow-up. Liver graft functions returned to normal levels, and the transplanted graft was saved. Thus, re-transplantation was not performed. The child had no signs of liver or heart failure. A written consent was obtained from the patient's parents for the publication of this case report and any accompanying images.

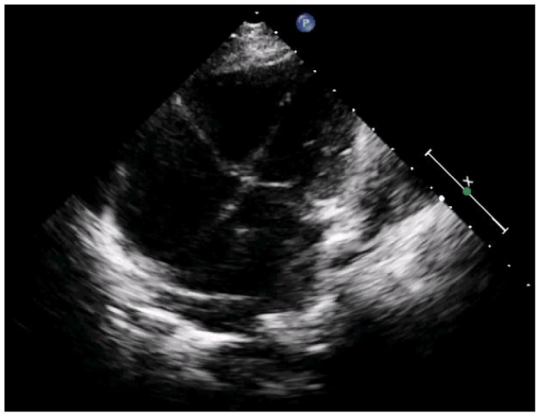

Figure 1. *Biatrial dilatation in 4-chamber view on 2 D echocardiography.*

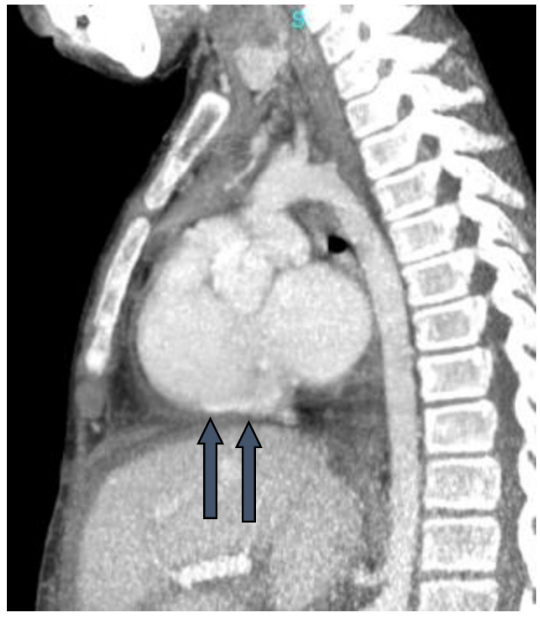

Figure 2. *Computed tomography revealed pericardial thickening and microcalcifications.*

3. DISCUSSION

Constrictive pericarditis is a clinical syndrome occurring due to chronic inflammation causing fibrotic thickening, calcification and adhesion of the pericardial layers which causes impaired diastolic filling of the ventricles. Tuberculosis, radiation, uremia, connective tissue diseases, trauma, heart surgery, and malignancy may be found as the underlying etiology; however, the majority of cases are idiopathic [4,5]. Liver transplantation related constrictive pericarditis is reported in only one adult patient previously [6]. Constrictive pericarditis may present with sign and symptoms of chronic renal disease, hepatomegaly and ascite may develop due to right heart failure. Other causes of graft dysfunction following liver transplantation may be bacterial or fungal peritonitis, obstruction of hepatic and portal veins or graft rejection [3,7-9]. On the other hand, several adult cases with CP has been reported following renal transplantation resulting graft dysfunction [10]. Absence of infectious causes and venous obstruction and dramatic improvement after pericardiectomy suggests constrictive pericarditis as the cause of liver dysfunction in our patient. It is difficult to understand if the initial liver dysfunction leading to liver transplantation was also related to constrictive pericarditis or constrictive pericarditis has developed as a consequence of the first transplantation in our patient retropectively since the cardiac evaluation before the first operation has been performed in another center and data at hand is inadequate. However, to our knowledge this is the first pediatric case with CP following liver transplantation.

As the pericardium becomes rigid in CP, ventricular filling is restricted, cardiac compliance decreases, systemic, hepatic, and pulmonary venous congestion occurs due to diastolic

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dysfunction. Compromised blood flow results in equalization of diastolic pressures in the right and left ventricles and the pressure in the splanchnic system raises [2]. In the subsequent stages, hepatic congestion may progress to fibrosis and then to cirrhosis, resulting in organ loss.

The clinical findings of CP can be highly variable, usually patients with CP present with weakness, easy fatigue, dyspnea, cough, weight loss, and chest pain. Jugular vein distension, ascites, hepatosplenomegaly, pretibial edema, muffled heart sounds and pericardial knock may be found in physical examination [11]. Our patient also had early fatigue and shortness of breath, and physical examination revealed the abdominal distension and pretibial edema.

Transthoracic echocardiography is helpful in the differential diagnosis of right heart failure. Decreased pericardial movement and increased pericardial echogenicity can be seen on echocardiography in patients with CP. Enlargement of the atria and vena cava superior and inferior, and attenuation of respiratory movements of the vena cava inferior may be detected [2,12]. Enlargement of both atria was present on echocardiography in our case which may suggest either CP or restrictive cardiomyopathy (RCMP). Doppler echocardiography is a useful method for differentiating CP and RCMP. A marked change occurs in CP, with respiration in the early ventricular diastolic volume rates, with tricuspid velocity increasing with inspiration and mitral flow velocity decreasing. A change exceeding 25% in mitral flow velocity with respiration is suggestive of CP. This finding is extremely uncommon in RKMP [12]. A marked change in mitral inflow was observed with respiration in the present case.

Differential diagnosis of CP and RCMP is a challenge in cardiology, which was also the case in our patient since some infiltrative diseases may cause both liver disease and restrictive cardiomyopathy. Diastolic dysfunction causes similar clinical and hemodynamic features in CP and RCMP. Atrial dilatation is more marked and ventricular systolic dysfunction may be present in RCMP. Presence of pericardial thickening, septal bouncing, prominent respiratory velocity changes in diastole suggests CP. Tissue Doppler velocity of lateral mitral annulus in diastole is normal or increased in CP while decreased in RCMP. E/E' decreases in CP.

Computed tomography and magnetic resonance imaging are useful non-invasive diagnostic tools with excellent sensitivity (88%) and specificity (100%) permitting direct visualization of pericardial thickening and calcifications [13]. Thoracal CT revealed pericardial thickening and calcifications in our patient.

Catheter angiography demonstrated elevated and equalized end-diastolic pressures of both ventricles and elevated pulmonary artery wedge pressure in our case that also supported the diagnosis of CP.

Pericardiectomy is the treatment of choice for symptomatic and severe CP, with surgical mortality rates of 6% and seven-year survival of approximately 88% [14]. Our case was referred for surgery and pericardiectomy and pericardial biopsy was performed at the same time. Diagnosis was confirmed when chronic fibrinous pericarditis was reported in histologic examination of pericardial tissue. Clinical condition of our patient improved dramatically after surgery, and the liver graft was saved.

Constrictive pericarditis must be considered and searched for in patients with symptoms of liver failure either before and after liver transplantation since it is a treatable condition by surgery. Our case is the first reported case of CP following LT.

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

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Authors' Contributions: NC: Coordinated and supervised data collection, drafted the initial manuscript, reviewed and revised the manuscript, BSC: Conceptualized and designed the case, coordinated and supervised data collection, EE, KA and ET : Had active roles in the diagnosis and treatment of the patients, FA : Critically reviewed and revised the manuscript. All authors read and approved the final version of the article.

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Case Report

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An unusual acute onset hard and small volume epidural blood clotting after anterior cervical discectomy with tetraparetic neurological findings

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ABSTRACT

Anterior cervical discectomy and fusion (ACDF) is one of the most commonly used surgical method to treat a variety of disorders in the cervical spine. Although, the incidence of complication related to ACDF is low, hematomas can be confronted after surgery. We report a 52-year-old male patient presented with complaints of paraesthesia and sensory loss in the upper extremities. Magnetic resonance imaging (MRI) showed a large central-right paracentral protrusion resulting in narrowing of the spinal channel at the level of C5-C6 accompanied by spinal cord compression and bilateral foraminal stenosis. ACDF was performed at this level. Five hours after surgery, sudden onset paraesthesia and tetraparesis developed. Urgent cervical MRI depicted acute filling half of the discectomy cavity, compressing the dural sac and spinal cord. The patient underwent revision surgery and the symptoms improved postoperatively. The patient received conservative therapy and was discharged without any nerological problem. Despite being a rare condition, sudden onset of neurological symptoms such as tetraparesis after ACDF surgery should remind the presence of hematoma at the surgical site.

Keywords: Discectomy, Magnetic Resonance Imaging, Hematoma

1. INTRODUCTION

Anterior cervical discectomy and fusion (ACDF) were first described by Smith and Robinson in 1958 [1]. It is a gold standard treatment method for many degenerative cervical spine diseases including disc herniation, radiculopathy, myelopathy, instability caused by degeneration or trauma, infection and tumours [2]. It is a relatively easy, reliable method with minimal risk [3]. ACDF is a widely used treatment technique which includes decompression of neural elements and stabilization when necessary, either through an anterior, posterior or combined approach. The approach selection depends on the location of the compressive element, type of fracture or ligament injury and the overall alignment [4].

Although, the incidence of complication related to ACDF is low, problems such as neural injury (recurrent laryngeal, superior laryngeal, hypoglossal nerve), organ injuries (oesophagus, trachea, vertebral and carotid artery, spinal cord and nerve roots, hoarseness, wound infection, CSF leakage) and hematomas can be seen after surgery [4-6].

Complications may be fatal if airway is involved due to cervical swelling or a hematoma and bleeding in the epidural space that may result in neurologic injury after ACDF surgery [7]. Therefore, the immediate diagnosis is crucial.

In this paper, clinical and imaging findings of an epidural hematoma that developed shortly after the operation with sudden onset of neurological deterioration, are presented.

2. CASE REPORT

A 52-year-old male patient presented with right-sided upper extremity radiating pain, right-sided sensation loss and muscle weakness ongoing for the last six months in his right arm. The neurological examination revealed 2/5 right biceps muscle weakness and paresthesia compatible with right C6 nerve root dermatomal zone. Cervical magnetic resonance imaging (MRI) examination showed a large central-right paracentral extrusion which compressed the right C6 nerve root. Upper extremity electromyography test demonstrated acute denervation on the right C6 nerve root. The patient underwent surgical treatment. Anterior cervical C5-C6 microdiscectomy with implantation of anterior interbody cage and demineralized bone matrix was performed to establish fusion. The duration of the surgery was

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45 minutes with no bleeding and neural structure damage. The patient was followed in the recovery room for one hour postoperatively. Vital findings were in the physiologic rate. He was transferred to neurosurgery patient ward after final examination by anaesthesiologist. His situation was uneventful till the five hours postoperatively, but he had complaints of numbness in the whole body, feeling as radiating electricity and could not move his upper and lower extremities. The physical examination revealed tetrapareses (upper extremities 2/5, lower extremities 4/5 muscle weakness). He was urgently transferred to obtain the whole spinal MRI. There were no brain functional disorders.

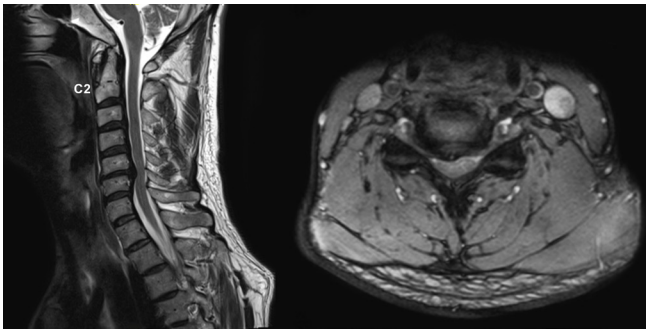

Figure 1. *Sagittal and axial MRI images show a large central-right paracentral protrusion resulting in narrowing the spinal channel at the level of C5-C6 with posterior longitudinal ligament thickening and spinal cord compression. Bilateral foraminal stenosis at this level is present.*

Urgent cervical MRI showed acute hematoma which was isointense in axial and sagittal T1-weighted images (WI), hyperintense in T2-WI; filling of the posterior space of the implanted cage and half of the discectomy cavity occupying more than half of the spinal canal in the C5-C6 level. It was measured 13.4 x 6.3 x 21 mm (0.886 ml) below one millilitre. This acute small hematoma compressed the dural sac and spinal cord at the level of C5-C6 (Figure 2). The patient was urgently transferred to operating room to evacuate haematoma. About 2 ml hematoma was evacuated by aspiration and serum physiologic irrigation. The hematoma was very hard as if stone, which was not irrigable during the surgery. Haemostasis was gentelly done using haemostatic fibrin agents and bipolar cauterization. An interbody cage and demineralized bone matrix were re-implanted in C5-C6 intervertebral body space and then hemovac drainage catheter was placed. No active vascular or bone sponges focus of bleeding was found at revision surgery. In the postoperative first day, muscle weakness of the upper and lower extremities decreased and his condition improved.

Blood analysis of activated partial thromboplastin time (28 seconds), prothrombin time (10,8 seconds), international normalized ratio (INR: 0.98) and platelet count were in physiologic ranges. Further haematological studies were done in order to clarify the underlying pathology (von Willebrand factor deficiency, factor XII deficiency, haemophilia A), no remarkable finding was present.

Physical treatment protocol was performed by physical medicine physicians. Four days postoperatively, the ambulation of the patient was done with walker and cane. The patient fully recovered ten days postoperatively and discharged.

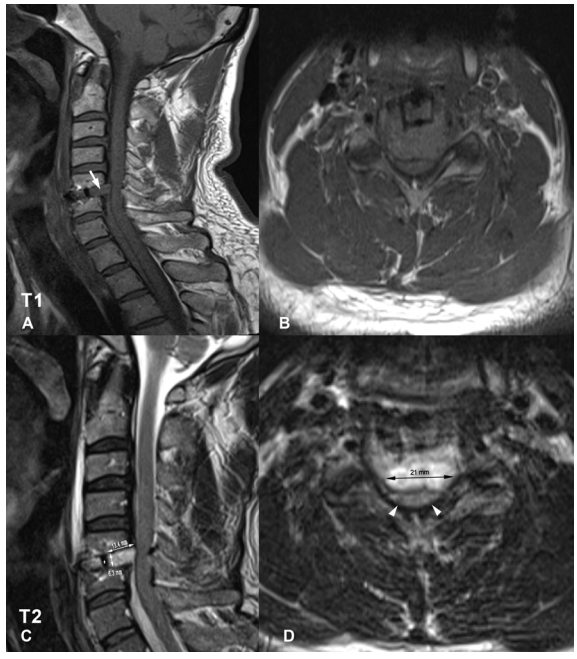

Figure 2. *Obtained urgent MRI five hours later postoperatively. Sagittal and axial T1 and T2-weighted MR images demonstrate acute hematoma which fills the half of the discectomy cavity, compressing the dural sac and spinal cord at C5-C6 disc level. The hematoma is isointense on T1-WI and hyperintense on T2-WI (A,D arrows). Metallic cage is shown (C, asterisk). The size of the hematoma is shown in T2-weighted images (C,D).*

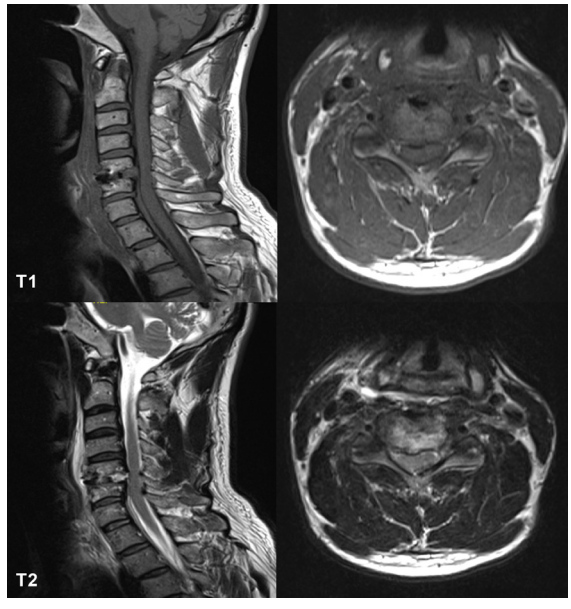

Figure 3. *shows resorption of hematoma (postoperative 4th day).*

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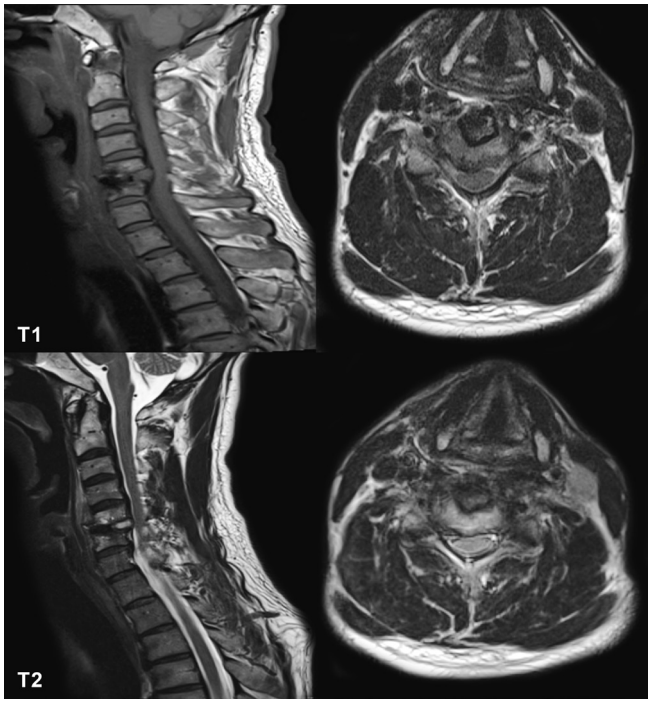

Figure 4. *shows resorption of hematoma (postoperative 11th day).*

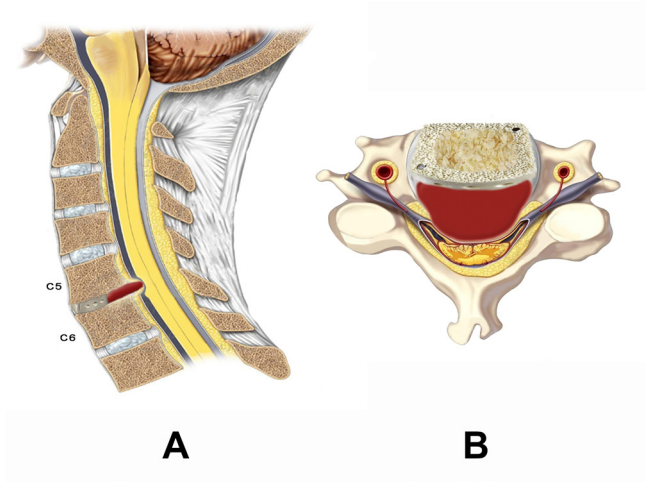

Figure 5. *Illustration of the present case shows the hematoma at C5-C6 level compressing the dural sac and spinal cord. Metallic cage and inside demineralized bone matrix are shown.*

3. DISCUSSION

The characteristic of current case is that after ACDF, neurological impairment and tetraparesis developed as a result of a rigid, immobile and stone-like small hematoma.

Possible complications such as epidural bleeding may be life threatening. Etiologic factors include trauma, invasive procedures (lumbar puncture, permanent spinal catheters, spine surgery), hematologic conditions (anticoagulation therapy, blood dyscrasias), infections, vascular malformations and pregnancy [8,9]. Studies revealed that all potentially life-threatening complications occur in the first six hours and patients should be discharged after the six hours observation period [10]. Among postoperative complications, dysphagia and hematoma are the two most common conditions which require intensive care during or after the operation. Some other complications such as airway oedema and respiratory involvement may peak at the second and third postoperative day [11].

The incidence of spinal epidural hematomas after surgery is very low (0.24%) [6,8]. Mean occurrence age of patients is between 50 – 80 years and the male dominant ratio is present (Male/female ratio = 1.4/1) [12,13].

There are some case studies and case series of ACDF that reported the complications of ACDF as well as hematoma in the literature (Table I). Although, the overall complication rates were low (1.71%) compared to other studies, in a meta-analysis study by Ben et al., four cases with hematoma were reported. They also mentioned dysphagia as the most common complication. Patients with hematoma were reported to have swelling, infection and pain [14]. This is an important point which indicates hematoma can occur early postoperatively, independent of complication rates.

Table I. *Bleeding complication in outpatient surgery*

Author	Total Cases	Cases with complication	Complication rate (%)	Complication
Adamson, et al. [22]	629	13	2.07	Hematoma (n=1)
Lied, et al. [23]	96	4	4.17	Hematoma (n=1)
Ben, et al. [14]	1693	29	1.71	Hematoma (n=4)

Yi et al., reported a series of postoperative epidural hematomas resulting in neurologic deterioration [6]. They retrospectively reviewed the database of 3720 cases over 7 years. Nine patients with postoperative neurologic in whom required surgical decompression were identified. They described the incidence of postoperative epidural hematoma as 0.24%. After decompression, clinical outcome revealed complete recovery in 3 cases (33.3%), incomplete recovery in 5 cases (55.6%) and no change in 1 case (11.1%). Factors increasing the risk of postoperative bleeding were defined as coagulopathy or anticoagulation therapy (44.4%) and highly vascularized tumour (33.3%). The time interval to evacuation of complete recovery group (29.3 hours) was shorter than incomplete recovery group (66.3 hours). Patients with coagulopathy and highly vascularized tumour were more vulnerable to develop spinal epidural hematoma. The postoperative outcome was related to the preoperative neurological deficit and the time interval

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to the decompression. In our case, the time of evacuation of hematoma after symptoms beginning to treatment was done less than six hours with complete recovery. There was no underlying pathology such as coagulopathy, tumour or AVM in our case.

Similar to our study Kim et al. [15] reported a case (36-year-old male) with sudden onset of cervical epidural bleeding which had developed within 5 minutes after surgery. The total surgery time was 108 minutes, and estimated blood loss was 50 cc. After surgery, five minutes later, he had developed tetraparesis. MRI showed an extensive epidural hematoma from C2 to T5, ventral to the spinal cord. The patient underwent an immediate revision operation and fresh blood was evacuated. On neurological exam after the revision ACDF, neurological symptoms had recovered. The post-op epidural hematoma had completely disappeared on follow-up imaging. In the article, the authors did not mention any finding about bleeding disorders or underlying pathology. Similar to this study as in our case, the hematoma was evacuated immediately, however, time of onset of symptoms in our case was 3 hours after surgery.

Jankowski et al. reported a 32-year-old female patient with huge acute cervical epidural hematoma as a complication developed after anterior cervical discectomy, resulting in complete loss of motor functions in the upper and lower limbs within 6 hours postoperatively. However, in our case, only a hematoma smaller than 1 ml caused the same clinical findings [16].

Imaging plays important role in diagnosis. MRI is the first-line diagnostic modality in detection [17]. It is able to demonstrate the exact location, extension, distribution, size of the hematoma as well as its relation with spinal cord and the severity of the cord compression [18].

The hematoma shows isointense signal on T1-WI, high signal on T2-WI in acute stage (within 24 hours) and hyperintensity on both T1 – and T2 – weighted images in subacute stage (36 hours after symptom onset) [19,20]. Spinal angiography is not valuable particularly for those patients with progressive neurological deterioration [21].

In presented case, before the neurological symptoms occurred after the surgery in the earliest postoperative period, we thought it was a huge hematoma occupying the larger space as we wait in cervical surgeries on the base of literature knowledge and neck surgeons' experiences. However, cervical MRI study demonstrated only below 1 ml of hematoma (Figure 2) resulting in the acute onset of tetrapareses. The cause of these symptoms was not due to the size of the hematoma, but was owing to its nature of the clot. The stone-like hematoma, compressed the Dural sac as well as spinal cord resulting in the symptoms. If the hematoma distributed homogenously and was not organised, the symptoms would have not prominent due to spread to larger area; therefore, destructive force of haematoma on spinal cord is decreased. Just despite of our case that it was very hard and cumulative haematoma as a bullet.

Conclusion

It is important to keep in mind that in a patient with sudden onset of neurological deterioration after ACDF surgery, the surgeon should consider the possibility of epidural hematoma at the surgical region and urgent MRI is required to clarify the pathology.

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Calyceal diverticular stones: Does the insistence on less invasive technique keep the patient away from a major complication?

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ABSTRACT

Calyceal diverticular stones are rare clinical entities without a clearly defined consensus on the treatment. Treatment indications include recurrent urinary tract infections, chronic pain and renal impairment. Treatment modality of surgical approach is usually based on the location of calyceal diverticulum.

A 23-year-old woman was presented with a calyceal diverticular stone of 16.5 mm. Her medical history revealed multiple urinary tract infections caused by extended spectrum beta-lactamase (ESBL)-producing Escherichia coli of which the stone was predicted to be the reason. The patient was treated ureterorenoscopically, a decision of which was taken intra-operatively.

In conclusion, insisting on performing nephroscopy following an intra-operative retrograde pyelography revealed no apparent ostium which could be interpreted as ureterorenoscopic retriaval would fail and development of postoperative bacteremia despite all precautions taken pre-and intra-operatively were two lessons indicating that all efforts must be exercised to reach the most minimal invasive method for the treatment of calyceal diverticular stones.

Keywords: Calyceal kidney stone, Endoscopic treatment, Flexible ureteroscopy

1. INTRODUCTION

Calyceal diverticular stones are rare clinical entities without a clearly defined consensus on treatment [1]. Treatment indications include recurrent urinary tract infections, chronic pain, and renal impairment [2]. The location of the diverticulum in the kidney usually determines the treatment modality or surgical approach for stones, and infundibular length and opening of the diverticulum may affect not only the treatment modality, but also the success rates [3]. Ureterorenoscopic retrieval of the stone in a calyceal diverticulum is recommended for small-sized stones located in the upper/mid and anterior locations, whereas, percutaneous nephrolithotomy (PCNL) is recommended for larger stones and lower and posterior locations, yielding higher stone-free rates [4]. However, infectious and major complications rates are still higher with PCNL.

Herein, we report a 23-year-old patient presenting with a calyceal diverticular stone treated by ureterorenoscopic stone retrieval technique.

2. CASE REPORT

A 23-year-old female patient with a body mass index of 20,7 kg/m^2 was referred to our urology outpatient clinic due to recurrent urinary tract infections for the past two years. During her physical examination, left costovertebral angle sensitivity was detected. An ultrasonographic evaluation revealed an intraparenchymal, hyperechogenic lesion in the upper posterior pole, measured maximally at 16.5 mm in size. The hyperechogenic lesion was suspicious for intra-parenchymally located kidney stone trapped behind the narrowed infundibula of the diverticulum. The parenchyma over the stone was very thin. A non-contrast computed tomography (CT) was performed for further evaluation, as the patient refused intravenous (IV) contrast media injection. A very small infundibular opening of the diverticulum was barely predicted at the CT cross-sections (Fig. 1). Decision of surgical intervention for the stone was taken and a written informed consent was obtained from the patient for both antegrade and retrograde approach.

The patient was given 1 g of meropenem (IV) preoperatively as prophylactic antibiotherapy, as the patient had a history of multiple urinary tract infections caused by resistant bacteria, although preoperative culture was sterile. Under general anesthesia, retrograde pyelogram (RPG) was performed using an 8F open-end ureteral catheter inserted to the left collecting system. Particular care was exercised not to increase the

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intrapelvic pressure, as the patient was still carrying a risk for bacteremia. Despite waiting for 3 to 4 min, the diverticulum was seen not to be filled with contrast media, which could be an indicator of stenotic infundibulum under fluoroscopy (Fig. 2). Subsequently, diagnostic flexible ureterorenoscopy (fURS) was performed to confirm the anatomy. An 8-10F ureteral access sheath of 35 cm in length was inserted into the upper ureter under fluoroscopic guidance over the guidewire. A millimetric punctuate ridge, which was thought to be the possible ostium of the diverticulum, was able to be visualized at the upper posterior pole during fURS (Fig. 3). A 0.035-inch guidewire was advanced through this ridge, entering the diverticulum and passing as confirmed by fluoroscopy. Using holmium laser, a precise cut of the mucosa beneath the guidewire and close to the infundibular ostium was done, allowing the entrance into the diverticulum. Lithotripsy was performed with a 270-micron holmium laser probe (Ho: Yttrium Aluminum Garnet (YAG) Laser; Dornier MedTech GmbH, Munich, Germany), after the stone was monitored with flexible ureterorenoscopy (Flex-X2, Karl Storz SE & Co. KG, Tuttlingen, Germany). Stone basketing was performed by tipless nitinol baskets (Zero Tip™; Dakotta™ Boston Scientific Corp., MN, USA). During the operation, the following settings were used for the laser energy: 10-20 Hz frequency and a power of 0.4-1.0 Joule. Dusting, popcorn and fragmentation modes were used for stone management. No residual stone fragment was left. Nephroscopic evaluation was also performed to confirm that no stones were migrated from the diverticulum to the other calyces and, finally, RPG revealed an intact infundibulum of the diverticulum (Fig. 4). The procedure was ended by inserting a 4.8F, 26 cm DJ stent with a magnetic tip (Black-Star; Urotech GmbH, Achenmühle, Germany) and catheterization of the bladder with a 16F Foley catheter.

At the fifth postoperative hour, the patient developed serious hypotension (70/40 mmHg), high fever (38.5°C), and shivering. Complete blood count and laboratory analysis revealed a white blood cell count of 19,000/mm^3 and elevated C-reactive protein (CRP) values. The platelet count also decreased with abnormal liver function tests. After taking blood culture samples, continuous high-flow (250 ml/h) IV fluid was given to achieve an optimal blood pressure. As the patient was clinically accepted as systemic inflammatory response syndrome (SIRS) with infection, IV antibiotic regimen was continued with meropenem (1 g t.i.d.). With supportive treatment, the patient recovered gradually without any sequelae. At postoperative day 5, all biochemical and clinical parameters of SIRS resolved, and the patient was discharged with oral antibiotherapy for 21 days.

On postoperative day 15, ultrasonographic evaluation showed a normal left kidney without a stone inside and the DJ stent was removed. Stone analysis revealed a mixed stone type containing calcium oxalate and carbonate apatite. Diet and proper recommendations according to the stone type was given to the patient. At three months of follow-up, there was no stone in the left kidney and a very small-sized diverticulum was visualized on ultrasonography. The patient is still free from symptoms and urinary tract infection at eight months of follow-up.

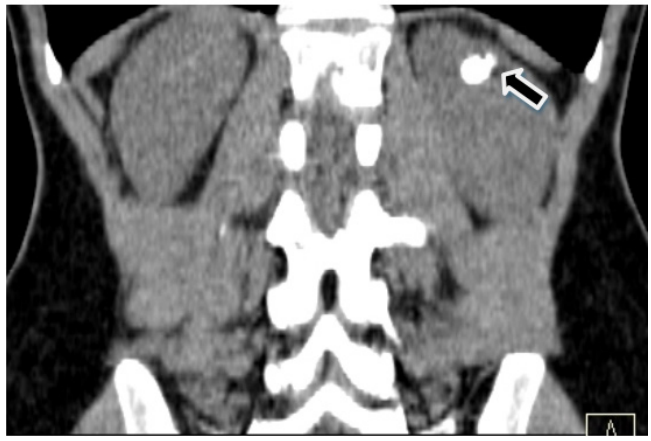

***Figure 1**. A non-contrast computed tomography image showing very small infundibular opening of the diverticulum.*

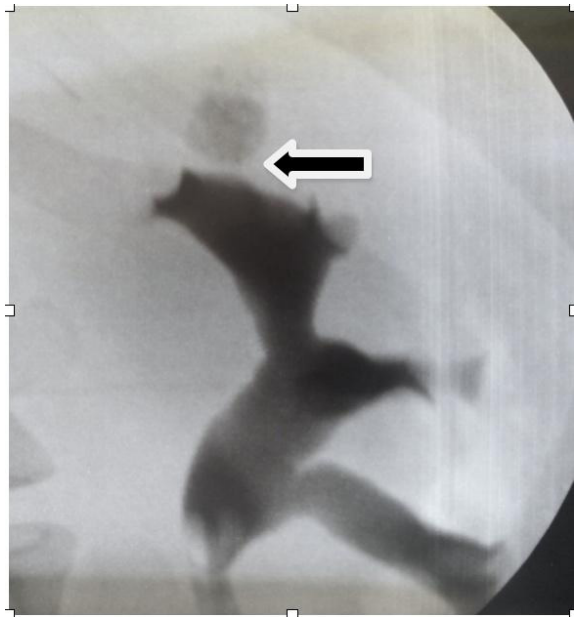

***Figure 2.** Diverticulum not filled with contrast agent.*

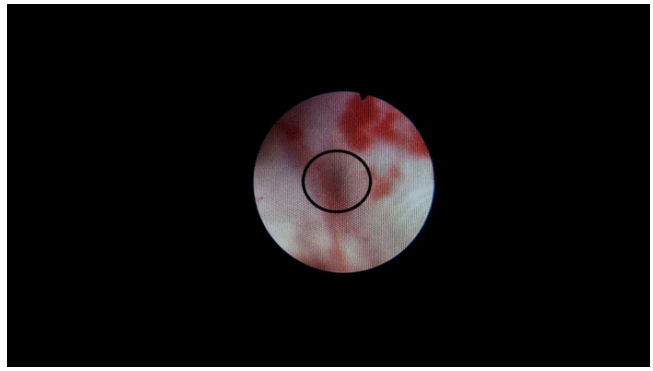

***Figure 3.** A view of ostium-like ridge at upper pole posteriorly during flexible ureterorenoscopy.*

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Figure 4. *Retrograde pyelography showing an intact infundibulum of the diverticulum after removal of stones.*

3. DISCUSSION

Calyceal diverticula are very rare abnormalities with an incidence of less than 1% [1]. They communicate with the collecting system through an infundibulum [2], and the diameter and length of the infundibulum are the main determinants of complications. The most common indication for surgical treatment of a calyceal diverticulum is the presence of calculus, which accounts for 9.5 to 39% of cases [2]. The presented case had a stone in the diverticulum, but indication for surgery was recurrent urinary tract infections with resistant bacteria, which is one of the indications for the treatment [3].

Although, preoperative non-contrast CT imaging may have resulted in inadequate evaluation of the diverticular infundibulum, we believe that this did not affect our treatment strategy at all for two main reasons. First, as in case of a narrow or stenotic infundibulum, contrast agent cannot fill these non-secretory cavities and, therefore, retrograde filming is still needed. Second, we planned to decide the treatment modality intraoperatively via RPG in our case. The reason for this was the preference of ureterorenoscopic intervention rather than percutaneous approach due to possible infectious complications and the fact that the patient had a history of multiple urinary tract infections with resistant bacteria. Thus, we recommend ureterorenoscopic treatment to avoid infectious complications in eligible cases.

In general, PCNL is preferred for such stones to achieve a stone-free status, particularly for a large diverticulum with a large stone burden [4]. Although, PCNL is more invasive than ureteroscopy (URS), it has the highest stone – and symptom-free rates (>90%) [5]. The reported incidence rates of infectious complications following PCNL are as follows: bacteremia 23%; endotoxemia 34%; fever 25%; and septic shock 0.3 to 2.5% [4]. On the other hand, postoperative infectious complications of ureterorenoscopy were reported in 2.97% of cases, of which 0.3% developed severe sepsis, which is slightly lower than PCNL with the latter, also having a higher rate of major complications up to 5.5% (i.e., hemo-pneumothorax, pneumothorax, and hemorrhage).

In the presented case, RPG was not successful to reveal the infundibular neck intraoperatively (Fig. 3). Therefore, PCNL decision could be made without a nephroscopic evaluation, as the major drawback of fURS is locating the diverticulum, since its neck may be narrow and even concealed [6]. In such a case, the fURS procedure must be converted to PCNL without any need for nephroscopy. However, insiting on fURS treatment was our drive to do nephroscopy in our case. Therefore, we recommend performing nephroscopy to check the diverticular orifice, even the RGP did not reveal it. During ureterorenoscopic stone retrieval, using the ureteral access sheath and keeping the irrigation pressure low, we attempted not to increase the intrarenal pressure, which may cause pyelovenous backflow and absorption, which are both proven risk factors for infectious complications. The holmium laser was manipulated very gently to avoid bleeding for the same reason. In addition, as sterile preoperative urine culture does not correspond with the infection present in the upper urinary tract, we used a potent, broad-spectrum antibiotic for preoperative prophylaxis. However, despite all these intraoperative efforts, including the empiric use of a broad-spectrum antibiotic, our case developed clinical signs and symptoms of postoperative urosepsis and was diagnosed with urosepsis based on the Sequential Organ Failure Assessment (SOFA) score. We believe that the reason for the negative urine and blood cultures obtained during this period may be due to the use of prophylactic antibiotherapy, namely meropenem.

Nonetheless, complete stone-free and infection-free status of our patient confirmed that the cause of the resistant urinary tract infections was the calyceal diverticular stones, although, infections were treated with the most appropriate antibiotic regimen. In a percutaneous approach, complications would be expected to be higher in this complicated case.

Conclusion

In conclusion, treatment of calyceal diverticular stones can be challenging in some cases. Minimally invasive management of this clinical scenario with retrograde intrarenal surgery is more effective than other treatment modalities in selected cases. Treatment must be tailored according to the anatomical features of each individual patient and experience of the surgical team. This case highlights the utility of less invasive techniques with careful handling to avoid more serious complications, keeping

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in mind that serious complications still may occur, despite all precautions and maneuvers.

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Orbital inflammatory disease in a child caused by a ruptured dermoid cyst misdiagnosed as orbital cellulitis

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ABSTRACT

One of the most common orbital tumors in childhood is orbital dermoid cysts. Cyst rupture or leakage of the cyst content is one of the rare causes of orbital inflammations and needs to be caught early. A five and a half year old boy was referred to us with the diagnosis of orbital cellulitis resistant to antibiotic therapy. Regarding his detailed medical history, physical examination and laboratory tests findings, he was diagnosed as orbital inflammatory disease caused by dermoid cyst rupture. He underwent surgical excision of the cyst after the inflammation was resolved with a short course of anti-inflammatory treatment. Although, it is rare, dermoid cyst rupture is one of the important causes of orbital inflammatory diseases and it must be kept in mind in the differential diagnosis of orbital infections. A detailed history and a careful examination helped us to make the correct diagnosis and avoided diagnostic delay and unnecessary treatment.

Keywords: Inflammation, Dermoid cyst, Child, Orbital cellulitis

1. INTRODUCTION

When we look at the etiology of orbital inflammatory disease (OID), we see a wide range of specific disease diagnoses to non-specific inflammation [1]. The most important steps in the management of OID are to identify the etiology and select an appropriate treatment. From idiopathic inflammatory disease to systemic or local inflammatory conditions, to other related conditions such as trauma, infection, neoplasm or congenital malformation should be remembered in the differential diagnosis of OID [1,2].

Dermoid cysts constitute 5% of all orbital lesions in childhood [3]. These lesions are assumed to originate from ectodermal tissue deviating from sequencing that occurs when suture lines are closed during embryonic development [4,5]. Cyst rupture or leakage of the cyst content is one of the rare causes of orbital inflammation. Dermal appendages such as sebaceous material, oil, hair, and cholesterol crystals can mount as an intense inflammatory response in the surrounding tissue. We herein, present a child who had recurrent orbital inflammation caused by dermoid cyst rupture, initially misdiagnosed as orbital cellulitis.

2. CASE REPORT

A five and a half year old boy was consulted to our ophthalmology department for evaluation of right eyelid and periorbital swelling. He had been referred to pediatrics clinic from another medical center with the diagnosis of orbital cellulitis with resistant to antibiotic therapy.

His medical history before the admission of our center was obtained from medical records; he has been suffering from transient right eyelid swelling, each episode lasting about 1-2 weeks, 4-5 times over the last year. He had been admitted to another medical center a week ago for the same complaints. The laboratory and radiologic investigation results were obtained from medical records; haemogram, ESR, CRP was normal. Orbital computed tomography (CT) scan showed edematous and inflammatory thickening of the right eyelids and periorbital area. There was no evidence of sinusitis on CT. Intravenous medical therapy (Ceftriaxon 100 mg/kg, Metronidazole 30 mg/kg per day) was given for 5 days, with the diagnosis of orbital cellulitis. Then he was referred to our center because of no improvement.

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On admission to our center, some diagnostic tests were done for differential diagnosis; haemogram and blood chemistry were in normal limits. Cultures for infection, C1q esterase inhibitor level for hereditary angioeudema, IgE level for allergic disease were evaluated but all results were normal (IgE :6,2 IU/ml, C1q est inh:31 mg/dL).

On ophthalmic examination of the right eye, severe periorbital edema was evident that makes the eyelids difficult to open . There was no local temperature rise. There was neither conjunctival hyperemia nor ocular motility restriction. A subcutaneous, semi-mobile, hard mass about 1 cm diameter was palpated on the right lateral orbital area around the frontozygomatic suture. Visual acuity measurement and posterior segment examination were not possible. On examination of the left eye, visual acuity was 20/20 and there were no abnormal findings.

There was no history of trauma or systemic illness. A new CT scan was not needed because no evidence other than inflammation was seen in the previous CT. The preoperative CT findings show, irregular borders of ruptured dermoid cyst and edema, and it appears to be completely recovered after the operation on the postoperative CT (Figure 1a, Figure 1b).

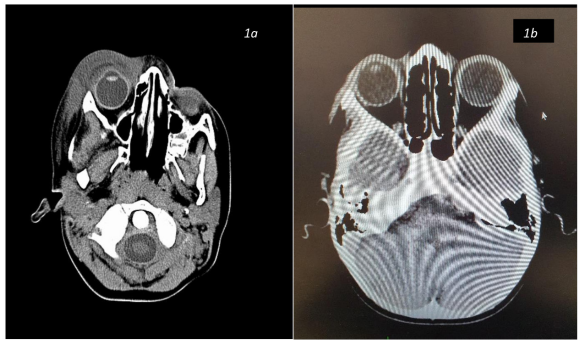

Figure 1a. *Preoperative CT, dense inflammation and thickening of the eyelid and periorbital area on CT.*
Figure 1b. *Postoperative CT*

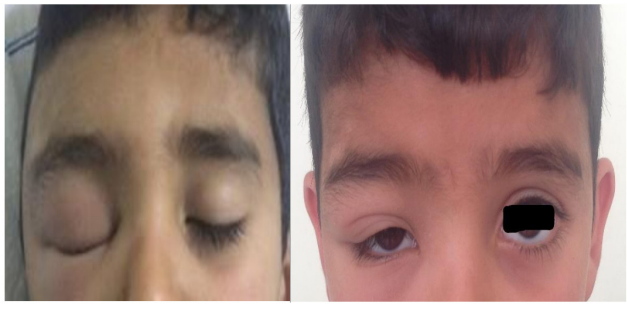

Figure 2. *Appearance of patient (before and after) the anti-inflammatory therapy*

Based on the history of recurrent periorbital swelling, spontaneous resolution and clinical findings, the patient was diagnosed as orbital inflammation related to ruptured dermoid cyst and medical treatment was rearranged. Nonsteroid antinflammatory agent (Ibuprofen 10 mg/kg.day orally) was started while antibiotic treatment was chased. The symptoms and signs started to resolve rapidly beginning from the first day of the anti-inflammatory therapy (Figure 2a, Figure 2b). On orbital magnetic resonance imaging (MRI) obtained after the inflammation was resolved to a large scale there was a mild thickening and condensation around the lateral orbital area, interpreted as a cyst wall. Then the patient underwent surgery to remove all the cystic components. A lid crease incision was used to approach the lesion and cyst wall and other parts of lesion removed totally (Figure 3). Histopathological examination of the excision material was revealed the cyst wall and inflammatory and fibrous alteration surrounding the wall. After the first week of the surgery, the patient was nearly normal. In our 6-month follow-up, we could not detect recurrence in our patient.

Written consent was obtained from the patient's parents for the publication of this case report and any accompanying images.

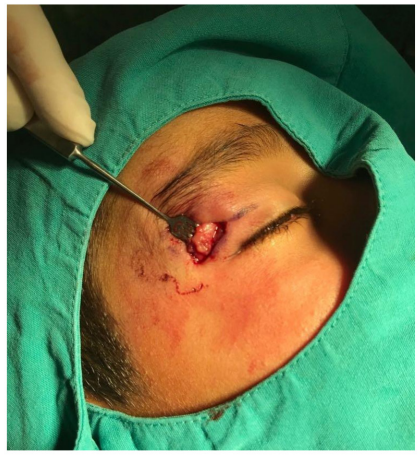

Figure 3. *Intraoperative appearance of the cyst wall.*

3. DISCUSSION

Orbital inflammation is due to the immune system's response, and the underlying cause is a clinical condition that needs clarification. It is important to investigate the etiology of autoimmune, structural, infectious, neoplastic and idiopathic causes. The clinician should carefully review all causes [1,6].

The clinician needs to look over five main groups of diseases in differential diagnosis of orbital inflammation;

1) Systemic diseases are the most common cause, including thyroid orbitopathy, Wegener granulomatosis, sarcoidosis, and other connective tissue diseases like systemic lupus erythematosis, some hematologic diseaseS and vasculitis [1,7].

2) Infections, especially orbital cellulitis, are life-threatening complications and require early diagnosis and treatment. In any

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doubt, patients should be hospitalized for parenteral treatment as soon as possible and empirical intensive-broad spectrum antimicrobial therapy should be initiated without waiting for culture results [1]. In orbital infections, symptoms such as fever, acute phase reactant elevation and leukocytosis are encountered, whereas these are rare in non-communicable OID [8,9].

But in our case, serological and orbital findings did not support the diagnosis of orbital infection.

3) Neoplastic diseases include lymphoproliferative disease, orbital metastasis, and primary malignant tumors such as rhabdomyosarcoma. Ruptured dermoid cysts can be classified in this group.

4) Vascular etiologies such as carotico-cavernous fistula or veno-lymphatic malformations also cause orbital inflammation.

5) Trauma and foreign body must be questionized in all OID patients.

In our patient's history, the intermittent nature and spontaneous resolution of the disease was remarkable. No evidence about systemic diseases was obtained from detailed medical and family history. The most important finding leading to our diagnosis was a palpable hard mass on lateral orbital area, suspicious of the cyst wall. Indeed, this has been confirmed by the way of surgery and histopathological examination of excised material.

Dermoid cysts are one of the rare but important causes of inflammation that are mostly seen in childhood. Depending on the location, it may vary clinically. There is a case of deeply located dermoid cyst in a 3-month-old baby reported from Nigeria in the literature [10]. The more superficially located ones can be overlooked until adulthood with false diagnoses such as cellulite. He was initially misdiagnosed with repeated hospital applications.

In a retrospective multicentric study, cases from 3 oculoplastic centers (Queen Victoria Hospital, East Grinstead, United Kingdom; King Abdulaziz University Hospital, Riyadh, Saudi Arabia; South Australian Institute of Ophthalmology and Visual Sciences, Australia) over a 10-year period, 29 of the total 86 cases were ruptured and only 2 were reported as spontaneous rupture. In the same study, it was pointed out that persistent inflammation was more pronounced after spontaneous rupture[11]. Our case was compatible with inflammation by ruptured dermoid cyst condition, in which infection and other causes are eliminated.

Dermoid cysts are defined as benign neoplasms and can cause complications such as spontaneous rupture and inflammation when not surgically removed. In a large ophthalmology clinic with 7 years of experience in Italy, 30 cases in the child age group were examined, by CT and / or MRI or echography which were suitable for diagnosis, and the best treatment was total excision, not biopsy. No recurrence has been reported after total removal [12]. We performed total excision due to the fact that our patient was a child and in the light of the literature. In our 6-month follow-up, we could not detect recurrence in our patient.

In conclusion, although it is rare, dermoid cyst rupture is one of the important causes of OID. A detailed history and a careful examination helped us to make the correct diagnosis and avoided diagnostic delay and unnecessary treatment.

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

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Authors' Contributions: HG.: Idea/Consept. HBB.: Design. HG.:Control/Supervision. HBB.: Data collection. HG.: Literature review and writing. HBB.: Critical review. HBB.: References. reviewed and revised the manuscript. Both authors read and approved the final version of the article.

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Case Report

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Coexistent intracranial midline cysts: Persistent cavum septum pellucidum, cavum vergae, cavum velum interpositum, and a pineal cyst

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ABSTRACT

This case is presented after a concurrent cavum septum pellucidum, cavum vergae, cavum velum interpositum, and a pineal cyst prevailed following a magnetic resonance imaging evaluation for a temporary blindness the patient reported that she recently had.
Keywords: Cavum septum pellucidum, Cavum vergae, Cavum velum interpositum, Congenital, Midline cranial cysts, Pineal cyst

1. INTRODUCTION

Persistent cavum septum pellucidum, cavum vergae, and cavum velum interpositum are considered as normal variants of the ventricular system. These anterior midline intracranial cysts may be asymptomatic, however, they may lead to cysts in considerable size that compress surrounding parts causing neuropathological conditions.

Septum pellucidum is a two-layered transparent membrane in the midline stretching vertically between the corpus callosum and the fornix. It separates the anterior horns and bodies of the lateral ventricles [1]. The cavity between two septa is known as cavum septum pellucidum (CSP). The two septa usually fuse within three to six months of postnatal life and CSP disappears [2], however, it may persist after 3-6 months of postnatal life by either remaining asymptomatic or leading to complications such as compression to surrounding structures, obstructive hydrocephalus, schizophrenia, post-traumatic disorder, or chronic brain trauma depending the size of the cavity [1,2]. Persistent CSP is at least 1mm in width and occurs about 15% of adults [3]. It is bounded superiorly by the body of the corpus callosum; anteriorly by the genu of the corpus callosum; inferiorly by the rostrum of the corpus callosum and anterior commissure; posteriorly by the anterior limbs of fornix, and laterally by the two layers of the septum pellucidum [3]. It is important to assess CSP during the embryonic development of fetus, as it can be a good monitor to observe any congenital malformations of the central forebrain during the early stage of development [4]. Failure to observe CSP can be associated with brain malformations [5] as it normally presents in all fetuses.

The cavum vergae (CV) is a posterior extension of the CSP and bounded superiorly and posteriorly by the body and splenium corpus callosum, respectively. Body of the fornix bounds it inferiorly, and the septum pellucidum bounds it, laterally (Figure 1). The CV is persistent in less than 1% of adults [3]. CSP and CV are located above the fornix.

The cavum velum interpositum (CVI) is a space located vertically in between the fornix and the choroid plexus of the third ventricle within the tela choroidea. It may or may not be connected to the cistern of the great cerebral vein (CGCV) (also known as cisterna ambiens or quadrigeminal cistern) [1, 3] (Fig. 1). It is situated superior to the internal cerebral veins and if connected to CGCV, it may be considered as an anterior extension of the CGCV [3]. Leucio and Dossani have reported prevalence of CVI as 5.54% of the population who is older than 2 years old [6].

Neither of these variants (CSP, CV, CVI) is included within the ventricular system of the brain, as they do not have choroid plexus, however they are filled with the cerebrospinal fluid (CSF) transferred from the ventricles [2].

The pineal cysts (PC) are rarely symptomatic intracranial findings and they are usually found incidentally [7]; mostly

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they are smaller than 1 cm [1] with a prevalence of 1% of the population, however the size might increase by the time (particularly in the age group 6-12 years) and may become a surgical indication depending the underlying reasons [8].

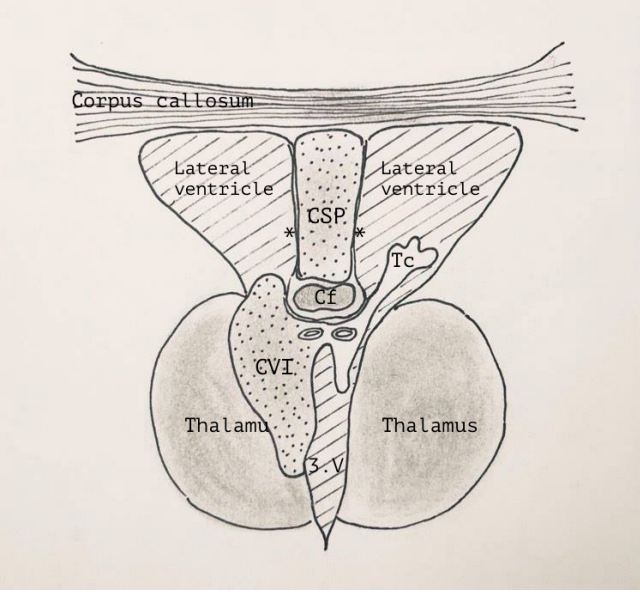

Figure 1. *Presented case illustrated in coronal section*
*CSP: Cavum septum pellucidum, CVI: Cavum velum interpositum, *: Septum pellucidum, 3.V: Third ventricle, Tc: Tela choroidea, Cf: Columns of fornix*

2. CASE REPORT

After 10-15 min of a partially blackened visual area on the left lower quadrant, 58 years old female patient went through some tests for evaluating the short episode of blindness she had. While, there was not any particular reason diagnosed for it, the radiology results stated that she had CSP, CV, CVI, and PC as normal variants after the performed magnetic resonance imaging (Figures 2, 3, 4, 5). The CSP and CV were about 5-10 mm wide depending on their exact location, while the CVI was seen on the right side to the midline and it was about 9-10 mm in width (Figures 1, 4). The widest transverse diameter of the PC was about 14 mm.

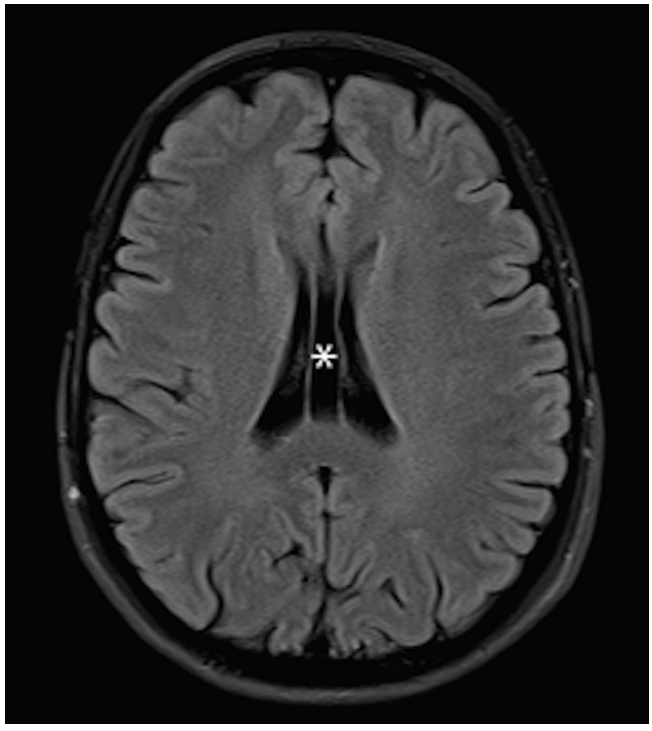

Figure 2. *CSP and CV in transverse section*

Figure 3. *CSP, CV, and PC in sagittal section*

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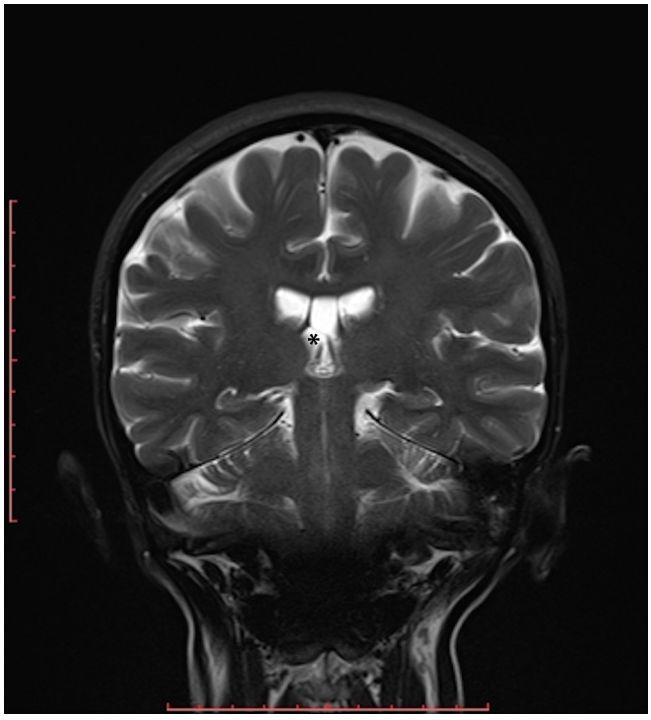

Figure 4. *CVI on the right side of the coronal section*

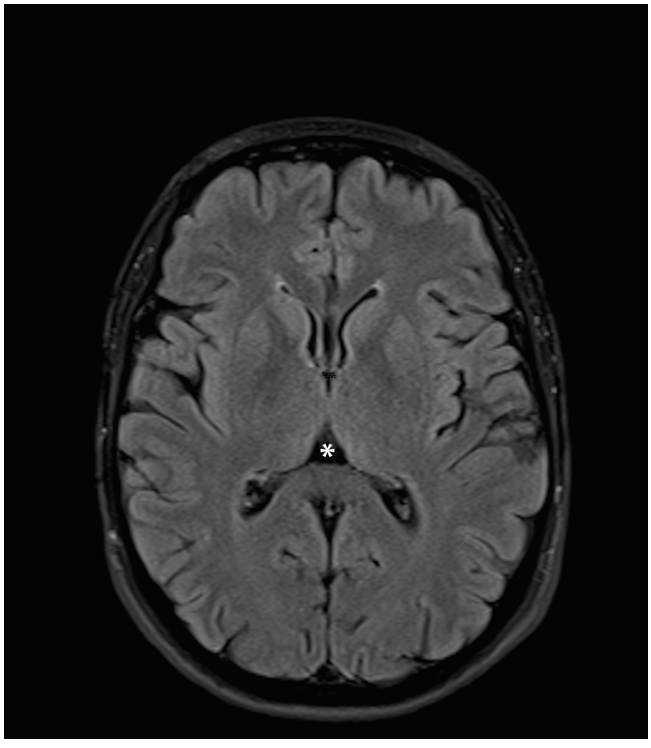

Figure 5. *CVI in the transvers section*

3. DISCUSSION

The CSP and CV develop from a common cavity under the corpus callosum, yet they may persist individually with a much wider occurrence of individual CSP (15%) than an individual CV (1%) [6]. They usually are asymptomatic except for rare situations such as cysts, obstructive hydrocephalus, and compression of surrounding structures that can cause psychiatric disorders and/or compression of optic chiasm and related tracts [1, 2, 9]. In the presented case, the patient was told that these normal variants are not susceptible to cause the patient's symptoms.

De Leucio and Dossani have reported the CVI with a prevalence of 5.54% of the population older than 2 years old. It is usually asymptomatic unless it is rarely large enough to push surrounding structures [6].

In this case, the patient had a history of migraine episodes since her childhood, yet the episodes became ocular only with no headaches as of the age of late 40s. She has never been diagnosed with any other potential symptoms of the concurrent persistent CSP, CV, CVI, and PC she had. They have been detected by coincidence after a brain MRI performed to evaluate the symptoms the patient had and there is not any similar case reported with these cysts to be found, concurrently.

These normal variants may remain with some questions to be followed to figure whether there is a possibility to relate the migraine attacks to these variants the patient has been having since her childhood [10] or the recent patient history with partial and temporary blindness after bending forward for a while is related to these variants.

These questions may find less subtle answers if the cysts would be reevaluated to see whether there is a change in the size of them.

The case suggests a consideration of the prevalence of coexisting midline cranial variants to be studied, as there is not any information yet to be found.

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