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Expression of sialic acid binding receptors (siglecs) in human trophoblast cell line

İnsan trofoblast hücre hattinda sialik asit bağlayici reseptörlerin (siglecs) ekspresyonu

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Abstract

Purpose: Cell surface proteins known as Siglecs exhibit a specific affinity for sialic acid. Primarily located on the surface of immune cells, Siglecs belong to the subset of lectins called I-type lectins. Siglecs have important roles in maternal-fetal immune tolerance. We aimed to analyze the expression of Siglecs on Human Villous Trophoblasts (HVT) cells.

Materials and methods: Total RNA was extracted from the HVT cell line, cDNA was synthesized, and real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR) was performed the determine the changes in Siglec -3, -5, -6, -7, -8, -9, -10, -11, and -16 mRNA levels. In addition, Siglec levels were assessed by using immunohistochemical staining. Immunoreactivity against Siglec-6 and Siglec-9 was evaluated separately according to the intensity of brown color.

Results: Expression levels of Siglec genes by qRT-PCR and melting curve analyses were performed using RNA extracted from the HVT cell line. Siglec -3, -5, -6, -7, -9, -10, -11, and -16 genes were found to be expressed in the HVT cell line. Differently, Siglec-8 results were undetected after cycle 40, which was considered a negative result. The immunocytochemical examination of the HVT cell line revealed that Siglec-6 expression was moderate in the cytoplasm (Score:2). Siglec-9 expression was prominent in the cytoplasm (Score:3).

Conclusion: The results showed that Siglec-6 and Siglec-9 were expressed more than other Siglec proteins in the human trophoblast cells. Immunocytochemistry results also support these findings. Our study is the first to show Siglec-9 expression in human trophoblast cells.

Keywords: Siglecs, human villous trophoblasts, sialic acid.

Cil N, Fenkci IV, Abban Mete G, Mutlu D, Kabukcu C, Cabus U. Expression of sialic acid binding receptors (siglecs) in human trophoblast cell line. Pam Med J 2024;17:195-203.

Öz

Amaç: Siglecler olarak bilinen hücre yüzeyi proteinleri, sialik asit için özel bir afinite sergilemektedir. Öncelikle bağışıklık hücrelerinin yüzeyinde bulunan Siglec'ler, I-tipi lektinler adı verilen lektin alt kümesine aittir. Siglecler maternal-fetal immün toleransta önemli rollere sahiptir. Bu çalışmada İnsan Villöz Trofoblast (HVT) hücre hatlarında Siglec tiplerinin belirlenmesi amaçlanmıştır.

Gereç ve yöntem: HVT hücre hattından total RNA ekstrakte edildi, cDNA sentezlendi ve Siglec -3, -5, -6, -7, -8, -9, -10, -11 ve -16 gen ekspresyonu Gerçek Zamanlı Kantitatif Ters Transkripsiyon Polimeraz Zincir Reaksiyonu (qRT-PCR) ile analiz edildi. Ek olarak, Siglec seviyelerini belirlemek için immünohistokimyasal boyamalar yapılmıştır. Siglec-6 ve Siglec-9' a karşı immünoreaktivite kahverengi rengin yoğunluğuna göre ayrı ayrı değerlendirilmiştir.

Bulgular: HVT hücre hattından ekstrakte edilen RNA kullanılarak Siglec genlerinin qRT-PCR ile ekspresyon seviyeleri ile erime eğrisi analizleri yapıldı. Siglec -3, -5, -6, -7, -9, -10, -11 ve -16 genlerinin HVT hücre hattında ifade edildiği bulundu. Farklı olarak, Siglec-8 sonuçları 40. döngüden sonra tespit edilemedi ve bu da negatif bir sonuç olarak kabul edildi. HVT hücre hattının immünositokimyasal incelemesinde Siglec-6 ekspresyonunun sitoplazmada orta düzeyde olduğu ortaya çıkmıştır (Skor:2). Siglec-9 ekspresyonu sitoplazmada belirgindi (Skor:3).

Sonuç: Sonuçlar Siglec-6 ve Siglec-9'un HVT hücre hattında diğer Siglec proteinlerinden daha fazla ifade edildiğini göstermiştir. İmmünositokimya sonuçları da bu bulguları desteklemektedir. Çalışmamız insan trofoblast hücrelerinde Siglec-9 ekspresyonunu gösteren ilk çalışmadır.

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Anahtar kelimeler: Siglecs, insan villöz trafoblast, sialik asit.

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Introduction

Infertility is the absence of conception following one year of unprotected and regular sexual intercourse [1]. The causes of infertility can be categorized simply as female only, male only, both male and female and unexplained causes. However, infertility is a complex, multifactorial condition. Therefore a more detailed evaluation for a definitive diagnosis is important to determine the most appropriate treatment for couples [2-4]. With the development of new treatment protocols in ovulation induction, the success rates in infertility treatment with assisted reproductive techniques have increased, considering the high rate of high-quality oocytes obtained and advances in fertilization and embryo development. Nevertheless, the desired level of embryo implantation, especially after high-quality embryo transfer, has not yet been achieved [5]. Although several parameters have been proposed to determine endometrial receptivity, no positive results have been obtained for clinical use [6]. Endometrial factors that increase endometrial receptivity and improve implantation rate may be new therapeutic targets [7].

Sialic acids are nine-carbon sugars synthesized in vertebrates. They are the last sugars complementing various glycosylation structures [8]. Some bacteria have developed to synthesize “Sialic acids mimic”, de-novo synthesize, and take up them or sialyl-coated structures from the host to provide a survival advantage. Cancer cells use a similar strategy to evade the immune system. There is extensive immunological suppression through sialylated mucins produced by the tumor. Indeed, sialic acids associated with abnormal glycosylation and increased sialylation have been demonstrated in many tumors, such as colon cancer, renal cell carcinoma, prostate cancer, head and neck squamous cell carcinoma, breast cancer, oral cancer, and have been shown in the progression and metastasis of cancer [9-14]. The primary role of sialic acids in the immune system is thought to be due to the fact that they contain ligands for Selectin or calcium-dependent *C-type* leukocyte motility regulating lectins [8]. Activation of immune cells seems to be related to decrease sialic acids in cell surface [15]. Sialic acids are ligands for cell adhesion molecules in the sialic acid binding immunoglobulin-like lectins (Siglecs) family, which regulate the immune response [8]. Monocytes, macrophages, and dendritic cells contain largely the same Siglec profile. Synthesis of *Siglec-3, -7, -9* are high and synthesis of *Siglec-1* and *Siglec-10* are low after stimulation with IFN-α [16-23]. An intracellular “immunoreceptor tyrosine-based inhibition motif (ITIM)” is present in most Siglecs. ITIM stimulates inhibitory signal when it binds Sialic acids [24]. *Siglec-5* to *Siglec-11* carrying ITIM in their cytoplasma are called inhibitory Siglecs [25]. In contrast, *Siglec-4* and *Siglec-16* do not contain an ITIM. Therefore they are called activating Siglecs. *Siglec-14* may be an inducer of an inflammatory response through activation of the MAPK pathway [26]. Siglecs are ligands expressed primarily on macrophages and dendritic cells that are involved in cell adhesion and the internalisation of sialic acid-expressing pathogens. In innate cells, most Siglec-associated ITIMs are thought to protect against the development of autoimmunity by attenuating inflammatory responses in various cell types, mainly through their cis-interacting with sialoglycoconjugates. However, pathogens and tumors can subvert these inhibitory responses for their own purposes. While immune stimulation is important for the treatment process in cancers, the overactive immune system in allergies and autoimmune diseases needs to be suppressed in some way [27].

It has been suggested that Sialic acids and Siglecs have important roles in maternal-fetal immune tolerance and, therefore, may have positive or negative effects on the implantation success of the embryo, depending on whether this system is functioning properly or not. In this context, it is essential to demonstrate

the presence of Siglecs in trophoblast cells. Evaluating the Siglec relationship may be clinically significant in women diagnosed with infertility, recurrent pregnancy loss, and recurrent implantation failure in assisted reproductive techniques treatments. In this study, we aimed to determine Siglec types in trophoblast cells.

Material and methods

Cell culture

Human Villous Trophoblasts (HVT) (Cat#910958, Innoprot) cell lines were obtained Cells cultured in DMEM/F12 (Sigma) supplemented with 10% Fetal Bovine Serum (FBS) (GIBCO), 1% penicillin/streptomycin (GİBCO) mix and were incubated at 37°C with 5% CO_2 [28].

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted using the innuPREP RNA Mini Kit 2.0 (Analytik Jena, Germany) according to the manufacturer's protocol. A total of 2.5 µg RNA was reverted to cDNA by using an A.B.T.™ cDNA Synthesis Kit according to the manufacturer's instructions. qRT-PCR was performed by using A.B.T.™ 2X qPCR SYBR-Green MasterMix (Atlas Biyoteknoloji, Türkiye) in an Applied Biosystems™ StepOnePlus™ Real-Time PCR System (Thermo, USA). The samples underwent two-step amplification with an initial step at 95°C (10 min), followed by 95°C (15 s), 72°C (30 s), and 60°C (30 s) for 40 cycles. The melting curve was analyzed. Each sample was performed in triplicate independently. GAPDH was used as an internal control (Table 1).

Table 1. Primer sequences of the genes used in this study

Gene Names	Primer Sequences	
	Forward	Reverse
GAPDH	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA
SIGLEC-3	GTGACTACGGAGAGAACCATCC	GCTGTAACACCAGCTCCTCCAA
SIGLEC-5	CTCACCTGTCAGATGAAACGCC	CCGTTCCTGAAGATGGTGATGG
SIGLEC-6	TTTCACCTGCCGTGCTCAGCAT	ACCAGGGTTGTGATGCTAGCTC
SIGLEC-7	CTGGTCTTCCTCTCCTTCTGTG	GCATCCTTCATGCCTATGTCTCC
SIGLEC-8	TGACTGTCTTCCAAGGAGATGCC	CTGTTGACAGCACAGACCAGGC
SIGLEC-9	CCACGAACAAGACCGTCCATCT	TCTGGGAGTGACAGAGATGAGC
SIGLEC-10	AACGGAGCGTTTCTGGGAATCG	TCTGAGTCCGTCTCTTCGGTAG
SIGLEC-11	AGAGTGGCTCTGTCTTCCAGCT	CTGAAGACGACAAGGCAGGAAC
SIGLEC-16	CAACCAGAGTCGAGAGGTGGAA	CACCCGAAAGAAGTACCATGCC

Immunocytochemistry

HVT cells were thawed by adding DMEM/F-12 medium containing 10% FBS and 1% penicillin/streptomycin mixture. Thawed cells were monitored every day. Cells reaching 80-90% confluency were passaged the following days. The replicated cells were seeded on chamber slides with 40.000 cells in each well. After the inoculated cells adhered, the medium was removed and fixed with methanol (-20°C). H_2O_2 was added to the dried cells, protecting them from light. After 30 minutes, they were washed with Phosphate Buffer Saline (PBS) (CAPRICORN). Secondary kit A solution was applied for 10 minutes. *Siglec 6* and *Siglec 9* (1:100) primary antibodies were applied and incubated at 4°C for one night. After washing with PBS, secondary kit B solution was applied for 1 hour. The chamber slide was rewashed with PBS and exposed to secondary kit C solution for 30 minutes. Stained with 3.3 diaminobenzidine (DAB) and hematoxylin as counterstaining, the chamber slide was covered with entellan. Immunoreactivity against *Siglec-6* and *Siglec-9* was evaluated separately according to the intensity of brown color. HVT cells were scanned at 40X magnification. *Siglec-6* and *Siglec-9* expression was evaluated in the cell nucleus and cytoplasm. Samples were evaluated by light microscopy according to the extent of coloured cells (0%=score 0, 1-10%=score 1, 11-50%=score 2; >51%=score 3) [29, 30].

Statistical analysis

Data are given as Mean ± Standard Deviation (for each data). The difference between the groups were analyzed using a one-way ANOVA test with GraphPad Prism 9 software.

Results

qRT-PCR findings

We observed the melting curves by qRT-PCR amplification targeting Siglec genes using RNA extracts from the HVT cell line. The amplicons specificity was verified by analysis (60 to 95°C) after 40 cycles, except for *Siglec-8* (Figure 1).

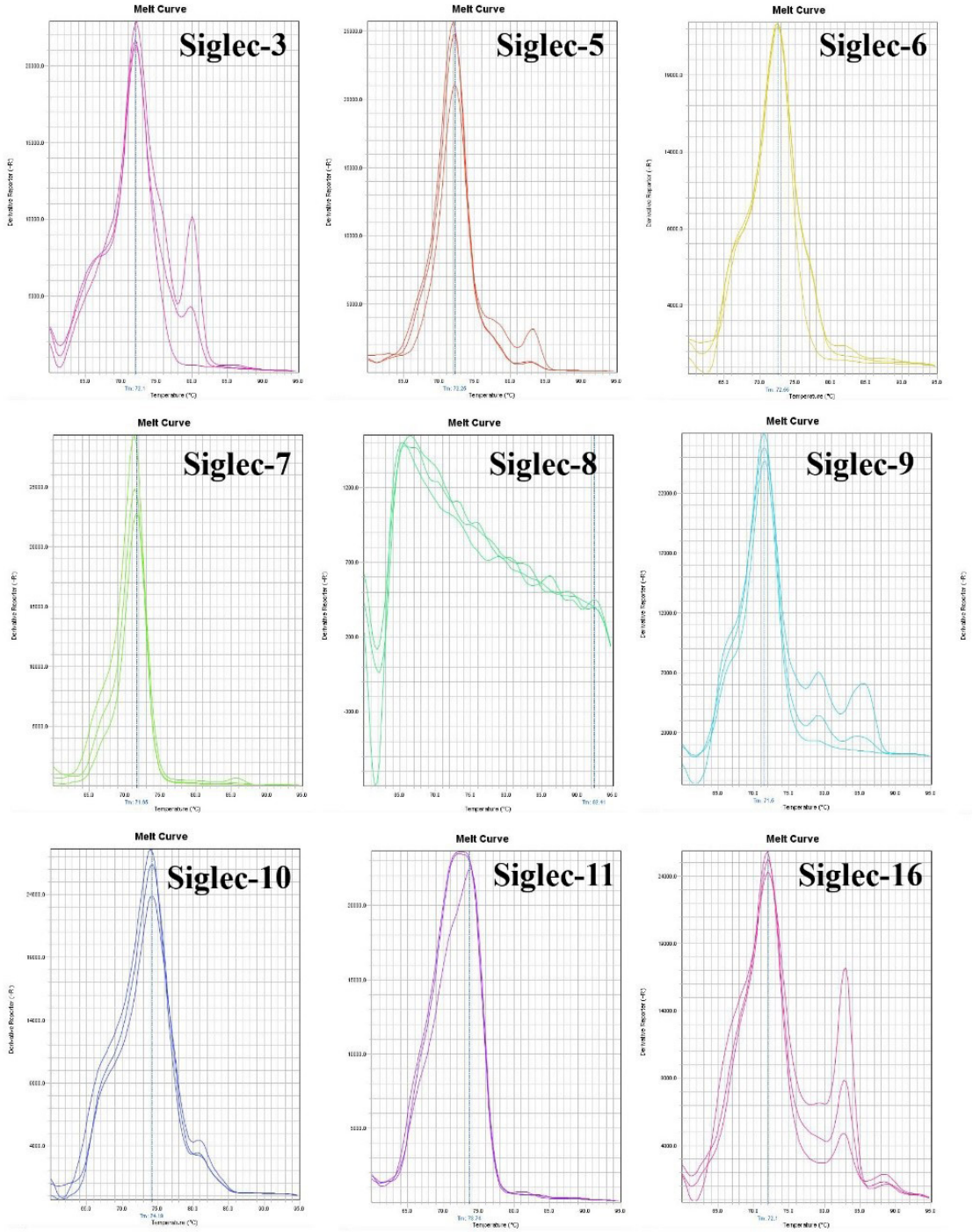

Figure 1. Melting curve profile of Siglec genes

The cycle threshold (Ct) values were obtained from each reaction with primer pairs. *Siglec -3, -5, -6, -7, -9, -10, -11*, and *-16* genes were found to be expressed in the HVT cell line. Differently, *Siglec-8* results were undetected after cycle 40, which was considered a negative result (Figure 2).

Immunocytochemical findings

In the immunocytochemical examination of the HVT cell line revealed that *Siglec-6* expression was moderate in the cytoplasm (Score:2), nuclear staining was weak, and there was no staining in some areas. Along with moderate staining in the cytoplasm and intense staining in some areas, moderate staining in the nuclear membrane in some areas was striking (Figure 3). In The immunocytochemical examination of the HVT cell line, *Siglec-9* expression was prominent in the cytoplasm (Score:3). Nucleus staining was weak, and some areas showed no staining. Nuclear membrane staining was moderate (Score:2) (Figure 3).

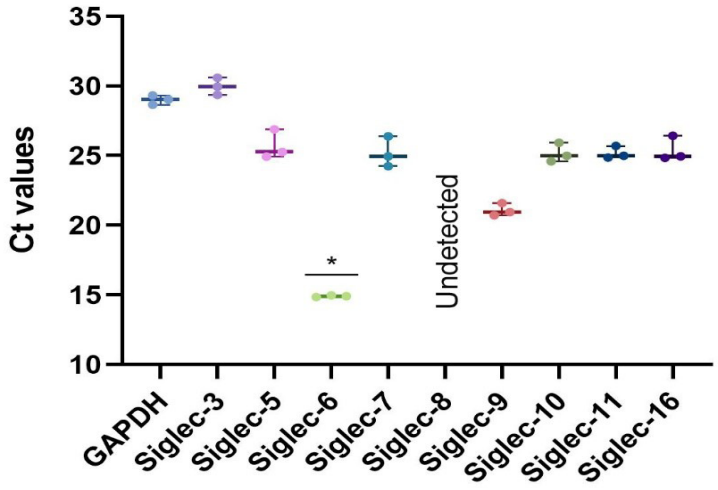

Figure 2. qRT-PCR Ct values for reference genes. Expression data is displayed as Ct values for each reference gene in HVT cells. Error bars represent the mean ± standard error of three replicates * indicates statistical difference (p<0.05, one-way ANOVA)

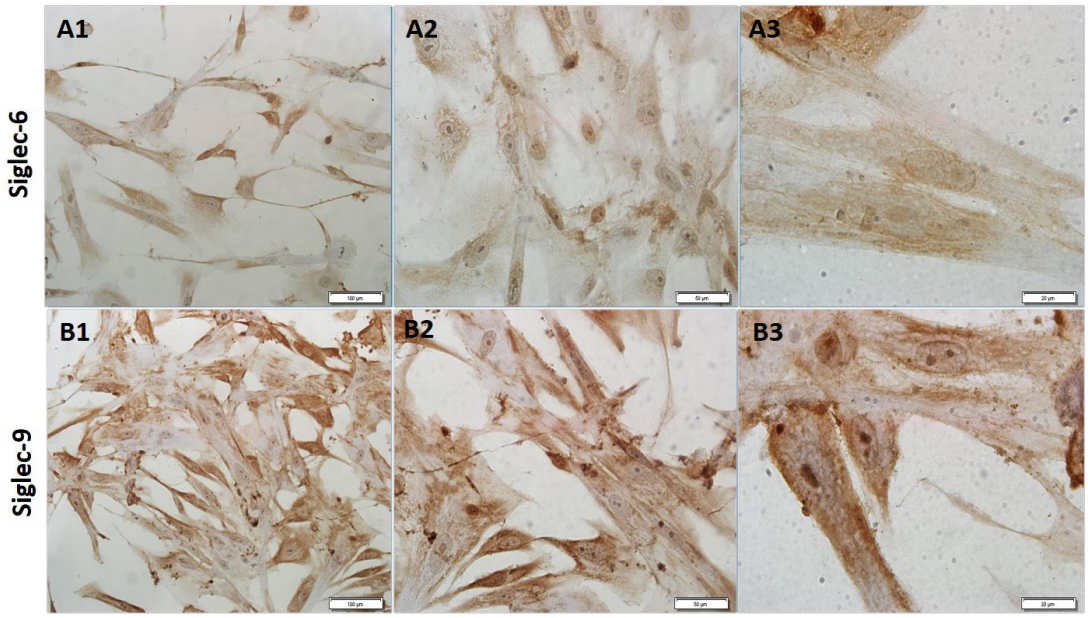

Figure 3. Immunocytochemical demonstration of Siglec-6 and Siglec-9 expression in HVT cells A1, B1: 20X; B2, B2: 40X; A3, B3: 100X, Immunoperoxidase & Hematoxylin

Discussion

The sialic acid-Siglec axis has been partially explored in reproductive system function and embryo implantation. Sialic acids are found in circulating glycoprotein hormones (FSH, LH, hCG). The N-glycans of FSH and hCG are coated with sialic acid, whereas in LH, a different modification occurs with the addition of sulfate. The fate of circulating LH is determined by these glycosylation changes; a specialized receptor in the liver clears the sulfated molecules. This clearance determines the half-life of the hormones in circulation. As a result, the reproductive cycle is optimized [27]. Sialic acids affect fertilization during sperm-egg contact [31]. It has been shown that these glycans contribute to fertilization during interactions with the surfaces and various fluids of the female reproductive system before the sperm arrives the ovum [32]. The endometrium has an agglutinin that detects sialic acids [33]. Sialic acids appear to be affect fertilization [34]. Sialic acids also affect embryogenesis. Although cells in culture can survive and divide without sialic acids, early embryonic death occurred in mice that were genetically engineered to eliminate sialic acid production [35]. Tecle et al. [36] investigated the expression of various Siglecs in pre-menopausal human uterine tissues. They showed that the epithelium primarily expressed *Siglec-10*. They investigated Ishikawa and HEC-1B, for the expression of Siglec on the cell surface. Their analysis revealed that HEC-1B and Ishikawa express *Siglec-10* and *Siglec-11* and activate other Siglecs. Importantly, these two cell lines were found to express *Siglec-16* highly [36]. In a study, despite *Siglec-11* and *Siglec-16* being expressed by endometrial cell lines, *Siglec-11*-Fc and *Siglec-16*-Fc proteins did not bind to human semen [37]. In our study, the presence of *Siglec-10, -11*, and *-16* in HVT was demonstrated in gene analysis using the Real-Time PCR method.

Siglec-8 is expressed on human mast cells and eosinophils and has low expression on basophils. These cells contribute to allergic and non-allergic diseases. They contribute to the inflammatory response by releasing mediators that attract other cells and activate inflammation. Early studies found that *Siglec-8* binds to monoclonal antibodies or selective sialoglycan ligands, ultimately inducing cell death by eosinophils and inhibiting degranulation of the mast cells. In vivo administration of anti-*Siglec-8* antibodies to the in vitro results were confirmed by transgenic mice expressing *Siglec-8* on eosinophils and mast cells [38]. We did not demonstrate Siglec-8 expression in trophoblast cells in our study.

Siglec-6 is expressed in syncytiotrophoblasts, cytotrophoblasts and extravillous trophoblasts in the human placenta. It is expressed in B cells of primates. However, placental expression of human is specific. Overexpression of *Siglec-6* was observed in placentas with preeclampsia (PE) patients [39]. Another study showed that expression of *Siglec-6* was higher in PE placentas compared to controls at preterm period. *Siglec-6* expression was approximately twice as high in PE samples in the basal plate and chorionic villi. With this study, *Siglec-6* was thought to have a role in trophoblast differentiation defects. Therefore, it was concluded that overexpression of *Siglec-6* in PE placentas may be a marker of PE [40]. Another study was reported that while *Siglec-6* is expressed in the immune cells of both humans and great apes, its expression in the placenta is human-specific. In the monkey placenta, its expression is either very low or absent. Natural ligands for *Siglec-6* are expressed in the human placenta. Ligands have also been located in the uterine endometrium and trophoblastic or endometrial-origin cell lines. As a result of the immunohistochemical analysis for *Siglec-6* localization in human placentas, a wide expression range was recorded in placentas obtained with normal delivery, with the highest expression [41]. Our study supports these studies, as shown both by Real-Time PCR results and immunocytochemically. In the images obtained immunocytochemically, a prominent reaction was observed in the cytoplasm and nuclear membrane.

Myeloid cells, B cells, NK cells, T cells express *Siglec-9*. It is a cell surface transmembrane receptor. When *Siglec-9* binds to its sialic acid-containing ligands, it initiates a negative signaling cascade that inhibits the function of immune cells. *Siglec-9*-mediated inhibition of the immune response is independent of MHC. Therefore, cancer cells can use this mechanism to evade the host immune response. These results indicate that *Siglec-9* is an important glycoimmune negative checkpoint against cancer and virally infected cells [42]. In a meta-

analysis, it was reported that expression of *Siglec-9* is altered in cancers and is associated with patient survival. The study revealed a correlation between *Siglec-9* expression and clinical characteristics of tumor patients. *Siglec-9* is strongly associated with the tumor immune microenvironment. *Siglec-9* expression levels have been shown to be highest in blood, spleen, and lung tissues and lowest in muscle and bone marrow [43]. Another study showed that *Siglec-9* was expressed in mesothelial cells and connected with glycan epitopes in endometrial cells. Inhibition of *Siglec-9* function decreases the connection between the endometrium and mesothelium [44]. According to Real-Time PCR results, *Siglec 9* expression was found to be considerably higher than other Siglecs, such as *Siglec 6* in our study. Immunologically, it was supported significantly by cytoplasm and nuclear membrane staining.

In conclusion, *Siglec -3, -5, -6, -7, -9, -10, -11*, and *-16* genes were found to be expressed in the HVT cell line in the study. The results showed that *Siglec-6* and *Siglec-9* were expressed more than other Siglec proteins in the HVT cell line. Immunocytochemistry results also support these findings. Our study is the first to show *Siglec-9* expression in human trophoblast cells. Experimental and human placental studies are needed to elucidate the effects of Siglec genes, which are thought to promote cell-cell interactions and regulate the immune system through glycan recognition, on these mechanisms.

Conflict of interest: No conflict of interest was declared by the authors.

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Ethics committee disclosure: In the our study “Expression of Sialic Acid Binding Receptors (Siglecs) in Human Trophoblast Cell Line”, human first trimester trophoblast cells (Human Trophoblast Cell Line) that we have in stock were used. Cells are cultured in medium. Cells that become confluent between 2 and 3 days are multiplied by changing the medium, and experimental groups of cells that have reached sufficient density are formed. Ethics committee approval is not required for cell culture studies and cells are in our stock.

Contributions of the authors to the article

I.V.F. constructed the main idea and hypothesis of the study. I.V.F., N.C. and D.M. developed the theory and arranged/edited the material and method section. D.M. evaluated the data of qRT- PCR in the results section. G.A.M. and N.C. contributed to the histological and immunohistochemical evaluation of the results. The article written by I.V.F., N.C., U.C., C.K. and D.M.

I.V.F. and G.A.M. reviewed the article and made the necessary corrections and approved it. In addition, all authors discussed the entire study and approved the final version.

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Impact of laparoscopic sleeve gastrectomy on periodontal status in obese patients

Obez hastalarda laparoskopik tüp mide ameliyatının periodontal duruma etkisi

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Abstract

Purpose: This study aimed to determine the effects of laparoscopic sleeve gastrectomy surgery on clinical periodontal parameters at 6 months in the postoperative period.

Materials and methods: Fifty-four obese patients with periodontitis between 18 and 70 years of age were evaluated for obesity-related parameters and periodontal status before and 6 months after bariatric surgery. Correlations of the changes in periodontal parameters and the total weight loss and body mass index (BMI) loss at 6 months after bariatric surgery were determined with Spearman correlation analysis. *P*<0.05 was considered as the statistical significance value.

Results: Significant improvements were achieved at 6-month follow-up compared to baseline in BMI values and several systemic health-related serum biomarkers such as C-reactive protein, high-density lipoprotein, low-density lipoprotein, and albumin levels. Periodontal parameters; plaque index (*p*=0.000), gingival index (*p*=0.004), and bleeding on probing (*p*=0.007) were decreased significantly at 6 months after surgery. However, no significant changes in probing depth and clinical attachment level values were observed. The changes in plaque index values were positively correlated with the percent of total weight loss (r=0.301, *p*=0.027). Also, there was a positive significant correlation between the changes in the percent of bleeding on probing levels and the percent of total weight loss and the percent excess BMI loss (r=0.637, *p*=0.000 and r=0.370, *p*=0.006 respectively).

Conclusion: Laparoscopic sleeve gastrectomy surgery resulted in a reduction in periodontal inflammation in parallel with the decrease in BMI levels and obesity-related biochemical markers during the 6-month follow-up period.

Keywords: Bariatric surgery, body mass index, obesity, periodontitis, prospective studies.

Lektemur Alpan A, Torumtay Cin G, Aykota MR. Impact of laparoscopic sleeve gastrectomy on periodontal status in obese patients. Pam Med J 2024;17:205-212.

Öz

Amaç: Bu çalışmada, laparoskopik tüp mide ameliyatının operasyon sonrası 6. ayda klinik periodontal parametreler üzerindeki etkilerinin belirlenmesi amaçlandı.

Gereç ve yöntem: Yaşları 18 ile 70 arasında değişen, periodontitisli 54 obez hasta, obezite ile ilişkili parametreler ve bariatrik cerrahi öncesi ve sonrasındaki periodontal durumları açısından değerlendirildi. Obezite cerrahisi sonrası 6. ayda periodontal parametrelerdeki değişiklikler ile toplam kilo kaybı ve vücut kitle indeksi (VKİ) kaybı arasındaki korelasyonlar Spearman korelasyon analizi ile belirlendi. *P*<0,05 istatistiksel anlamlılık değeri olarak kabul edildi.

Bulgular: 6 aylık takipte VKİ değerlerinde ve C-reaktif protein, yüksek yoğunluklu lipoprotein, düşük yoğunluklu lipoprotein ve albümin seviyeleri gibi sistemik sağlıkla ilgili çeşitli serum biyobelirteçlerinde başlangıca kıyasla önemli iyileşmeler elde edildi. Plak indeksi (*p*=0,000), gingival indeks (*p*=0,004) ve sondlamada kanama (*p*=0,007) gibi periodontal paramatreler ameliyattan 6 ay sonra anlamlı derecede azaldı. Ancak sondlama derinliği ve klinik ataçman seviyesi değerlerinde anlamlı bir değişiklik gözlenmedi. Plak indeksi değerlerindeki değişiklikler toplam kilo kaybı yüzdesi ile pozitif korelasyon gösterdi (r=0,301, *p*=0,027). Ayrıca sondlamada kanama yüzdesindeki değişiklikler ile toplam kilo kaybı yüzdesi ve aşırı VKİ kaybı yüzdesi arasında pozitif anlamlı bir korelasyon tespit edildi (sırasıyla r=0,637, *p*=0,000 ve r=0,370, *p*=0,006).

Sonuç: Laparoskopik tüp mide ameliyatı, 6 aylık takip döneminde VKİ seviyelerinde ve obezite ile ilişkili biyokimyasal belirteçlerde azalmaya paralel olarak periodontal inflamasyonda da azalma sağlamıştır.

Anahtar kelimeler: Bariatrik cerrahi, vücut kitle indeksi, obezite, periodontitis, prospektif çalışmalar.

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Introduction

Obesity is a condition that is a significant global health concern with increasing prevalence rates [1] characterized by excess body fat. Body Mass Index (BMI) which is used to define obesity of 30 or higher is considered indicative of obesity [2]. Obesity is related to many health risks and can have a significant impact on a person's overall well-being. The pathogenesis of type 2 diabetes mellitus (T2DM), cancer, cardiovascular disease, and osteoporosis is linked with obesity [3, 4].

Periodontitis is a chronic inflammatory oral disease that involves a complex interplay between oral bacteria and the person's immune response, resulting in the irreversible destruction of the periodontium [5]. The 2017 periodontal disease classification of the link between these two conditions underscores the idea of comorbidity, where the presence of obesity increases the risk and severity of periodontitis [6]. The relationship between obesity and periodontitis is multifaceted and can involve various mechanisms, including inflammation, altered immune response, and systemic factors [7, 8].

Bariatric surgery (BS), is a set of surgical procedures performed to help individuals with severe obesity lose weight by reducing their BMI. The principal types of BS are laparoscopic sleeve gastrectomy (LSG), adjustable gastric band, biliopancreatic diversion with duodenal switch, and Roux-en-Y Gastric Bypass (RYGB) [9]. LSG is a procedure that allows the stomach to be turned into a narrow tube or sleeve by completely removing the fundus part of the stomach with a vertical incision along the larger curvature (outer curve) of the stomach [10]. LSG has an additional benefit beyond its restrictive effect. It reduces the production of an appetite-stimulating hormone called ghrelin produced in the fundus of the stomach and plays a role in regulating hunger [11]. Research related to the effects of BS on periodontal conditions has produced conflicting results [12-16]. While some studies have stated that BS has a negative effect on periodontal status [12, 13], others have emphasized that the periodontal condition becomes better [15]. Therefore, our study aims to evaluate the 6-month changes in periodontal parameters in obese individuals undergoing LSG. Our study hypothesizes that obesity decreases due to weight loss after LSG and this may lead to improvement in periodontal parameters at the follow-up at 6 months.

Materials and methods

Study population

This prospective observational study evaluated a total of 54 obese patients diagnosed with periodontitis (AAP/EPF classification) [17] who were being monitored in the obesity clinic at the Faculty of Medicine in Pamukkale University and eligible for LSG surgery, during the preoperative and 6-month postoperative period. The indication for BS was made by the experienced bariatric surgeon (MRA) if a patient had a BMI >40 kg/m^2 or BMI >35 kg/m^2 and obesity-related comorbidity [18].

The ethical approval was obtained from the Ethics Committee of the Faculty of Medicine at Pamukkale University with the protocol number 2018/19 and was carried out by the Declaration of Helsinki. Each participant was asked to provide written informed consent. Patients were excluded if they were <18 or >70 years of age, pregnant, underwent periodontal treatment within a 6-month period, and had <15 teeth. After detailed periodontal examination, individuals who underwent sleeve gastrectomy surgery were followed up for a 6-month period after the operation.

Demographic and metabolic variables

Demographic characteristics such as age, sex, education level, and smoking habits were collected from each subject at the baseline of the study. Participants' medical conditions were determined from their medical records. The history of hypertension (systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥90 mmHg or receiving treatment for hypertension), dyslipidemia (high-density lipoprotein level (HDL): female, <50 mg/dL and males, <40 mg/dL; triglyceride level ≥150 mg/dL or receiving treatment for dyslipidemia) and diabetes (fasting plasma glucose ≥126 mg/dl or receiving treatment for diabetes) was defined according to the related criteria. C reactive protein (CRP), HDL, low-

density lipoprotein (LDL), triglyceride, albumin, glycated hemoglobin (HBA1c) levels, aspartate transaminase (AST), alanine transaminase (ALT) and gamma-glutamyl transpeptidase (GGT), levels were recorded at baseline and 6 months after surgery.

BMI was calculated by taking the weight, in kilograms divided by the height in meters squared. According to the Edmonton Obesity Staging System, participants were categorized according to BMI, such as obesity class I (30 to 34.9), obesity class II (35 to 39.9), obesity class III (≥40) [19]. Weight was measured at baseline and 6th month after the surgery. The percentage of original weight loss and the percent excess BMI loss (EBMIL%) were calculated [20].

Periodontal examinations

Full-mouth periodontal measurements of the patients were made by the same clinician. Intra-examiner calibration was calculated with the Intraclass Correlation Coefficient. The intra-examiner ICC values were 0.90 (PD) and 0.88 (CAL). The number of existing teeth in the patients was recorded. Plaque index (PI) [21], gingival index (GI) [22], the presence of bleeding on probing (BOP) [23], probing depth (PD), and clinical attachment level (CAL) were measured using the Williams periodontal probe (Hu-Friedy, Chicago IL). PD and CAL were calculated at six surfaces per tooth, whereas PI and GI were evaluated at four surfaces per tooth. The periodontitis severity of patients was determined based on the Classification of Periodontal and Peri-Implant Diseases and Conditions stated in the 2017 World Workshop.

Sample size calculation

Power analysis was performed to calculate the sample size of the study. Sample size was calculated using the G*Power statistical program for α=0.05 and d=0.4. The analyses revealed that a total of 52 subjects achieved a power of 80% with 95% confidence.

Statistical analyses

The data were analyzed using a statistical program (SPSS 21 Inc., Chicago, IL). Continuous variables were presented as mean ± standard deviation and categorical variables as numbers and percentages. Kolmogorov-Smirnov test was used to detect data's normality. Wilcoxon test was applied to compare the differences in metabolic and periodontal parameters at baseline and 6 months postoperatively. Spearmen correlation analysis was applied to investigate the associations between periodontal changes within a 6-month period and total weight loss and BMI loss. The statistical significance value was considered as $p<0.05$.

Results

A total of 54 patients who indicated to undergo LSG surgery completed the study throughout the 6-month follow-up period. The baseline demographic characteristics of the study population were presented (Table 1). The majority of patients were female (66.7%), and the mean age of the study population was 41.1±8.89. The prevalence of obesity level III was observed in 68.5% of all participants. While stage 2 periodontitis was diagnosed in 40.7% of participants, the percentage of stage 3 periodontitis was 29.6%.

The metabolic and periodontal parameters were compared between the preoperative period of sleeve gastrectomy and post-operative 6th months after the surgery and were indicated in Table 2. BMI values, CRP, HDL, LDL, and albumin levels were significantly decreased 6 months later compared to the baseline. Regarding periodontal parameters, while there were no significant changes in PD and CAL measurements, PI, GI, and BOP% levels significantly decreased in the 6th month.

The correlations between changes in periodontal parameters, the percent of total weight loss, and the percent excess BMI loss in the 6-month post-operative period of sleeve gastrectomy were shown in Table 3. A positive significant correlation was observed between the changes in PI and the percent of total weight loss (r=0.301, p=0.027). Also, the changes in BOP% levels showed a positive significant correlation both with the percent of total weight loss and the percent excess BMI loss (r=0.637, p=0.000 and r=0.370, p=0.006 respectively).

Table 1. Demographic characteristics of the study population

Baseline characteristics	Number (n)	Percent (%)
Age (mean ± SD)	41.1±8.89	
Sex		
Female	36	66.7
Male	18	33.3
Education level		
Elementary	6	11.1
High school	24	44.4
Collage and PhD	24	44.4
Smoking status		
Smoker	22	40.7
Non-smoker	32	59.3
Obesity level		
II (35 to 39.9)	17	31.5
III (≥40)	37	68.5
The number of present teeth (mean ± SD)	25.17±3.48	
Periodontitis severity		
Stage I	16	29.6
Stage II	22	40.7
Stage III	16	29.6
Presence of hypertension	21	38.9
Presence of diabetes mellitus	22	40.7
Presence of dyslipidaemia	18	33.3

Table 2. Metabolic and periodontal parameters of sleeve gastrectomy patients before and 6 months after the surgery

Metabolic Parameter	Pre-op	Post-op	*p* value
BMI kg/m^2	41.58±5.25	32.15±4.38	**0.000**
CRP (mg/L)	7.54±6.21	3.91±3.94	**0.048**
AST (U/L)	23.30±15.03	20±8.38	0.661
ALT (U/L)	23.59±14.25	22.63±11.77	0.201
GGT (U/L)	27.44±16.37	25.50±14.28	0.067
HDL (mg/dL)	46.24±10.14	47.97±9.43	**0.003**
LDL (mg/dL)	116.65±26.08	111.72±24.86	**0.022**
Triglyceride (mg/dL)	135.20±55.9	128.22±42.03	0.112
Albumin (g/L)	44.88±6.61	41.69±3.44	**0.000**
HBA1c (%)	6.22±0.76	6.10±0.54	0.063
Periodontal Parameter			
PI	1.27±0.57	1.07±0.41	**0.000**
GI	1.37±0.49	1.33±0.44	**0.004**
BOP (%)	76.80±15.80	74.93±14.81	**0.007**
PD	2.67±0.85	2.65±0.84	0.104
CAL	3.16±1.02	3.16±1.02	0.482

BMI: body mass index, CRP: C-reactive protein, AST: aspartate transaminase, ALT: alanine transaminase
GGT: gamma-glutamyl transpeptidase, HDL: high-density lipoprotein, LDL: low-density lipoprotein, HBA1c: glycated haemoglobin
PI: plaque index, GI: gingival index, BOP: bleeding on probing, PD: probing depth, CAL: clinical attachment level
All variables did not present normal distribution (Kolmogorov-Smirnov test $p<0.001$)
Wilcoxon test, Statistically significant differences are indicated in bold ($p<0.05$ and $p<0.001$)

Table 3. Correlations of the changes in periodontal parameters and the total weight loss and BMI loss at 6 months after bariatric surgery

Periodontal variables	Total weight loss (%)	The percent excess BMI loss (% EBMIL)
PI		
p	0.027	0.057
r	0.301*	0.261
GI		
p	0.797	0.845
r	0.036	-0.027
BOP (%)		
p	0.000	0.006
r	0.637**	0.370**
PD		
p	0.355	0.924
r	0.128	0.013
CAL		
p	0.487	0.187
r	0.097	0.182

PI: plaque index, GI: gingival index, BOP: bleeding on probing, PD: probing depth, CAL: clinical attachment level
p: *p* value, r: correlation coefficient value, * statistical significance at $p<0.05$,** statistical significance at $p<0.001$

Discussion

Obesity and periodontitis are prevalent and significant health issues that affect populations worldwide [24]. Both conditions are associated with low-grade inflammation and can have systemic effects on overall health. Additionally, there is emerging evidence suggesting a bidirectional relationship between obesity and periodontitis, meaning that obesity can increase the risk of periodontitis, and periodontal inflammation can contribute to obesity-related complications [7, 25].

LSG is primarily viewed as a restrictive weight loss procedure, but it has also been found to have significant benefits in treating metabolic derangements and improving metabolic health, beyond its role in achieving weight loss [26]. LSG can have a significant and often more effective impact on the management and treatment of T2DM and its associated complications when compared to the best available medical therapies [26].

CRP is an acute-phase reactant, which is a protein produced by the liver in response to various inflammatory signals, including interleukin-6 (IL-6). CRP is a sensitive inflammation marker in the body, and its levels can increase rapidly in response to inflammation or tissue damage [27]. Increased CRP levels have been associated with central obesity, which, in turn, is related to various health concerns, including atherosclerosis and other macrovascular disorders [28]. Also, many systematic reviews indicated elevated CRP levels in patients with periodontitis [29]. During the 6-month observation period of our study, it was observed that serum CRP levels decreased to almost half of the baseline levels. This decrease in CRP levels indicates that systemic inflammation has decreased. The decrease in circulating CRP levels may have led to improvements in periodontal inflammation.

Dyslipidemia refers to a group of lipid metabolism disorders characterized by abnormalities in the levels of various lipids in the blood, particularly lipoproteins such as high levels of total cholesterol, triglycerides, LDL, and decreased levels of HDL [30]. The suggestion of a two-way relationship between dyslipidemia and periodontal disease highlights the potential bidirectional influence of these two conditions on each other [31]. Possible mechanisms play roles such as a direct effect of oral bacteria or an indirect effect on the elevation of pro-inflammatory mediators, promoting lipolysis and the subsequent elevation of circulating TG and consequently the development of atherosclerotic cardiovascular diseases [32, 33]. In our study, an increase in HDL levels and a decrease in LDL and triglyceride levels were observed in the patients. This result may be another factor that

causes a decrease in periodontal inflammation and an improvement in periodontal parameters. According to our findings, LSG caused a reduction in BMI resulting in a decrease in PI, GI, and BOP which represents periodontal inflammation. Furthermore, no changes were observed in CAL and PD. Consistent with our study, BMI, PI, BOP, and PD were found to decrease significantly according to the results of the 1-year follow-up cohort study. As a difference, the decrease in PD levels was not significant in our study. The reason for this may be that the 6-month follow-up period is not sufficient for PD reduction [34]. In contrast with our study results; de Moura Grec et al. [13] observed an improvement in clinical systemic conditions 6 months after BS, nevertheless, PD and CAL increased after surgery significantly compared to baseline. They associated this situation with nutritional deficiencies caused by bariatric surgery, changes in eating habits, or inadequate periodontal status of the patients before surgery. According to the results of a study, a decrease in BMI caused an increase in BOP for 12 months. The authors stated that at least 2 years are required after surgery for the stabilization of physiological processes and inflammatory profile, and that the nutritional imbalance that occurs after the surgical process may cause this result. Additionally, unlike our study, this study focused on patients who had laparoscopic RYGB. Postoperative recovery of these patients develops differently from LSG. In concordance with our study Jaiswal et al. [15] found a significant decrease in bleeding score, plaque, and gingival index as a result of 6-month follow-up of patients with BS, they did not find a significant change in PD and CAL levels.

Ghrelin is a peptide hormone and has roles in various physiological processes, including growth hormone secretion regulation, energy metabolism, and food intake [35]. With the help of LSG, the fundus region of the stomach, that is, the area where the ghrelin hormone is predominantly secreted, is removed LSG provides a decrease in ghrelin concentrations [36] compared to other restrictive techniques [37] or RYGB [38]. Two different forms of ghrelin (des-acylated ghrelin and acylated ghrelin), have been studied for their distinct roles in these processes [35]. Serum total ghrelin concentration was positively correlated with pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and IL-6 [39]. Total and acylated ghrelin levels in serum were elevated in chronic periodontitis patients [40]. The levels of ghrelin in gingival crevicular fluid in patients with both periodontitis and T2DM, ghrelin levels in GCF were higher compared to periodontally healthy individuals with T2DM [41]. The observation that levels of TNF-α and IL-6, tend to decrease at 6 months after BS [42]. The increase in circulating levels of adiponectin is known to have anti-inflammatory and insulin-sensitizing properties that have been demonstrated after BS [43]. The observed decrease in GI and BOP may be due to a decrease in inflammatory cytokines and decreased ghrelin concentration after LSG. The acknowledgment of the inability to determine which specific factor or factors contributed to the improvements in periodontal status following LSG is an important limitation of the present study. This limitation underscores the complexity of the interactions between systemic health, lifestyle, and oral health.

The reduction of body fat causes a decrease in systemic inflammation and a decrease in the progression of all diseases linked to obesity (hypertension, diabetes, cardiovascular diseases). According to our findings in this present study, a reduction in BMI led to a decrease in periodontal inflammation but no changes were observed in terms of CAL.

Conflict of interest: No conflict of interest was declared by the authors.

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Author contributions

A.L.A. was responsible for drafting the manuscript. A.L.A., G.T.C. and M.R.A. were responsible for Data collection and Interpretation and for drafting the manuscript. A.L.A. was responsible for conceptualization, funding acquisition, design of the study, supervision, and drafting of the manuscript. M.R.A. was responsible for surgical intervention. A.L.A. and G.T.C. were responsible for data analysis and interpretation. All authors reviewed the article critically for important intellectual content and approved the final version to be submitted.

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Evaluation of children with dysfagia

Disfajisi olan çocukların değerlendirilmesi

Sevinç Garip

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Abstract

Purpose: Difficulty in swallowing; It is a symptom that occurs as a result of mechanical inhibition of the transfer of ingested food from the mouth to the stomach. It can be due to acute and chronic diseases. It is presented because of the limited number of studies comparing the characteristics of both types of oropharyngeal and esophageal dysphagia in healthy and chronically ill children in the pediatric population.

Materials and methods: 201 pediatric patients admitted with dysphagia between May 2019 and November 2020 were included. They were grouped according to the types of dysphagia.

Results: Group 1 consisted of 80 pediatric patients with oropharyngeal dysphagia, while Group 2 consisted of 121 pediatric patients with esophageal dysphagia. 51.7% of the patients were female, mean age was 9.4 years. While all patients in Group 2 had solid food dysphagia, Group 1 had 27% liquid and 53% solid-liquid dysphagia. Percutaneous endoscopic gastrostomy tube for 50 pediatric patients who could not be fed safely; a nasogastric feeding tube was placed in 4 children. Esophagogastroduodenoscopy was performed in 72.6% of the patients, and esophageal pathology was detected in 55.4%.

Conclusions: Although the incidence of dysphagia is high in children with chronic diseases, it should not be forgotten that it can also be seen in healthy children and may be associated with treatable.

Keywords: Child, dysphagia, gastroenterology, swallowing.

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Öz

Amaç: Yutma güçlüğü; alınan gıdanın ağızdan mideye transferinin mekanik olarak engellenmesi sonucu oluşan semptomdur. Akut ve kronik hastalıklara bağlı olabilir. Pediatrik popülasyonda sağlıklı ve kronik hastalığı olan çocuklarda, orofarengeal ve özofageal her iki tip disfajiyi kapsayan, özelliklerini karşılaştıran çalışma sayısı az olması nedeniyle sunulmuştur.

Gereç ve yöntem: Mayıs 2019 ve Kasım 2020 arasında disfaji şikayeti ile başvuran 201 çocuk hasta dahil edildi. Disfaji tiplerine göre gruplandırıldı.

Bulgular: Grup 1 orofarengeal disfaji olan 80 çocuk hastadan oluşurken Grup 2 özofageal disfaji olan 121 çocuk hastadan oluşmaktaydı. Hastaların %51,7 kız, ortalama yaş 9,4 yıldı. Grup 2'de tüm hastalarda katı gıda disfajisi varken, Grup 1'de %27 sıvı %53 katı-sıvı disfaji vardı. Güvenli oral beslenemeyen 50 çocuk hastaya perkütan endoskopik gastrostomi tüpü; 4 çocuk hastaya nazogastrik beslenme tüpü yerleştirildi. Hastaların %72,6 özofagogastroduodenoskopi yapıldı, %55,4'ünde özefagus patolojisi saptandı.

Sonuç: Disfajinin kronik hastalığı olan çocuklarda görülme sıklığı yüksek olsa da sağlıklı çocuklarda da görülebileceği ve tedavi edilebilir hastalıklarla ilişkili olabileceği unutulmamalıdır.

Anahtar kelimeler: Çocuk, disfaji, gastroenteroloji, yutma.

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Introduction

Swallowing is a complex function made possible by the coordinated work of the oral cavity, pharynx, larynx and esophagus. For safe swallowing, the oral cavity, pharynx muscles, cranial nerves, upper and lower esophageal sphincter, esophageal muscles and stomach must work in coordination. Dysphagia, which means difficulty in swallowing, is a symptom that occurs as a result of mechanical inhibition of the transfer of ingested food from the mouth to the stomach, decreased strength of the muscles that provide the swallowing movement, or deterioration of coordination [1].

There are 3 stages of swallowing; oral, pharyngeal and esophageal. Oral stage; is the transfer of food to the pharynx by chewing and breaking it into small pieces. The pharyngeal stage is involuntary. Food is safely passed into the esophagus in less than a second. In the esophageal stage; the bite is transmitted to the stomach by primary and secondary peristaltic movements of the esophagus. All three components of swallowing in infants are reflex and involuntary, and the oral stage becomes controlled over time [2]. Transfer dysphagia occurs in the oropharyngeal stage and results from neurological, myopathic and metabolic causes. Nontransfer dysphagia, on the other hand, is often caused by intrinsic diseases of the esophagus, mechanical or motility disorders in the esophageal stage [3]. Dysphagia in children can be seen at any age, and it can adversely affect growth and development, most commonly with feeding and/or respiratory problems. It is necessary to define and manage appropriately in a multidisciplinary manner. There are few studies comparing the characteristics of both types of oropharyngeal and esophageal dysphagia in the pediatric population. Therefore, we aimed to present the common causes, types, clinical features, laboratory, imaging techniques and results of dysphagia in patients with neuromuscular, neurometabolic, allergic or syndromic abnormalities and healthy children, together with the current literature.

Material and methods

201 pediatric patients who applied to the Pediatric Gastroenterology outpatient clinic of Health Sciences University Adana City Training and Research Hospital between May 2019 and November 2020 with the complaint of dysphagia were included. They were grouped according to the types of dysphagia. Group 1 consisted of 80 patients with oropharyngeal dysphagia and group 2 consisted of 121 patients with esophageal dysphagia. Age, gender, type, characteristics and duration of dysphagia, respiratory system and gastrointestinal system complaints, accompanying allergic, neurological, metabolic diseases, height and weight percentiles were evaluated. To detect the presence of iron deficiency anemia, hemogram, serum iron, iron binding capacity, ferritin; Vitamin B12 levels and folic acid levels for vitamin deficiency, thyroid function tests, total Ig E for allergic diseases, and food and respiratory tract-specific antigen test results were examined retrospectively. Esophagography was performed to detect anatomical abnormalities. Multiple biopsies were taken by esophagogastroduodenoscopy. Since there is no study in the literature that includes both oropharyngeal and esophageal type dysphagia in the pediatric age group, all data were evaluated in the light of the current literature. The study was approved by the scientific research ethics committee of our university.

The parametric descriptive statistics of the numerical data in the study group were calculated as the mean, standard deviation, and the median (min-max) of the non-parametric ones, and categorical data were given as percent (%). Chi-square test was used for comparison between groups. The limit of significance was accepted as $p<0.05$.

Results

Two hundred and one pediatric patients were included in the study, 51.7% of them were women and the mean age was 9.4 years. The rate of dysphagia in pediatric patients was 40% for oropharyngeal and 60% for esophageal. The characteristics of the patients are presented in Table 1. Eighty percent (64 patients) of 80 patients with oropharyngeal dysphagia were followed by pediatric neurology and metabolism clinics. Those with hemoglobin and hematocrit levels below 2 age-appropriate standard deviations in the complete blood count were defined as iron deficiency anemia. Those with vitamin B12 levels below 200 pg/ml were defined as vitamin B12 deficiency. Of 121 patients with esophageal dysphagia, 55.4% had esophageal pathology. There was

no neurometabolic disease in the esophageal dysphagia group. Esophageal symptoms such as nausea, vomiting and pyrosis were 91% in the esophageal dysphagia group. Of the 85 patients with respiratory system symptoms such as cough, hoarseness, cyanosis while feeding, wheezing, and frequent lung infection, 74% were children with neurometabolic disease in the oropharyngeal dysphagia group. Fourteen percent of those with dysphagia were accompanied by odynophagia. When pediatric patients with odynophagia are examined; Reflux esophagitis was detected in 8 pediatric patients, a history of drinking corrosive substances in 6 pediatric patients, foreign body ingestion in 3 pediatric patients, ulcers in 3 pediatric patients, candida esophagitis in 2 pediatric patients, and eosinophilic esophagitis in 2 pediatric patients. When the growth of the patients was evaluated, the weight was below the 3rd percentile in 33% of 201 patients. The percentiles of cases with acute dysphagia, which developed especially after ingestion of foreign bodies and ingestion of corrosive substances, were normal. Nutritional support was provided by placing a percutaneous endoscopic gastrostomy tube in 50 pediatric patients who could not be fed safely due to chronic dysphagia, and a nasogastric feeding tube was placed in 4 pediatric patients. In the esophageal dysphagia group, patients with a weight below the 3rd percentile had esophageal taste and ulcers due to different reasons. Pathology was detected in 30 of 160 patients who underwent contrast-enhanced esophagography. Esophageal stricture was found in 12 of 18 pediatric patients with esophageal pathology, gastroesophageal reflux stage two in 5, and hiatal hernia in 1 patient. Esophageal symptoms such as nausea, vomiting and pyrosis were 91% in the esophageal dysphagia group. Respiratory system symptoms such as cough, hoarseness, cyanosis when feeding, wheezing and frequent lung infections were present in 85 patients. Of these, 74% were children with neurometabolic disease in the oropharyngeal dysphagia group. The accompanying complaints according to the type of dysphagia are presented in Figure 1. 14% of those with swallowing difficulties were accompanied by odynophagia. Eight patients with odynophagia had reflux esophagitis, 6 patients had corrosive substance ingestion, 3 patients had foreign body, 3 patients had ulcers due to stenosis due to achalasia, 2 patients had candida esophagitis, 2 patients had eosinophilic esophagitis. High resolution monometry was performed in 3 pediatric patients who could adapt to manometry, which was thought to be achalasia due to esophageal stenosis, and a diagnosis of type 2 achalasia was made. Peroral endoscopic myoromy was performed in 3 patients diagnosed with type 2 achalasia. Dilation was performed on young patients with cricopharyngeal achalasia and other stenoses such as Shatzki ring. Esophagogastroduodenoscopy was performed under anesthesia in 72.6% of 201 patients with dysphagia. The diagnoses of the patients according to the groups are shown in Table 2; concomitant diseases are presented in Table 3.

Table 1. Characteristics of the patients

	All patients	**Group 1 (Oropharyngeal)**	**Group 2 Esophageal)**
Number of patients (n)	201	80	121
Gender (M / F)	97/104	44/36	53/68
Age (months)	112	66	144
Type of dysphagia			
Solid	107	14	93
Liquid	30	27	3
Solid+Liquid	64	42	22
Dysphagia Duration (months)	11.3	13.8	9.4
Extraesophageal Symptoms	85	63	22
Esophageal Symptoms	183	76	107
Allergic Disease	24	0	27
Neurometabolic Disease	64	64	0
Weight <3 p	67	48	19

Table 2. Diagnosis of patients by groups

Oropharyngeal Dysphagia (n:80)		Esophageal Dysphagia (n:121)	
64	Neurometabolic Disease	31	Reflux esophagitis
4	Cleft Palate	13	Heterotopic gastric mucosa
2	Crichopharyngeal Achalasia	13	Gastroesophageal reflux
2	Shatszki Rings	13	Vitamin B12 deficiency
2	Esophageal Ulcer	13	Vitamin B12 deficiency+ Iron deficiency
2	Globus hysterical	7	Eosinophilic esophagitis
1	Bicuspid Aorta	6	Pulmunary Winson Syndrome
1	Reflux esophagitis	6	Ccorrosive substances
1	Iron deficiency anemia	5	Gastritis
1	B12 vitamin deficiency	3	Foreign body ingestion
		3	Type 2 Achalasia
		2	Candida esophagitis
		1	Scleroderma
		1	Congenital Heart Disease
		1	Esophageal polyp
		1	Hypothyroidism
		1	Pyloric stenosis

Table 3. Concomitant diseases

Neurometabolic Genetic Diseases Associated with Oropharyngeal Dysphagia (n)	Concomitant Diseases in Esophageal Dysphagia (n)
Down Syndrome (2)	Scleroderma
Syndromic Patient (2)	Familial Mediterranean Fever
Nieman Pcik Type B (1)	Celiac Disease (1)
Cerebral palsy+ Epilepsy (34)	Crohn’s Disease (2)
Pontocerebellar hypoplasia type10 (1)	
West Syndrome (5)	
Sandorff Syndrome (3)	
MSUD	
Cri de Cat Syndrome	
Neurometabolic (3)	
SMA (3)	
Canavan Syndrome	
Propionic Acidemia	
Jaberi elahi syndrome	
Trisomy 18	
Glutaric Acid type	
Gikohen Warehouse type 1b	
Bochledek Syndrome	

Discussion

Dysphagia is seen in every age group for very different reasons. Oropharyngeal and esophageal distinction should be made accurately and quickly, and life-threatening conditions should be controlled and treated. Children with neurometabolic or neuromuscular disorders and chronic gastrointestinal disease are at higher risk of dysphagia than healthy children. Swallowing problems can prevent development by preventing adequate energy and nutrient intake. In necessary patients, feeding should be done with a gastrostomy tube.

In a study by Bhattacharyya [4], in the United States, the annual incidence of pediatric swallowing problems was found to be 0.9%. In different studies, swallowing disorders are seen at a rate of 20-40% in healthy children, while the rate increases to 80% in children with neuromuscular, neurometabolic disease, traumatic brain injury or airway malformations [5-7]. Of the 201 children in our study, 55.2% (111 patients) were healthy and 44.7% (90 patients) had neuromuscular, neurometabolic, genetic, allergic and rheumatological diseases. When past studies on children with oropharyngeal and esophageal swallowing difficulties are examined; Svystun et al. study [8] 54% male, 46% female (128 patients), Lefton Greif et al. study [9] 68.5% male 32.5% female (19 patients), Sheikh et al. study [10] 69.3% were male and 30.7% were female (13 patients). Differently, our study consisted of 51.7% female patients. The number of female patients in the esophageal dysphagia group was 68 and 36 in the oropharyngeal dysphagia group. Female gender was 1.8 times higher in the esophageal dysphagia group. History of eating and swallowing, motor and language developmental stages, medical history in a child presenting with dysphagia; especially respiratory system history, presence of gastroesophageal reflux disease, medications used, history of surgery, presence of allergy, communication of the feeder, nutritional environment should be questioned in detail, body weight and weight gain should be recorded [6, 11]. In our study, the number of patients in group 1 consisting of oropharyngeal dysphagia patients was 80, and group 2 consisting of esophageal dysphagia patients was 121. In group 1; 80%, 64 patients, were followed by pediatric neurology and pediatric metabolism clinics, and their neuromotor development was slower than their age. Due to the structure of oropharyngeal dysphagia, liquid foods cannot be advanced to the esophagus despite chewing and repeated swallowing [12].

Laryngeal penetration occurs when food enters the laryngeal vestibule. Aspiration occurs when the food fall below the level of the vocal cords. Asphyxiation occurs when a bolus physically blocks the airway and can be life-threatening [13]. Cough, drooling, increased secretion, regurgitation, apnea, unexplained respiratoryproblems, and vomiting may ocur [14]. Consistent with the study of Steele and Cichero [12], especially in our oropharyngeal group there was dysphagia to liquid foods. Eighty percent of the patients in this group were children who could not take solid food due to chewing and swallowing problems and were fed only liquid or blenderized foods. In our study, Lefton Greif et al. [14] similar to the studies, laryngeal penetration and aspiration findings such as choking, coughing attacks, increased secretion, drooling, regurgitation, cyanosis while feeding, and frequent respiratory tract infections were present in 79% of our patients, but they could not be demonstrated by swallowing tests. In our study, it was performed in only 3 patients, since the swallowing center was not located in our city in pediatric patients with oropharyngeal dysphagia. It was one of the shortcomings of our study, it shows the need for studies with more VFYC and FEES.

In esophageal dysphagia; during the passage of food into the esophagus, the upper esophageal sphincter cannot relax. There may be coughing, throat clearing, pyrosis, burping, vomiting, abdominal pain, and unexplained weight loss [7]. The rate of solid food dysphagia was 86.7% in the esophageal dysphagia group. Esophageal symptoms such as vomiting, abdominal pain and pyrosis were accompanying 9% of 121 patients. In group 2, 23% of the patients who developed ulcers in the esophagus as a result of ingestion of corrosive substances, foreign body ingestion, candida esophagitis and type 2 achalasia stricture were accompanied by odynophagia.

When the growth of the patients was evaluated, in 201 patients, the weight was 33.3% below the 3rd percentile and 10% was in the 3-10th percentile. In the literature, Svystun et al. [8] 9%, Sheikh et al. [10] 10%; Lefton Greif et al. [9] 10% found growth retardation due to malnutrition. Weight percentiles were found to be significantly lower in patients with oropharyngeal dysphagia. It was thought that the nutritional problems related to the primary disease were related to the long-term duration. Height percentiles of the patients were also found below the 3% percentile. On the other hand, height and weight percentiles were normal in patients with swallowing complaints after acute ischemic events such as traffic accident, falling and drowning. Patients with a weight below the 3rd percentile who could not be fed safely due to dysphagia were fed with a percutaneous endoscopic gastrostomy tube. Examining the studies, Sheikh et al. [10] placed a gastrostomy tube at a rate of 30.8% and Svystun et al. [8] at a rate of 12.6%. In our study, percutaneous endoscopic gastrostomy tube was placed in 24.8% of the patients, and feeding was provided safely by inserting a nasogastric feeding tube in 4 pediatric patients.

The most commonly used swallowing evaluation methods in the analysis of oral, pharyngealand esophageal phases in the evaluation of pediatric feeding and swallowing disorders; videofluoroscopic swallowing study (VFYC) and fiberoptic endoscopic swallowing study (FEES) [15]. In the literature, there are many pediatric nutrition and swallowing assessment tools developed for use in certain age ranges. VFYC and FEES developed by Benfer et al. [16] are both accepted as the gold standard in diagnosis when applied and interpreted by experienced clinicians. However, the lack of sufficient and experienced personnel to implement and interpret it, and the patient's inability to cooperate at a level to fulfill simple eating-swallowing instructions reduces its use [7]. In our study, only 3 patients underwent videofluoroscopic swallowing study, and they were trained because of thedifficult accessibility in our city. Oropharyngeal dysphagia is more common in neuromuscular and neurometabolic diseases because neuromuscular communication is not intact. My center is in the region where the rate of consanguineous marriage is common, this type of swallowing problem is common, but there is no center that evaluates swallowing. It again emphasizes the inadequacy of the necessary devices and the number of researchers and centers with appropriate knowledge and experience for the assessment of swallowing.

In iron deficiency and vitamin B12 deficiency, cytokine expression is impaired, oxidative enzymes are decreased, white matter myelination is impaired, webs are formed in the pharyngeal muscles as a result of atrophy and myopathy. Neurogenic difficulty in swallowing, nausea, and vomiting develop [17, 18]. Plummer-Vinson syndrome (PVS); a clinical condition characterized by dysphagia, upper esophageal web, and iron deficiency anemia [19]. Webs, a rare cause of upper cervical dysphagia, are seen in 5-15% of patients with dysphagia [20]. The webs may be multiple, and if the lumen is narrower than 2 cm, dysphagia is observed, so most of them are asymptomatic [21]. The diagnosis is clinically made with intermittent dysphagia to solid foods associated with chronic iron deficiency anemia, and esophageal webs are observed only in 10% of patients radiologically and endoscopically. For the diagnosis of PVS, esophageal web is not always necessary in a patient with iron deficiency, but dysphagia must be present. The first stage of treatment is the correction of iron deficiency and vitamin B12 deficiency. Although webs persist after iron therapy in non-obstructive PVS patients, esophageal motility returns to normal, resulting in significant improvement in dysphagia [21]. In our study, 201 pediatric patients with dysphagia were found to have 3% iron deficiency anemia, 6.5% vitamin B12 deficiency, 6% iron deficiency and vitamin B12 deficiency. All of the patients were in the esophagealdysphagia group and had no accompanying pathologies. In our study, similar to the Godino and Wong [20] study, no web was detected radiologically or endoscopically. Unlike the study of Novacek [22]. The rate of female patients was 71.5%, which was approximately three times higher than that of men. A different theory in its development is that it develops after an immunological process that triggers the formation of autoantibodies against the esophagus [21]. The reason why the rate of female patients was 3 times higher in our study suggested that it may be due to autoimmune etiopathogenesis. In our study,

other autoimmune diseases were not evaluated in patients diagnosed with PVS, leading to more comprehensive and larger studies to test these possibilities.

Another cause of dysphagia is muscular or mucosal rings in the lower part of the esophagus that narrow the lumen. Schatzki [23] ring is the most common type, its incidence is 0.2-15% in the literature; in children, it is 0.2%. Etiopathogenesis includes fibrotic changes in the distal esophagus due to acid reflux, Barrett's esophagus, and eosinophilic esophagitis [24]. Although patients are mostly asymptomatic, episodic dysphagia and food retention, especially against solid foods, occur. Diagnosis is made by barium radiography or upper endoscopy [25]. In our study, Schatzki [23] ring was detected with a rate of 1.5%, patients had difficulty in swallowing against solid foods, vomiting and pain when passing food in the distal esophagus. The stenosis of the distal esophagus was also detected in the esophagogastroscopy in barium X-ray. In two patients, biopsies were taken and dilatation was performed in the treatment in patients who could not progress to the stomach due to stenosis. Although Schatzki [23] ring is very rare in children, it should definitely be considered because it is an important treatable cause of dysphagia.

Dysphagia; can be oropharyngeal or esophageal after corrosive substance ingestion. Solid and powdery ones stick to the mouth, pharynx and larynx and cause oropharyngeal dysphagia, while those with liquid consistency due to esophageal damage; cause esophageal dysphagia [26, 27]. In the study of Kutlu et al. [28] 48% of children who underwent endoscopy after ingestion of corrosive substances were stage 1, 20% stage 2, 5.7% stage 3 esophagitis, in the study of Previtera et al. [29] 28% stage 1, 9.2% stage 2, 4.8% stage 3 esophagitis, only 2.5% esophageal stenosis was detected. In our study, the rate of dysphagia due to corrosive substance intake was 3%, while 50% of the patients were women, the mean age was 84 months. Similar to the study of Moulin et al. [27], all patients had complaints of esophageal dysphagia and odynophagia was accompanied by vomiting. In esophagogastroduodenoscopy, stage 3 esophagitis with esophageal stenosis in 50% of patients, stage 1 in 33%, and stage 2 esophagitis in 17% were detected. It was thought that the high rate of stenosis and esophagitis patients was due to the low number of patients and the fact that the patients came after taking corrosive substances for a long time and had severe feeding and swallowing problems.

Achalasia; develops due to primary motility disorders of the esophagus and is rarely seen in children. According to the region where the dysfunction originates; It is defined as cricopharyngeal achalasia above and thoracic achalasia distally [30]. Cricopharyngeal achalasia is a relaxation problem due to spasm in the cricopharyngeal muscle during swallowing, ganglion cell loss is absent in this type. It can occur for many reasons. Partial relaxations, premature contractions, or failures in upper sphinter relaxation cause dysphagia. In the esophageal passage graphy taken for diagnostic purposes, narrowing in the esophagus passage is detected at the level of the cricopharyngeal muscle [31]. In our study, cricopharyngeal achalasia was found in only 2 patients, and both patients were children with neurological disease, which is consistent with the literature. Patients with narrowing of the esophageal passage at the level of the cricopharyngeal muscle in the esophageal passage X-ray were referred to pediatric surgery for treatment.

Thoracic achalasia is a motility disease of the esophagus that occurs as a result of high resting pressure in the lower esophageal sphincter (LES) and insufficient relaxation of the LES during swallowing. The etiopathogenesis is not clear. The most common reason for admission is the difficulty in swallowing against solids and liquids, which has been increasing for several years. Regurgitation of undigested food, nocturnal cough due to microaspiration, recurrent pneumonia, chest pain, retrosternal burning and weight loss can be observed [32]. Domingeus et al. [33] detected progressive substernal dysphagia with solid and liquid foods, and Zhang et al. [34] and Hussain et al. [35] reported dysphagia as the most common symptom in pediatric patients with achalasia. In our study, 1.5% of the patients were diagnosed with achalasia. In accordance with the literature, all patients had esophageal dysphagia against solids and liquids, which increased over time for about a year. It was accompanied by retrostenal burning, vomiting, and weight loss. In the esophageal passage X-ray, which is the

first diagnostic test, a narrowing in the shape of a bird's beak at the extreme megaesophagus and esophagocardiac junction is diagnostic. Esophagogastroduodenoscopy may reveal enlargement of the esophagus, esophagitis due to food residues in the esophageal lumen, and ulcers. High-resolution impedance manometry is the gold standard diagnostic method, enabling its typing [36]. Zhang et al. [34] detecting goush-beak appearance in patients, performed esophagogastroduodendoscopy in 61.5% of the patients, and the diagnosis was confirmed by manometry. In our study, esophageal radiography was performed in 3 patients with distal achalasia, and distal stenosis, bird's beak appearance and megaesophagus were detected in accordance with the literature. Esophagogastroduodenoscopy revealed stenosis hyperemia and ulcer covered with white membrane in the distal esophagus. The diagnosis of type 2 achalasia was confirmed by high resolution monometry.

Inlet patch was first described by Schmidt in 1805. It is a heterotopic gastric mucosa located in the proximal esophagus as a result of a problem in the transformation of the esophageal mucosa from columnar epithelium to squamous epithelium or healing of the esophageal epithelium due to infection, trauma, and regurgitation [37]. It can be separated by sharp borders, varying in size from 2-3 mm to 4-5 cm, on the posterior or lateral wall, in single or multiple pieces [38]. In our study, 71% of 201 pediatric patients with dysphagia were treated with esophagogastroduodenoscopy and 13 children 1 to 3 HGM with diameters ranging from 10 mm to 25 mm were detected in the patient, located in the upper esophagus. Its frequency in pediatrics is 1.4-6%, and the frequency in our study was found to be 6.4% in line with the literature. Inlet patch is usually asymptomaticand dysphagia is seen in 15-39% of cases [39]. Other complaints are sore throat due to laryngopharyngeal reflux, globus sensation, retrosternal burning, regurgitation, chest pain, and symptoms due to acid production and colonization of Helicobacter pylori. In our study, the main complaint of all patients with HGM was dysphagia, which was against solids and liquids. Abdominal pain, vomiting, bad breath and stomach pain accompanied by the feeling of being stuck were other complaints. Although it is rarely seen in pediatric patients presenting with dysphagia, HGM should be remembered, therefore, it should be performed by experienced endoscopists, and it should be passed slowly and carefully when entering the upper esophagus with the endoscope.

Eosinophilic esophagitis (EoE); As a result of eosinophilic inflammation in the esophagus, it causes esophageal dysfunction and dysphagia [40]. Its frequency varies between 1% and 37% [41]. Cheung et al. [42] 88%; Desai et al. [43] 54.8% detected EoE. In our study, the rate of patients diagnosed with EoE was 3.4%, with more than 20 eosinophils, basal cell hyperplasia, and eosinophilic micro-abscesses detected only in esophageal biopsy samples in the histopathological examination. There are different clinical findings in eosinophilic esophagitis. Food refusal, vomiting, restlessness, and developmental delay are seen in young children. In older children, dysphagia against solid foods, food insertion, chest pain, pyrosis, abdominal pain, vomiting, chewing a lot, keeping food in the mouth are detected most frequently. In our study, 4 patients had esophageal dysphagia to solids and liquids, and 3 patients to liquids only. Gastrointestinal complaints such as abdominal pain, vomiting and pyrosis were accompanied by 57%. Upper gastrointestinal endoscopy should be performed for EoE in allergy patients with dysphagia. In the endoscopy, red lines, white exudates, hyperemic fragile mucosa, ringing are detected in the esophagus [44]. Liacouras et al. [40] and Prasad et al. [45] studies, one out of three patients with histological EoE was found to be normal by endoscopy. In our study, ring formation, white dot lesions and grooves in the esophagus were detected in 57.1 %. Hyperemic fragile mucosa was seen in 71%. Esophageal mucosa was normal in 28.5%. As in the studies of Liacouras et al. [40] and Prasad et al. [45], although the endoscopic appearance is normal, it supports the necessity of taking multiple biopsies for histopathological examination.

In conclusion, a comprehensive evaluation should be performed in children with dysphagia. Under the leadership of pediatric gastroenterologists, pediatric neurology, child metabolism, otolaryngology, swallowing-speech therapists should work in cooperation.

This study is retrospective. It would be useful to perform swallowing tests in patients

with difficulty swallowing. However, test can be performed on a limited basis due to the difficulty of performing tests in the pediatric age group and the small number of experienced specialists who will perform and interpret them. It could be performed on a limited number of patients in our study.

We believe that our series will contribute to the literature by being one of the few studies in the literature that includes both types of dysphagia in childhood, conducted in a single center with 201 patients. It will shed light on a well-designed study with prospective limitations.

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Research Article

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Complications in digital subtraction angiography: initial three years of experience

Dijital substraksiyon anjiografide komplikasyonlar: ilk üç yıllık deneyim

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Abstract

Purpose: In this study, we describe the complications we encountered during digital subtraction angiographies (DSA) in our initial three years of experience and evaluate the risk factors in our patient population.

Materials and methods: A series of 507 patients with different pathological processes were diagnosed via DSA in our institution from April 2019 through May 2022 and were retrospectively evaluated. During DSA, the date of the procedure, patient age, gender, comorbidities, catheter types, number of catheters used, and all procedure-related complications were recorded, even if they did not cause any neurological sequelae. Complications were categorized as neurological, non-neurological, or local.

Results: Our study included a total of 507 patients. Of these, 256 (50.5%) were male, and 251 (49.5%) were female. The mean age of patients was 49.2 years (range 5-91). The most preexisting comorbidity in patients was hypertension (22.5%). Of 507 patients, a total of 10 patients had either a neurological complication, radiological complication, or angio-site-related complication, and the overall rate of complications was 0.02%. In 6 patients with neurological complications, 3 (0.6%) had permanent neurological deficits, and 3 (0.6%) had transient deficits. In 4 patients with non-neurological complications, asymptomatic vasospasms were encountered in two cases; internal carotid artery (ICA) dissection was experienced in one case, and scrotal hematoma was observed in one case.

Conclusion: Complications following DSA are rare but must be minimized with knowledge of the characteristics of the patients and determining the proper indication. Although the risk is low, complications such as thromboembolism can cause permanent neurological deficits and even death.

Keywords: Digital subtraction angiography, neurological complication, non-neurological complication, catheter, vasospasm.

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Öz

Amaç: Bu çalışmada ilk üç yıllık deneyimimiz boyunca dijital substraksiyon anjiyografilerde (DSA) karşılaştığımız komplikasyonlar anlatılmış olup, hasta popülasyonumuzdaki risk faktörleri değerlendirilmiştir.

Gereç ve yöntem: Kurumumuzda Nisan 2019'dan Mayıs 2022'ye kadar farklı patolojilere sahip 507 hastaya DSA işlemi uygulandı ve sonuçlar retrospektif olarak değerlendirildi. Anjiyografi sırasında herhangi bir nörolojik sekel saptanmasa bile işlem tarihi, hastanın yaşı, cinsiyeti, yandaş hastalıkları, kateter tipleri, kullanılan kateter sayısı ve işleme bağlı tüm komplikasyonlar kaydedildi. Komplikasyonlar nörolojik, nörolojik olmayan veya lokal olarak kategorize edildi.

Bulgular: Çalışmamıza toplam 507 hasta dahil edildi. Hastaların 256'sı (%50,5) erkek, 251'i (%49,5) kadın olmakla beraber, ortalama yaşı 49,2 (5-91 arası) olarak saptandı. Primer patolojilere en çok eşlik eden hastalığın hipertansiyon olduğu görüldü (%22,5). 507 hastadan toplam 10 hastada nörolojik komplikasyon, radyolojik komplikasyon veya anjiyo bölgesine bağlı komplikasyon görüldü ve genel komplikasyon oranı %0,02 olarak bulundu. Nörolojik komplikasyon gelişen 6 hastanın 3'ünde (%0,6) kalıcı nörolojik defisit, 3'ünde (%0,6) ise geçici nörolojik defisit saptandı. Nörolojik olmayan komplikasyon gelişen 4 hastada iki olguda asemptomatik vazospazm, bir olguda internal karotid arter (İKA) diseksiyonu, bir olguda skrotal hematom gözlendi.

Sonuç: Dijital substraksiyon anjiografi esnasında komplikasyonlarla nadir olarak karşılaşılsa bile hastaların özelliklerinin bilinmesi ve uygun endikasyonun belirlenmesi ile komplikasyon oranı en az seviyeye indirilebilir. Risk oranının düşük olmasına ragmen tromboembolizm gibi komplikasyonlar kalıcı nörolojik defisitlere ve hatta ölüme neden olabilir.

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Anahtar kelimeler: Dijital substraksiyon anjiografi, nörolojik komplikasyon, nörolojik olmayan komplikasyon, kateter, vazospazm.

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Introduction

Efforts to visualize the vessels in the human body began with the discovery of X-rays by Roentgen [1]. In 1895, Haschek and Lindenthal obtained radiographs of blood vessels in cadavers. Afterward, Barberish and Hirsh began to visualize the arterial and venous system of the arm in 1923. Later, using a similar technique to Barberish and Hirsh, Brooks envisioned the blood circuit of legs [2, 3]. Finally, human intracranial circulation was successfully visualized by Egas Moniz in 1927 by using a radiopaque contrast agent [4]. Since then, with the improvements in catheterization techniques, injectors, subtraction, and magnification techniques, cerebral digital subtraction angiography (DSA) has been widely used to diagnose vascular abnormalities and cerebral pathologies and has become the gold standard imaging method to assess vascular pathologies [1, 5-11]. Although DSA was accepted as a safe way to visualize the cerebral vascular system, it is an invasive diagnostic tool and can cause complications [12].

The most severe complications caused by DSA are related to the neurological system. Although most neurological complications are transient, permanent neurological deficits can be seen because of cerebral infarction, and even death can be seen [13-17]. Large prospective series shows that permanent neurological complication rates change between %0.1-1.3 [18, 19]. Besides neurological complications, systemic and local complications such as contrast-induced allergy, contrast nephropathy, entrance side hematoma, amnesia, femoral artery occlusions, arterial dissections, or infections may seriously affect patients [15, 20-22].

In this study, we describe the complications we encountered during diagnostic cerebral angiographies during our initial three years of experience and evaluate the risk factors in our patient population.

Materials and methods

Patient data and outcome assessment

A series of 507 patients with different pathological processes were diagnosed via DSA in our institution from April 2019 through May 2022 and were retrospectively evaluated. The study did not include patients undergoing angiography as a part of interventional therapeutic procedures.

All patients underwent a detailed neurological examination at admission, during the DSA procedure, discharge, and follow-up. During the angiography, the date of the procedure, patient age, gender, comorbidities, and all procedure-related complications were recorded, even if they did not cause any neurological sequelae. Complications were evaluated as follows: neurological complications such as thromboembolism, intracranial hemorrhage, or ischemia, which generates new neurological signs or symptoms or worsening of preexisting deficit after the procedure; non-neurological complications which are radiologically revealed, such as asymptomatic vasospasm or dissection and local complications such as groin hematoma which are related to the puncture site.

Written informed consent was obtained from patients, implying that their medical records and images could be used for research in the future. The study was approved according to the ethical standards of the Declaration of Helsinki.

DSA technique

All angiographies were performed with the patient under local anesthesia or sedation via the femoral approach using Siemens Artis Zee monoplane angiography unit. Oxygen saturation and blood pressure levels were measured continuously. In our daily practice, internal carotid and vertebral arteries were selectively catheterized. Common carotid artery injections were performed before the selective catheterization. Selective catheterization was

not performed in the presence of severe arterial stenosis or plaque. 5F catheters were routinely used for adult patients and 4F catheters for children.

A non-ionic contrast agent (Omnipaque 300, GE Healthcare) was used in all procedures. Two-dimensional diagnostic images were obtained using hand injections, but three-dimensional DSA images were obtained with pump injections. Catheters were intermittently flushed by hand with heparinized saline. At the end of the procedure, hemostasis was achieved by manual compression for 15-20 minutes. A 5 kg sandbag placed over the femoral artery puncture site in the leg and remain there for 6 hours. The patients were followed immobile for 6 hours.

Statistical analysis

Statistical analysis was performed to identify relations between preexisting comorbidities, indications for DSA, number of catheters, catheter types, and complications. SPSS 25.0 program (IBM SPSS statistics 25 software (Armonk, NY: IBM Corp.) was used for analyses. Mann Whitney U test was used to compare the ages of patients. The Spearman Chi-Square test evaluated the distribution among the categorical variables. A statistically significant value was taken as $p<0.05$.

Results

Our study included a total of 507 patients. Of these, 256 (50.5%) were male, and 251 (49.5%) were female. The mean age of patients was 49.2 years (range 5-91). The most preexisting comorbidity in patients diagnosed with DSA was hypertension (22.5%), followed by diabetes mellitus (8.1%). Demographic features and systemic disease of the patients are listed in Table 1.

Of the 507 patients, the most common indication for the diagnostic DSA procedure was subarachnoid hemorrhage (SAH) (21.7%), and to confirm and evaluate the aneurysms detected in magnetic resonance imaging (MRI) or computerized tomography angiography (CTA) (21.7%). In 15 patients, a Simmons 1 catheter (SIM 1); in 277 patients, a Simmons 2 catheter (SIM 2); in 329 patients, a vertebral catheter; and in 20 patients, a head-hunter catheter was used. Single catheters were used in 393 patients (77.5%), and more than one catheter was used in 114 patients (22.5%).

Table 1. Demographic data and comorbidities of the population

Demographic data		Number of patients with comorbidities	Rate of comorbidities in all patients (%)
Age		49.2±17.49	
Gender	**Male**	256	50.5
	Female	251	59.1
Diabetes Mellitus		41	8.1
Hypertension		114	22.5
Thyroid Dysfunction		16	3.2
Chronic Obstructive Pulmonary Disease		6	1.2
Cardiovascular Disease		22	4.3
Hypercholesterolemia		10	2.0
Malignity		11	2.2
Others		12	2.4

Procedural complications

Of 507 patients, a total of 10 patients had either a neurological complication, radiological complication, or angio-site-related complication, and the overall rate of complications was 0.02%. Complications were seen in five males and five females. The mean age of the patients who encountered complications was 48.90±9.06. Only 30% of the patients with complications have comorbidities, which are mostly hypertension (30%), and the SIM 2 catheter was used in 80% of the patients with complications.

In 6 patients, neurological complications occurred. Of those six patients, 3 (0.6%) had permanent neurological deficits, and 3 (0.6%) had transient deficits. One patient had aphasia, which was improved 2 hours later; one had generalized tonic-clonic seizure; and one had left hemiparesis, which was dissolved one week later. Permanent neurological deficits were caused by thromboembolism, and one patient with MCA thromboembolism died 21 days after the procedure.

In 4 patients with non-neurological complications, three were included in complications shown radiologically. Asymptomatic vasospasms were encountered in two cases, and vasospasms were resolved after nimotop injection. In one patient, internal carotid artery (ICA) dissection was encountered (Figure 1). However, the flow was not limited due to dissection. Aspirin was given to the patient, and no neurological symptom was seen. In one case, scrotal hematoma was observed after DSA, which was included in angio-site-related complications. One month later, it was observed that the hematoma had resolved. SIM 2 catheter was used in 80% of the patients with complications. Detailed information regarding the characteristics of the patients with complications is summarized in Table 2.

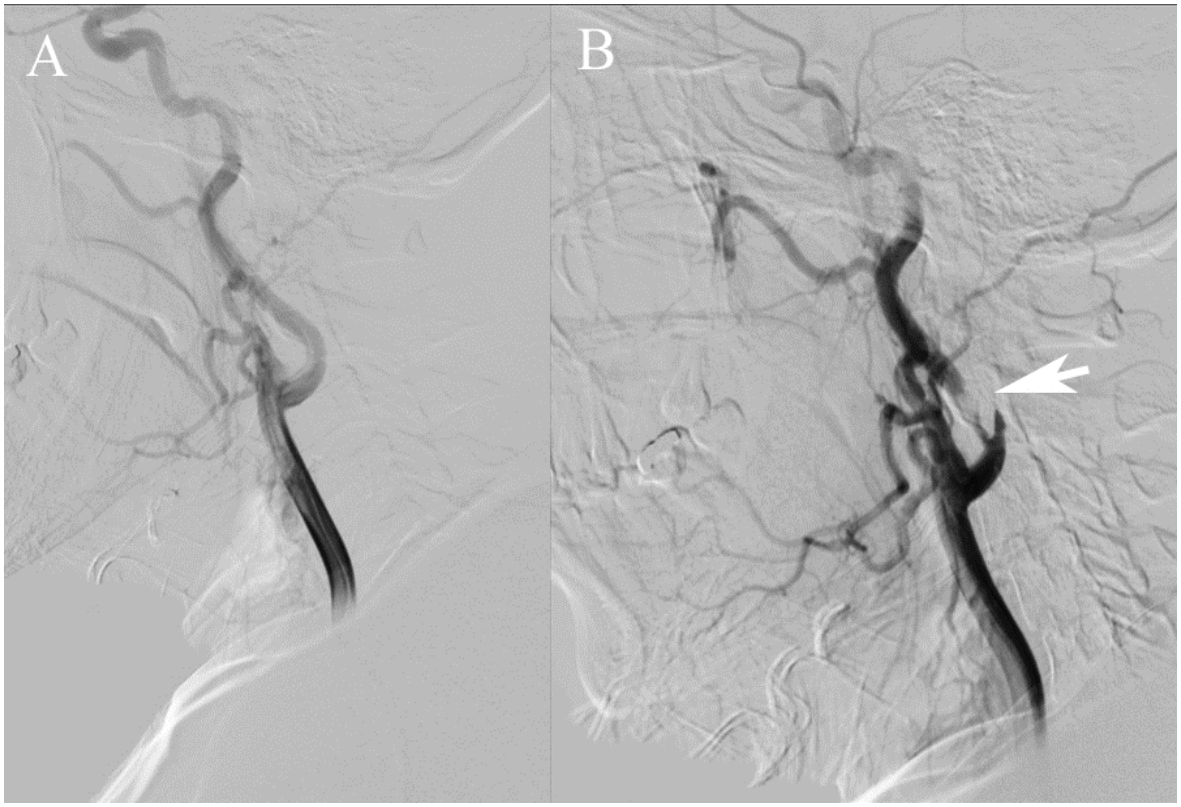

Figure 1. Following common carotid artery injection (A), internal carotid artery (ICA) was catheterized selectively. No pathology was detected following selective ICA injection, but a dissection in the cervical segment of ICA (B) was noticed during rotational imaging performed with the automatic injector. Since it did not cause any neurological deficit, no additional intervention was planned for the patient. The dissection site was indicated by an arrow

Table 2. Characteristics of patients with complications

Case No	Age	Gender	Comorbidity	Anesthesia	Indication	Catheter Type	No. Of Catheter Used	Complication	Type of Complication
1	63	F	HT	Sedation	ICH	Vertebral	1	MCA thromboembolism	Permanent
2	52	F	-	Local	Aneurysm on MRI or CTA	SIM1, SIM 2, vertebral	3	ICA posterior communication segment hromboembolism	Transient
3	54	M	HT	Local	ICH	Vertebral, SIM2	2	MCA thromboembolism	Permanent (exitus)
4	41	F	-	Local	SAH	SIM 2	1	GTC seizure	Transient
5	48	M	-	Local	ICH	SIM 2	1	Vasospasm	Radiological
6	53	M	-	Local	Aneurysm clip check	SIM 2	1	Scrotal hematoma	Transient
7	31	M	-	Local	ICA stenosis on MRI or CTA	SIM 2	1	Aphasia	Transient
8	57	M	-	Local	Stroke etiology	SIM2	1	MCA thromboembolism	Permanent
9	47	F	HT	Local	Aneurysm on MRI or CTA	SIM2, vertebral	2	ICA dissection	Radiological
10	43	F	-	Local	ICA stenosis detected on MRI or CTA	Vertebral	1	Vasospasm	Radiological

HT: Hypertension, MRI: Magnetic Resonance Image, CTA: Computerized Tomography Angiography, ICA: Internal Carotid Artery, MCA: Middle cerebral artery, GTC: Generalized Tonic-Clonic
SIM: The Simmons catheter, ICH: Intraparenchymal hematoma, SAH: Subarachnoid hemorrhage

Evaluation of the risk factors for complications

There were differences in the complication rates when they were analyzed for an association with age, anesthesia type, preexisting comorbidities, indication for DSA, and type of catheter used. The complication rate was similar in males (5 of 256, 1.96%) and females (5 of 251, 1.95%), and only hypertension was detected in patients with complications as preexisting morbidity. Gender (p=0.612), age (p=734), and comorbidities were not found to be statistically significant. The patients who underwent DSA to investigate the intracerebral hemorrhage (ICH) had the highest complication rate (9.1%), followed by the ICA stenosis detected on CTA or MRI (6.1%). ICH was associated with an increased risk in the DSA procedure (p=0.024). Although the P-value was insignificant for carotid stenosis (0.099), it can also be associated with an increased risk. Clinical indications for DSA and the rate of all complications are listed in Table 3.

In 5 patients with complications, only SIM2 catheters were used. In 3 patients, multiple catheters were used, including SIM2. In 2 patients, only vertebral catheters were used. Although the relationship of SIM2 catheters with complications is insignificant, its p-value is 0.093.

Table 3. Indications for DSA and rate of all complications and *P* values

Indication for DSA	Number of complications	Total number of angiograms for specified indication	Complication rate (%)	*p* value
ICH	3	33	9.1	0.024
SAH	1	110	0.91	0.324
Aneurysm on MRI-CTA	2	110	1.82	0.374
AVM on MRI-CTA	-	20	0	
Check after GKRS for AVM	-	18	0	
Check after the AVM operation	-	11	0	
Venous Angioma on MRI-CTA	-	17	0	
AVF on MR-CTA	-	4	0	
Vasculitis	-	3	0	
ICA dissection on MRI-CTA	-	10	0	
ICA stenosis on MRI or CTA	2	33	6.1	0.099
Check after Aneurysm clipping	1	32	3.1	0.482
Check after EVT	-	34	0	
Transient ischemic attack	1	27	3.7	0.424
Planning for GKRS	-	24	0	
Tumor Evaluation	-	5	0	
Others	-	16	0	
Total	10	507	1.97	

MRI: Magnetic Resonance Image, CTA: Computerized Tomography Angiography, AVM: Arteriovenous Malformation, AVF: Arteriovenous fistula ICA: Internal Carotid Artery, EVT: Endovascular Treatment, GKRS: Gamma Knife Radiosurgery, DSA: Digital Subtraction Angiography ICH: Intraparenchymal hematoma, SAH: Subarachnoid hemorrhage

Discussion

DSA is essential for diagnosing and evaluating intracranial vascular pathologies and tumors [18, 23]. Advancements in the catheter designs, contrast media, technical experience, and injection methods, complication rates continued to decrease [12, 24]. Although noninvasive imaging modalities such as MRI or CTA have reduced the use of DSA, these modalities had limitations as primary diagnostic tools in patients with SAH. Since the studies demonstrate that DSA can identify the lesion that causes SAH, in 4%-14% of patients with negative findings in CTA [6, 7] and the small vascular abnormalities cause intraparenchymal hematoma can be masked by the mass effect and hyperintensity of the hematoma [25], we performed diagnostic DSA for all patients with the suspected vascular pathologies which were detected on CTA or MRI.

In our series, the rate of neurologic complications associated with DSA was 1.2% (0.6% permanent; 0.6% transient). Our rate of permanent neurological disability is slightly higher than the other studies [12, 14, 19, 20]. This finding was expected as the numbers in this single-center experience need to be more significant to show similar results for the other studies and because of initial experiences in DSA. Dawkins et al. [15] show that ICH and SAH were associated with an increased risk of complications. In our study, the indication for DSA was ICH for the three patients with complications. Specific clinical and procedural factors are associated with an increased risk of complication. Studies define that patients with older age, coronary artery disease, peripheral arterial disease, or hypertension were also at high risk of complications during DSA procedures [19, 20, 26, 27]. Although the patient's age, gender, and comorbidities were not found significant, hypertension was the most common comorbidity, which was seen in our patients with complications.

Ischemic stroke and carotid stenosis were reported as risk factors in cerebral angiography [20, 26, 27]. Cloft et al. [28] defined that the neurological complication rate is higher for the patients investigated due to ischemic stroke compared to patients with SAH, aneurysm, or arteriovenous malformation (AVM). In our study, one patient with stroke etiology was evaluated with DSA, and MCA thromboembolism occurred as a complication with permanent neurological deficit. Besides being a risk factor, acute ischemic stroke is one the most severe complications that occur during DSA and is primarily caused by thromboembolism. It may be caused by multiple attempts to visualize stenotic vessels due to atherosclerotic plaque and plaque disruption by catheter or wire [12, 14, 26]. Also, a thrombus can form inside the catheters, or a hydrophilic coating can be formed over the catheter or wire [15, 29, 30]. Thromboembolism was the most devastating complication in our study-three permanent neurological deficits caused by thromboembolism.

Arterial dissections can also cause ischemic symptoms by limiting flow secondary to severe stenosis or occlusion or acting as a source of thromboembolism in DSA procedures. The intima layer of the vessel can be damaged by the manipulation of the catheter or the guidewire, and enlargement of the damaged intima can be seen because of injecting the contrast material under the intima [14, 23, 31]. Besides the interventionist's experience level, the catheter tip applying tension with cardiovascular pulsation against the arterial wall is important because it may damage the intima with the continuous pulsation [15, 31]. We encountered one carotid dissection during DSA procedure. In addition to catheter type, the number of catheters used for patients is important in evaluating the complications. Our study used more than one catheter in three patients with complications. Earnest et al. [13] found a higher correlation between neurological complications and the number of catheters used. Studies define that using smaller, softer catheters may decrease the risk of neurological complication rate [20, 32, 33]. Since only the SIM 2 catheter was used in 50% of our complicated patients, it was observed that this catheter type increased the risk of complications.

We realize that there were potential limitations in our study. Diffusion-weighted images (DWI) were not performed routinely after the DSA procedure. Therefore, subclinical events and silent thromboembolism were missed in our research and not counted as complications. However, subclinical events detected by DWI are much higher than expected, as shown in the

studies [34-36]. Another limitation of the study is that the significance of risk factors could not be demonstrated statistically due to the small sample size. Furthermore, the data analysis and accuracy depended on our clinical follow-up and retrospective evaluations.

In conclusion, complications following DSA are rare but must be minimized with knowledge of the characteristics of the patients and determining the proper indication. Although the risk is low, complications such as thromboembolism can cause permanent neurological deficits and even death. As we know, using non-invasive imaging modalities has decreased the use of DSA, endovascular treatments, and therapeutic catheter-based procedures continuously increasing. In our initial 3-year experience in DSA, we found a 1.2% rate of complications leading to neurological deficits (0.6% permanent; 0.6% transient).

Conflict of interest: No conflict of interest was declared by the authors.

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Ethics committee approval: Permission was obtained from Pamukkale University Non-Interventional Clinical Research Ethics Committee for the study (permission date:10.10.2023, permission number:16).

Authors' contributions to the article

S.C. and F.Y. have constructed the main idea and hypothesis of the study. S.C. and B.B.B. developed the theory and arranged/edited the material and method section. S.C., E.T. and M.E.C. have done the evaluation of the data in the results section. Discussion section of the article was written by S.C., F.Y., E.T. and M.E.C. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

Research Article

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The relationship of postoperative tramadol activity with the CYP2D6*17 genome in total knee artroplasty patients

*Total diz artroplastisi uygulanan hastalarda postoperatif tramadol etkinliğinin CYP2D6*17 genomu ile ilişkisi*

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Abstract

Purpose: In our study, we examined the effect of tramadol maintenance on the VAS score in geriatric patients. We observed them until the postoperative 60th minute. We investigated the incidence of pain in patients who underwent knee arthroplasty in the study. Our aim was to examine the effect of the *17 allele of the CYP2D6 genome on postoperative tramadol activity.

Materials and methods: In our study we examined 110 patients who underwent total knee arthroplasty in the Department of Orthopedics and Traumatology at our facility, along with 100 healthy individuals without complaints who served as the control group. Each patient received a 100 mg dose of intravenous tramadol (Contramal). The postoperative VAS scores of the patients were recorded at 0-15-30-45-60 minutes.

Results: The average age of the patients was 62.36 years. In our study, 86.4% of the patients were female, while this rate was 46% in the control group. We found that 3.65% of individuals (*17 carriers) possessed the *17 allele in both the patient group (n=7) and the control group (n=7). At the postoperative 0th minute, the VAS score for patients in the *1/*1 group was 91.07, while for the *1/*17 group, it measured 95.0. There was no statistically significant difference between the genomes (p>0.050). Likewise, no statistically significant difference was found between the genomes at the postoperative 15th, 30th, 45th, and 60th minutes (p>0.050). However, we observed a statistically significant decrease in the postoperative VAS score between 0-60 minutes in both groups, indicating time-dependent variation (p=0.000).

Conclusion: When examining diverse literature on tramadol classification as intermediate metabolizer (IM) or extensive metabolizer (EM) concerning the *17 allele, our study indicates that the *17 allele should be regarded as both extensive metabolizer (EM) and normal metabolizer (NM).

Keywords: Total knee arthroplasty CYP2D6, Tramadol, CYP2D6*1/*17, postoperartive pain.

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Öz

Amaç: Geriatrik hastalarda tramadol idamesinin VAS skoruna etkisini incelediğimiz çalışmamızda ameliyat sonrası 60. dakikaya kadar gözlem yaptık. Çalışmada diz artroplastisi uygulanan hastalarda ağrı insidansını araştırdık. Ameliyat sonrası tramadol etkinliğine CYP2D6 genomunun *17 alelinin etkisini incelemeyi amaçladık.

Gereç ve yöntem: Bu çalışmaya Pamukkale Üniversitesi Tıp Fakültesi Ortopedi ve Travmatoloji Anabilim Dalı'nda servisimize total diz antroplastisi uygulanan 110 hasta ve kontrol grubu olarak şikâyeti olmayan sağlıklı 100 kişi dahil edildi. Her hastaya intravenöz yoldan 100 mg Tramadol (contramal) uygulandı. Hastaların post-op 0-15-30-45-60. dk VAS skorları kaydedildi.

Bulgular: Hastalar ortalama 62,36 yaşındaydı. Çalışmamızdaki hastaların %86,4'ü kadınken kontrol grubunda bu oran %46 olarak bulundu. *17 alelinin varlığı hasta (n=7) ve kontrol grubunda (n=7) toplamda %3,65 oranında (n=14) *17 taşıyıcısına rastladık. VAS postoperartive 0. dakikada *1/*1 grubundaki hastaların VAS skoru 91,07 ve *1/*17 genomunun VAS skoru 95,0 şeklindeydi ve genomlar arasında istatistiksel olarak anlamlı bir farklılık yoktu (p>0,050). Benzer şekilde ameliyat sonrası 15., 30., 45. ve 60. dakikada da genomlar arasında istatistiksel olarak anlamlı bir farklılık yoktu (p>0,050). Zamana bağlı değişimde her iki gruptada post-op VAS skorunun 0-60 dakika arasında istatistiksel olarak anlamlı şekilde düştüğünü gördük (p=0,000).

Sonuç: Tramadolun *17 aleli ile ilişkisinde IM veya EM olarak sınıflandırıldığı literatürdeki farklı sonuçlar alınmış çalışmaları incelediğimizde bizim çalışmamızın sonuçlarına göre: *17 aleli EM ile NM şeklinde değerlendirilmesi gerektiğini düşünüyoruz.

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Anahtar kelimeler: Total diz artroplastisi, CYP2D6, Tramadol, CYP2D6*1/*17, ameliyat sonrası ağrı.

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Introduction

Tramadol is rapidly and nearly entirely absorbed after oral administration [1]. After a single oral dose, tramadol has an average bioavailability of around 68%, which escalates to over 90% following multiple doses [2, 3]. IV administration reports a single dose with 90% bioavailability [1]. Although CYP2D6 accounts for only about 2-4% of hepatic CYP enzymes, as one of the most extensively researched CYPs, it metabolizes approximately 25% of drugs processed in the human liver [4]. The liver metabolizes tramadol through O- and N-demethylation processes, alongside conjugation reactions that result in the production of glucuronides and sulfate metabolites. Cytochrome P450-2D6 mediates the (O-) demethylation of dexmethitramadol [5-7].

While tramadol is generally categorized as 'opioid-like,' previous studies have often included it indiscriminately with other opioids when describing the effects of opioids on postoperative outcomes [8-10]. It's commonly accepted that tramadol reaches peak effectiveness approximately 180 minutes after total knee arthroplasty (TKA) [10]. However, in some reports, this timeframe has been reported to decrease to 60 minutes, with patients experiencing a reduction in their VAS score to zero [11, 12]. In a study examining tramadol's efficacy after 60 minutes, the VAS score showed an average decrease of 70 units [12]. This study explores the effect of tramadol maintenance on the VAS score in geriatric patients, observed until the postoperative 60th minute. The aim of this study is to investigate the occurrence of pain in patients who underwent knee arthroplasty, examining the impact of the *17 allele of the CYP2D6 gene on postoperative tramadol efficacy.

Materials and methods

The study was conducted at Pamukkale University Faculty of Medicine, specifically within the orthopedics and traumatology service. The study group consisted of patients who had undergone total knee arthroplasty, a procedure recognized for its association with elevated levels of postoperative pain. For this study, we selected 110 patients who had undergone total knee arthroplasty, and the control group comprised 100 healthy individuals without pain. A total of 210 individuals participated in the study, and their CYP2D6 genes were analyzed through DNA isolation. Pain assessment was conducted using the Visual Analog Scale (VAS), which employed a scale ranging from 0 to 100 (no pain: 0, excruciating severe pain: 100).

We performed genomic DNA isolation from blood samples obtained from the study participants using the standard phenol-chloroform method. We utilized the Polymerase Chain Reaction method to amplify specific genomic regions from the isolated DNA genomes. We examined the presence of the *1/1 and *1/17 alleles within the isolated CYP2D6 genomes.

The drug doses administered were as follows: Tramadol (Contramal) 100 mg was diluted in 150 ml of saline and intravenously administered over 20 minutes at the onset of the postoperative period. We recorded pain scores during the initial 60 minutes and closely monitored patients for any potential side effects. Thankfully, no complications were noted, and the study concluded at the 60-minute postoperative interval. The patient group excluded individuals who had taken analgesics within the past 6 hours, those with kidney or liver diagnoses, individuals sensitive to Tramadol, and those who declined to participate.

Research Termination Criteria: No drug-related side effects were noted throughout the study. The study concluded upon reaching the specified number of participants.

Statistical analysis

IBM SPSS for Windows version 25 statistical package program was used for analysis. Number(n) and percentage (%) were used for categorical data, mean and standard deviation were used for numerical variables. Since the

ratio of the number of patients between alleles did not support the normal distribution, it was analyzed with the Mann Whitney U test. *P*<0.05 was considered significant.

Permission was obtained from Pamukkale University Non-Interventional Clinical Research Ethics Committee for the study 18/01/2022 date and 2022TIPF006 permission number.

Results

The average age of the patients was 62.36±13.62 years. In our study, 86.4% of the patients were women, while this percentage was 46% in the control group. Upon examining the presence of the *17 allele, we identified a total of 7% carriers of the *17 allele in both the patient and control groups (Table 1).

The comparison of the *17 allele with the normal metabolizer *1 allele in the CYP2D6 genome is given in the Table 2. At the 0th minute postoperative VAS assessment, patients with the *1/*1 genotype had a VAS score of 91.07, whereas those with the *1/*17 genotype scored 95.0. Notably, there was no statistically significant difference observed between the genomes (p>0.050). Likewise, no statistically significant difference was observed between the genomes at the 15th, 30th, 45th, and 60th minutes postoperatively (p>0.050). We observed a statistically significant decrease in the postoperative VAS score between 0 and 60 minutes in both groups, indicating a time-dependent variation (p=0.000).

Table 1. Demographic and clinical data

		Patient (n=110)	Control (n=100)
Gender	Female	95 (86.4%)	46 (46%)
	Male	15 (13.6%)	54 (54%)
CYP2D6	*1/*1	103 (93.6%)	93 (93%)
	*1/*17	7 (6.4%)	7 (7%)

Table 2. VAS score variation of CYP2D6 *1/*1 and *1/*17 alleles

VAS score	*1/*1 (n=103)	*1/*17 (n=7)	*p*
Postoperative 0. min	91.07±9.31	95.0±5.0	0.306
Postoperative 15. min	70.92±16.87	78.57±8.99	0.144
Postoperative 30. min	52.04±23.47	42.86±17.04	0.198
Postoperative 45. min	37.33±20.81	27.14±18.00	0.207
Postoperative 60. min	29.47±19.28	22.86±14.96	0.369
p*	0.000	0.000	

*Change between 0-60 minutes, VAS: Visual Analog Scala

Discussion

In our study, 86.4% of the patients who underwent knee osteoarthritis prosthesis were predominantly women. The average age of these patients, 67.19, aligns with the geriatric patient population. While investigating the influence of the CYP2D6 genome on the population, including healthy individuals, the average age of the healthy participants was 57.05, notably lower than that of the patients. Given the stability of the CYP2D6 genome in patient genetics and its independence from age-related changes, we excluded the age difference between the groups from the statistical analysis.

The IM phenotype is characterized by a combination of a null allele and an allele with reduced function (*10, *14, *17, *18, *36, *41, *47, *49, *50, *51, *54, 55, and 57). Reduced enzymatic activity can stem from factors such as diminished protein stability, alterations in

substrate recognition, or reduced substrate affinity [4]. A multicenter study demonstrated a 93.1% reduction in tramadol clearance among individuals with CYP2D610, whereas those with CYP2D617 exhibited a 64% reduction in tramadol sensitivity [13]. Remarkably, the diminished substrate-enzyme affinity observed with other CYP2D6 substrates interacting with the *10 and *17 variants was absent in this case [13].

Dagostino et al. [14] compiled the association between CYP2D6 alleles and metabolizer classification, categorizing the *1/*17 and *2/*17 alleles as extensive metabolizers (EM), while *3, *4, *5, *6, *7, *8, *9, *10, *11, *15, *17, *29, *35, and *41 haplotypes were considered intermediate metabolizers (IM). In their meta-analysis, Magarbeh et al. [15] designated the *17 allele as an extensive metabolizer and compared it to other studies. These studies encompassed two investigations examining the *17 allele's involvement in codeine toxicity and tramadol activity, suggesting that the *17 allele might exhibit greater efficacy compared to the *1/*1 genotype in these scenarios [15-17]. Nevertheless, due to the relatively lower frequency of studies focusing on CYP2D6 compared to other alleles in its group, it is frequently compared in the literature. It's important to note that it doesn't enjoy the same level of prominence as other alleles such as *2, *3, *4, *6, *10, and *41, for which extensive data on the mechanism of action is available.

According to a study conducted by Aynacioglu et al. [18] in Türkiye with 404 participants, the incidence rate of the *17 allele in the Turkish population is 1.11%. Even in the Indian population, where the *17 allele is most prevalent globally, it has been reported to occur in 3.3% of the population [19]. Its prevalence was reported to be 0.8% in the African population [20]. In our study, we identified a higher occurrence of the *17 allele, present in 14 patients (3.65%) out of a total of 210 participants, which contrasts with the frequencies commonly reported in the literature.

The activation and metabolism of tramadol's primary active metabolite are largely mediated by CYP2D6 [21, 22]. Therefore, CYP2D6 plays a crucial role in tramadol pharmacokinetics [5]. Previous studies have investigated the influence of the CYP2D6 genotype on plasma levels of tramadol and its metabolites, as well as on the efficacy and adverse reactions associated with tramadol (such as nausea, vomiting, sweating, pruritus, constipation, and headache) [21, 23, 24]. Oscarson et al. [19] observed that the *17 allele alone did not exhibit any effect on enzyme activity. However, their findings indicated that the CYP2D6*17 allele had a distinct impact, as it represents a polymorphic variant of cytochrome P450. This variant requires a combination of substitutions to modify the catalytic properties of the enzyme.

In conclusion, our study revealed that patients possessing the *17 allele reported less pain compared to those without it. However, all patients who received tramadol after total knee arthroplasty had pain scores below 50, considered the pain threshold at 60 minutes. We hypothesize that the absence of a statistically significant difference could be attributed to the limited number of patients carrying the *17 allele in our study. Nonetheless, our observed carrier rate of 3.65% was notably higher than the rates commonly reported in the literature.

At the 30th minute, patients with the *17 allele exhibited a VAS score below 50, while patients with the *1/*1 genotype maintained a VAS score above 50.

Upon reviewing literature studies categorizing tramadol as IM or EM concerning the *17 allele, our study's findings suggest that the *17 allele should be regarded as both EM and NM.

Conflict of interest: No conflict of interest was declared by the authors.

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Authors' contributions to the article

M.E.G. and N.O. constructed the main idea and hypothesis of the study. M.E.G. and A.K. developed the theory and arranged the material and method section. N.O. and A.K. have done the evaluation of the data in the results section. Discussion section of the article written by N.O. and M.E.G.

A.K. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

Research Article

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Potential effects of parietin on apoptosis and cell cycle related genes in SH-SY5Y neuroblastoma cells

Parietinin SH-SY5Y nöroblastom hücrelerinde apoptoz ve hücre döngüsü ile ilgili genler üzerindeki potansiyel etkileri

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Abstract

Purpose: Ingredients obtained from natural products have been used in cancer treatments for years. High diversity and non-toxicity compared to chemotherapeutic agents are the main reasons for their preference. Lichens having potential for treatment of cancer consist of fungus and 1-2 species of algae. Under the name of lichen substances, many of them have also been synthesized as specific substances. The secondary metabolites in lichens are generally insoluble in water, and have many biological activities such as antiviral, antitumor, antibacterial, and antioxidant; they store in the fungal cell or on the surface of the hyphae and can only be extracted with organic solvents. Parietin extracted from lichen species such as xanthoria parietina is an anthraquinone pigment and a secondary metabolite. In our study, the effects of parietin on cytotoxicity, gene expression, migration, invasion, and colony formation in neuroblastoma cells treated with parietin were investigated. SH-SY5Y cell line without parietin was used as the control group.

Materials and methods: The IC_{50} value of the parietin was determined using XTT assay. The total RNA extractions were performed from the cells using the Tri-Reagent kit. The expressions of BAX, CASPASE3, CASPASE8, CASPASE9, P53, PUMA, NOXA, TIMP1, TIMP2, BCL2, BCL-XL, CASPASE10, BID, CYCLIND1, CDK6, P21, MMP2, MMP9, TRADD and FADD genes were investigated by Lightcycler 480 (Roche) using SYBR Green dye. Migration analysis of the control and the dose group cells were performed in accordance with the Wound-healing assay protocol. Invasion activities were determined using the “Invasion Chamber” (BD Biosciences) protocol. Colonies were treated with crystal violet and observed under the light microscope.

Results: The IC_{50} value of the parietin used for 48-hour treatment on the cells was determined as 35 μM. It was found that the expression levels of BCL-XL, BCL-2, MMP2, MMP9, P21, and CYCLIN D1 mRNA were downregulated, and it was also shown to be upregulated the expression levels of CASPASE3, CASPASE9, BAX, P53, PUMA, and NOXA to be upregulated. It was determined that parietin suppressed both cell invasion and migration, and colony formation in the neuroblastoma cells.

Conclusions: Thus, it can be possible parietin to be used as an alternative, complementary, and supportive agent together with the other drugs in the treatment of neuroblastoma. However, more comprehensive studies supporting these significant effects of parietin will increase its potential in the application.

Keywords: Parietin, neuroblastoma, cell culture, gene expression, migration.

Dodurga Y, Secme M, Elmas L, Gundogdu G, Cekin A, Gunel NS. Potential effects of parietin on apoptosis and cell cycle related genes in SH-SY5Y neuroblastoma cells. Pam Med J 2024;17:243-253.

Öz

Amaç: Doğal ürünlerden elde edilen bileşenler yıllardır kanser tedavisinde kullanılmaktadır. Kemoterapötik ajanlara göre çeşitliliğinin fazla olması ve toksik olmaması tercih edilmelerinin başlıca nedenleridir. Kanser tedavisi potansiyeli olan likenler mantar ve 1-2 tür algden oluşmaktadır. Liken maddeleri adı altında birçoğu spesifik maddeler olarak da sentezlenmiştir. Likenlerdeki sekonder metabolitler genellikle suda çözünmez ve antiviral, antitümör, antibakteriyel ve antioksidan gibi birçok biyolojik aktiviteye sahiptir; mantar hücresinde veya hifanın yüzeyinde depolanırlar ve yalnızca organik çözücülerle ekstrakte edilebilirler. Xanthoria parietina gibi liken türlerinden ekstrakte edilen parietin, bir antrakinon pigmenti ve ikincil bir metabolittir. Çalışmamızda parietin ile tedavi edilen nöroblastoma hücrelerinde parietin'in sitotoksisite, gen ekspresyonu, migrasyon, invazyon ve koloni oluşumu üzerine etkileri araştırılmıştır. Parietin tedavisi uygulanmayan SH-SY5Y nöroblastom (NB) kontrol grubu olarak uygulanmıştır.

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Gereç ve yöntem: Parietin'in IC_{50} değeri XTT testi kullanılarak belirlendi. Total RNA ekstraksiyonları, Tri-Reaktif kiti kullanılarak hücrelerden gerçekleştirilmiştir. BCL-XL, BCL-2, MMP2, MMP9, P21, CYCLIN D1, CA3, CASAPASE-9, BAX, P53, PUMA ve NOXA genlerinin ekspresyonları Lightcycler 480 (Roche) ile SYBR Green boyası kullanılarak araştırılmıştır. Kontrol ve doz grubu hücrelerinin invazyon, yara iyileşmesi testi protokolüne uygun olarak gerçekleştirilmiştir. İnvazyon, "İnvazyon odacıkları" (BD Biosciences) protokolü kullanılarak belirlenmiştir. Koloni testi için hücreler kristal viyole ile muamele edilmiş ve ışık mikroskobu altında gözlenmiştir.
Bulgular: Hücrelerde 48 saatlik tedavide kullanılan parietin'in IC_{50} değeri 35 μM olarak belirlenmiştir. BCL-XL, BCL-2, MMP2, MMP9, P21 ve CYCLIN D1 mRNA'nın ekspresyon seviyelerinin downregüle edildiği; CASPASE3, CASPASE9, BAX, P53, PUMA ve NOXA'nın upregüle edildiği tespit edilmiştir. Parietin'in nöroblastoma hücrelerinde hem hücre invazyonu ve migrasyonu hem de koloni oluşumunu baskıladığı belirlenmiştir.
Sonuç: Böylece parietin'in nöroblastom tedavisinde diğer ilaçlarla birlikte alternatif, tamamlayıcı ve destekleyici bir ajan olarak kullanılması mümkün olabilir. Ancak parietin'in bu önemli etkilerini destekleyen daha kapsamlı çalışmalar uygulamadaki potansiyelini artıracaktır.

Anahtar kelimeler: Parietin, nöroblastoma, hücre kültürü, gen ekspresyonu, migrasyon.

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Introduction

Neuroblastomas (NB), malignant solid tumors that arise from out-of-control primitive cells of the sympathetic nervous system, are often seen in early childhood; It is believed these tumors to stem from primitive neural crest cells. Some of the primitive cells undergo changes and form tumors, and the reason for the changes is genetic or environmental factors. The disease usually occurs at an early age. The reason for this is that the cells are of embryonal origin and the faulty development in nerve cells starts before the baby is born. Chromosomal changes and/or faulty gene regulation cause this faulty development in nerve cells [1]. Interestingly, the occurrence of neuroblastoma does not vary across different racial groups.

Neuroblastoma develops mainly in the adrenal medulla or paravertebral sympathetic ganglia at any level from the neck to the pelvis [2]. It can occur anywhere along the sympathetic nervous system and the most common primary site is an adrenal mass [3]. Compared with other tumors; The primary tumor may show different clinical manifestations of metastatic disease and paraneoplastic syndromes. Spontaneous regressions and differentiation into benign disease can be given as examples of different behaviors of neuroblastoma. It may be more aggressive in older children [4]. The SH-SY5Y NB cells are a triple cloned sub-colony of the SK-N-SH cell line (SK-N-SH-> SH-SY-> SH-SY5-> SH-SY5Y-CRL-2266). SH-SY5Y, showing dopamine-β-hydroxylase activity, can convert glutamate to the neurotransmitter Gamma-Aminobutyric acid (GABA). GABA, or Gamma-Aminobutyric Acid, is widely recognized as a key presynaptic inhibitor in both the central nervous system and the retina. This neurotransmitter holds significant importance in regulating brain metabolism and functioning. For these reasons, the SH-SY5Y cell line was chosen.

In our study, the expression levels of apoptosis, cell cycle and tumor suppressor genes were investigated. Many genes are involved in the orientation of the cell to apoptosis. The first of these is the Bcl2 family. The Bcl2 family is divided into two groups as proapoptotic and antiapoptotic members, and the excess of proapoptotic members increases the apoptosis tendency of the cell. Bad, Bax, Noxa, and Puma genes are proapoptotic genes and cause programmed death of cells by inducing apoptosis. Bcl-XL and Bcl2 are antiapoptotic genes. These genes suppress apoptosis and cancer cells affected by suppression continue to proliferate uncontrollably [5].

Caspases are essential proteins required for apoptosis. It consists of two pathways, intrinsic and extrinsic. While the intrinsic pathway starts with caspase 9 activation, the extrinsic pathway starts with caspase 8 activation and both pathways converge in caspase 3 [6].

The studies carried out in recent years shows that natural compounds have been used in the treatment of cancer as well as chemotherapeutic agents due to their non-toxicity.

Since time immemorial, plants and lichens have been used as drugs for medicinal

purposes in many countries within the scope of traditional treatment. There are studies that lichens are used as therapeutic agents in some diseases such as cancer, arthritis, diabetes, eczema, respiratory and circulatory tract and have a therapeutic effect thanks to secondary metabolites such as cystic acid, gyrophoric acid, and norstic acid from lichen species. Anthraquinones (AQs) are the predominant type of quinones found in nature and are abundant in various sources [7]. Parietin, an anthraquinone pigment, is located in the upper part of the upper cortex of lichens. They are localized as small extracellular crystals and play a protective role against sunlight, giving them a strong orange-brown color.

The antifungal and antibacterial activities of parietin were investigated in various studies. It has been reported that parietin exhibits significant antiangiogenic activity in breast cancer by inhibiting subcytotoxic concentrations, migration of endothelial cells and tube formation [8].

The objective of this study is to investigate the anticancer effects of parietin on SH-SY5Y cells. In addition, it was also aimed to reveal the molecular mechanisms such as therapeutic activity, cell proliferation, invasion, colony formation, cell cycle and apoptosis.

Material and methods

Cell Culture-Cytotoxicity Test (XTT)

SH-SY5Y cells were obtained from ATCC (American Type Culture Collection, USA). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 2mM L-Glutamine, Penicillin-streptomycin (20 units/mL-20 µg/mL), and 10% Fetal Calf Serum under 5% CO_2 and 37°C conditions. The cytotoxicity assays were performed when the cells multiplied with DMEM reached a sufficient number. The cytotoxicity assays were made using 1×10^4 cells. Cytotoxicity experiments and the determination of IC_{50} of Parietin were done by XTT method in accordance with the protocol. The cells were seeded into 96-well plates using the DMEM being prepared.

In order for the cells to adhere to the bottom of the plate, they were incubated in the oven for 24 hours under appropriate conditions. At the end of the incubation, certain doses (10-250 µM) of parietin were added to the wells. 24 and 48 hours after the addition of parietin, 10 µL of XTT Reagent was added per well in accordance with the XTT protocol and incubated for 2 hours in an oven with 5% CO_2 and 37°C conditions.

At the end of the incubation period, absorbance detections at a wavelength of 450 nm were performed by a microplate reader (Reference wavelength=630 nm). The percentage of the cell viability was determined using the equation below:

The measured mean optical densities (MOD) of the sample was used in the equation below to determine the percentage of viable cells: % viability = [(MOD of sample) / (MOD of control)] x100

Total RNA isolation and PCR

Total RNA was extracted from the SH-SY5Y cells and exposed to the IC_{50} dose of parietin from the 5×10^6 cell flasks, so as to be 1000 µl per flask, the cells were removed from the base using Trizol, and cell scrapers and transferred to eppendorf tubes (1 mL).

After adding 200 µL of chloroform to each eppendorf tube, the tubes were incubated for 15 min at 25°C. Centrifugation was performed at 15,000 rpm and 4°C for 20 minutes with a refrigerated centrifuge; after that, the collected supernatants were placed to new eppendorf tubes.

After adding 500 µL of isopropanol to the collected supernatant, this mixture was incubated at 25°C for 10 minutes. After that, the resultant mixture was centrifuged at 15,000 rpm, 4°C for 30 minutes. After adding 70% ethanol to the pellet, centrifugation was performed at 12,000 rpm and 4°C for 10 minutes. The supernatants were discarded, and the pellet was dissolved with 40 µL of RNase-DNase free water. The concentration and purity of total RNAs were measured using the Thermo Nanodrop device.

The process of cDNA synthesis was carried out utilizing the First-Strand cDNA Synthesis Kit. Gene expressions of BAX, BCL-2, BCL-XL, CASPASE3, -8, -9, -10, BID, TRADD, CYCLIND1, CDK6, P53, P21, FADD, PUMA, NOXA, MMP-2, MMP-9, TIMP-1, and TIMP2 were analyzed using specific primers and the

Real-Time RT-PCR Applied Step One Plus instrument following the SYBR Green qPCR Master Mix protocol. The obtained expression results were compared with the expression levels of the GAPDH gene, which served as the housekeeping gene in the study.

Cell migration, invasion, colony analysis

a. Migration

Migration analysis of the control and the experiment group cells were performed in accordance with the wound-healing assay protocol. Its working principle is based on the Culture-Insert 2 Well placed on the surface of the petri dish, which provide two cell culture reservoirs separated by a 500 µm wall, and thus the migration was observed [9, 10].

Adding the cell suspension to the reservoirs allowed the cells to grow only in the designated growth areas. $3x10^5$ cells/mL per reservoir were seeded and 24 hours later after seeding, the inserts were first removed, and then the determined IC_{50} dose of Parietin was applied to the cells.

Only medium with serum was given to the control group. A gap of approximately 500 µm formed after the insert was removed. An inverted microscope was used to evaluate the migration process. The images at different times (0h and 24h) were compared during the evaluation.

b. Invasion

Percent invasion ratio of the control and the experiment group cells were determined using 2.5 $x10^5$ cells/mL on 24-well culture plates in accordance with the "Invasion Chamber" (BD Biosciences) protocol. According to the kit protocol, the filters were established with methanol for 10 minutes and dyed with crystal violet dye. The cells reaching the lower surface of the filter were counted under a light microscope. Migration analysis was confirmed using analysis of the wound healing assay. Percent invasion formula was calculated using the following equation:

Invasion (%) = (Number of cells in matrigel matrix basement membrane) / (Number of cells in control membrane) x 100.

c. Colony analysis

The cells were seeded in 6-well culture dishes for colony formation analysis. The parietin-treated cell group and the control group cells were incubated in incubators, and in a humid environment containing 5% CO_2 at 37°C. The cells were kept under constant observation and checked with a light microscope. The medium was removed by an aspirator and washed with 1X PBS at the end of the 48th hour and this process was repeated 3 times. The cells were detached with trypsin and centrifugation was used at 500 g for 5 min to collect the cells and the parietin - treated cell group and the control group cells were inoculated into 24-well plates at $2x10^5$ cells/mL per well and incubated in a 37°C incubator, and a humid environment containing 5% CO_2 for 1-2 weeks.

When the incubation period concluded, the cells fixed by 100% methanol for 20 min were treated with crystal violet for 5 min. After staining, the cells washed with 1X PBS were allowed to dry overnight. After all these procedures, the cells were observed under the light microscope.

Statistical analysis

Analysis of the results was performed by the ΔΔCT calculation method. The comparison of the two groups, and the evaluation of the expression changes were performed by the "Volcano Plot" analysis from "RT^2ProfilesTMPCR Array Data Analysis". The student t-test was used to analyze the results, and to determine statistical significance; the p-value that meets the condition of $p<0.05$ was considered to be statistically significant.

Since our study is an *in vitro* study, ethics committee approval is not required.

Results

Cytotoxicity analysis with XTT method

Parietin was implanted to the SH-SY5Y neuroblastoma cells at the doses of 10-20-35-50-75-100-200-250 µM. The cells without parietin were considered as the controls. The IC_{50} dose of the parietin in the 48th hours were determined as 35 µM by the XTT assay. The dose and the time graph of percentage cell viability are shown in Figure 1.

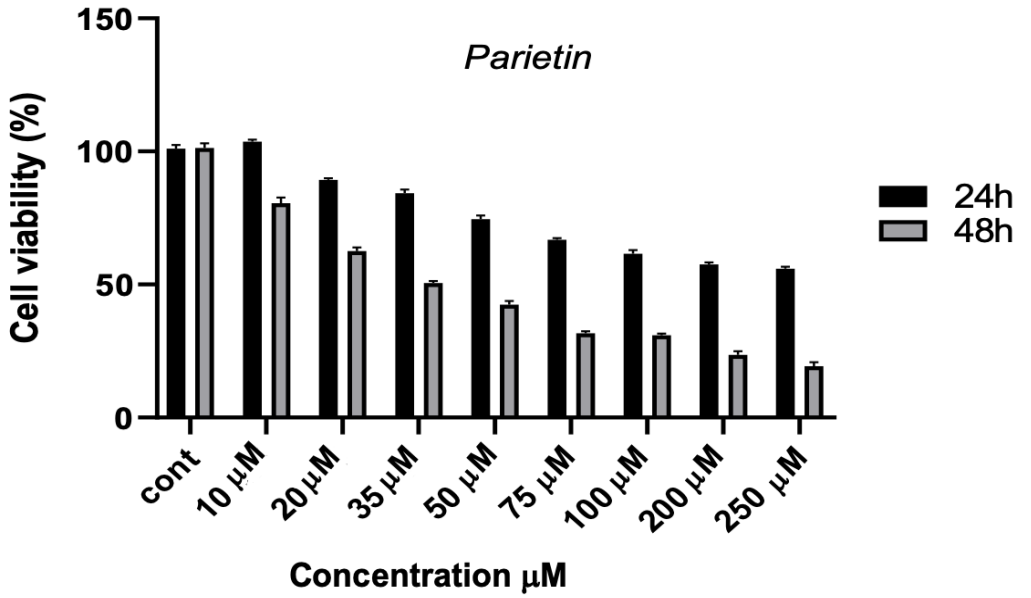

Figure 1. The effect of parietin on the viability of SH-SY5Y neuroblastoma cells was studied. The cells were exposed to various concentrations of parietin over different time intervals, and their growth was evaluated using the XTT assay. The presented data represents the averaged outcomes from three separate experiments

Invasion

In the SH-SY5Y cells treated with the IC_{50} dose of parietin, the invasion rate was reduced three times compared to the control group; the % invasion rates are shown in Figure 2. The percentage invasion rate of 70% in the control group decreased to 40% in the cells in the parietin-treated group. 10 representative fields were used to count the cells passing through the membrane.

Migration

Migration analysis was performed on the SH-SY5Y cells treated with 35 μM Parietin. The results obtained were compared with that of the control group. Inverted microscope images, taken at the 0 and the 24 hours, are shown in Figure 3. It was found that the parietin-treated SH-SY5Y cells suppressed migration compared to the control group.

Colony formation

Parietin decreased colony- forming ability of neuroblastoma cells. The effect of parietin on neuroblastoma cells is shown in Figure 4. While an average of 82 colonies were counted in the control group, this number decreased to an average of 40 in the parietin group (Figure 5).

It was shown parietin to reduce colony formation in the SH-SY5Y neuroblastoma cells.

Real-Time PCR

Based on the Real-Time PCR results, it was revealed that the mRNA expression levels of BCL-XL, BCL-2, MMP2, MMP9, P21 and CYCLIN D1 were downregulated in the parietin-treated groups of the cells. Conversely, it was revealed the expression levels of CASPASE3, CASPASE9, BAX, P53, PUMA and NOXA to upregulate in the same group.

The mRNA expression levels of PUMA and NOXA were doubled compared to the control group; BAX, CASPASE9 triple; p53 about four times; CASPASE3 increased fivefold. The mRNA expression level of BCL-2 was observed to decrease twofold when compared to the control group.

The effect of parietin on fold changes in mRNA level of genes in neuroblastoma cells is shown in Figure 6.

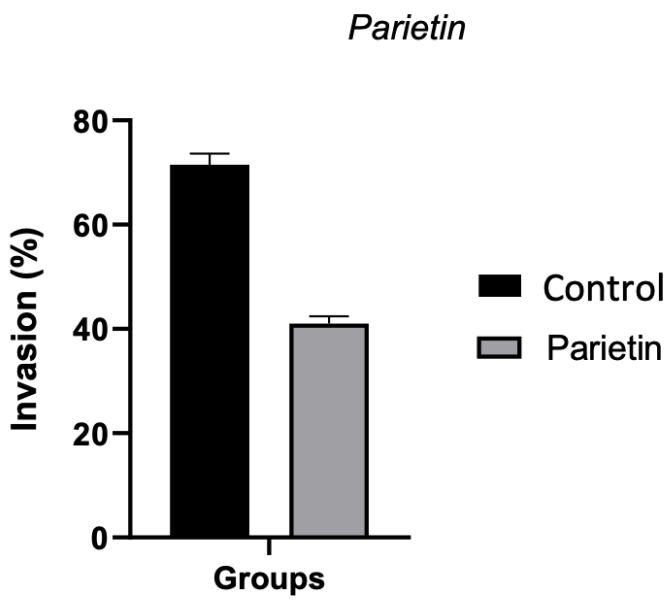

Figure 2. The invasion ratio of SH-SY5Y cells in the control group and the dose group was calculated as the percentage of cells that successfully passed through the membrane. The counting was conducted in 10 specific areas chosen as representatives

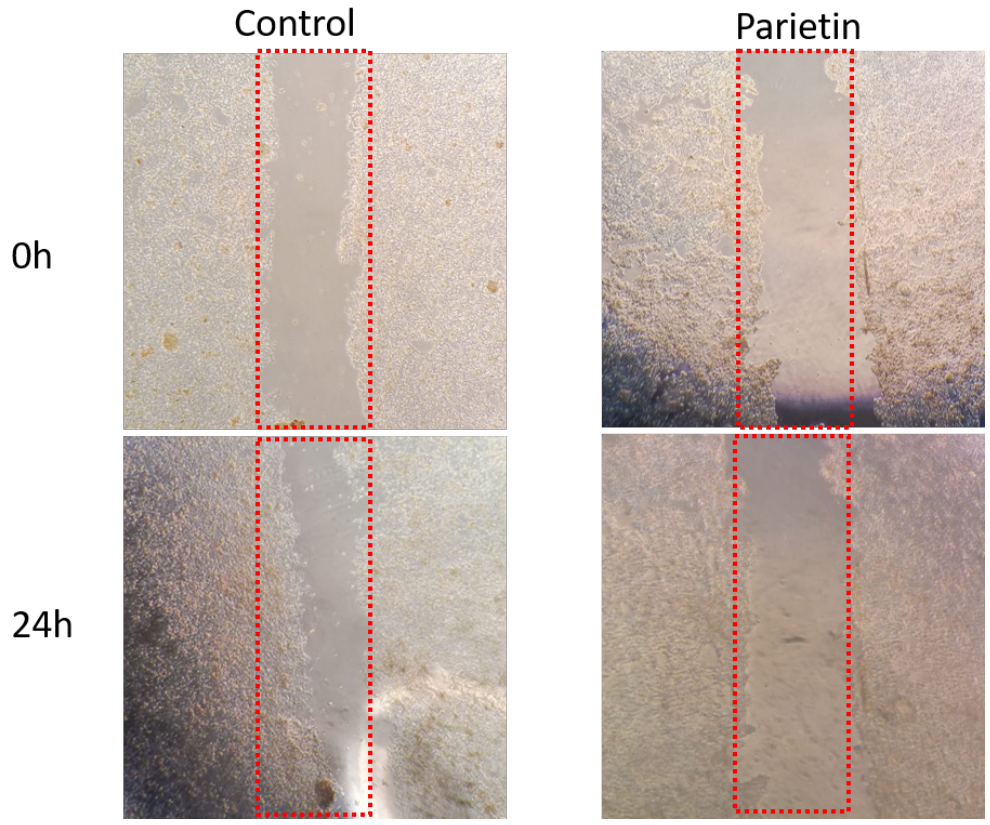

Figure 3. The findings from the wound-healing assay indicated that parietin effectively decreased cell migration. Images from both the control and dose groups were captured at 0 hours and 24 hours to observe the progression of the wound closure over time

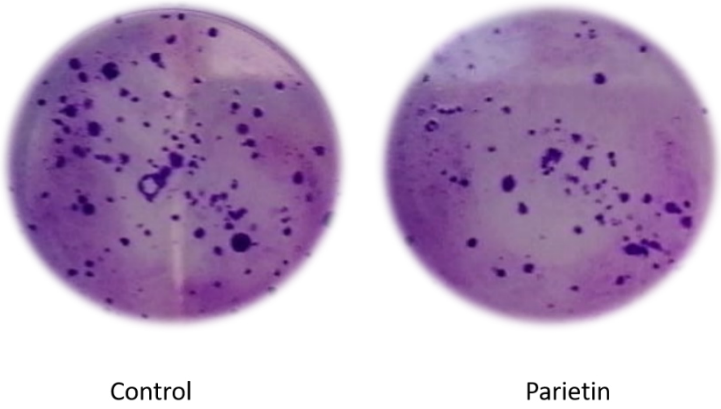

Figure 4. Parietin decreases colony formation. The colonies were stained with crystal violet

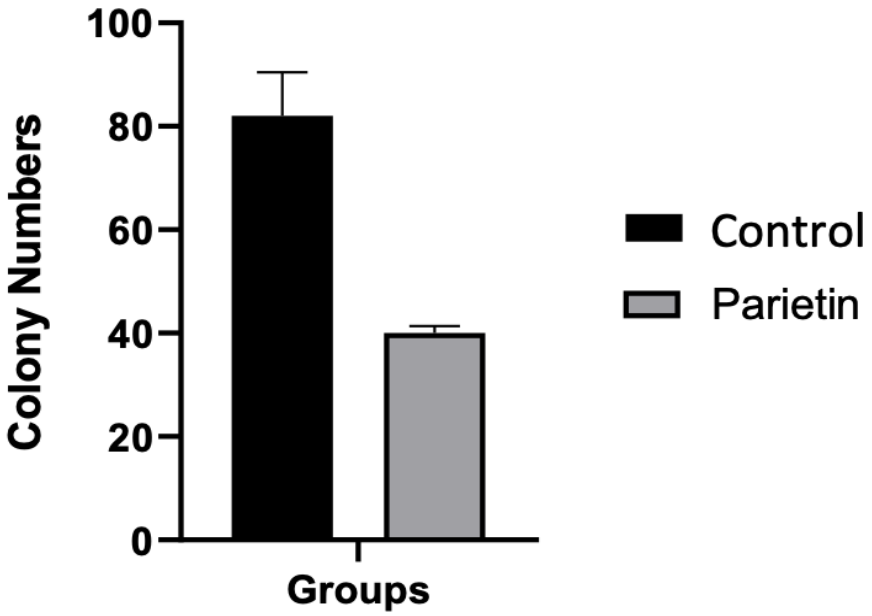

Figure 5. The number of colonies were significantly decreased in the cells treated with ferulic acid compared with the control cells

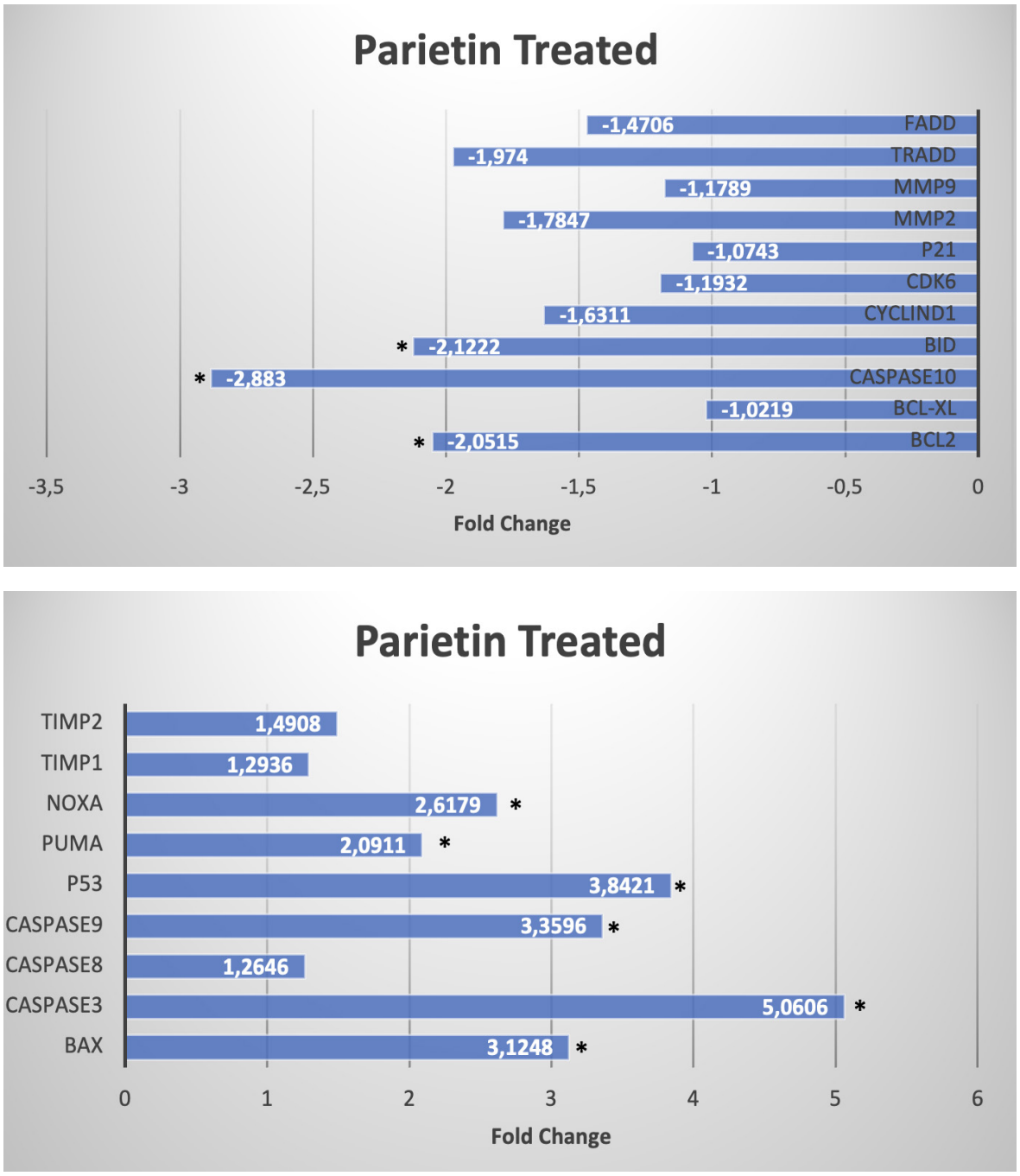

Figure 6. *mRNA* expression changes in parietin treated SH-SY5Y dose group
* $p<0.05$

Discussion

Cancer consists of cells that are unlimited and capable of dividing very rapidly. Cells have a certain number of divisions under normal conditions, but muscle and nerve cells do not have this feature.

Normal cells may turn into tumor cells over time due to genetic or environmental factors. When tumor cells come together, first a bulk and then cancer occurs. Neuroblastoma is malignant solid tumor originating from the sympathetic nervous system. It usually occurs in early childhood.

In a study, by isolating parietin from Rheum ribes L, it was obtained in different concentrations ranging from 1-100 µM and the cytotoxic effect of Parietin on Human Dermal Fibroblast cells was determined by MTT method. Concentrations in the range of 5-250 µM were chosen for parietin, and doses higher than 50 µM at 24 hours were found to have cytotoxic effects [11]. In our study, however, we found the IC_{50} value of parietin in the SH-SY5Y NB cells at a lower concentration to be 35 µM at the 48th hour.

In another study, the cytotoxic effect of parietin on cisplatin-resistant BRCA2-mutated human breast cancer (HCC1428), human breast ductal carcinoma (T-47D), and (HUVEC) cells were investigated and its effect on ROS accumulation and caspase 3 activation were determined.

Cell viability assays were performed by AlamarBlue method. The experiments, performed with parietin the calculated IC_{50} values of which for 48 hours treatment on the cells were higher than 600 µM, showed parietin to have less cytotoxic effects on healthy HUVEC cells than HCC1428 and T-47 D cells depending on concentration and time. Parietin showed significant anti-angiogenic and apoptotic activity at low concentrations, which are less or equal to 100 µM [12]. This result is in direct proportion to our study result.

Based on the results obtained from this study, it can be stated that it has been proven that parietin can be used in cancer treatment.

In the one study, the cytotoxic effect of Parietin isolated from Rheum ribes L. plant on the HepG2 cell line was examined. HepG2 liver cancer cells were cultured under appropriate conditions and incubated for 24 to 48 hours by applying parietin at concentrations ranging from 25-1000 µM to the cells. The cell viability rate was determined as 25 µM at 48th hour by XTT method depending on dose and time [13]. The results are also in direct proportion to those obtained in the present study. We determined IC50 value of parietin in the SH-SY5Y cells as 35 µM using XTT viability assay. In another study, parietin was isolated from Xanthoria Parietina (L.). The effect of parietin ethanolic extract (XpE) on mouse skin fibroblast cells L929 was investigated. The cell viability test was performed using the XTT method and the IC50 value in the L929 cell lines was determined to be 768.01 mg/mL [14].

In another study, the anti-proliferative and cytotoxic effects of the lichen compounds (parietin, atranorin, usnic acid, and gyrophoric acid) on nine human cancer cell lines (A2780, HeLa, MCF-7, SK-BR-3, HT-29, HCT-116 p53(+/+), HCT-116 p53(-/-), HL-60 and Jurkat) were evaluated. Parietin was obtained from Xanthoria Parietina.

The cell viability was tested by MTT analysis. In the cytotoxicity analysis performed depending on the dose and time, the IC50 value of the parietin was determined as 50 µM. Other cell lines were found to be parietin-resistant [15]. Excessive glutamate release causes prolonged activation of glutamate receptors, leading to excitotoxicity. Excitotoxicity is a process by which nerve cells are damaged due to their overstimulation. In another study, the neuroprotective effects of parietin in primary cortical neuron cultures against glutamate toxicity were investigated. It was shown that parietin increases cell viability/ migration and speed up wound healing [16]. The most significant increase was observed at 5-10 µM parietin concentrations by 92% and 96.87%, while the least increase was observed at 500 µM parietin concentration by 67% [16].

In a study, the neurotoxicity and molecular mechanism of Difenoconozal in the SH-SY5Y cell line were evaluated in vitro. Difenoconozal is a triazole fungicide. The IC50 value of Difenoconozal in SH-SY5Y NB cells was found to be 55.41 µM at the 24 h exposure. Difenoconozal has been shown that it can cause apoptosis of SH-SY5Y cells [17].

The cytotoxic effects of vulpinic acid, one of the lichen metabolites, on CaCo2, HepG2, Hep2C, RD Wehi cancer lines and normal cells and the gene expression levels were investigated. It was shown that the IC50 value of vulpinic acid changed the mRNA levels of Bax, Bcl2, and P53 genes in the cancer cells being examined, and the increase in Bax gene expression was more than the Bcl-2 and P53 genes in all cell lines [18].

Usnic acid is a secondary metabolite of lichens like parietin, and it has been used in many studies. The effects of usnic acid on lung cancer cell lines A549 were investigated. The expression levels of the genes (1-10-50 µg/L) in usnic acid at different concentrations were evaluated by Reverse Transcriptase Polymerase Chain Reaction method.

ß-actin was used as a control while determining the expression levels of APOPT1, cytochrome C, APAF1, CASPASE3, CASPASE9, TNF, BCL2, BCL2L1, and AIFM1 genes.

It was obtained from the analysis that usnic acid at concentration of 1 µg/mL showed no significant effect compared to ß-actin, except for CASPASE3. APOPT1, cytochrome C, APAF1, CASPASE3. CASPASE9 genes were found to have higher gene expression results at 10 µg/mL and 50 µg/mL concentrations of usnic acid compared to ß-actin. It was observed that there were no significant differences in the RT-PCR analyzes of TNF, BCL2, BCL2L1 and AIFM1 [19].

In another study, it was found that olivetol at the doses of 50 and 100 µM decreased MMP2 and MMP9 gene expressions of SH-SY5Y NB cell line [20]. ß-actin has been used as a control to compare data from the experimental groups and gene expression analysis evaluated by RT-PCR method.

Considering the gene expression studies, it is understood that there is limited information about Parietin. Considering the results of the studies related to parietin-like lichen metabolites, significant differences have been observed in the results regarding BAX, BCL-2, P53, MMP2, MMP9, CASPASE3, and CASPASE9 genes in direct proportion to our study results.

Usnic Acid, atranorine, physodic acid, ramalin, and physciosporin are secondary metabolites obtained from lichens such as Parietin. The migration and invasion analyze of these metabolites were performed on various cell lines. The results from the experiments results, performed on lung, liver, colon, breast, and prostate cancer cell lines, have shown that the metabolites suppress migration and invasion in these cancer lines [21].

Parietin is also a secondary metabolite and we showed it to likewise suppress migration, invasion formation when compared with our results on the SH-SY5Y NB cells.

Conflict of interest: No conflict of interest was declared by the authors.

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Ethics committee approval: Since our study is an in vitro study, ethics committee approval is not required.

Authors' contributions to the article

Y.D. have constructed the main idea and hypothesis of the study. M.S. and L.E. developed the theory and edited the material and method section. M.S. and G.G. have done the evaluation of the data in the results section. A.C. and N.S.G. have done the evaluation of the Discussion section of the article written by Y.D., A.C., N.S.G. and G.G. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

Research Article

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Investigation of DNA damage and inflammatory marker profile in patients after bariatric surgery

Obezite ameliyatı sonrası hastaların DNA hasarı ve inflamatuar marker profilinin araştırılması

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Abstract

Purpose: Obesity is a significant risk factor in the development of many serious diseases. The most prominent ones among them are diabetes and coronary heart disease. Elevated blood sugar levels in obese individuals lead to increased susceptibility to infections due to the suppression of the immune response. Moreover, fatty skin folds can cause topical infections, ulcers, and delayed/impaired wound healing. Therefore, inflammatory and pro-inflammatory markers in the serum have gained importance in diseases such as diabetes and obesity. Furthermore, in obesity, reactive oxygen species (ROS) lead to DNA damage. 8-oxo-dG, which is the primary product of DNA oxidation, can be detected in the serum, saliva, and urine, making it an ideal biological marker for DNA damage in large population-based studies. The Comet assay analysis is a method used to demonstrate the double-strand breaks in DNA. Induction of γ-H2AX in tissue indicates the initiation of a well-regulated mechanism to reverse double-strand breaks in DNA. Potential benefits of monitoring the genomic health in obesity include creating a sense of urgency for personalized intervention measures and evaluating their progress. DNA damage in obesity is believed to be a reversible condition. Chronic inflammation is an etiological factor known to support DNA damage and neoplastic transformations in cells. Cytokines secreted from adipose tissue, especially TNF-alpha, IL-6, and IL-1β, promote the accumulation of various cells, including neutrophils, macrophages, and dendritic cells, and it indicates the initiation of an inflammatory process. In this project, it was aimed to investigate possible changes in inflammation markers and DNA damage in individuals undergoing bariatric surgery, who were beginning to improve endocrine and metabolic syndrome markers.

Materials and methods: For this purpose, blood and urine samples were collected from 45 obese patients, who had undergone bariatric surgery and 45 healthy volunteers matched for age and gender. The levels of inflammatory markers (IL-1β, IL-6, IL-8, and TNF-α) and the DNA damage marker γ-H2AX in serum, as well as the amount of 8-oxo-dG in urine, were determined using ELISA. Additionally, the percentage of DNA damage was determined using the Comet assay analysis.

Results: Weight control achieved through bariatric surgery and the subsequent reduction in fat tissue resulted in a significant decrease in the levels of γ-H2AX and 8-oxo-dG, as well as a parallel significant reduction in the percentage of DNA damage in the Comet assay results. The significant decrease in inflammatory markers IL-1β, IL-6, IL-8, and TNF-α levels indicated that bariatric surgery also affects inflammation indirectly.

Conclusion: Although there are numerous studies in the literature on individual parameters related to DNA damage in various diseases and obesity, it is believed that the present study determining DNA damage, oxidation, and repair mechanisms simultaneously with inflammatory marker levels serves as a guiding example for comparing genomic health and stability in pre-obese, post-obese, and non-obese individuals.

Keywords: Obesity, DNA damage, inflammation, bariatric surgery.

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Öz

Amaç: Obezite, birçok ciddi hastalığın gelişmesinde önemli bir risk faktörüdür. Bunlar arasında en büyük payı diyabet ve koroner kalp hastalıkları oluşturmaktadır. Yüksek kan şekeri immün yanıtı baskıladığından obezlerde enfeksiyona yatkınlık artmaktadır. Ayrıca yağlı deri katlantıları topikal enfeksiyon, ülser ve gecikmiş-bozulmuş

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yara iyileşmesi sorunlarına neden olmaktadır. Bu nedenle diyabet ve obezite gibi hastalıklarda serumdaki inflamatuar ve pro-inflamatuar markerlar önem kazanmıştır. Ayrıca obezitede reaktif oksijen türleri (ROS), DNA hasarına neden olur. DNA oksidasyonunun başlıca ürünü olan 8-oxo-dG serum, tükürük ve idrarda tespit edilebilir, bu da büyük popülasyon bazlı çalışmalar için ideal bir DNA hasarı biyolojik belirleyicisidir. Komet assay analizi DNA çift zincir kırıklarının gösterilmesi için kullanılan bir yöntemdir. Dokuda γ-H2AX indüksiyonu, DNA'nın çift iplik kırıklarının tersine çevrilmesi için iyi düzenlenmiş bir mekanizmanın başladığını göstermektedir. Obezitede genom sağlığını izlemenin potansiyel faydaları, kişiselleştirilmiş müdahale önlemlerinin aciliyetini oluşturmak ve ilerlemelerini değerlendirmek için önemlidir. Obezitedeki DNA hasarının, geri dönüşümlü bir durum olduğu düşünülmektedir. Kronik inflamasyon, DNA hasarı ve hücrelerde neoplastik dönüşümlerin destekleyicisi olarak bilinen bir etiyolojik faktördür. Adipoz dokudan salgılanan sitokinler, özellikle TNF-alfa, IL-6 ve IL-1β, nötrofiller, makrofajlar ve dendritik hücreler dahil olmak üzere çeşitli hücrelerin toplanmasını teşvik eder ve bu da inflamatuar bir sürecin başladığını göstermektedir. Biz bu proje ile obezite operasyonu ile endokrin ve metabolik sendrom belirteçleri düzelmeye başlayan bireylerde inflamasyon markerları ve DNA hasarı üzerine olası değişimlerini araştırmayı amaçladık.

Gereç ve yöntem: Bu amaçla 45 adet obezite ameliyatı geçirmiş hasta ve bu hastalarla yaş ve cinsiyet açısından uyumlu olan 45 adet sağlıklı gönüllüden kan ve idrar örnekleri alınmıştır. İnflamatuar marker IL-1β, IL-6, IL-8 ve TNF-α ve DNA hasar markerı γ-H2AX'in serum miktarı ve ayrıca idrarda 8-oxo-dG miktarı ELISA ile tespit edilmiştir. Komet assay analizi ile de DNA hasar yüzdesi tespit edilmiştir.

Bulgular: Obezite ameliyatı ile sağlanan kilo kontrolü ve dolayısı ile yağ dokusundaki azalma, DNA hasarı üzerinde γ-H2AX ve 8-oxo-dG miktarının azalması ve buna paralel olarak komet assay sonuçlarında da DNA hasar yüzdesinin anlamlı derecede azalması ile sonuçlanmıştır. İnflamasyon markerları IL-1β, IL-6, IL-8 ve TNF-α miktarlarının anlamlı derecede azalması obezite ameliyatının dolaylı olarak inflamasyon üzerine de etkili olduğunu göstermiştir.

Sonuç: Literatürde DNA hasarına ilişkin tekil parametrelerle ilgili birçok hastalıkta ve obezitede çalışma bulunmakla birlikte, DNA hasarının, oksidasyonunun ve tamir mekanizmasının belirlenmesi ve inflamasyon markerlarının da eş zamanlı seviyelerinin belirlenmesi sayesinde genom sağlığı ve stabilitesinin obezite öncesi, sonrası ve non-obez bireylerde karşılaştırılması açısından çalışmamızın yol gösterici olduğunu düşünmekteyiz.

Anahtar kelimeler: Obezite, DNA hasarı, inflamasyon, obezite ameliyatı.

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Introduction

Obesity arises from the disparity between calorie intake and expenditure, leading to the accumulation of excess fat when more food is consumed than the energy consumed in the body [1]. Obesity is a serious health issue in today's world. It is estimated that there are approximately 700 million obese individuals in the world. It is projected that, as of the year 2030, 2.16 billion individuals (38%) will be overweight, and 1.12 billion individuals (10%) will be considered obese [2].

Obesity is a significant risk factor for the development of several serious diseases. Diabetes and coronary heart disease have the largest share among them. The treatment of obesity, especially for patients with morbid obesity (BMI >40), is often more costly and time-consuming than expected. Bariatric surgery is offered as a preferred treatment due to its long-lasting effects and low risk. Various surgical techniques with varying efficiency levels have been developed for this purpose [3]. Individuals with a BMI of 40 kg/m² or a BMI of 35 kg/m² with comorbidities (such as diabetes, hypertension, dyslipidemia, cardiovascular disease, and respiratory diseases) despite the diet and exercise can be treated with bariatric surgery [4]. Laparoscopic Sleeve Gastrectomy (LSG) and Roux-en-Y Gastric Bypass are currently the most widely preferred surgical methods nowadays [5]. In the LSG method, the procedure starts with the dissection of the omentum attached to the greater curvature of the stomach. Starting from 2.5 to 4 cm proximal to the pylorus, the greater curvature is resected up to the angle of His at the cardioesophageal junction. The gastrocolic and gastrosplenic ligaments are cut first. To completely resect the stomach fundus, where ghrelin, also known as the hunger hormone, is secreted, the omentum is freed up to the left diaphragmatic crus. With an average calibration of 36 French (1.2 cm), the orogastric tube is inserted into the stomach by the anesthesia team. Resection is performed by using cutting and stapling devices, ensuring that there is neither stricture nor excessive volume in the remaining stomach portion [6].

In obesity, the disruption of the balance between reactive oxygen species (ROS) mediated signaling mechanism and antioxidant defense causes comorbid diseases. ROS are known to be mutagenic, and they cause DNA damage and altered protein expression, as well as interfering with signaling pathways that support tumor formation [7]. 8-oxo-deoxyguanosine (8-oxo-dG) is a product of free radical-induced oxidation of guanosine. 8-oxo-dG can be detected in serum, saliva, and urine; this makes it an ideal biological marker for DNA damage in large population-based studies [8]. The comet assay analysis is a method used to demonstrate double-strand breaks in DNA. Tail length and the percentage of DNA within the tail are considered measures of DNA damage [9].

The induction of nuclear γ-H2AX foci indicates the initiation of a significant repair process potentially following a carcinogenic DNA lesion. The induction of γ-H2AX in tissues suggests the initiation of a well-regulated mechanism for reversing double-strand DNA breaks [10].

Previous studies reported a positive correlation between BMI and DNA damage. The potential benefits of monitoring “genomic health” in obesity are important in order to emphasize the urgency of personalized intervention measures and assess their progress. It is believed that DNA damage in obesity is a reversible condition [11].

Since high blood sugar suppresses the immune response, obese individuals are more susceptible to infections. Moreover, fatty skin folds can lead to topical infections, ulcers, and delayed/impaired wound healing. Therefore, in diseases such as diabetes and obesity, inflammatory and pro-inflammatory markers in the serum have gained importance. Bariatric surgery has become a widely preferred method for individuals with reduced responsiveness to medical treatment, compromised vital functions, and affected vital organs, leading to decreased mobility [12].

Chronic inflammation is an etiological factor known to support DNA damage and neoplastic transformations in cells. In the case of obesity, the release of pro-inflammatory molecules from adipose tissue, including CRP, TNF-α, and IL-8, was reported in previous studies [13]. It is known that plasma levels of IL-6, TNF-α, and TNF receptors increase in obese individuals [14]. Cytokines released from adipose tissue, especially TNF-alpha, IL-6, and IL-1β, promote the accumulation of various cells, such as neutrophils, macrophages, and dendritic cells, which indicates the initiation of an inflammatory process [15].

With this project, it was aimed to examine potential changes in inflammatory markers and DNA damage among individuals, in whom endocrine and metabolic syndrome markers started improving with bariatric surgery.

Material and methods

Sample collection

In the present study, 45 obese patients, who had undergone bariatric surgery, and 45 healthy volunteers matching with these patients in terms of age and sex were included. Approximately 5 ml of peripheral venous blood samples were collected from both healthy controls and obese patients before the surgery and one year after the surgery by using biochemical tubes (yellow cap), 3 ml of peripheral blood samples were collected into hemogram tubes (purple cap), and approximately 3 ml of urine samples were obtained.

DNA damage detection with the comet method

A 3 ml blood sample from the hemogram tube was added to a sterile 15 ml falcon tube after gently pipetting it following the addition of 3 ml histopaque. Following the centrifugation performed at 3000 rpm for 30 min. at 12°C, the intermediate phase containing lymphocytes was transferred to a new sterile 15 ml falcon tube. Subsequently, 3 ml of PBS was added, followed by centrifugation at 3000 rpm for 10 minutes at 12°C. The isolated lymphocytes were then stored at -80°C until the completion of other study samples.

DNA damage in lymphocytes was detected by using the classic alkaline comet assay method. In this method, lymphocytes were seeded on coverslips coated with agarose solution with a low melting temperature after being covered with agarose solution with a

high melting temperature. After treatment with a lysis solution, electrophoresis was performed in the electrophoresis solution at +4°C. After the electrophoresis process, neutralization was performed. Then, the coverslips treated with methanol were prepared for staining. Ethidium Bromide dye was used to stain the coverslips, and imaging was performed using a fluorescent microscope. DNA damage was evaluated based on parameters such as head length, head intensity, tail length, tail intensity, and tail moment of cells using a fluorescent microscope.

Detection of the serum levels of inflammatory markers IL-1β, IL-6, IL-8, TNF-α, and DNA damage marker γ-H2AX by using ELISA

Blood samples in the biochemical tube obtained from patient and control groups were centrifuged at 2500 rpm for 10 min. at +4°C to obtain serum. The sera obtained were stored at -80°C until the completion of the samples.

The quantities of inflammatory markers IL-1β, IL-6, IL-8, and TNF-α, as well as the DNA damage marker γ-H2AX, were determined by using the ELISA (BT Lab, China) method in all serum samples. In this system that is based on the biotin double-antibody (sandwich) principle, the wells were coated with specific antibodies. Standards were prepared in serial dilutions, starting from the stock standard, with concentrations of 1x, 1/2, 1/4, 1/8, 1/16, and 1/32. To the standard well, 50µl of standard and 50µl of Streptavidin-HRP were added. For the sample wells, 40µl of sample + 10µl of antibody + 50µl of Streptavidin-HRP were added. After incubation at 37°C for 60 minutes, washing process was performed with the washing solution. Then, 50µl of chromogen solution A and 50µl of chromogen solution B were added to the wells sequentially. After incubating in the dark at 37°C for 10 minutes, 50µl of stop solution was added (the blue color will turn yellow). The absorbance changes were measured at 450 nm wavelength with the resulting color change.

Detection of the level of 8-oxo-dG in urine by using ELISA

Urine samples obtained from patient and control groups were transferred to sterile 15 ml falcon tubes and centrifuged at 3000 rpm for 5 minutes at room temperature. The supernatant was transferred to sterile Eppendorf tubes and stored at -80°C until the completion of samples.

The amount of 8-oxo-dg indicating DNA damage due to oxidative stress was determined in all urine samples using the same ELISA (BT Lab, China) procedure.

Permission was obtained from Pamukkale University Non-Interventional Clinical Research Ethics Committee for the study.

Statistical analyses

A strong effect size (dz=1.6) was observed in the reference study. Considering that a lower effect size could be obtained (dz=0.4), a power analysis was performed, indicating that a 95% confidence level with 80% power could be achieved when at least 41 participants were included in the study. In order to make comparisons with the control group, a similar number of participants were included in the patient group (minimum n=41). Data were analyzed by using SPSS 25.0 software. Continuous variables were presented as mean ± SD, whereas categorical variables were presented as numbers and percentages. Independent group differences were compared with the Two-Sample t-test when parametric test assumptions were met, and the Mann-Whitney U test was used when parametric test assumptions were not met. For dependent group comparisons, the Two-Sample Paired t-test was used when parametric test assumptions were met, and the Wilcoxon test was used when parametric test assumptions were not met. Moreover, correlations between continuous variables were examined by using Spearman or Pearson correlation analyses, while differences between categorical variables were examined using the Chi-Square analysis.

Results

DNA damage between groups

Examining the results of comet analysis for DNA damage detection among groups, it was observed that the DNA damage of patients had significantly decreased at the post-surgery control performed 1 year after the surgery (p=0.0081). When evaluated in terms of head length, tail length, head intensity, tail intensity, and tail moment at the 1-year follow-up, a statistically significant difference was found in head length (the median value before surgery was 69.05 with an interquartile range of 14.37, while the median value was 84.35 with an

interquartile range of 23.74 in the 1st year control, *p*=0.010). No statistically significant difference was observed between the control group and the post-surgery group (median value for the control group was 89.35 with an interquartile range of 21.74, *p*=0.285) (Figure 1).

Serum levels of inflammatory markers IL-1β, IL-6, IL-8, TNF-α, and the DNA damage marker γ-H2AX among the groups

Comparing the IL-1β levels of the groups, it was observed that the IL-1β levels of patients significantly decreased at the 1st-year follow-up after bariatric surgery (*p*=0.003). No significant difference was determined between the control group and the 1st-year post-surgery control group (*p*=0.763) (Table 1).

Examining the IL-6 levels of the groups, significant differences in IL-6 levels of patients were observed at the 1st-year follow-up after bariatric surgery (*p*=0.001). No significant difference was observed between the control group and the 1st-year post-surgery control group (*p*=0.873) (Table 2).

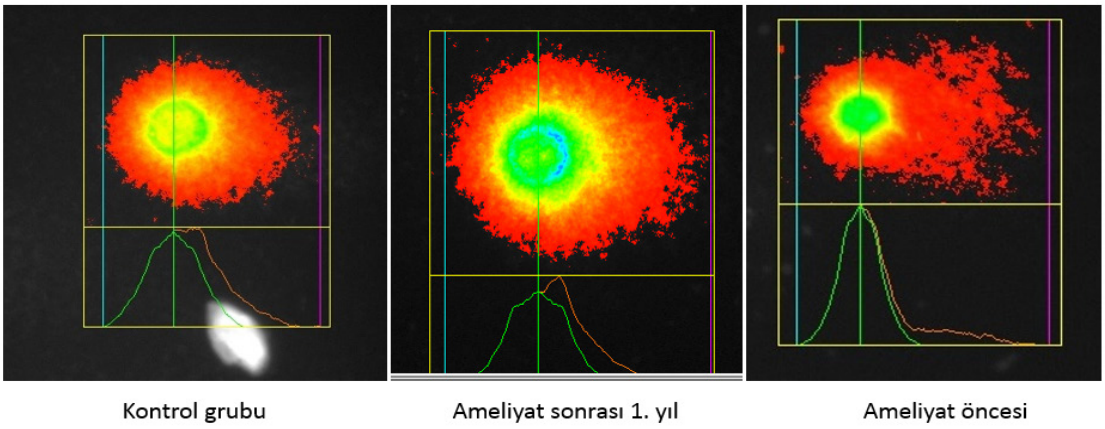

Figure 1. Comet images of control group, pre surgery and 1st year follow up

Table 1. Pre-surgery, 1st year postop, and control groups' IL-1β levels (pg/ml)

Group	Mean	Standard Deviation	Standard Error	*p*
Pre-surgery	498.96	352.91	52.64	0.003
1st year follow-up	333.58	207.31	30.902	0.763
Control	303.165	180.7	23.44	

Table 2. Pre-surgery, 1st year follow-up, and control groups' IL-6 levels (ng/L)

Group	Mean	Standard Deviation	Standard Error	*p*
Pre-surgery	60.45	37.56	5.59	0.001
1st year follow-up	31.42	7.63	1.138	0.873
Control	28.23	6.75	1.03	

Analyzing the IL-8 levels of the groups, it was determined that there was a statistically significant difference in IL-8 levels of patients at the 1st-year follow-up after bariatric surgery (p=0.001). While there was a significant difference in IL-8 levels between the control group and pre-surgery IL-8 levels, no significant difference was observed at the 1st-year post-surgery follow-up (p=0.6996) (Table 3).

Examining TNF-α levels of the groups, significant differences in TNF-α levels of patients were observed at the 1-year follow-up after bariatric surgery (p=0.000). While a statistically significant difference was found TNF-α levels of the control group and pre-surgery group, no significant difference was observed at the 1st-year post-surgery follow-up (0.723) (Table 4).

Considering the γ-H2AX levels of the groups, a significant difference was found in γ-H2AX levels of patients at the 1st-year follow-up after bariatric surgery (p=0.000). Although a significant difference was determined between γ-H2AX levels of the control group and pre-surgery γ-H2AX levels, no significant difference was observed at the 1st-year post-surgery follow-up (p=0.124) (Table 5).

Urinal 8-oxo-dG levels of the groups

Examining the 8-oxo-dG levels of the groups, there was a significant difference in 8-oxo-dG levels of patients at the 1st-year follow-up after bariatric surgery (0.002). Even though a significant difference was found between 8-oxo-dG levels of the control group and pre-surgery 8-oxo-dG levels, there was no significant difference in the 1st-year post-surgery follow-up (p=0.378) (Table 6).

Table 3. Pre-surgery, 1st year follow-up, and control groups' IL-8 levels (ng/L)

Group	Mean	Standard Deviation	Standard Error	*p*
Pre-surgery	233.2966	104.18029	15.53028	0.001
1st year follow-up	106.5141	31.60977	4.71211	0.6996
Control	90.85	22.03	2.36	

Table 4. Pre-surgery, 1st year follow-up, and control groups' TNF-α levels (ng/L)

Group	Mean	Standard Deviation	Standard Error	*p*
Pre-surgery	98.50	57.14	8.51	0.000
1st year follow-up	63.50	9.76	1.45	0.723
Control	61.25	10.85	2.43	

Table 5. Pre-surgery, 1st year follow-up, and control groups' γ-H2AX levels (ng/ml)

Group	Mean	Standard Deviation	Standard Error	*p*
Pre-surgery	7.35	5.81	0.866	0.001
1st year follow-up	2.87	1.52	0.227	0.124
Control	3.23	2.13	0.452	

Table 6. Pre-surgery, 1st year follow-up, and control groups' 8-oxo-dG levels (ng/ml)

Group	Mean	Standard Deviation	Standard Error	*p*
Pre-surgery	7.2	4.8	0.763	0.002
1st year follow-up	4.85	2.22	0.492	0.378
Control	3.12	1.73	0.397	

Discussion

The relationship between obesity and genotoxic damage is a relatively new research subject, and previous studies reported an accumulation of DNA damage among individuals with obesity and its association with obesity-related diseases [16, 17]. The results of a previous study carried out on the potential effects of weight loss on DNA damage by using the single-cell gel electrophoresis technique showed that weight loss can lead to a decrease in DNA damage levels in the body [18]. Furthermore, weight loss was shown to reduce inflammation markers such as C-reactive protein (CRP), Tumor Necrosis Factor-Alpha (TNF-α), and interleukin-6 (IL-6), as well as a decrease in oxidative stress [19]. In addition, high levels of proinflammatory cytokines, including prostaglandin E2, TNF-α, IL-2, IL-8, IL-10, and monocyte chemoattractant protein-1, were associated with increased levels of body fat. It was claimed that inflammation triggered by the activation of the nuclear factor kappa light chain-enhancer of activated B cells (NF-κB) complex may promote cancer development. Inflammation and immunological changes can also cause malignancy and progression by affecting DNA repair mechanisms, gene functions, and cellular mutation rates [20, 21]. In addition to endogenous antioxidant systems, a diet rich in antioxidants can protect DNA and enhance cellular resistance to oxidative stress [22, 23]. Consuming a diet rich in fruits and vegetables was shown to reduce the risk of metabolic diseases and cancer [24]. Furthermore, the quality and quantity of dietary fat were shown to be correlated with DNA stability and a diet rich in polyunsaturated fatty acids might reduce DNA damage [25].

It is known that obesity alters the DNA double-strand break repair mechanism caused by genotoxic chemicals [26]. Previous scientific studies have revealed that individuals with obesity have double-strand breaks, single-strand breaks, oxidized bases, and DNA damage that are twice as high as in normal-weight individuals [27, 28]. Obesity can lead to an increase in DNA damage or disruptions in DNA repair mechanisms, resulting in the accumulation of DNA damage in cells, leading to inflammation, changes in gene expression, and disruptions in cellular metabolism [26]. Studies carried out on experimental animals reported an increase in genotoxic damage to mitochondrial DNA (mtDNA) in animals fed a high-fat diet [29, 30]. During the development of obesity, the accumulation of T lymphocytes and macrophages in adipose tissue promotes the production of reactive oxygen species (ROS) by NADPH oxidase and nitric oxide 2 protein [31-33]. ROS formation can lead to increased insulin levels, fatty acids, and glucose levels, and indirectly contribute to inflammation [34]. Disruptions in the oxidant/antioxidant balance can negatively affect cellular biomolecules, including DNA [35]. According to the results of a previous study, obesity was associated with inflammation and oxidative stress, which induce DNA damage, and the prevention of DNA repair results in the accumulation of DNA damage in adipocytes [26]. A study carried out on young adults aged between 18 and 30 years revealed a negative correlation between BMI and nucleotide excision repair (NER) capacity [36]. The results of a study comparing the evaluation of DNA damage in peripheral lymphocytes of obese and normal-weight adolescents in cell culture environments indicated that obesity can alter the repair mechanism of DNA double-strand breaks caused by genotoxic agents [37].

In this study, comparing the preoperative and postoperative results of DNA damage markers, γ-H2AX, and 8-oxo-dG, in both whole blood and serum, it was demonstrated that there was a reduction in the amount of DNA damage among obese patients after bariatric surgery. In addition, comparing the levels of inflammation

markers, IL-1β, IL-6, IL-8, and TNF-α, before and one year after the surgery, a decrease was observed in inflammation markers after surgery, reaching similar levels to the control group.

In conclusion; weight control achieved through sleeve gastrectomy, which is a restrictive method in morbidly obese individuals, resulted in a reduction in DNA damage and inflammation. Although there are many studies carried out on individual parameters related to DNA damage in various diseases and obesity, it is believed that the present study provides valuable insights into genome health and stability, both before and after obesity, and in non-obese individuals, by determining DNA damage, oxidation, and repair mechanisms simultaneously, as well as evaluating inflammation markers.

Conflict of interest: No conflict of interest was declared by the authors.

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Evaluation of final heights in patients with congenital adrenal hyperplasia

Konjenital adrenal hiperplazili hastalarda final boyun değerlendirilmesi

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Abstract

Purpose: Congenital adrenal hyperplasia (CAH) is an autosomal recessive disease that occurs as a result of a deficiency of any of the enzymes required for the synthesis of glucocorticoids, mineralocorticoids and sex steroids from cholesterol in the adrenal cortex. In this study, we have aimed both to evaluate the final heights in patients with CAH secondary to 21-hydroxylase and 11β- hydroxylase deficiency and also to investigate the factors affecting the final heights.

Material and methods: The anthropometric, clinical, and laboratory findings of patients diagnosed with CAH in the Pediatric Endocrinology Clinic were evaluated retrospectively. Among patients who reached their final heights and adhered to their regular control visits, a total of 39 CAH patients without precocious puberty, and any additional disease diagnosed during their follow-up were included in the study.

Results: Among cases with 21- hydroxylase deficiency, mean final heights of female, and male patients with classic simple virilizing CAH were 158.2±5.46 cm, and 168.8±11.67 cm, while in salt-wasting CAH the corresponding final heights were 152.2±5.94 cm, and 156.5±6.2 cm, respectively. In the group with non-classic CAH, mean final heights of female, and male patients were 155.9±7.59 cm, and 157 cm, respectively. The final height SD of all classic CAH cases was - 1.41±1.45, and it was calculated as -0.81±1.12 (-2.30-0.80) in cases with simple virilizing type classic CAH and -1.79±1.53 (-3.70-0.70) in cases with salt-wasting type classic CAH. In non-classic CAH cases, the final height SD was calculated as -1.65±1.69. When patients with salt-wasting CAH and simple virilizing CAH were compared in terms of final height SDs and genetically adjusted height SDs, the final heights of patients with simple virilizing CAH were significantly higher (p<0.05), and the final heights of cases with 11 β- hydroxylase deficiency were significantly shorter than all groups (p<0.05). In CAH, both hyperandrogenism resulting from inadequate treatment and high-dose glucocorticoid treatment may result in a comparatively shorter final height. For this reason, patients should be evaluated at regular intervals in terms of early recognition of CAH through CAH screening programs, administration of glucocorticoid therapy in appropriate doses (10-15 mg/m²/day), and metabolic control monitoring.

Conclusion: In our study, the best average final height was found in the group using hydrocortisone dose of 10-15 mg/m²/day. We have revealed that when daily doses ranging between 5-10 mg/m² were used, androgens were not suppressed sufficiently and the epiphyses closed prematurely, and in cases where daily doses exceeding 15 mg/m² were administered, the final heights were relatively shorter due to the use of excess doses of glucocorticoids.

Keywords: Congenital adrenal hyperplasia, 21-OH deficiency, final height, corticosteroid treatment.

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Öz

Amaç: Konjenital adrenal hiperplazi (KAH), adrenal kortekste kolesterolden glukokortikoid, mineralokortikoid ve seks steroidinin sentezi için gerekli olan enzimlerden herhangi birinin eksikliği sonucu ortaya çıkan otozomal resesif bir hastalıktır. Bu çalışmada 21-hidroksilaz ve 11 Beta hidroksilaz eksikliğine bağlı KAH hastalarında son boy uzunluğunun değerlendirilmesi ve bunu etkileyen faktörlerin araştırılması amaçlandı.

Gereç ve yöntem: Çocuk Endokrinoloji Kliniğinde KAH tanısı konulan hastaların antropometrik, klinik ve laboratuvar bulguları retrospektif olarak değerlendirildi. Çalışmaya düzenli kontrolleri olan, takiplerinde erken puberte geçirmeyen, takiplerinde ek hastalığı olmayan ve son boya ulaşan 39 KAH hastası dahil edildi.

Bulgular: 21 hidroksilaz eksikliğine bağlı klasik basit virilizan KAH'lı kadın olgularda final boy 158,2±5,46 cm, erkek olgularda final boy 168,8±11,67 cm, tuz kaybettiren tip kadın olgularda final boy 152,2±5,94 cm, tuz kaybettiren erkek olgularda final boy ise 156,5±6,2 cm idi. Non-Klasik kadın olgularda final boy 155,9±7,59 cm, 1 erkek olguda ise final boy 157 cm olarak tespit edildi. Klasik tip KAH olgularının tamamının final boy SD-1,41±1,45 SD olup, basit virilize tip klasik KAH olgularında -0,81±1,12 (-2,30-0,80), tuz tüketen tip klasik

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KAH vakalarında -1,79±1,53 (-3,70-0,70) olarak hesaplandı. Klasik olmayan KAH vakalarında son boy SD'si -1,65±1,69 SD olarak hesaplandı. Tuz kaybettiren KAH ve basit virilize KAH'lı hastalar final boy SD'si ve genetiğe göre düzeltilmiş boy SD'si açısından karşılaştırıldığında, basit virilize KAH'lı hastaların final boyları anlamlı derecede yüksek (p<0,05) ve 11 Beta hidroksilaz eksikliği olan hastaların final boyları ise vakaları tüm gruplara göre anlamlı olarak daha kısaydı (p<0,05).
KAH'ta hem yetersiz tedaviden kaynaklanan hiperandrojenizm, hem de yüksek doz glukokortikoid tedavisi boy kısalığına neden olabilir. Bu nedenle KAH tarama programları ile KAH'ın erken tanınması, uygun dozlarda (10-15 mg/m²/gün) glukokortikoid tedavisinin uygulanması ve metabolik kontrol takibi açısından hastaların düzenli aralıklarla değerlendirilmesi gerekmektedir.
Sonuç: Çalışmamızda en iyi ortalama final boy uzunluğu 10-15 mg/m²/gün hidrokortizon dozu kullanan grupta bulundu. 5-10 mg/m²/gün kullanıldığında androjenlerin yeterince baskılanmadığını ve epifizlerin erken kapandığını, >15 mg/m²/gün kullanıldığında ise glukokortikoid fazlalığına bağlı olarak son boyun kısaldığını saptadık.

Anahtar kelimeler: Konjenital adrenal hiperplazi, final boy, 21-OH eksikliği, kortikosteroid tedavisi.

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Introduction

Congenital adrenal hyperplasia (CAH) is an autosomal recessive disease that occurs as a result of deficiency of any of the enzymes required for the synthesis of glucocorticoids, mineralocorticoids, and sex steroids from cholesterol in the adrenal cortex [1-7]. While CAH due to 21-hydroxylase enzyme deficiency constitutes 90-95% of all cases, 11 β - hydroxylase deficiency is the second common cause of CAH. Clinical findings vary depending on the type and degree of enzyme deficiency. In our treatment, we have aimed to replace the unsynthesized cortisol, as well as to prevent the increase in androgen secretion by suppressing the adrenocorticothyropic hormone (ACTH) secretion. The gap between the doses used in undertreatment and overtreatment is extremely narrow, and the risk of complications increases if the doses used are not individualized. In cases where inadequate doses used for the treatment of CAH, excessive androgen exposure causes bone age progression and precocious puberty, increasing the adult final heights. On the other hand, treatment with higher doses of glucocorticoid also suppresses linear growth by adversely affecting the growth hormone-IGF-1 axis. As a result, final heights may remain short in those receiving low or high doses of glucocorticoids. The glucocorticoid dose that is used for treatment should be high enough to suppress the excessive production of sex steroids and low enough to minimize the side effects that may develop due to hypercortisolism [8, 9].

The dose of glucocorticoids required to normalize ACTH levels in patients with CAH may be much higher than used in other forms of adrenal insufficiency. Therefore, when planning treatment in classic CAH, a balance should be maintained between avoiding overtreatment, which may cause many negative side effects on growth rate, metabolic, cardiovascular status and bone health, or undertreatment, which carries the risk of life-threatening adrenal crisis and virilization [10].

In this study, we have aimed to evaluate the final heights in patients with CAH due to 21-hydroxylase or 11 β-hydroxylase deficiency and to investigate the factors affecting the final heights.

Materials and method

The anthropometric, clinical and laboratory findings of patients diagnosed with CAH in the Pediatric Endocrinology Clinic of our university hospital were evaluated retrospectively. Among patients who reached their final heights and adhered to their regular control visits, a total of 39 CAH patients without precocious puberty, and any additional disease diagnosed during their follow-up were included in the study. Based on patients' complaints on admission (suspicious genitalia, signs of salt wasting CAH, pubic hair growth, accelerated somatic development, early puberty), genotypic sex determination by karyotype analysis, levels of basal or ACTH-stimulated serum 17-OHP, adrenal androgens (DHEAS, androstenedione), serum testosterone and ACTH, results of

genetic tests performed to diagnose CAH due to 21-hydroxylase deficiency, patients were grouped as follows: Classic CAH due to 21-hydroxylase deficiency (13 salt-wasting and 7 simple virilizing CAH patients), non-classical CAH (n:13) and 11 β-hydroxylase-deficient group (n:6). The diagnosis of congenital adrenal hyperplasia due to 11 β-hydroxylase deficiency is based on raised serum 11-deoxycortisol and 11- deoxycorticosterone levels together with increase in the levels of adrenal androgens.

All patients received hydrocortisone treatment. The treatment doses received by the patients and their stages of puberty were recorded.

Anthropometric evaluations (height, body weight, BMI, waist circumference, growth rate) of the patients were made at each control visit. Body mass indices were calculated according to the formula: BMI=weight(kg)/height(m)}^{2}. The SD value of the relevant anthropometric value for each case was calculated by taking into account the standards of our country. Final height was defined as bone age ≥15 years in girls, and ≥16 years in boys, or growth rate of <1cm/year.

For metabolic monitoring, serum basal 17-OHP, ACTH, testosterone, androstenedione, and in salt-wasting CAH patients' plasma renin activity levels were measured. During the follow-up, serum 17-OHP and ACTH measurements were made at least 3 times a year to check whether the desired levels were achieved. The desired level was accepted as <10 nmol/lt (3.3 ng/ml) for 17-OHP and <70 pg/ml for ACTH. In all control visits, the patients who achieved the desired metabolic control values of 70% or higher for ACTH and 17-OHP levels were interpreted as having good metabolic control, while the others were interpreted as having poor metabolic control. In salt wasting CAH patients, serum Na and K and plasma renin levels were measured at each follow-up visit, and subtle signs of salt wasting were investigated. In addition, care was taken to ensure that testosterone and androstenedione levels remained within normal ranges in patients with good metabolic control. In routine controls, blood samples were taken from the patients between 08:00 and 09:00 AM, following an 8-hour fasting period. Glucose, cortisol, 17-OHP, ACTH, aldosterone, PRA, DHEAS, androstenedione and testosterone levels were studied from the blood samples taken. Glucose levels were examined using an Architect c16000 autoanalyzer. Total testosterone and DHEAS levels were measured by a chemiluminescent immunoassay method (Advia Centaur® XP). ACTH measurement was also performed with the chemiluminescence method (Immulite® 2000XPI). Serum androstenedione, 17-OHP, and plasma renin levels were examined by radioimmunoassay method.

The mean daily hydrocortisone (HC) or HC-equivalent glucocorticoid doses (mg/m^2) used during the whole follow-up period were also calculated. Bone age assessment was done annually according to the Greulich Pyle radiographic atlas of skeletal development of the hand and wrist.

Target heights (THs) of the study participants were calculated by the sum of the average heights of the parents as follows: Boys: Sum of mother's and father's heights+13 cm)/2, Girls: (Sum of mother's and father's heights - 13 cm)/2. TH SDs were calculated as follows: For girls (target height-163 cm)/5.93, and for boys (target height-176 cm)/6.3 was used. Adjusted height SD ≥0 was interpreted as appropriate height according to genetic height potential, and <0 as height below genetic height potential. The height of the patients who reached the final height was also evaluated considering that their target height was within ±5 cm of their final heights. Based on our assessments, patients whose final heights were at least 6 cm shorter than their target heights did not reach their target heights Approval for the study was received from the Educational Planning Board of Dr. Sami Ulus Gynecology, Child Health and Diseases Training and Research Hospital.

Statistical analyses

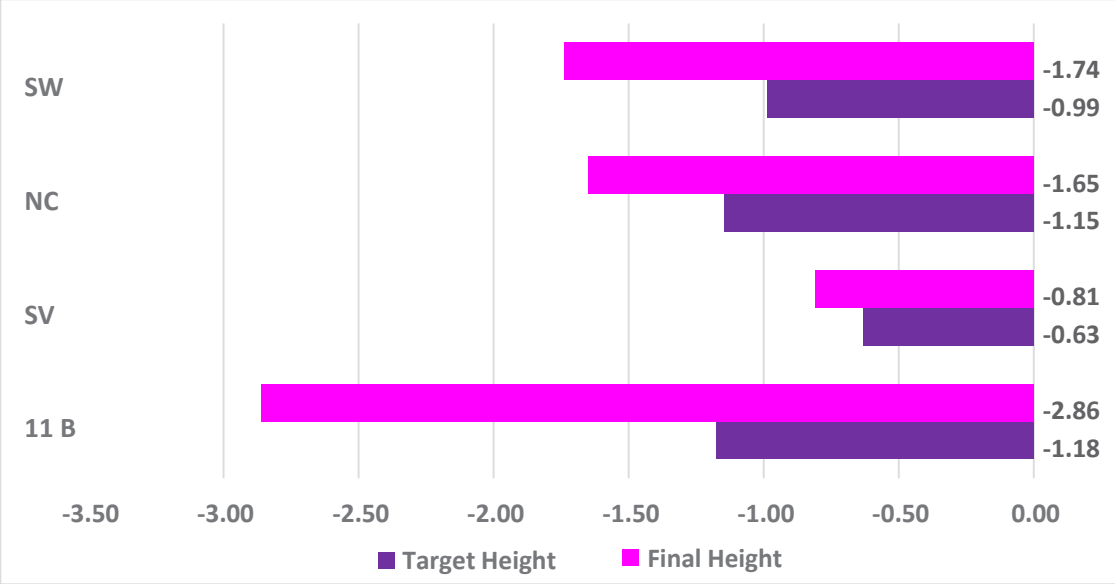
All statistical analyses were performed using the IBM SPSS for Windows Version 22.0 package program. Numerical variables were summarized as mean ± standard deviation, and median [minimum-maximum] values. Categorical variables were shown in numbers and percentages. Differences between both groups in terms of numerical variables (if any) was investigated with the Mann-Whitney U

test. Spearman correlation coefficient was used to examine the correlation between different variables. According to Pearson correlation analysis, correlation coefficients (r) of 0.25, 0.26-0.50, 0.26-0.50, and 0.76-1 indicated the presence of weak, moderate, strong, and very strong correlations between different variables The level of statistical significance level was accepted as $p<0.05$.

Results

Our study population consisted of 30 (76.9%) female, and 9 (23.1%) male patients. The average age at diagnosis of all cases was 3.0±5.46 years (0-17 years) and 13 of them were diagnosed in the neonatal period. Mean ages at diagnosis of cases with classic type 21-hydroxylase deficiency (1.7±2.7 years), non-classic type 21-hydroxylase deficiency (11.3±3.8 years,) and 11 β-hydroxylase deficiency (2.4±1.4 years) were as indicated cases diagnosed with CAH had 21-hydroxylase deficiency (n:33; 84.6%), 11 β- hydroxylase deficiency (n:6; 15.4%), while patients with 21-hydroxylase deficiency had salt-wasting classic CAH (n:13; 33.3%), simple virilizing classic CAH (n:7; 17.9%), and non-classic CAH (n:13; 33.3%).

The mean final heights were 158.2±5.46 cm in female and 168.8±11.67 cm in male cases with classic simple virilizing CAH due to 21-hydroxylase deficiency. Female, and male cases with salt-wasting classic CAH had mean final heights of 152.2±5.94 cm and 156.5±6.2 cm, respectively. In female cases cases with non-classic CAH mean final height was 155.9±7.59 cm. and 157 cm in 1 non-classic male case. The final height of all classic type CAH cases was - 1.41±1.45 SD, and they were -0.81±1.12 (-2.30-0.80) in cases with simple virilizing type classic CAH and -1.79±1.53 (-3.70-0.70) in cases with salt-wasting type classic CAH. In non-classic CAH cases, final height SD was calculated as -1.65±1.69. When patients with salt-wasting CAH and simple virilizing CAH were compared in terms of final height SD scores and genetically adjusted height SD scores, the final heights of patients with simple virilizing CAH were significantly higher ($p<0.05$), and the final heights of 11 β-hydroxylase deficiency cases were significantly shorter than all groups ($p<0.05$). Graph 1 shows the final height SDS and target height SDS by CAH types.



Graph 1. Final height SDs and target height SDs by CAH types

SW: Salt wasting, NC: Non-classic, SV: Simple Virilizating

The average daily hydrocortisone doses used were 13.14±5.06 mg/m² in all cases. While average daily hydrocortisone doses used were 15.06±3.78 mg/m² in the classic CAH, 14.42±3.49 mg/m², in the simple virilizing CAH, 15.40±4.02 mg/m² in the salt-wasting type CAH, 7.90±2.74 mg/m² in o non-classic CAH and 18.08±2.59 mg/m²/g in 11 β-hydroxylase deficiency groups. The average daily HC doses were 5-10 mg/m² in 11 (28.2%), 10-15 mg/m² in 12 (30.8%), and ≥15 mg/m² in 16 (41%) patients. Among all groups the highest dose of hydrocortisone was used for patients with 11 β-hydroxylase deficiency (p<0.05).

Table 1 shows the distribution of patients' characteristics at diagnosis and clinical follow-up.

Indicated numbers of cases reached their target heights in groups of classic salt-wasting type CAH (n:10; 76.8%) simple virilizing classic type CAH (n:7; 100%), non-classic CAH (n:10; 76.8%), and 11 β-hydroxylase deficiency (n:3; 50%). Table 2 shows the clinical and demographic characteristics of the patients who could not achieve their target heights.

When final height SDs of the cases were evaluated in consideration of average hydrocortisone doses used, we have noticed that respective mean final height SDs, and genetically adjusted height SDs of the cases who used average daily hydrocortisone doses of 5-10 mg/m² (n:11) (-1.72±1.58, and -0.55±1.3) or 10-15 mg/ m² (n:12) (0.75±0.98, and -0.22±1.0) or ≥15 mg/m² (n:16) (-2.18±1.29, and 1.17±0.8) were as indicated in parentheses (Graph 2). Final height SD was found to be significantly lower in those using daily hydrocortisone doses of ≥15 mg/m². The final height of the patients whose daily HC doses were in the range of 10-15 mg/m²/day reached a statistically significantly higher average final height compared to the other patients (p<0.05). In addition, the patients using daily hydrocortisone doses of 10-15 mg/m² had a statistically significantly higher genetically adjusted mean height than the patients using daily hydrocortisone doses of 15 mg/m² (p<0.05).

Nineteen (48.7%) of our cases had achieved good and 20 (51.3%) cases had poor metabolic control. The mean final height, and genetically adjusted height SD SDs of the patients with good metabolic control were -1.15 SD and -0.34 SD, respectively, while the corresponding SDs for patients with classic CAH with poor metabolic control were -2.25 SD and -1.08 SD, respectively (Graph 3). The final height SD and genetically adjusted height SD of patients with CAH with good metabolic control were significantly higher when compared to those with classic CAH with poor metabolic control (p<0.05).

Table 1. Diagnosis and follow-up data of patients

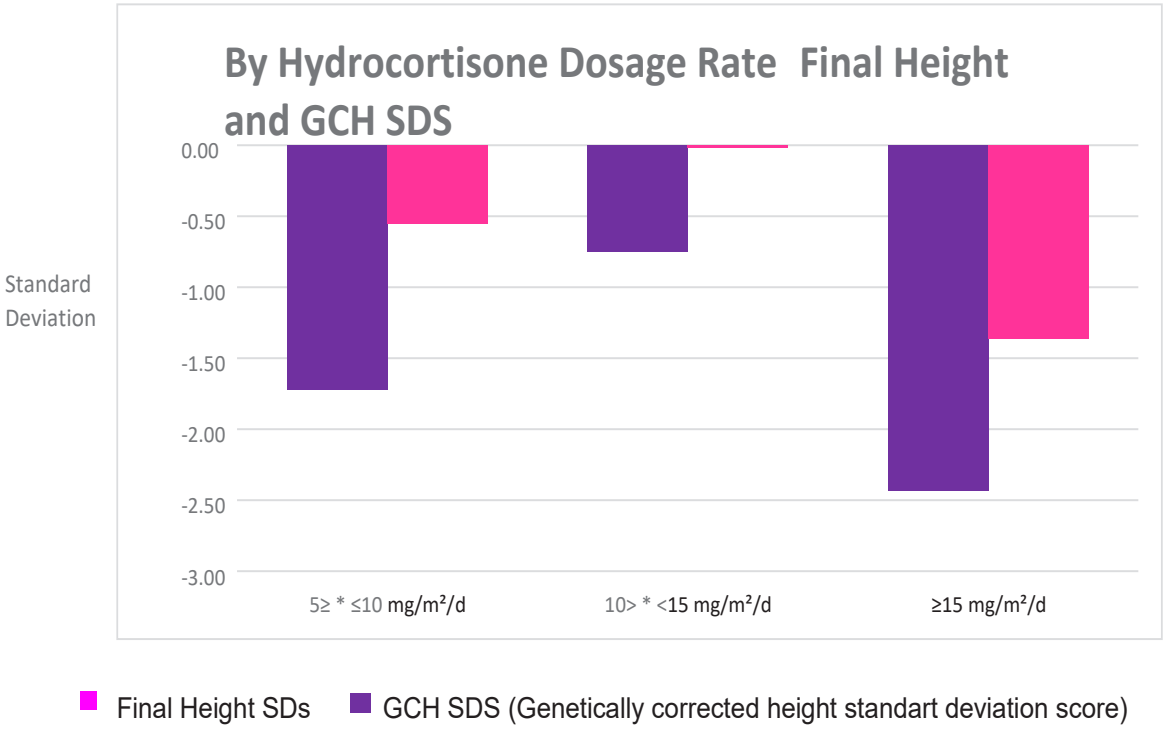
Gender	
Famele	30 (76.9%)
Male	9 (23.1%)
Age at diagnosis	3.0±5.46 years (yd-17 years)
Newborn	13 (33.3%)
>1 month	26 (66.7%)
CAH type	
21 OH	33 (84.6)
Salt-wasting	13(33.3%)
Simple virilizing	7 (17.9%)
Non-classic	13 (33.3%)
11B	6 (15.4)
Average HC dose (mg/m²/day)	
All cases	13.14±5.06 (5.1-27.0)
21 OH (n:33)	12.25±4.89 (5.1-27.0)
Classic CAH (n:20)	15.06±3.78 (10.1-27.0)
Simple-virilizing (n:7)	14.42±3.49 (10.1-18.3)
Salt-wasting (n:13)	15.40±4.02 (10.3-27.0)
Non-Classic CAH (n:13)	7.90±2.74 (5.1-15.3)
11B (n:6)	18.08±2.59 (15.2-22.0)
HC dose (mg/m²/day)	
5-10	11 (28.2%)
10-15	12 (30.8%)
≥15	16 (41.0%)
Metabolic control	
Good	19 (48.7%)
Poor	20 (51.3%)
Final Height SDS (by diagnosis)	
All cases	-1.41±1.45 (-3.70-0.80)
21 OH (n:33)	-1.51±1.53 (-4.60-0.86)
Classic CAH (n:20)	-1.41±1.45 (-3.70-0.80)
Simple-virilizing (n:7)	-0.81±1.12 (-2.30-0.80)
Salt-wasting (n:13)	-1.79±1.53 (-3.70-0.70)
Non-Classic CAH (n:13)	-1.65±1.69 (-4.60-0.86)
11 β (n:6)	-2.86±1.53 (-5.40 - -1.30)
Final Height (by gender)	
Genotypic female (46, XX)	155.60±7.79 cm (139.7-168.0)
Genotypic male (46, XY)	160.36±7.52 cm (150.6-177.1)

CAH: Congenital adrenal hyperplasia, HC: Hydrocortisone, 21 OH: 21 Hydroxylase deficiency, 11 β: 11 Beta-hydroxylase deficiency
SDS: Standard Deviation Score

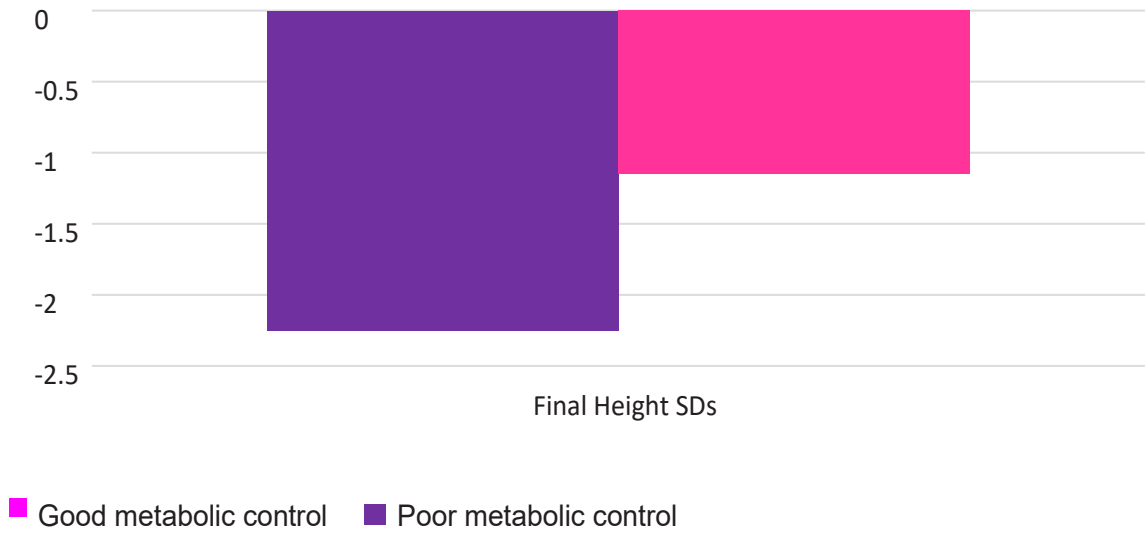
Table 2. Clinical and demographic characteristics of patients who could not achieve their target heights

Case	CAH type	Gender	Age at diagnosis (years)	Age at final height (years)	Calendar Age (years)	HC Dose (mg/m^2/ day)	Final Height (cm)	Target Height (cm)	Final Height SD	Target Height SD	GGDB SD	Body weight (kg)	BMI (kg/m^2)	BMI SD	Metabolic/ Control
1 ÖB	SW	46XX	NB	11	18	15.3	148.0	154.2	-3.40	-1.50	-1.90	57.30	24.00	0.920	Poor
2 HU	SW	46XY	5	12	16	18.3	150.6	168.0	-3.70	-1.30	-2.40	63.00	27.60	1.500	Poor
3 TH	SW	46XY	0	16	20	12.2	155.9	165.0	-3.00	-1.78	-1.22	80.40	33.30	2.450	Poor
4 DY	NC	46XX	7	13.5	15	15.3	148.1	154.0	-3.40	-1.53	-1.87	43.05	19.60	-0.450	Good
5 FA	NC	46XY	10	15	20	5.1	157.0	166.5	-2.85	-1.54	-1.31	60.00	22.20	0.660	Good
6 FT	NC	46XX	15	15	19	7.5	144.7	158.5	-4.60	-0.78	-3.82	50.65	24.10	0.800	Poor
7 MS	11 β	46XX	3	14	18	19.0	140.6	156.2	-3.71	-1.20	-2.51	44.00	22.13	0.250	Good
8 HO	11 β	46XY	3	13.1	25	22.0	155.3	169.3	-3.00	-0.99	-2.01	65.00	26.60	-0.990	Good
9 MS	11 β	46XX	4	14	16	15.6	139.7	166.5	-5.40	-1.54	-3.86	45.10	23.30	0.330	Poor

CAH: Congenital adrenal hyperplasia, HC: Hydrocortisone, SD: Standard Deviation, GGDB: Genetically adjusted height, BMI: Body mass index, NB: Newborn, SW: Salt wasting, NC: Non-Classic; 11 β:11 beta-hydroxylase deficiency



Graph 2. Final height SDs and GCH SDs by hydrocortisone doses



Graph 3. Metabolic control-final height SDs

Discussion

In our study, the final heights of 39 patients followed up with the diagnosis of congenital adrenal hyperplasia and the factors affecting their final heights were evaluated, and 60% of the cases achieved their target heights. Although the best final height average was achieved in patients with simple virilizing CAH, and in 11 β-hydroxylase deficiency, the final height average of the former group was lower when compared with 21-hydroxylase deficiency cases. The age at diagnosis, hydrocortisone dose, and metabolic control were important factors affecting the achievement of final and target heights.

Congenital adrenal hyperplasia is an autosomal recessive disease that occurs as a result of the deficiency of any of the enzymes required for the synthesis of glucocorticoids and mineralocorticoids from cholesterol in the adrenal cortex, causing glucocorticoid and mineralocorticoid deficiency. However, the selection of metabolic criteria to be used as a parameter in monitoring the patients with good and poor metabolic control, and the determination of the ideal treatment dose are still controversial issues [8]. Recent studies have emphasized that metabolic status evaluated by serum 17-OHP levels lead to the use of higher doses of steroids, and attention is drawn to the use of serum testosterone and androstenedione levels as metabolic control measures. Despite all these suggestions, there is no consensus on the gold standard monitoring parameter to be used for the adjustment of the treatment dose in CAH.

In different studies, along with heart rate monitoring, anthropometric measurements, and bone age assessments, serum 17-OHP and/or ACTH levels were measured [11]. Serum 17-OHP, ACTH and testosterone levels were evaluated in prepubertal patients [8], and serum 17-OHP and pregnanetriol and 17-ketosteroid were measured in 24-hour urine samples [12]. Although it is recommended to measure serum 17-OHP levels at 08:00 AM, considering the diurnal variations, it is emphasized that it is more appropriate to evaluate serum 17-OHP levels using the 17-OHP profile determined based on measurementa at different time points (08:00 AM, 12:00 AM, 05:00 PM, and 10:00 PM) [13]. The most important points to be emphasized in the treatment of CAH are to normalize ACTH secretion, inhibit the excessive secretion of adrenal androgens, and replace steroids that are not synthesized in the adrenal gland [1-4, 8]. Therefore, the dose of corticosteroid used should be kept in the dose range that will suppress adrenal androgens and at the same time minimize the negative effects of the long-term steroid treatment. If the patient has received excessive or insufficient treatment doses, relatively shorter final height may be achieved, and maintained due to the failure to control early pubertal findings, the acceleration in somatic development and the resulting epiphyseal closure [6]. Due to all these disadvantages encountered in the management of the disease and the complications that may develop, it is extremely important to follow up CAH patients conscientiously. In our study, we evaluated serum ACTH, 17-OHP, testosterone and androstenedione levels, as well as anthropometric parameters, to evaluate metabolic control in CAH patients. If our patients' growth rate decreased and they became overweight during follow-up period, we reduced the steroid dose. On the other hand, rapid progression in growth rate and bone age was taken as an evidence of insufficient maintenance doses of steroids used, and increase in androgen levels urged us to increase the steroid dose. We meticulously adjusted and individualized the steroid doses of our patients during their follow-up, taking into account both anthropometric and hormonal changes. In our study population we used serum ACTH and 17-OHP levels as metabolic control measures. Therefore, during the follow-up of these patients, serum 17-OHP levels between 1-10 ng/ml and ACTH levels below 71 pg/ml were used as criteria for good metabolic control. Patients who achieved 70% of the desired values in all measurements were included in the good metabolic control group.

Accordingly, 52% of the cases were evaluated as having good metabolic control. Although the final height deviations of the cases in the good control group - though not statistically significantly-were better than those in the poor control group. When the metabolic controls and average heights of our cases were examined, it was seen that patients with good metabolic control reached a better final height than those with poor metabolic control.

The final height SDs of patients with good, and poor metabolic control were 0.15±0.51 SD, and -2.25±0.89 SD, respectively ($p<0.05$).

Wasniewska et al. [14] reported that hydrocortisone treatment did not significantly affect height outcomes in children without classic CAH. However, they commented that wide differences in the number of patients included in their study groups could affect the reliability of the results. In our study, we found that hydrocortisone treatment used in appropriate doses favorably affected final heights of the patients.

Although the required physiological daily doses of hydrocortisone range between 6-7

mg/m², in cases with CAH, hydrocortisone treatment should be given at daily doses of 10-15 mg/m² tid to suppress androgen production from the adrenal cortex [4, 7, 8]. In the literature, widely different average daily HC doses used in studies conducted in children and adolescent patient groups were reported by Cordeiro et al. [15]; (13.7 mg/m²), Volkl et al. [16]; (14.8±4.76 mg/m²), Ambroziak et al. [17] (18.55±4.8 mg/m²), and Aycan et al. [18] (19.7±2.9 mg/m²). As expected in our study, the mean HC dose used was lower in the non-classic CAH group than in the classic CAH group. In the CAH group due to 11 β-hydroxylase deficiency, the mean HC dose was higher than in all other groups. The mean HC doses calculated in our study were consistent with the literature.

In the treatment of congenital adrenal hyperplasia, uncontrolled use of glucocorticoids exceeding the physiological doses is known to disrupt the growth process with its negative effects on both the growth hormone/IGF-1 axis and the bone cartilage [19-22]. Sarafoglou et al. [23] found that daily HC doses of 104 patients had negatively affected estimated adult height, with a 0.37cm decrease from the final height for each mg/m² increase in daily doses. In their retrospective study with 92 patients, Hargitai et al. [24] emphasized that the daily hydrocortisone doses should not exceed 17 mg/m² during puberty in order to optimize the final height of patients with classic adrenal hyperplasia. In a study performed with 31 cases with 21-hydroxylase deficiency Cordeiro et al. [15] used average daily HC dose of 13.7 mg/m² (dose range: 10.9-40 mg/m²/day) and the final height SD of these patients achieved was -2.13±1.11 SD, without any significant difference between the final height, gender, clinical form of CAH and hormone control of the treated groups. However, they found a significant negative correlation between final height and hydrocortisone dose

The common conclusion of all these studies is that the most important factor affecting the increases in the heights of the patients in CAH is the high doses of steroid therapy. Girgis et al. [25] reported that 32 patients with CAH did not develop short stature after at least 4 years of treatment with hydrocortisone at daily doses of 10-15 mg/m². In our study, the effect of the hydrocortisone dose and target height on the final height was examined by linear regression analysis, and it was seen that the target height variable had a positive, while the HC dose had a negative effect on the final height, and optimal final height was achieved with daily HC doses ranging between 10-15 mg/m².

In a study on 124 patients with CAH, Hargitai et al. [24] found the height SD -1.55 in male and 1.25 SD in female patients. In our previous research we conducted in 2006, the average daily hydrocortisone dose was 17.64±3.60 mg/m², and the average height SD was -1.77 SD [26]. In the study we conducted in 2009, final height and final height SD were 152.2±7.2 cm and -1.0±1.1 SD in girls; and 163.1±6.6 cm and -1.2±1.0 SD in boys, while the average daily hydrocortisone dose received by the patients was 19.7±2.9 mg/m² [18]. In this study, we reported that 79.1% of the cases could not achieve their target height, and obesity developed in 54% of the cases. In these studies, we attributed higher rates of inability to achieve final target heights and the development of obesity in these cases to the use of excessive doses of hydrocortisone used, and strongly suggested use of lower doses of hydrocortisone in these patients, as we applied in our clinical practice. As a matter of fact, our most recent study included the results of treatment with lower doses of hydrocortisone in which we observed better final height results relative to our previous studies. While only 20% of the cases with classic CAH in the previous study achieved the genetic height potential, in our current study 60% of the cases achieved their genetic height potential. Smaller number of our cases diagnosed with 11β hydroxylase deficiency, who had to receive HC treatment at higher doses achieved their mean final and target heights compared to the cases with classic type 21-hydroxylase deficiency which demonstrated unfavorable effects of hydrocortisone overdose

In a multicenter study by Hargitai et al. [24], 341 of 598 CAH cases were followed longitudinally from birth to the time they reached their final heights. According to the data obtained from this study, it was determined that the final heights of all cases were shorter than both country references and target heights. In the longitudinal follow-up, it was emphasized that patients with simple virilizing CAH were taller in early childhood when compared to their gender- and age-matched groups, while

those with salt-wasting CAH were shorter than their peers at 0-3 years of age. In this study, they attributed relative tallness of the patients with simple virilizing CAH in early childhood to delayed diagnosis and advanced bone age and suggested that earlier diagnosis and use of high doses of steroids in the salt-wasting CAH may negatively affect growth rate. In case of delayed treatment, advanced bone age should be taken into consideration as another factor that negatively affects height rate. Advanced bone age in cases of delayed diagnosis and steroid overload together with poor monitoring have the same negative effect. Hargitai et al. [24] suggested that short stature diagnosed especially in infancy is commonly related to poorly monitored steroid therapy. Similarly, in our study, the average final height and percentage of achieving the target height in cases with simple virilizing type CAH were higher than those with salt-wasting type CAH which may be due to the fact that patients with salt-wasting type CAH were exposed to high-dose HC treatment in early infancy. Although the enzyme activity in patients with non-classic CAH is higher than the enzyme activity in simple virilizing patients, the shorter mean final height is associated with a longer exposure to hyperandrogenemia due to the delayed diagnosis. There is little data on the final height of patients with 11 β-hydroxylase deficiency. In our study, the mean final height SDS of 6 cases diagnosed with 11 β-hydroxylase deficiency who achieved their final heights was lower than the cases diagnosed with 21-hydroxylase deficiency (-5.40±1.30 vs -2.86±1.53) which was attributed to the delayed diagnosis of cases with 11 β-hydroxylase deficiency and the fact that these patients received higher doses of hydrocortisone.

In conclusion, in patients with CAH, both inadequate and high-dose glucocorticoid treatment may result in relatively shorter final height. For this reason, early recognition of CAH through CAH screening programs, application of glucocorticoid therapy in appropriate doses (10-15 mg/m²/day) and regular evaluation of patients for metabolic control monitoring can ensure achievement of adequate final height. In our study, the best average final target height was found in the group using daily hydrocortisone doses of 10-15 mg/m². Indeed, when daily doses of 5-10 mg/m² were used, androgens were not suppressed sufficiently and the epiphyses closed prematurely, and in cases where daily doses of >15 mg/m² were used, the age-adjusted targeted final height was not achieved due to the excess glucocorticoid dose.

Conflict of interest: No conflict of interest was declared by the authors.

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Authors' contributions to the article

Z.A. and F.B.K. have constructed the main idea and hypothesis of the study. They developed the theory and arranged/edited the material and method section. E.B. and M.K. have evaluated the data in the Results section. Discussion section of the article was written by F.B.K.

Z.A. reviewed, corrected and approved the final version of the manuscript. In addition, all authors critically reviewed the entire study and approved the final version of the manuscript.

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Secondary pseudotumor cerebri in the pediatric population: clinical features, treatment, and prognosis

Pediatrik popülasyonda sekonder psödotümör serebri: klinik özellikler, tedavi ve prognoz

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Abstract

Purpose: Pseudotumor cerebri syndrome (PTCS) is characterized by elevated intracranial pressure (ICP) without intracranial mass, hydrocephalus, or abnormalities in cerebrospinal fluid (CSF) composition. In secondary PTCS (sPTCS), there is a reason that increases the CSF pressure. The aim of this study is to evaluate the diagnosis, treatment, and follow-up of pediatric patients diagnosed with sPTCS.

Materials and methods: This is a retrospective study conducted in a single-center tertiary pediatric hospital. We included patients aged 1-17 years who were diagnosed with sPTCS in a tertiary hospital between 2018 and 2023 and met the current diagnostic criteria for PTCS. We evaluated the complaints, etiology, ophthalmological evaluations, and treatment results of the cases.

Results: Seventeen patients with a diagnosis of PTCS were included in the study. The mean age was 9.82 (±4.6). Of the patients, 9 (56.2%) were male and 8 (43.7%) were female. The most common symptoms were headache in 10 patients (62.5%), nausea/vomiting in 6 patients (37.5%), and double vision in 5 patients (31.2%). All patients had papilledema and 7 (43.7%) patients had sixth nerve palsy. Recurrence was observed in 3 (16%) patients. Optic nerve fenestration was performed in three patients as a second-line treatment.

Conclusion: In cases that do not respond to medical treatment, optic nerve sheath fenestration may be a good treatment option.

Keywords: Optic nerve, pseudotumor cerebri, child.

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Öz

Amaç: Psödotümör serebri sendromu (PTSS), intrakranyal kitle, hidrosefali veya beyin omurilik sıvısı (BOS) bileşiminde anormallikler olmadan yüksek kafa içi basıncı (KİB) ile karakterizedir. İkincil PTSS'de (sPTSS), BOS basıncını artıran bir neden vardır. Bu çalışmanın amacı sPTSS tanısı alan pediatrik hastaların tanı, tedavi ve takiplerinin değerlendirilmesidir.

Gereç ve yöntem: Bu çalışma tek merkezli üçüncü basamak bir pediatri hastanesinde gerçekleştirilen retrospektif bir çalışmadır. 2018 ile 2023 yılları arasında üçüncü basamak bir hastanede sPTSS tanısı konan ve PTSS için mevcut tanı kriterlerini karşılayan 1-17 yaş arası hastaları dahil ettik. Olguların şikayetleri, etiyolojisi, oftalmolojik değerlendirmeleri ve tedavi sonuçları değerlendirildi.

Bulgular: Çalışmaya PTSS tanısı alan 17 hasta dahil edildi. Ortalama yaş 9,82 (±4,6) idi. Hastaların 9'u (%56,2) erkek, 8'i (%43,7) kadındı. En sık görülen semptomlar 10 hastada (%62,5) baş ağrısı, 6 hastada (%37,5) bulantı/kusma, 5 hastada (%31,2) çift görme idi. Hastaların tamamında papilödem, 7 (%43,7) hastada ise altıncı sinir felci vardı. 3 (%16) hastada nüks görüldü. Üç hastaya ikinci basamak tedavi olarak optik sinir fenestrasyonu uygulandı.

Sonuç: Medikal tedaviye yanıt alınamayan durumlarda optik sinir kılıfı fenestrasyonu iyi bir tedavi seçeneği olabilir.

Anahtar kelimeler: Optik sinir, psödotümör serebri, çocuk.

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Introduction

The definition of pseudotumor cerebri syndrome includes both primary and secondary PTCS. IIH (idiopathic intracranial hypertension) is synonymous with primary PTCS and its etiology is unknown. sPTCS has a known cause and its treatment may differ from PTCS [1, 2]. When all diagnostic criteria are met, PTCS is called definite primary/secondary PTCS. When all diagnostic criteria are not met, it is classified as possible primary/secondary PTCS. In the pediatric population, some of the most common causes of sPTCS are intracranial tumor, sinus venous thrombosis, intracranial hemorrhage, hydrocephalus, CNS (central nervous system) infection, severe anemia from drug use, discontinuation of chronic corticosteroids, use of antibiotics such as synthetic growth hormone and tetracycline [3]. There are cases of sPTCS occurring as a result of the discontinuation of corticosteroid therapy. Accordingly, it suggests that abnormal glucocorticoid metabolism plays a role in the pathophysiology of PTCS. Cortisol level may play a triggering role for PTCS by affecting the 11-β-hydroxysteroid dehydrogenase type 1 and 2 (HSD1 and HSD2) enzyme complex in the choroid plexus, which regulates the functions of cortisol. Headache is the most common symptom of primary and sPTCS. Acetolzamide, topiramate, prednol are given in medical treatment. Optic nerve sheath fenestration (ONSF) and ventriculoperitoneal shunt (VPS) are used in surgical treatment. Most importantly, lowering ICP and revealing its etiology requires a multidisciplinary approach [4, 5].

Materials and methods

Between January 2018 and 2023, 17 pediatric patients with sPTCS, aged 1-17 years, were included. The causes of secondary PTCS included in the study are infectious, endocrinological diseases and drug exposure, more rarely hematological diseases, trauma, arachnoid cyst rupture, some syndromic diseases, and chronic renal failure (CRF). The body mass index (BMI) of the patients was recorded. We classified the patients into age groups 0-14 years and 15-17 years. We evaluated the patients according to their phenotype, age, gender, comorbidities, and BMI status. IIH is diagnosed according to the ICHD-III criteria and revised Friedman criteria [6]. Papilledema scores were categorized as mild, moderate, and severe, respectively (mild degree 0-1), (moderate degree 2-3), (severe degree 4-5). Visual impairment was defined as mild (visual acuity 20/40-20/80) or severe (visual acuity <20/80). Patients who met the updated diagnostic criteria for PTCS and were found to have a cause for high ICP were included in the study [6]. Despite medical or surgical treatment, mild or severe visual impairment and optic atrophy were considered unresponsive to treatment. This study was approved by the Pamukkale University Non-Interventional Clinical Research Ethics Committee.

Statics

IBM SPSS 21.0 software was used for data analysis. Mean ± standard deviation was calculated for continuous variables. Two-way analysis of variance (ANOVA) was employed to compare continuous variables among groups. Discrete variables were compared using the appropriate Pearson chi-square test or Fisher's test. A *p*-value <0.05 was considered statistically significant.

Results

There were 17 children with sPTCS in our study, 8 females and 9 males. The underlying causes were infection in 3 patients (17.6%), drug exposure in 3 patients (17.6%), endocrine disease in 4 patients (23.5%), and less common causes are summarized in Table 1. The median age of the cases is 10.7 (2.6 between 17.6). Headache was the most common symptom 10 (58.8%), nausea or vomiting in 6 patients (35.2%), diplopia in 5 patients (29.4%), blurred vision in 5 patients (29.4%), and the patient (11.7%) had tinnitus. Papillary edema was present in all patients. Opening CSF pressure was 38±4.7 H_2O. There was no difference in prognosis, incidence, and etiology between adolescent and non-adolescent children. Papilledema was detected in all patients at the time of diagnosis. There was 7 (41.1%) abducens paralysis (5 unilateral, 2 bilateral). All patients received primary and/or secondary treatment. Primary treatment was acetazolamide in 13 (76.4%) patients and topiramate in 4 (23.5%) patients. Secondary treatment was applied to 3 of the cases and ONSF was applied to all of these cases. No complications were observed

Table 1. Clinical features of children with secondary pseudotumor cerebri syndrome

Case	Year/ gender	Etiology	Papil edema	Field of vision	Treatment	Comorbodite	Result
1	15/M	Mastoiditis	Severe	None	Acetazolamide	CVT	Normal
2	11/M	CRY (cystinosis)	Mild	None	Topiramate/optic nerve sheath fenestration	IDA Hypertension	Chronic papilledema
3	7/F	Head trauma	Mild	None	Acetazolamide	None	Normal
4	14/M	Mastoiditis	Severe	Mild visual field loss	Acetazolamide/ONSF	CVT	Normal
5	10/M	Pansinusitis	Mild	None	Acetazolamide	CVT	Normal
6	16/F	Andersen-Tawil Syndrome	Mild	Mild visual field loss	Acetazolamide	Pernicious anemia	Normal
7	4/F	Hypothyroid	Mild	None	Topiramate	Obesity	Normal
8	15/F	Steroid stop	Mild	None	Topiramate	Obesity	Normal
9	7/F	Coats Plus Syndrome	Severe	Mild visual field loss	Acetazolamide/ ONSF	IDA	Normal
10	2/M	Achondroplasia	Moderate	None	Acetazolamide	None	Normal
11	8/M	Growth hormone treatment			Acetazolamide	None	Normal
12	16/M	Retinoic acid treatment	Mild	None	Topiramate	Obesity	Normal
13	5/F	Acute lymphoblastic leukemia	Moderate	None	Acetazolamide	Steroid stop	Normal
14	15/F	Arachnoid cyst rupture	Mild	Mild visual field loss	Topiramate/ ONSF	Obesity	Normal
15	7/M	Hypothyroid	Mild	None	Acetazolamide	IDA	Chronic papilledema
16	5/M	Hypothyroid	Severe	None	Acetazolamide	IDA	
17	10/F	Turner syndrome	Mild	None	Acetazolamide	None	Normal

M: Male F: Female, CVT: Cerebral venous thrombosis, IDA: Iron deficiency anemia, ONSF: optic nerve heath fenestration

in the patients who received ONSF. VP shunt was not applied to any patient. Papilledema was completely resolved in 15 patients (88.2%) after a mean follow-up of 4.5 months (1.0 between 27.1 months). The other 2 patients (11.7%) had chronic papilledema that did not progress for 13 months and was not treated. There was no patient whose visual impairment continued after treatment. The most common comorbidity associated with sPTCS in our population was anemia 5 (29.4%).

Discussion

In our study, infectious diseases, endocrinological pathologies, and drug exposure were found more frequently in the etiology of PTCS. This finding shows similar results to the literature. Secondary pseudotumor cerebri can cause mortality and morbidity if not diagnosed and treated early. Especially in the treatment of sPTCS, the etiologic cause should be treated as well as reducing the ICP [1, 2]. In children, approximately 20% of cases of sPTCS have a poor long-term prognosis. These include visual impairment and optic atrophy. In our study, 4 of 17 patients had a mild visual loss. There was no patient whose vision loss continued at the end of primary and secondary treatment. In studies, mild long-term visual impairment was found in 9-10% of patients with primary and sPTCS. In addition, surgical treatment was not common in PTCS cases in these studies [2]. In our study, optic nerve fenestration was performed in 3 of 17 patients with secondary PTCS to lower ICP and the results were quite good. Because we followed up our patients very frequently (2 days a week) and in case of non-response to medical treatment, we thought that our results could be good due to surgical treatment without wasting time. Optic nerve sheath decompression is a surgical procedure performed to relax the optic nerves and relieve papilledema, which causes visual impairment due to increased intracranial pressure. A study investigated the efficacy and complications of ONSF. In this study, 525 ONSF procedures were performed on 341 patients during the follow-up period of 42.3 months. Accordingly, they concluded that ONSF could reduce papilledema and improve vision. Papilledema improved in 95% of patients, visual acuity improved in 67%, and visual fields improved in 64%. However, the effect of ONSF in relieving headache was insufficient (41%). A second fenestration operation was required in 11% of the cases [7]. Our patients did not require a second surgical procedure. It may be due to our continued medical treatment after surgical treatment. In another study, visual acuity and visual fields improved in 95% of patients who underwent ONSF without reporting any intraoperative complications.

The average follow-up period of the cases was 18.7 months. Among the postoperative complications, ocular deviation was detected (6%) and corneal Dellen was detected [8, 9]. In another retrospective study, unilateral ONSF showed papilledema and bilateral improvement in vision [8, 10]. In the literature, it has been found that ONSF in IHH cases, and especially in cases with acute papilledema, papilledema regresses in more than 90% of the patients. Repeated fenestrations have been shown to provide significant improvement in visual function. In a recent study, 13 of 53 IIH patients with acute papilledema and vision loss were treated with ONSF. However, 11 of them required secondary or tertiary decompression treatments. As a result, visual fields improved [8, 11]. Publications on the long-term outcomes of sPTCS in children are limited, and usually primary and secondary cases have been evaluated together. There is more work in primary PTCS. In one study, primary and secondary cases were evaluated together. While the vision of all 12 sPTCS patients was normal, 2 patients did not respond to medical treatment and VP shunt was applied to one patient [12]. In another study, moderate visual field loss was found in only 1 (4%) of 23 patients with sPTCS [13].

The most common comorbidities accompanying the causes of sIH in our cases were obesity and anemia. Obesity is a well-known risk factor for both secondary and primary PTCS [2]. In a study evaluating obesity in the pediatric population, it was reported that the incidence of IIH was equal and there was no clear relationship with obesity [14]. In another study, obesity was evaluated as a risk factor [1]. In our study, we did not find a relationship with obesity in terms of prognosis. However, as the number of cases increases, there may be a significant difference between obesity. It is well-documented that corticosteroid withdrawal can induce sPTCS [15, 16]. In one study, eight of 15 subjects with sPTCS were induced after

discontinuation of corticosteroids. Anemia is thought to be a risk factor for sPTCS by increasing cerebral blood flow [17]. Anemia and tissue hypoxia alters cerebral hemodynamics, increasing cerebral capillary permeability and thus increasing ICP, which causes sPTCS [18]. The literature review did not find an answer to the question of how severe anemia should be, although anemia was considered a cause or possibly an additional risk factor for sPTCS. This is because there are different study methods and different criteria for anemia. Iron deficiency anemia and hemolytic anemia have been identified as the most common risk factors for PTCS, and we found anemia due to iron deficiency and B12 deficiency in our cases [2]. Cerebral venous thrombosis (CVT) can be seen in the comorbidity of patients with secondary PTCS. In their study on this subject, they reported that visual acuity decreased in 3 (7%) of 37 children with cerebral venous sinus thrombosis. Additionally, surgery was performed in 19% of children with decreased visual acuity [1, 19]. One of our patients with CVT underwent ONSF and vision loss was not permanent. Treatments for sPTCS and primary PTCS are different. Since the etiology is very diverse in the treatment of sPTCS, a multidisciplinary approach is essential. For example, anticoagulation therapy should be arranged by a pediatric stroke team for children with cerebral venous sinus thrombosis.

If sPTCS has developed due to drug use or exposure, once this agent is identified, necessary precautions should be taken. When treating the underlying cause of sPTCS, it is more important to lower ICP and monitor visual function and papilledema. If there are signs of advanced papilledema/visual impairment, direct treatment of ICP may be indicated. There are cases where papilledema does not subside despite treatment of the underlying etiology. For example, in some studies, 2 patients with cerebral venous sinus thrombosis required a VP shunt due to severe or chronic papilledema despite anticoagulation therapy [1].

Limitations, the small number of our cases, the lack of longer follow-up periods and the small number of patients who underwent surgical treatment may limit our evaluation of the prognosis.

Since sPTCS has many different causes, classifying all causes into a single group reduces the reliability of the study. This was due to the low number of cases due to specific causes and insufficient risk factors and subgroup analyses. Due to the small number of cases in each sPTCS group in our study, we could not establish a strong association between different risk factors and sPTCS. It is thought that a multidisciplinary study for surgical options of patients who do not respond to medical treatment. Multidisciplinary work with clinicians will increase awareness of possible risk factors for sPTCS and provide better treatment of patients.

Conflict of interest: No conflict of interest was declared by the authors.

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Authors' contributions to the article

O.G. constructed the main idea and hypothesis of the study. O.G and E.S.U. developed the theory and arranged/edited the material and method section. O.G., B.K.Y. and O.P. have done the evaluation of the data in the results section. Discussion section of the article written by O.G. and E.S.U. and, B.K.Y. and O.P. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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Changes in structure during the corpus luteum's formation

Korpus luteum oluşumu sırasında meydana gelen yapısal değişiklikler

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Abstract

Purpose: Our study aims to investigate whether new follicles form among fibroblast-like cells in the region after the restructuring of the surface epithelium and tunica albuginea adjacent to the corpus luteum. Additionally, another goal is to demonstrate the structures developed from the follicles and the histological changes in the ovary.

Materials and methods: Histological sections of the corpus luteum, formed from the Graafian follicle after ovulation, were prepared from ovarian tissues of 12-14 months old Wistar albino rats.

Results: When the corpus luteum reaches a high volume, the number of fibroblast-like cells in the tunica albuginea is quite low. Subsequently, it has been observed that the number of fibroblast-like cells in the tunica albuginea rapidly increases, and these cells form concentric arrangements of collagen fibers. Additionally, the formation of primordial and primary follicles between the surface epithelium and the tunica albuginea adjacent to the corpus luteum has been observed.

Conclusion: Examining the ovary as a whole and investigating the developmental processes of structures can assist in gaining a better understanding of the dynamics of this organ.

Keywords: Corpus luteum, ovary, tunica albuginea, follicles.

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Öz

Amaç: Çalışmamız, korpus luteum'un bitişiğindeki yüzey epiteli ve tunika albugineanın yeniden yapılanmasından sonra bu bölgedeki fibroblast benzeri hücrelerin arasında yeni foliküllerin oluşup oluşmadığını araştırmayı amaçlamaktadır. Ayrıca, foliküllerden gelişen yapıları ve ovaryumdaki histolojik değişiklikleri göstermek de bir başka hedefimizdir.

Gereç ve yöntem: Ovulasyondan sonra graaf folikülünden oluşan korpus luteum'un histolojik kesitleri, 12-14 aylık Wistar albino tipi sıçanların ovaryum dokularından alınarak hazırlanmıştır.

Bulgular: Korpus luteum yüksek bir hacime ulaştığında, tunika albugineada bulunan fibroblast benzeri hücrelerin sayısı oldukça düşüktür. Daha sonrasında, tunika albuginea bulunan fibroblast benzeri hücrelerin sayısının hızla arttığı ve bu hücrelerin oluşturduğu kollajen liflerin konsantrik dizilim oluşturdukları gözlemlenmiştir. Ayrıca, korpus luteumun bitişiğindeki yüzey epiteli ve tunika albuginea tabakası arasında primordial ve primer foliküllerin oluştuğu izlenmiştir.

Sonuç: Ovaryumu bir bütün olarak ele almak ve yapıların gelişim süreçlerini incelemek, bu organın dinamiklerini daha iyi anlamamıza yardımcı olabilir.

Anahtar kelimeler: Korpus luteum, ovaryum, tunika albuginea, folikül.

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Introduction

The ovary is an organ with endocrine and exocrine functions and is covered by an epithelium that varies from single-layered squamous epithelium to cuboidal epithelium. In the lower part of the basement membrane, there is a tight connective tissue called the tunica albuginea. Tunica albuginea is rich in collagen fibers and is restructured after ovulation. Follicles are structural units located in the stroma of the cortex layer in the ovary consisting of the cortex and medulla. In embryonic life, primordial germ cells appear in the endoderm of the dorsal wall of the embryonic yolk sac, migrate towards the gonad outline, and are called oogonium. After the formed oogoniums, the primordial follicle structures are formed. Folliculogenesis is regulated by some factors including endocrine, paracrine, and autocrine factors [1, 2].

The oocyte plays an active role in the growth of follicles by secreting paracrine growth factors (such as GDF9 and BMP15) and directing the differentiation of granulosa cells. On the other hand, granulosa cells regulate the development of oocytes by communicating between germ and somatic cells [3]. The outer surface of the follicle cells surrounding the oocyte is bounded by the basal lamina and surrounded by stromal cells. Type IV collagen is the main component of the basement membrane in the follicle. The oocyte formed in the primordial follicle is surrounded by a single layer of flat follicle cells. The number of primordial follicles in the ovaries determines how long the reproductive ability will continue, and these structures are considered reserves. After puberty, primordial follicles are selected and begin to grow. Since primordial follicles do not contain FSH receptors, FSH is not required for their activation. In each cycle, 6-12 follicles begin to develop and only one continues to develop as a mature follicle, while the others undergo atresia [4]. While atresia in preantral follicles generally occurs by autophagy, in antral follicles, atresia mostly occurs by apoptosis mechanism [5].

Follicles continue their development as primary, secondary, and Graaf follicles, respectively. It can also undergo atresia at any stage of its follicular development. In some atretic follicles, the granulosa layer degenerates and the theca interna layer expands, and these atretic follicles resembling the corpus luteum are called corpora lutea atretica [6]. Although there are views that there is a certain fixed number of oocytes in the ovary and no new follicle production occurs, there are also studies suggesting that postnatal oogenesis production occurs [6].

The endocrine functions of the corpus luteum and the hormones it secretes have been extensively researched. However, there are relatively few studies that focus on the changes in the adjacent tunica albuginea and the restructuring of the ovarian surface epithelium. The presence of capillary vessels in the tunica albuginea adjacent to the corpus luteum, along with the observation of primordial follicles, has raised suspicions regarding postnatal oogenesis. Therefore, a thorough examination of the morphological changes in the ovule, Graafian follicle, and corpus luteum is necessary [1, 7, 8]. The developing Graaf follicle changes its location towards the outer part of the ovarian cortex towards ovulation. The theca layer in the Graaf follicle is divided into two parts as theca interna and externa. Theca interna consists of secretory cells of epithelioid character and is rich in terms of vascularization. It contains fibroblasts and type III collagen fibers. In the theca externa layer, there are myofibroblasts, type I and type III collagen fibers. After ovulation, the theca externa layer of the Graaf follicle completely degenerates; significant morphological changes occur in the tunica albuginea and the surface epithelium. Collagen fibers found in the theca externa layer, collagen fibers and fibroblast-like cells in the tunica albuginea are broken down with proteolytic enzymes. Then, reconstruction occurs not only in the tunica albuginea and surface epithelium part where the oocyte-cumulus complex is separated, but also throughout the tunica albuginea and surface epithelium of the entire corpus luteum. The reconstructed surface epithelium is of the squamous epithelium type and the tunica albuginea is a thin layer consisting of fibroblast-like cells containing one-two rows of concentrically arranged collagen fibers. Afterwards, the tunica albuginea thickens; fibroblast-like cells proliferate and become a layer containing five-six rows of concentrically

arranged collagen fibers. At this time, the surface epithelium also differentiates towards the cuboidal epithelium. New capillary vessels are formed in the thickened tunica albuginea [1, 7, 8].

The appearance of primordial follicles in the tunica albuginea adjacent to the corpus luteum in the sections suggests that VSELs cells, whose existence has been shown in previous studies, may come to this area from the bone marrow through capillary vessels and differentiate. VSELs cells in the bone marrow head towards the relevant organ by coming out to peripheral vessels in situations that cause tissue damage such as stroke, acute myocardial infarction, hypoxia where the inflammatory process is intense [9]. A similar inflammatory process emerges with the degeneration of collagen fibers and fibroblast-like cells in the theca externa and tunica albuginea as the Graaf follicle differentiates into the corpus luteum after ovulation. There are claims that VSELs cells coming out to peripheral vessels can reach the newly formed capillary vessels in the tunica albuginea of the corpus luteum and can turn into primordial follicles by receiving signals from the surrounding fibroblast-like cells. The finding supporting these views is that primordial follicles, which are densely located in the cortex and medulla of the ovary in the first four weeks after birth in rats, start to be seen mostly in the cortex region and the tunica albuginea during the reproductive period [10].

The aim of our study is to investigate the source of the primordial follicles seen in this region during the formation of the corpus luteum and the reconstruction of the tunica albuginea. On the other hand, we see it as a correct approach to examine the changes the structure undergoes, the cell types it contains, and its secretions with a holistic approach in order to better understand the functions of the corpus luteum.

Materials and methods

Ethical approval for our study was obtained from Pamukkale University Animal Experiments Ethics Committee with the decision no PAUHDEK. The ovaries of 6 Wistar albino-type rats (12-14 months old) in the ovarian aging process were removed under sterile conditions and general anesthesia. The right ovaries of all rats were fixed in 10% neutral buffered formaldehyde for 72 hours. After fixation, the routine tissue follow-up protocol was applied and embedded in paraffin blocks (FFPE). Hematoxylin-eosin staining was performed by taking 5 µm sections from FFPE ovarian tissues. Masson's trichrome staining was performed according to manufacturer instructions (Bio-Optica, Milano, Italy). Images of the ovarian surface epithelium were taken under light microscopy (BX51 Olympus, Japan). Using the explant culture method, a mixed cell culture was produced from the left ovaries [11]. Surface epithelial cells that were proliferating alongside ovarian stromal cells in a mixed cell culture were locally tagged, and these cells were extracted by using local trypsinization. The adhering ovarian surface epithelial cells were removed using trypsin enzyme 0.25% (Hyclone, USA) and injected into two fresh culture dishes with full media after they had multiplied and reached confluence (70-80%). Dulbecco's Modified Eagle's Medium (DMEM) (Capricorn Scientific, Germany), Fetal Bovine Serum (FBS) (Capricorn Scientific, Germany), and Penicillin-Streptomycin are all included in the complete medium (Pan Biotech, Germany).

Results

After ovulation, the basement membrane between the mural granulosa cells and theca layer was destroyed and it was observed that the mural granulosa cells rapidly hypertrophied and filled the entire structure. In the formed corpus luteum, initially, the number of granulosa lutein cells is high, but later the migrating theca lutein cells seem to provide the majority. With the formation of new vessels in the structure, blood supply also increases. The corpus luteum volume is at its highest when it is newly formed (Figure 1-2). In some areas, the ovarian surface epithelium transforms into pseudostratified epithelium. Primordial follicles were observed to form in this remodeled tunica albuginea (Figure 3-11). Meanwhile, the surface epithelium is squamous, few in number, and the underlying tunica albuginea is rather thin. Later, fibroblast-like cells in the surface epithelium and tunica albuginea proliferate. The squamous surface

epithelium increases in number and turns into cubic epithelium in places. The tunica albuginea thickens and restructures (Figure 12-15). In some sections, vascular structures were observed in atretic follicles. In the lumen of another atretic follicle, a primary follicle was observed (Figure 16). It was observed that while the structure regressed, the presence of granulosa lutein cells did not continue and the darker stained theca lutein cells filled the structure. In the late stage, the corpus luteum is formed by theca lutein cells, which are known to secrete progesterone and androgen hormones (Figure 17). In the sections, it was observed that the granulosa cells in the primary and secondary follicles, which were partially atresia, degenerated, and the theca interna cells continued their functions in the structures that turned into glandular structures (Figure 7).

We consider that VSELs cells found in the bone marrow reach the tunica albuginea adjacent to corpus luteum via newly formed capillary vessels, and these cells develop by receiving signals from fibroblast-like cells and sometimes differentiate by entering among proliferating surface epithelia. When we isolate and culture the ovarian surface epithelium, the observation of structures similar to primordial follicles among proliferating ovarian surface epithelia has increased our suspicions in this direction (Figures 18).

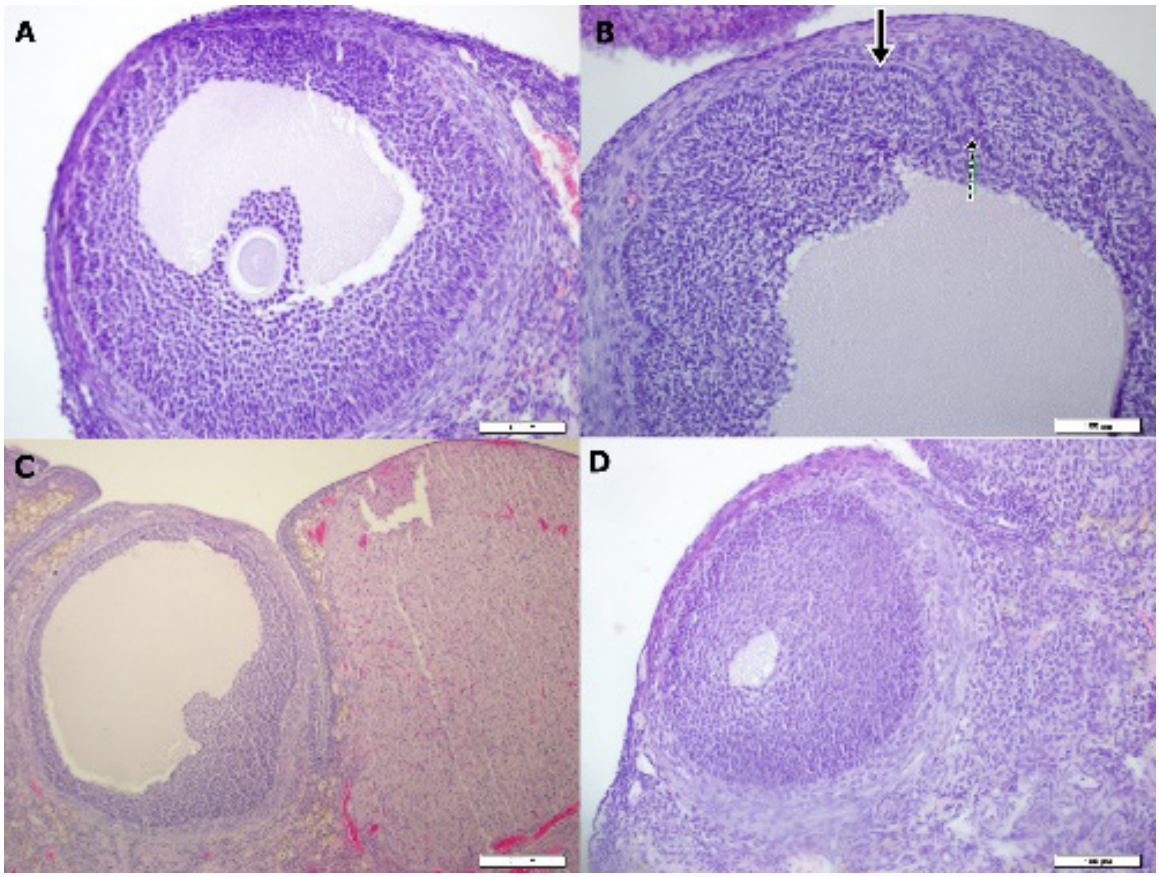

Figure 1. A- Graafian follicle B- (→) Granulosa cells, (- - >) Invading theca interna cells C- Ovulated graafian follicle D- Ovulated graafian follicle in which granulosa cells that have begun to hypertrophy fill the antrum (Magnification: 200X)

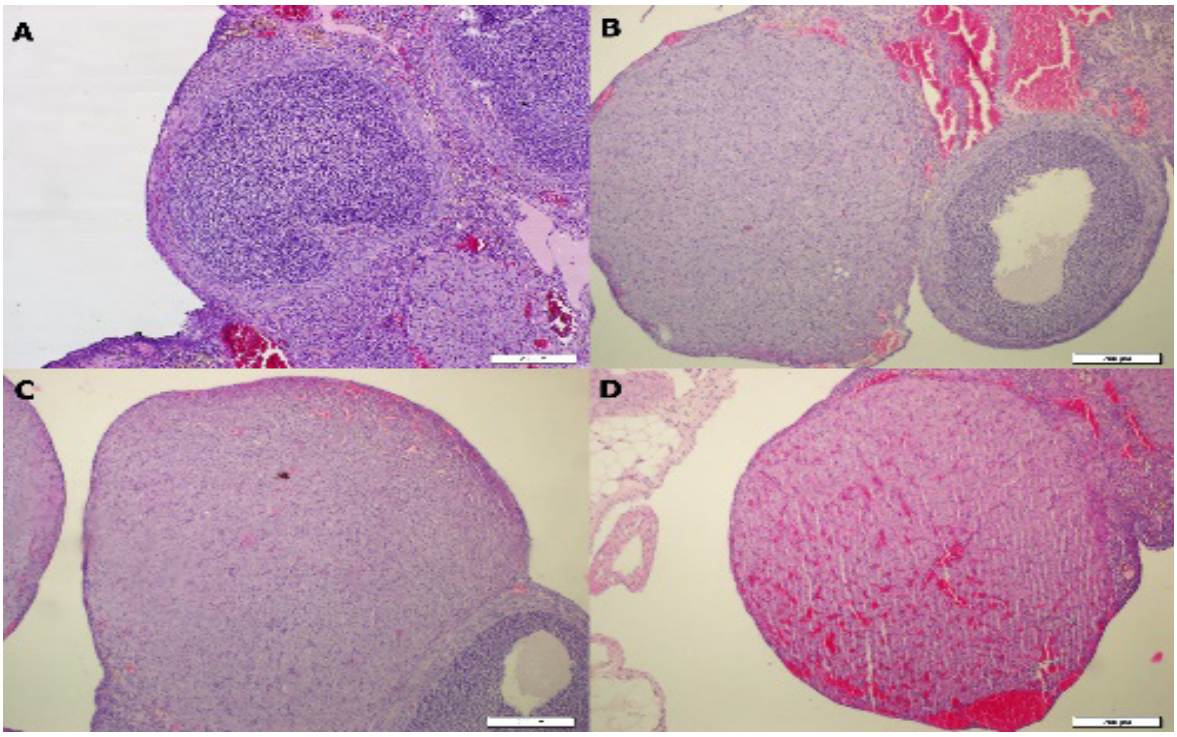

Figure 2. A- Ovulated graafian follicle, in which the antrum is entirely filled with granulosa cells that have started to hypertrophy. B- A large-volume Graafian follicle. C- The corpus luteum's developing vascularization. D- The corpus luteum, where vascularization has significantly enhanced (Magnification: 200X)

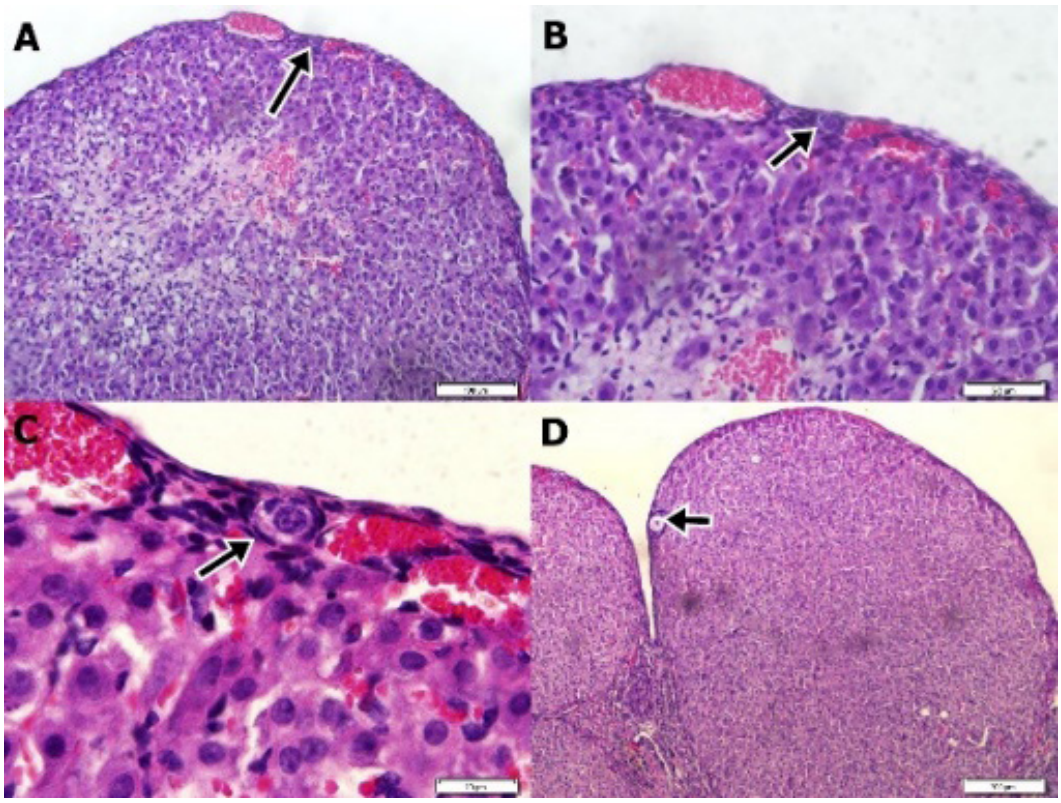

Figure 3. A (Magnification: 200X) - B (Magnification: 400X) - C (Magnification: 1000X) - D (Magnification: 200X)- (→) Primordial follicle that occurs between the completely regenerated tunica albuginea and the surface epithelium after the Graafian follicle has ovulated. D- (→) primary

follicle

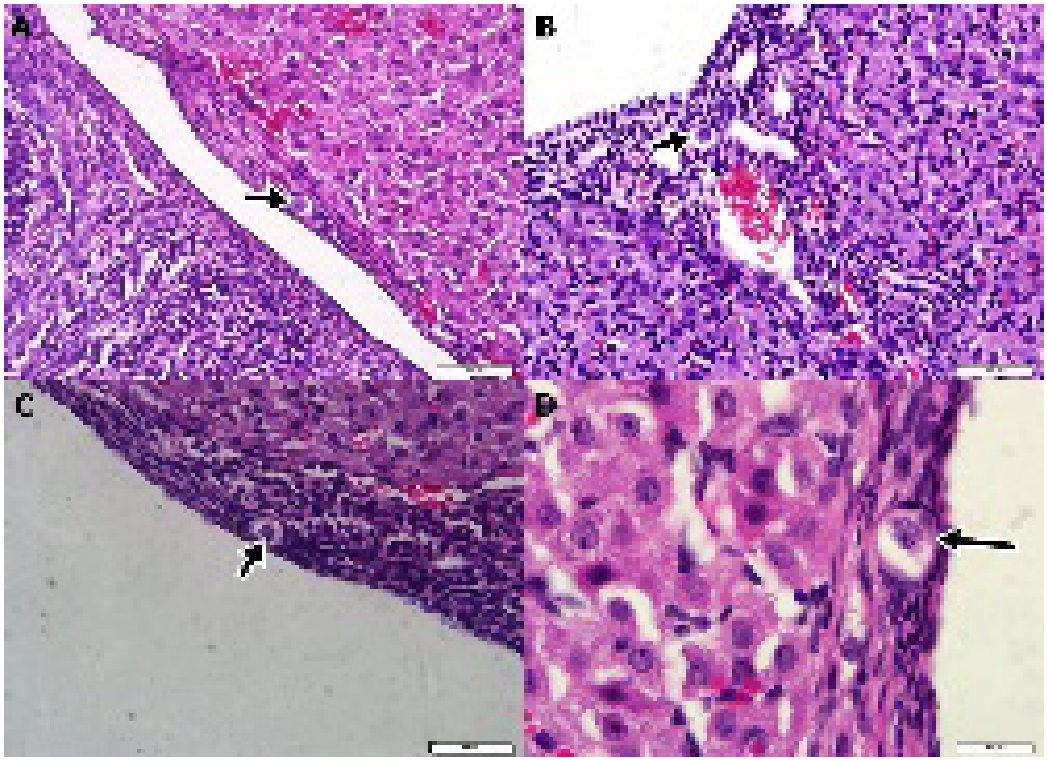

Figure 4. A- (→) Primordial follicle in the tunica albuginae of the corpus luteum (Magnification: 200X) B- (→) Primordial follicle in the tunica albuginae of the corpus luteum (Magnification: 200X) C- (→) Primordial follicle in the tunica albuginae of the corpus luteum (Magnification: 400X) D- (→) Primordial follicle in the tunica albuginea of the corpus luteum (Magnification: 400X)

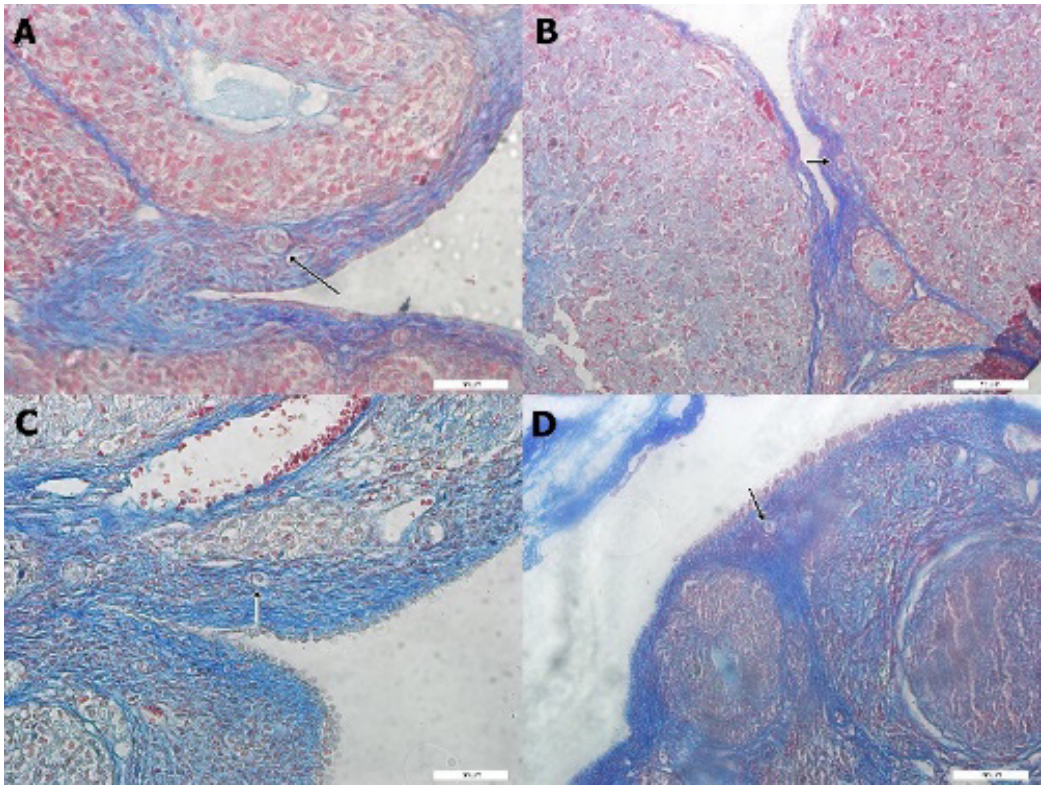

Figure 5. A (Magnification: 400X)- B (Magnification: 400X)-C (Magnification: 400X) - D (Magnification: 200X)- (→) Primordial follicles in the tunica albuginea (Masson trichrome staining)

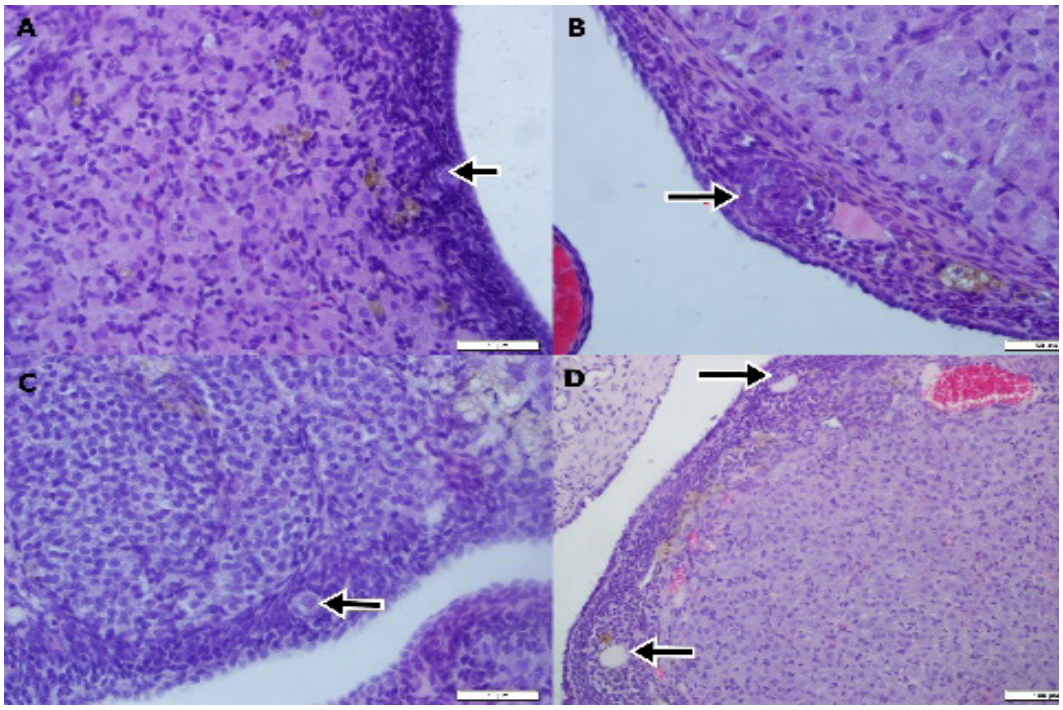

Figure 6. A- (→)Primordial follicle in the tunica albuginea part of the corpus luteum (Magnification: 400X), B- (→)Primary follicle in the tunica albuginea part of the corpus luteum (Magnification: 400X), C- (→)Primordial follicle in the tunica albuginea part of the regressed corpus luteum (Magnification: 400X), D- (→) atretic follicle in the tunica albuginea part of the corpus luteum (gland structures that develop with apoptosis of granulosa cells in primary-secondary follicles and proliferation of theca lutein cells, Magnification: 200X)

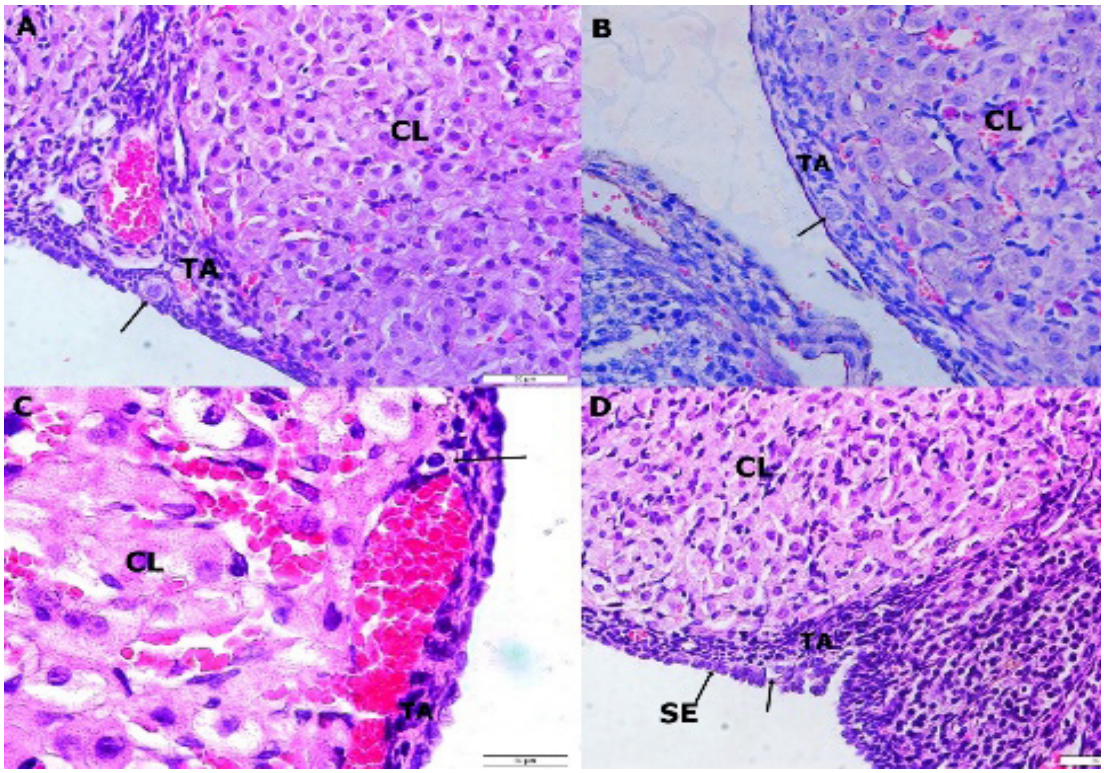

Figure 7. A-D- Primordial-primary follicles are seen just below the surface epithelium of the corpus luteum and in the tunica albuginea formed by fibroblast-like cells, (Magnification: 400X)
(CL: Corpus luteum, TA: Tunica albuginea, SE: Surface ephitelium)

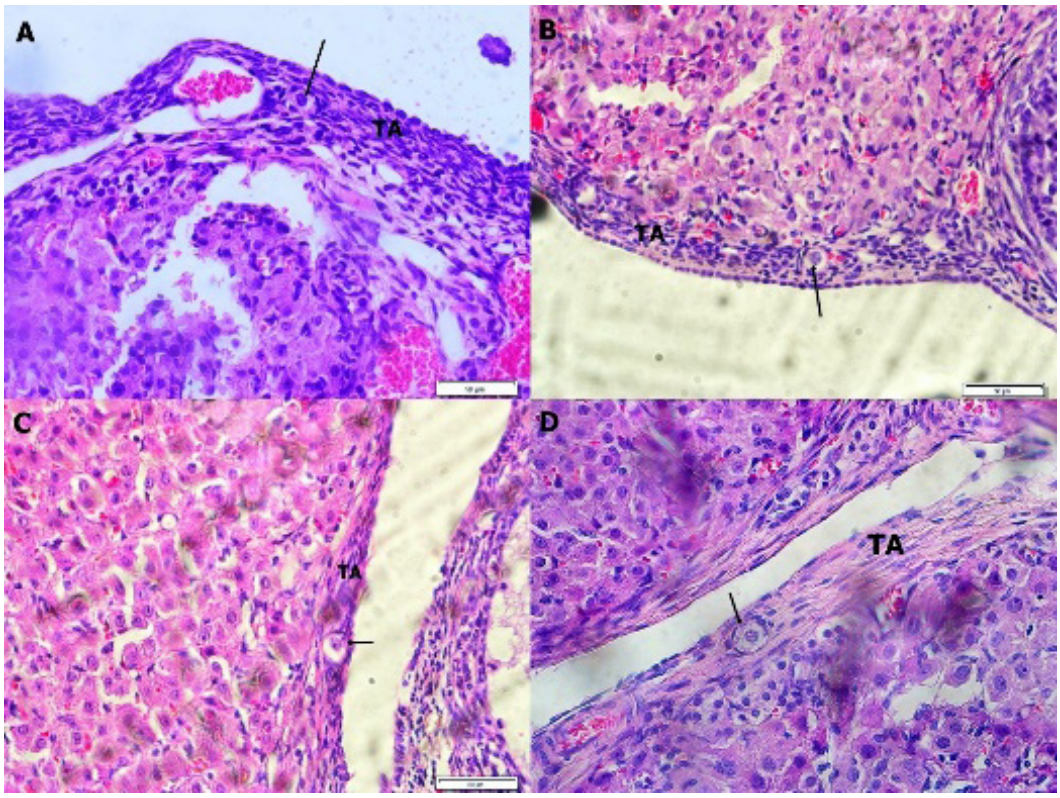

Figure 8. A-D- Primordial-primary follicles are seen just below the surface epithelium of the corpus luteum and in the tunica albuginea formed by fibroblast-like cells, (Magnification: 400X)
(TA: Tunica albuginea)

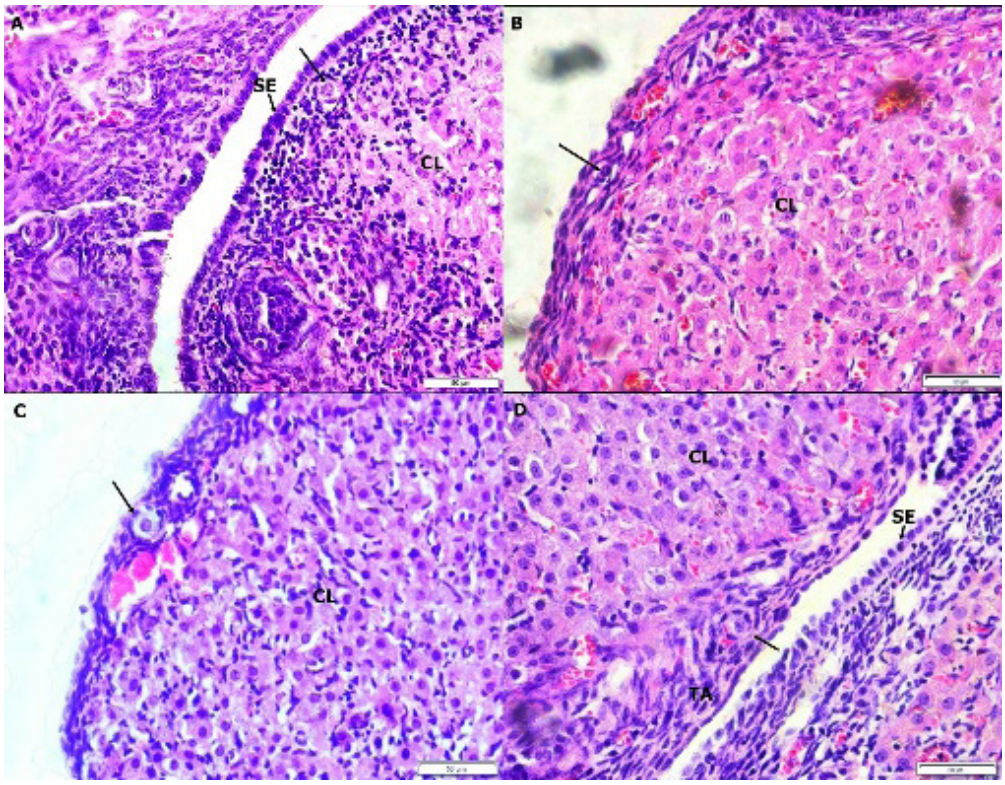

Figure 9. A-D- Primordial-primary follicles are seen just below the surface epithelium of the corpus luteum and in the tunica albuginea formed by fibroblast-like cells, (Magnification: 400X)
(CL: Corpus luteum, SE: Surface ephitelium)

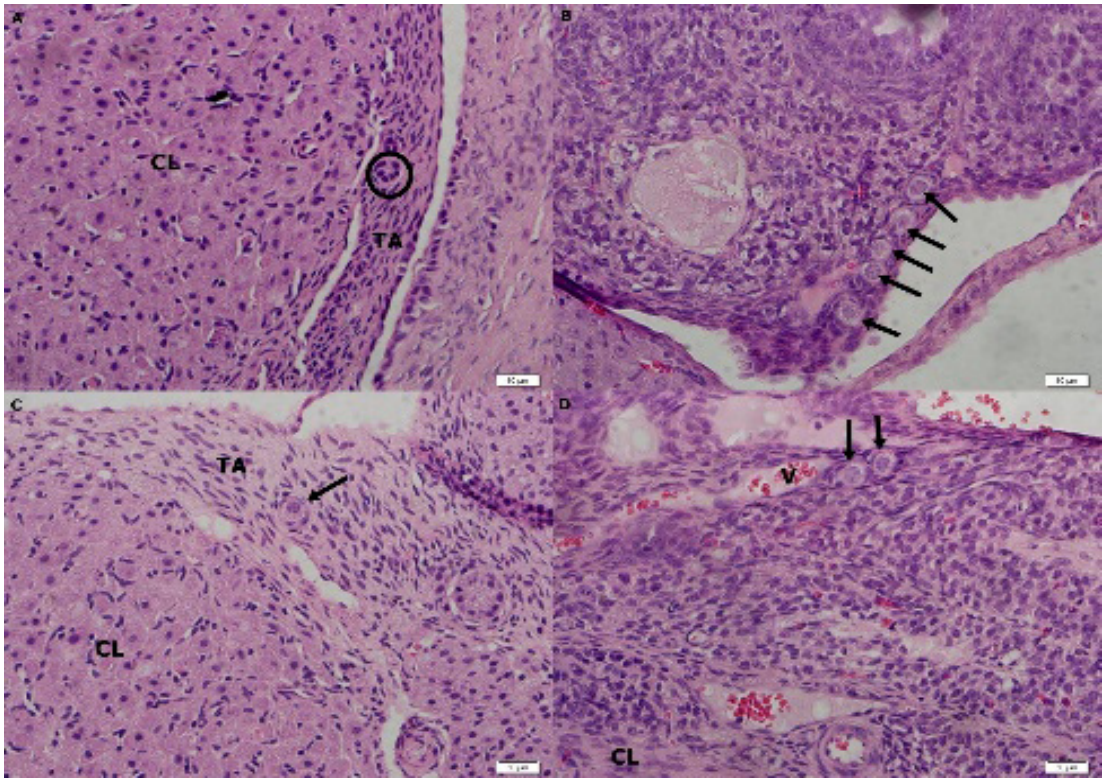

Figure 10. A- Primary follicles in the tunica albuginaea layer of the corpus luteum B- Primordial-primary follicles in the tunica albuginea C- Primordial follicles in the tunica albuginea formed by fibroblast-like cells just below the surface epithelium of the corpus luteum D- Primordial-primary follicles in the tunica albuginea, (Magnification: 400X)
(CL: Corpus luteum, TA: Tunica albuginea, V: Vessel)

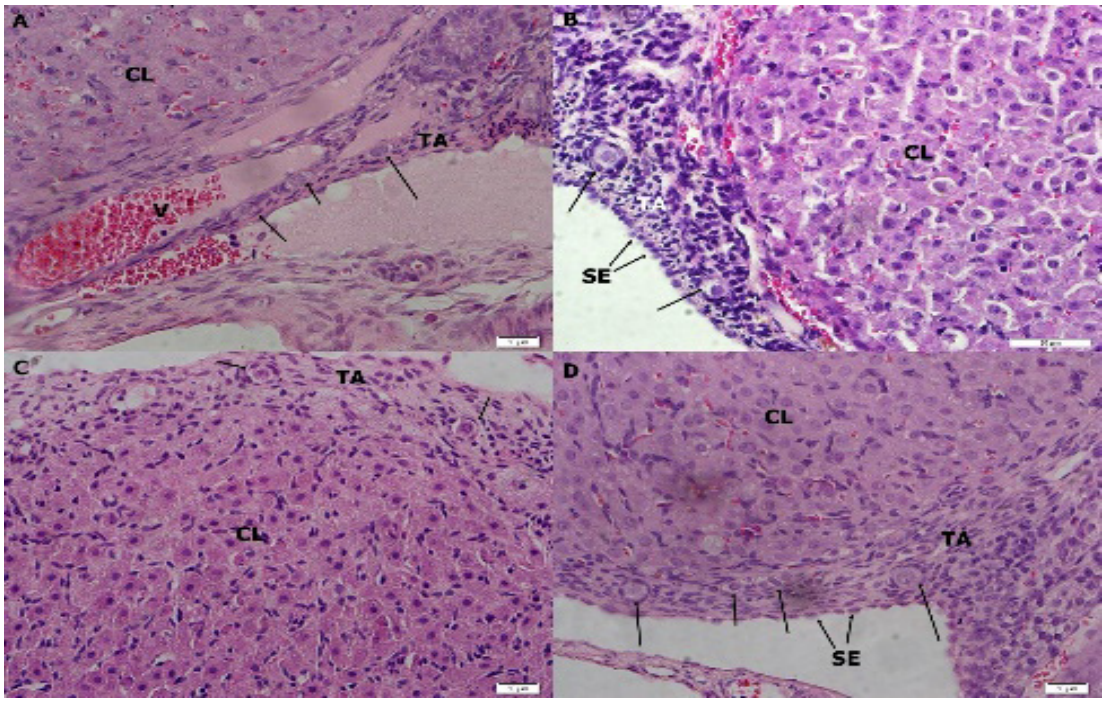

Figure 11. (A-D)- Primordial-primary follicles are seen just below the surface epithelium of the corpus luteum and in the tunica albuginea formed by fibroblast-like cells, (Magnification: 400X)
(CL: Corpus luteum, TA: Tunica albuginea, SE: Surface ephitelium, V: Vessel)

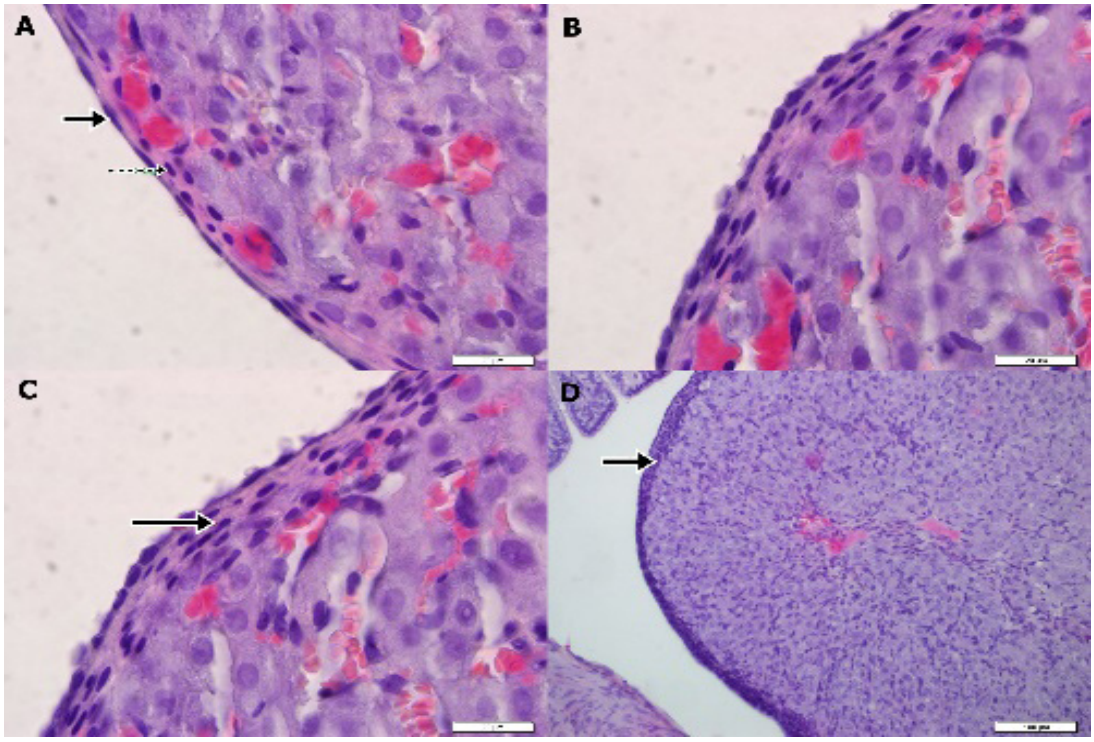

Figure 12. A- (→) Ovarian surface epithelium consisting of a single layer of squamous epithelium, (- - >) Fibroblast-like cell in the tunica albuginea B- Differentiation of surface epithelium into cubic epithelium C- (→) Proliferation of fibroblast-like cells D- (→) Thickening of the tunica albuginea Magnification (A,B,C:1000X, D:400X)

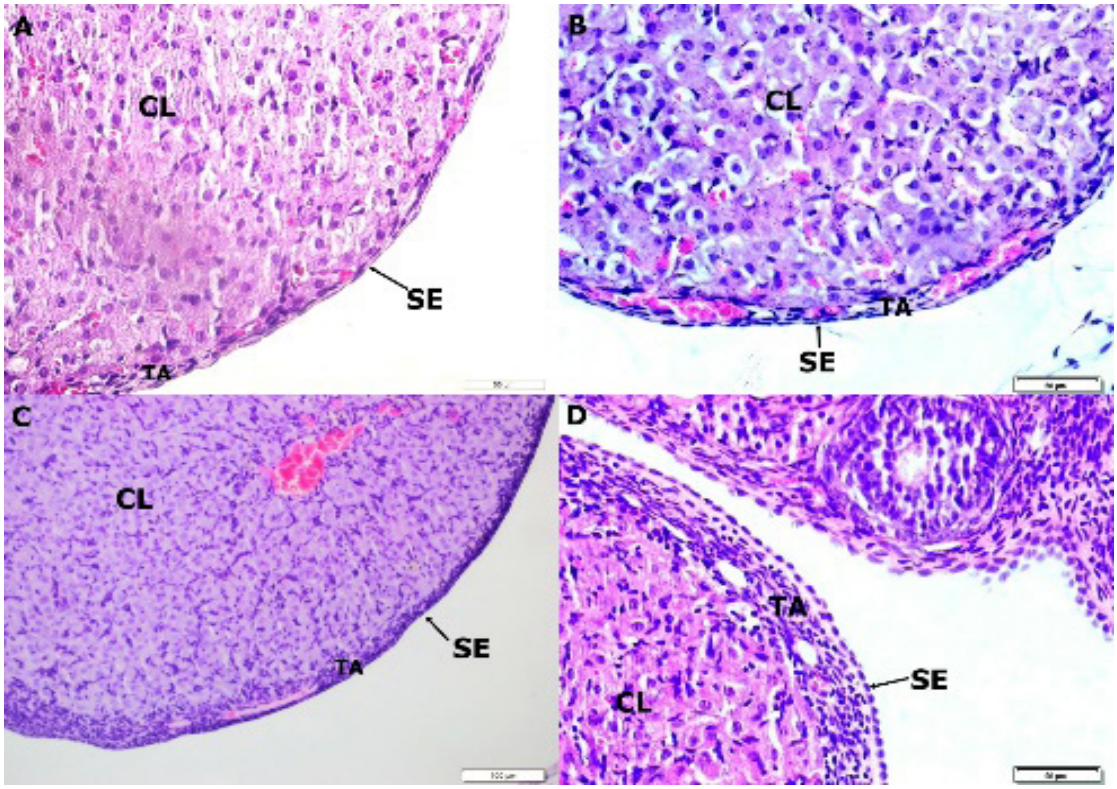

Figure 13. A-B- The surface epithelium of the corpus luteum and a thin layer of tunica albuginea are seen just below it. C-D- Fibroblast-like cells proliferate in the tunica albuginea and the layer thickens (CL: Corpus luteum, TA: Tunica albuginea, SE: Surface ephitelium) (Magnification: 400X)

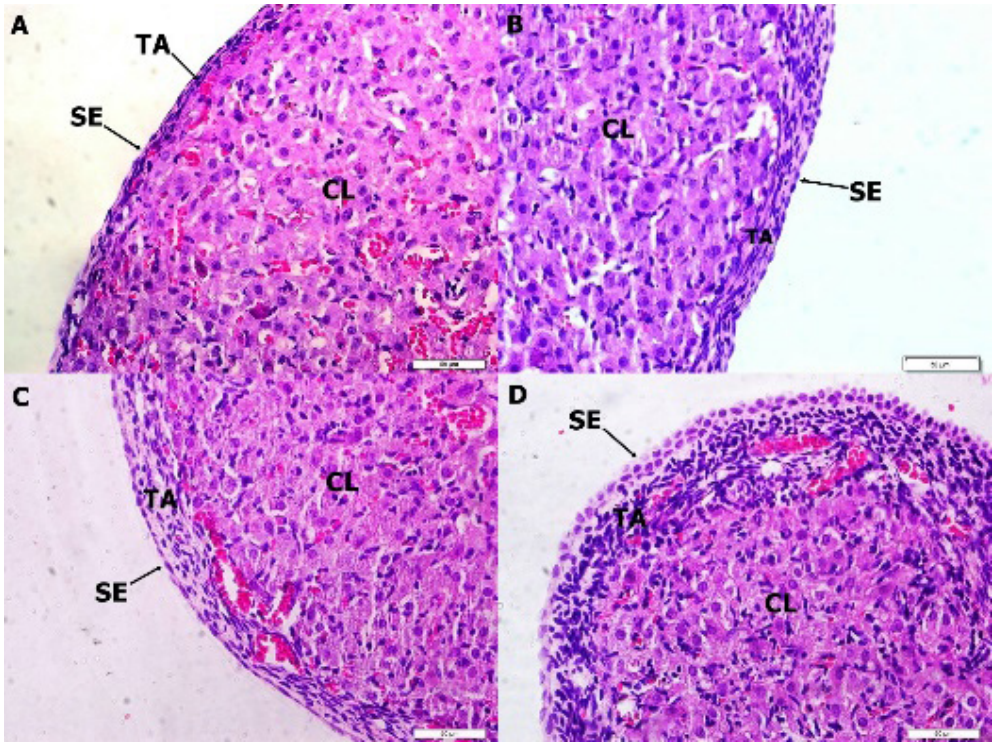

Figure 14. A-B- The surface epithelium of the corpus luteum and a thin layer of tunica albuginea are seen just below it. C-D- Fibroblast-like cells proliferate in the tunica albuginea and the layer thickens (Magnification: 400X), (CL: Corpus luteum, TA: Tunica albuginea, SE: Surface ephitelium)

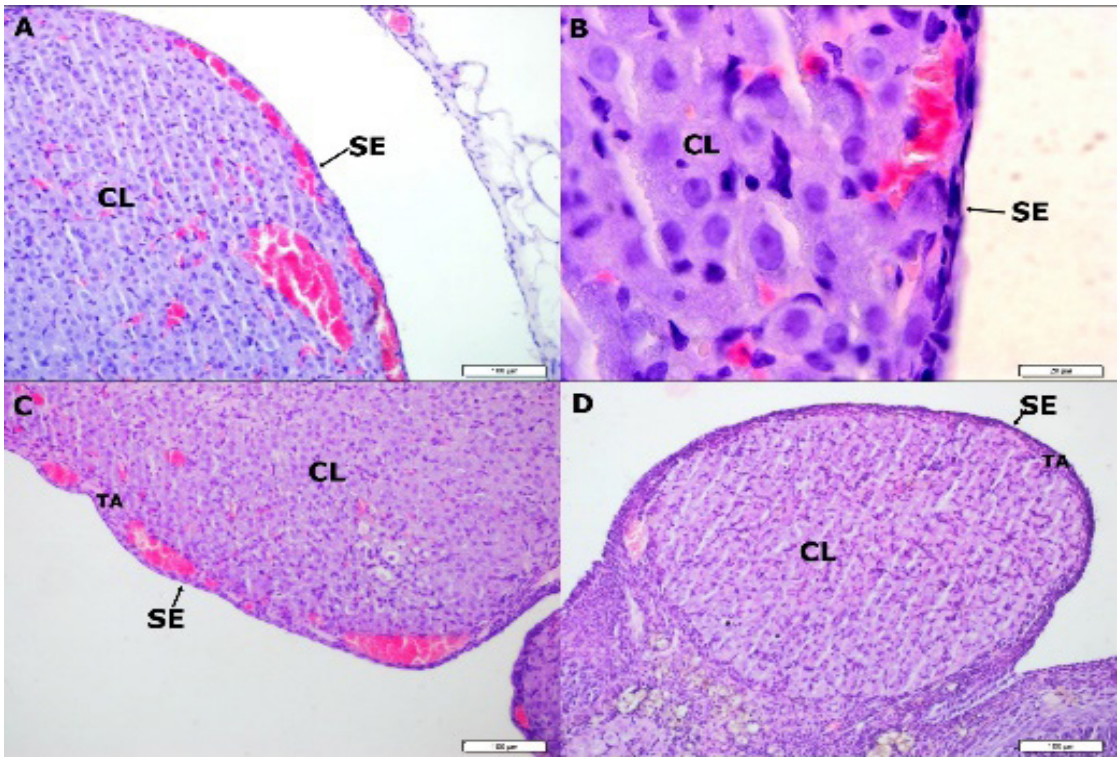

Figure 15. A (Magnification: 400X), B- The surface epithelium of the corpus luteum and a thin layer of tunica albuginea are seen just below it (Magnification: 1000X) C (Magnification: 400X), D- Fibroblast-like cells proliferate in the tunica albuginea and the layer thickens, (Magnification: 200X)
(CL: Corpus luteum, TA: Tunica albuginea, SE: Surface ephitelium)

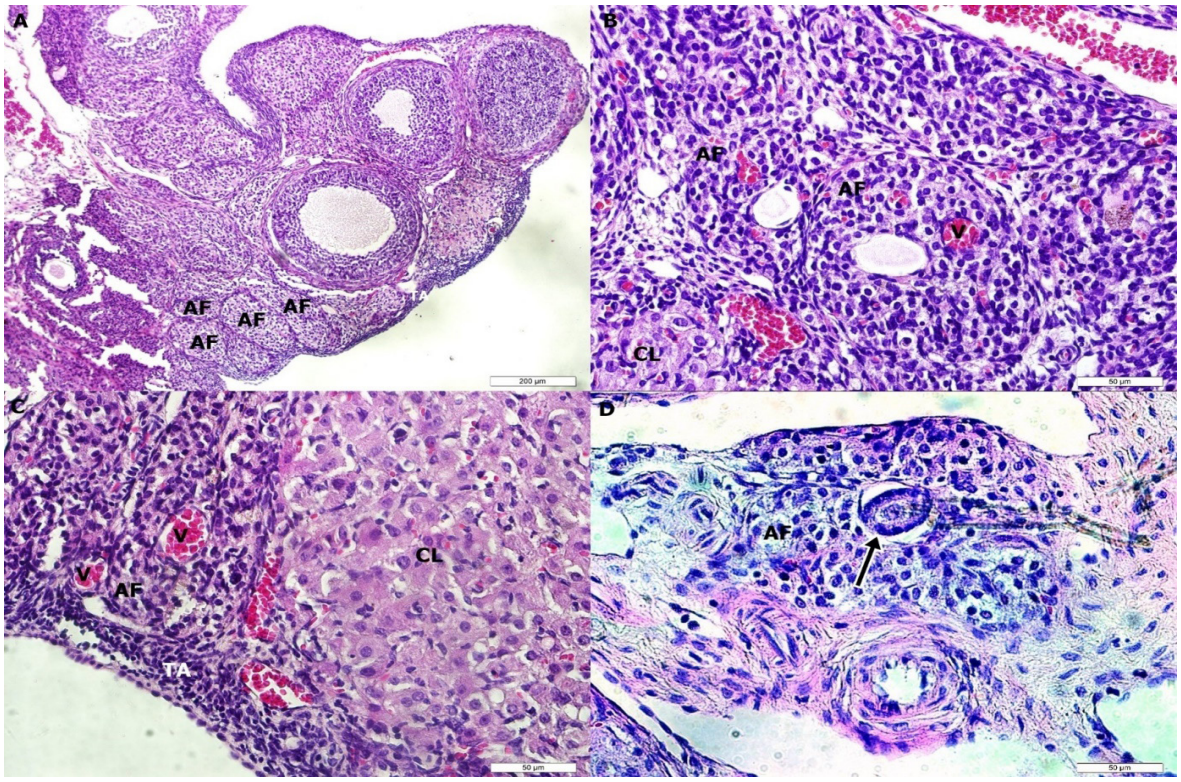

Figure 16. A (Magnification: 100X), B Atretic follicles C: Vascular formation in the lumen of the atretic follicle D: Primary follicle formed in the lumen of the atretic follicle, (B-D: Magnification: 400X) (AF: Atretic follicule)

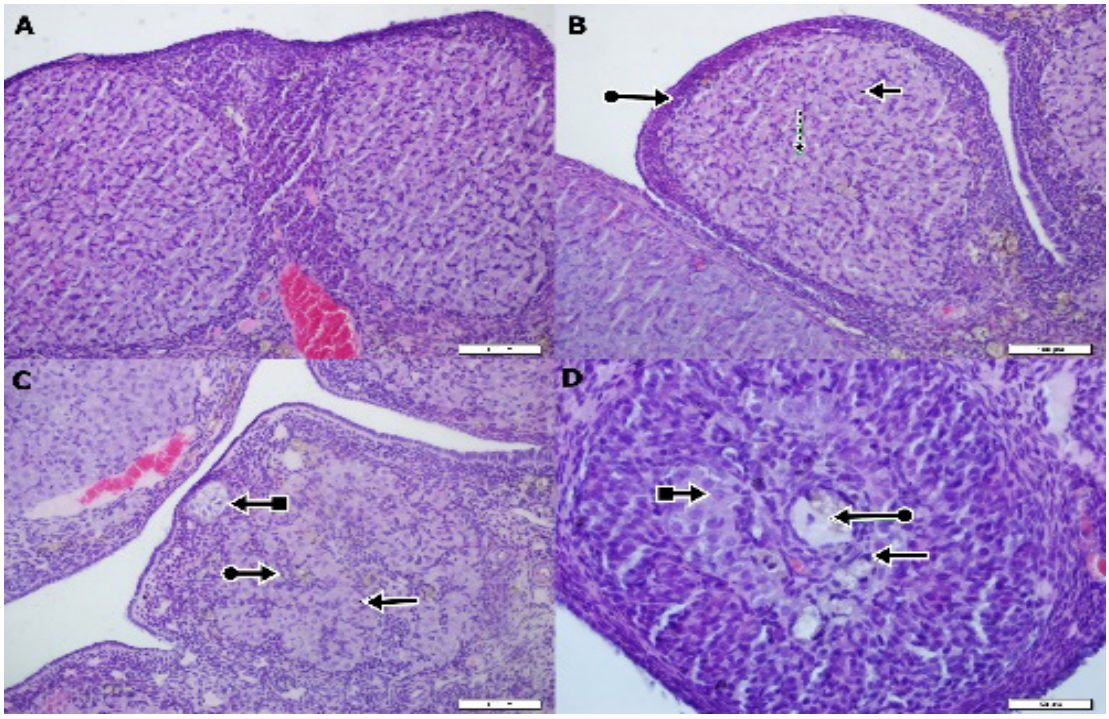

Figure 17. A- Theca lutein cells begin to appear in the corpus luteum as well as granulosa lutein cells B- **(●→)** Tunica albuginea, (- - >) Theca lutein cells, (→) Granulosa lutein cells C- **(■→)** Primary follicle formed in the tunica albuginea in the reconstructed corpus luteum, **(●→)** Theca lutein cells, (→) Granulosa lutein cells D- **(●→)** Lumen of corpus luteum, (→) Theca lutein cells, **(■→)** Granulosa lutein cells (A, B Magnification: 200X; C,D: Magnification: 400X)

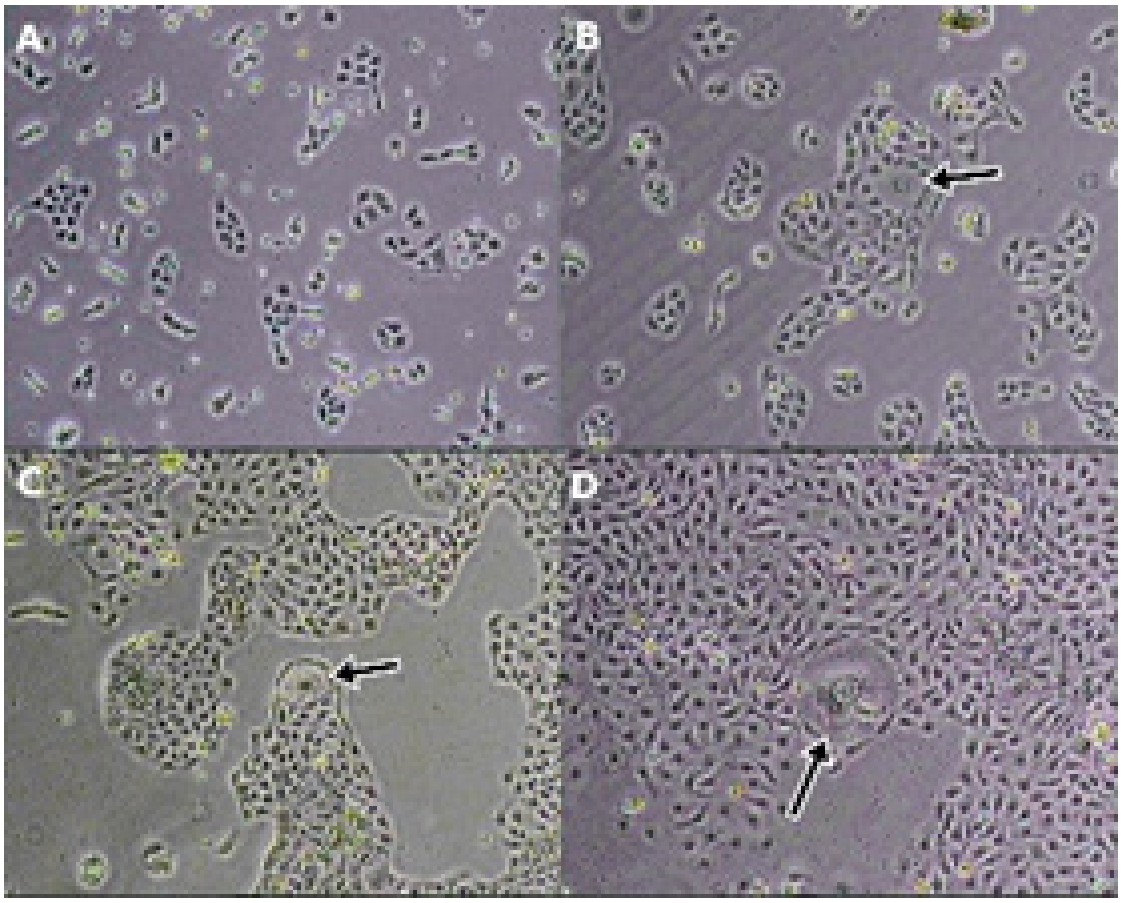

Figure 18. A- Morphological appearance of ovarian surface epithelium proliferating in cell culture (B-D) (→) Primordial follicle-like structures in ovarian surface epithelium (Magnification: 100X)

Discussion

The Graafian follicle is known as mature, and its wall consists of a granulosa cell layer and theca layer with basal lamina between them. In the region where it is associated with the oocyte, the cumulus granulosa cells form a mound called the cumulus oophorus [7]. In the theca interna layer adjacent to the mural granulosa cells, androstenedione, testosterone, and dihydrotestosterone are synthesized. Androstenedione is transported to granulosa cells and converted to estradiol (E2) by aromatase (CYP19A1). Mural granulosa cells do not have the enzymes necessary for the production of estrogens. The theca externa is a connective tissue containing collagen fibers and smooth muscle cells and is continuous with the stroma of the ovary. Granulosa cells in Graafian follicles acquire LH receptors as well as FSH receptors, which are necessary for the luteinization of the corpus luteum [4]. Ovulation is the process of expulsion of the secondary oocyte from the Graaf follicle. After ovulation, the basement membrane breaks down and the granulosa and theca interna cells are rearranged to form a gland called the corpus luteum. Blood vessels from the theca layer of the follicle invade the transforming granulosa cells. The cells of the epithelioid theca interna follow the blood vessels and become the theca lutein cells of the corpus luteum. Granulosa lutein cells are larger and centrally located. Theca lutein cells are smaller and located peripherally [1, 4, 12]. Immediately after ovulation, pericytes originating from the theca compartment are the first vascular cells invading the developing luteal parenchyma.

Vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), endocrine-derived (EG-VEGF), and angiopoietins (Ang) are required for molecular regulation of angiogenesis in the corpus luteum. All findings show that the functional life of the corpus luteum depends on paracrine and autocrine mechanisms [13]. Then, a rich vascular network forms in the corpus luteum, and the

cells it contains begin to secrete estrogen and progesterone. During the luteal phase, theca lutein cells do not hypertrophy, while granulosa lutein cells undergo extensive hypertrophy and contribute significantly to the growth of the corpus luteum. In rodents, granulosa lutein cells and theca lutein cells differ in basal progesterone secretion rates, and granulosa lutein cells produce more progesterone than theca lutein cells [1, 13, 14].

Progesterone and estrogen hormones stimulate endometrial growth and differentiation to prepare for embryo implantation. In the case of pregnancy, the pregnancy corpus luteum is formed and degenerates after 4-5 months of existence. If pregnancy does not occur, the corpus luteum degenerates, and corpus Albicans is formed. Prostaglandin F2 alpha (PGF2α) secretion from the endometrium has an important role in the regression of the corpus luteum. Prostaglandin regresses the corpus luteum and stops the secretion of progesterone hormone. After the regression of the corpus luteum, the theca lutein cells released in the stroma can secrete corticosteroids and they are called interstitial glands [4, 15, 16].

The mesoderm-derived surface epithelium undergoes morphological changes due to cyclic changes, proliferates, and shows pseudostratification in some areas. Stem cell markers such as Oct 4, Stella, C-Kit, and Nanog are also expressed in the ovarian surface epithelium, along with epithelial (cytokeratin) and mesenchymal markers (vimentin). After ovulation, the ovarian surface epithelium helps repair damaged areas. The extracellular matrix also plays an important role in the development of the follicle and the formation of the corpus luteum [17-20]. The extracellular matrix (ECM) is a complex system consisting of a collagen network (types I, III, IV, VI) associated with proteoglycans and glycoproteins, elastic and reticular fibers, fibronectin, and laminin. It plays an important role in ovarian functions by participating in processes such as cell migration, proliferation, growth, and differentiation. The ECM provides structural support for the follicle, maintains cellular organization and connectivity, and provides signals that support follicle development and maturation [21-23].

Fibroblast-like cells in the externa layer of the graaf follicle have lysosome-like granules. While type I collagen fibres are found in the theca externa layer, both type I and type III collagen fibres are found in the theca interna. In the tunica albuginea, type I and type III collagen fibres form bundles with concentric arrangement.

Type I collagen fibres are mostly seen in the superficial part of the tunica albuginea, while type III collagen fibres are found in deeper regions. During ovulation, collagen fibre bundles in the theca externa and tunica albuginea undergo collagenolysis. Proteolytic activity in the ovarian surface epithelium causes the breakdown of the ovarian surface epithelium and ovarian cortex during ovulation and also contributes to their reorganisation. The ovarian surface epithelium produces proteolytic enzymes such as urokinase plasminogen activator (uPA) matrix metalloproteases 2 and 9 [7, 24, 25].

The corpus luteum is a temporary endocrine gland containing different cells such as granulosa cells, theca cells, endothelial cells, pericytes, fibroblasts, and macrophages [13, 15, 26]. These cells have different morphological, endocrine, and biochemical features. FSHRs play an important role during follicular development but have no function when follicular cells differentiate. Their expression decreases after the LH peak in the luteinization process. In rodents, both the estrogen receptor α (Erα) and estrogen receptor β (Erβ) genes are expressed in the ovary as a single (6.5 kb) and multiple transcripts (1.0 kb to about 10 kb). ERβ is abundantly expressed in the follicle, especially in the granulosa cell layer. However, ERα is a receptor found at higher levels than ERβ in the corpus luteum [13].

Angiogenesis is a crucial process that helps establish and maintain the normal structure and function of the corpus luteum. Luteal development can be considered as an inflammatory response. There are many activated leukocytes such as macrophages, neutrophils, and eosinophils in the corpus luteum, and these cells are commonly involved in neovascularization [27, 28]. Studies show that cytokines secreted by immune cells regulate both luteotropic and luteolytic processes [29].

Vascular endothelial growth factor (VEGF) has been found to have an important role in the corpus luteum. VEGF is secreted by granulosa

cells in the antral follicle and granulosa lutein cells in the corpus lutem and has two receptors, VEGFR-1 and VEGFR-2. Since it cannot pass through the basement membrane of blood vessels in the theca layer of the follicle, it cannot reach the granulosa cell layer. After the destruction of the basement membrane, blood vessels invade the corpus luteum together with cells in the theca interna layer. Perivascular cells (pericytes) found in the follicle in the preovulatory phase and seen in the capillaries of the corpus luteum in the early stages of the luteal phase show a high proliferation rate [27]. Studies have shown that the administration of HCG to human granulosa lutein (hGL) cells increases VEGF expression. In the follicles of the ovary, TGF-β1 protein expression occurs in both granulosa and theca cells, while TGFβ2 occurs specifically in the theca cells of the follicles. VEGF is expressed by the stimulating effect of TGF-β1 on granulosa cells [30].

Theca lutein cells and granulosa lutein cells express progesterone receptors. Studies have shown that progesterone receptors are expressed in steroidogenic cells at all stages of the luteal phase. Among all steroidogenic cell types examined, progesterone expressions were found to be variable during the luteal phase [31]. Studies have shown that progesterone alpha receptors (PRA) are expressed in the theca lutein cells of the regressed corpus luteum and the interstitial gland cells proliferating from theca cells in atretic follicles [32]. In addition, membrane progesterone receptor expression, unlike nuclear progesterone receptor, has been described in the corpus luteum of rats and sheep. However, the function of this receptor in luteal cell formation and its signaling mechanism is unknown [13]. In some studies, mural granulosa cells obtained from IVF were isolated and cultured to form an in vitro corpus luteum model. Studies with these cells may lead to the identification of signaling pathways related to regulating the corpus luteum and developing new treatment strategies [33].

On the other hand, numerous studies have been conducted regarding postnatal oogenesis. Johnson et al. [34] proposed that bone marrow or peripheral blood serves as a reservoir for ovarian stem cells, which migrate towards the ovary and facilitate germ cell renewal in the adult mouse ovary. In their studies, they demonstrated that oocyte production was restored following bone marrow transplantation in mice that had undergone chemotherapy and had ataxia telangiectasia mutations, revealing the presence of donor-derived oocytes [34]. Bukovsky et al. [35], in their studies, demonstrated that human ovarian surface epithelial cells could differentiate into oocytes and granulosa cells in a culture environment. They maintained the cells derived from the ovarian surface epithelium in environments containing estrogenic stimulants such as phenol red for 5-6 days. They reported that cells cultured in the presence of phenol red directly differentiated into large oocyte-like cells (180 microns).

Virant Klun et al. [36] claimed to have isolated ovarian stem cells in postmenopausal women and women with premature ovarian insufficiency in their study. They isolated small round cells, ranging from 2 to 4 mm in diameter, from the material obtained by scraping. They determined that these cells expressed transcription factors such as SSEA-4, OCT4, NANOG, SOX2, and C-KIT. Bhartiya et al. [37], in their study with mouse ovaries, identified two different cell populations. They noted that VSEL cells were 3-5 μm in size and expressed nuclear OCT4, while OKH cells were larger than 8 μm and expressed cytoplasmic OCT4.

There are also some limitations in our study. The cells and structures developing in the tunica albuginea adjacent to the corpus luteum are observed to not always be fully stained due to their very small size and sometimes their locations. In addition, even in serial sections, while cells and structures are seen in one section, they could not be detected in the next section and most of the time they could only be observed at x400 and x1000 magnifications. Due to the rare encounter with these cells, the chance of imaging can be increased by conducting immunohistochemistry techniques in a multicentric manner and including a large number of samples.

The corpus luteum differentiates from the structure left behind as a result of the expulsion of the oocyte from the Graaf follicle. The collagen fibers and the cells they contain in the external theca layer of the ovulated Graaf follicle completely degenerate. After the collagen fibers and fibroblast-like cells in the tunica albuginea are broken down by proteolytic enzymes, this

region is largely rebuilt. In the sections, new capillary vessels have been observed to form in the tunica albuginea, which was previously known to be avascular. It can be thought that VSEL cells such as monocytes, leukocytes, and mast cells, which pass from the bone marrow to the peripheral circulation, also reach the region via capillary vessels and develop by receiving signals from fibroblast-like cells located here. The appearance of primordial follicles in the tunica albuginea adjacent to the corpus luteum during reconstruction has increased suspicions towards neofolliculogenesis, and further studies are needed in this direction.

Conflict of interest: No conflict of interest was declared by the authors.

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Authors' contributions to the article

M.S.U. constructed the main idea and hypothesis of the study. M.S. and S.T. developed the theory and arranged/edited the material and method section. M.S.U. and S.T. has/have done the evaluation of the data in the Results section. Discussion section of the article written by all authors reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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Complications of imaging-assisted port catheters and factors affecting complications

Görüntüleme eşliğinde takılan port kateterlerinin komplikasyonları ve komplikasyonlara etki eden faktörler

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Abstract

Purpose: This research investigates complications associated with Imaging-Assisted Port Catheters (IAPCs) to provide insights for healthcare professionals involved in their usage. The study aims to optimize patient safety, implement preventive strategies, and guide evidence-based decision-making in the clinical use of IAPCs.

Materials and methods: A retrospective study comprising 1247 patients who underwent IAPC placement in the Interventional Radiology Unit between 01.09.2012-01.09.2020 was conducted. Data, including indications for port placement, complications, duration of port stay, and reasons for explantation, were extracted from electronic medical records. Comparative analysis with surgically implanted ports was performed, and the efficacy and safety of imaging-guided port implantation were assessed.

Results: The study predominantly involved right internal jugular vein placement (93.8%). Major complications did not occur during the 7 to 1330 days of port usage. The average usage period was 243 days, totaling 310,503 catheter-days. Infectious complications were the most common (4.8%), significantly higher (13.9%) in hematological malignancies. Multivariate analysis revealed a significantly increased complication rate in hematological malignancy patients (p<0.001).

Conclusion: The research reveals an 8.6% overall incidence of complications in IAPC usage, with hematologic malignancy identified as a significant independent risk factor. The low complication rate per 1000 catheter days (0.36) aligns with recent studies, emphasizing the benefits of image guidance and procedural expertise. Notably, infectious complications, especially in hematologic malignancy patients, underscore the need for further research to refine IAPC management for long-term intravenous access. The study contributes essential insights for healthcare professionals involved in the field, emphasizing ongoing efforts in advancing IAPC management for the benefit of patients.

Keywords: Cancer treatment, catheterization, complication, totally implantable access port, ultrasound guidance.

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Öz

Amaç: Bu araştırma, Görüntüleme Destekli Port Kateterler (IAPC'ler) ile ilişkili komplikasyonları araştırarak, bunların kullanımında yer alan sağlık çalışanlarına öngörü sağlamaktadır. Çalışma, hasta güvenliğini optimize etmeyi, önleyici stratejiler uygulamayı ve IAPC'lerin klinik kullanımında kanıta dayalı karar verme sürecine rehberlik etmeyi amaçlamaktadır.

Gereç ve yöntem: Girişimsel Radyoloji Ünitesinde 01.09.2012-01.09.2020 tarihleri arasında IAPC yerleştirilen 1247 hastayı içeren retrospektif bir çalışma yapıldı. Port yerleştirme endikasyonları, komplikasyonlar, portun kalış süresi ve eksplantasyon nedenleri dahil olmak üzere veriler elektronik tıbbi kayıtlardan elde edildi. Cerrahi olarak implante edilen portlarla karşılaştırmalı analiz yapıldı ve görüntüleme kılavuzluğunda port implantasyonunun etkinliği ve güvenliği değerlendirildi.

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Bulgular: Çalışmada ağırlıklı olarak sağ internal juguler ven yerleştirildi (%93,8). Portun 7 ila 1330 günlük kullanımı sırasında majör komplikasyon görülmedi. Ortalama kullanım süresi 243 gün ve toplam 310.503 kateter günü idi. En sık enfeksiyöz komplikasyonlar (%4,8) görülmüş olup hematolojik malignitelerde anlamlı derecede yüksektir (%13,9). Çok değişkenli analiz hematolojik malignite hastalarında komplikasyon oranının önemli ölçüde arttığını ortaya koymuştur (p<0,001).
Sonuç: Araştırma, IAPC kullanımında genel komplikasyon insidansının %8,6 olduğunu ve hematolojik malignitenin önemli bir bağımsız risk faktörü olarak tanımlandığını ortaya koymaktadır. Her 1000 kateter günü başına düşen düşük komplikasyon oranı (0,36), görüntü rehberliği ve prosedürel uzmanlığın faydalarını vurgulayan son çalışmalarla uyumludur. Özellikle hematolojik malignite hastalarında görülen enfeksiyöz komplikasyonlar, uzun süreli intravenöz erişim için IAPC yönetimini iyileştirmeye yönelik daha fazla araştırma yapılması gerektiğinin altını çizmektedir. Bu çalışma, hastaların yararına IAPC yönetimini ilerletmek için devam eden çabaları vurgulayarak, bu alanda çalışan sağlık profesyonelleri için önemli bilgiler sunmaktadır.

Anahtar kelimeler: Kanser tedavisi, kateterizasyon, komplikasyon, tamamen implante edilebilir port kateter, ultrason rehberliği.

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Introduction

The use of subcutaneously placed imaging-assisted port catheters (IAPCs) has become increasingly common. They are preferred especially in patients receiving intermittent and long-term infusion therapy because of the patient comfort they provide and low infection rates [1]. The most common indication for port catheter is patients with malignancy requiring long-term chemotherapy [2]. In the past, venous port catheters were implanted in the operating room under general anesthesia by surgical departments. Venous port insertion in the angiography room with interventional radiology techniques was first described by Morris et al. [3] in 1992, and since then, radiological venous port insertion has been among the routine procedures of interventional radiology.

In adult patients, the port can be placed easily with local anesthesia. Existing radiological images should be examined before port placement, and the planned vein should be evaluated in detail with ultrasound (US). In order to reduce the risk of infection, the area where the port will be placed should be prepared and covered in a sterile manner. The procedure should be done under sterile conditions.

Central venous access complications are now seen rarely in imaging-guided interventions, however, complications can be seen due to the inadequate experience of the practitioner, the difficulty of venous anatomy, and the anatomical-morphological structure of the patient. The occurrence of IAPC-related complications can be influenced by a variety of factors. Patient-related factors, such as age, underlying comorbidities, immunosuppression, and nutritional status may impact the susceptibility to infections and the overall healing process. Procedural factors, including catheter insertion technique, catheter tip position, and the experience of the healthcare professional performing the procedure can influence the immediate and long-term success of IAPCs. Moreover, device-related factors, such as catheter material, design, and the use of antithrombotic coatings may play a significant role in determining the occurrence of complications. Understanding the spectrum of IAPC-related complications and their determinants is essential for optimizing patient safety, implementing preventive strategies, and guiding evidence-based decision-making in the clinical use of IAPCs. This research aims to provide valuable insights to interventional radiologists, nurses, and other health professionals by understanding IAPCs' factors affecting complications.

Material and methods

Study design

This article is a retrospective study aiming to investigate the factors affecting complications of IAPCs. Ethical approval was obtained from the Institutional Review Board before the commencement of the study. Patients who underwent IAPC placement in our Interventional Radiology Unit between 01.09.2012-01.09.2020 were included in our study. Indications for port placement, early or late complications seen at the venous access and port placement site, duration of port stay, and reasons for explantation were

reviewed from electronic medical records. The results obtained were compared with the data of surgically implanted ports in the literature and the efficacy and safety of imaging-guided port implantation were evaluated.

IAPC placement prosedure

All implantation procedures were performed under the guidance of US and angiography device. Implantations were performed under local anesthesia. During the procedure, the requirements of surgical sterility were absolutely fulfilled. Patients with high risk and/or absolute neutropenia (WBC <500/mm3) were given antibiotic prophylaxis with 1 g IV cefazolin sodium (Sefazol®, Mustafa Nevzat İlaç Sanayi AŞ, Istanbul, Türkiye) 30 minutes before the procedure. Patients who were not hospitalized were followed up for 4-6 hours and sent home. A procedure note including the follow-up procedure was written for the hospitalized patients, and then they were followed up in the service and discharged under appropriate conditions. All patients were called for control one week after the procedure, and it was checked whether there was redness, swelling, temperature increase, hematoma, and separation at the suture site in the port-inserted area. The images of the process steps are given in Figure 1 as a summary.

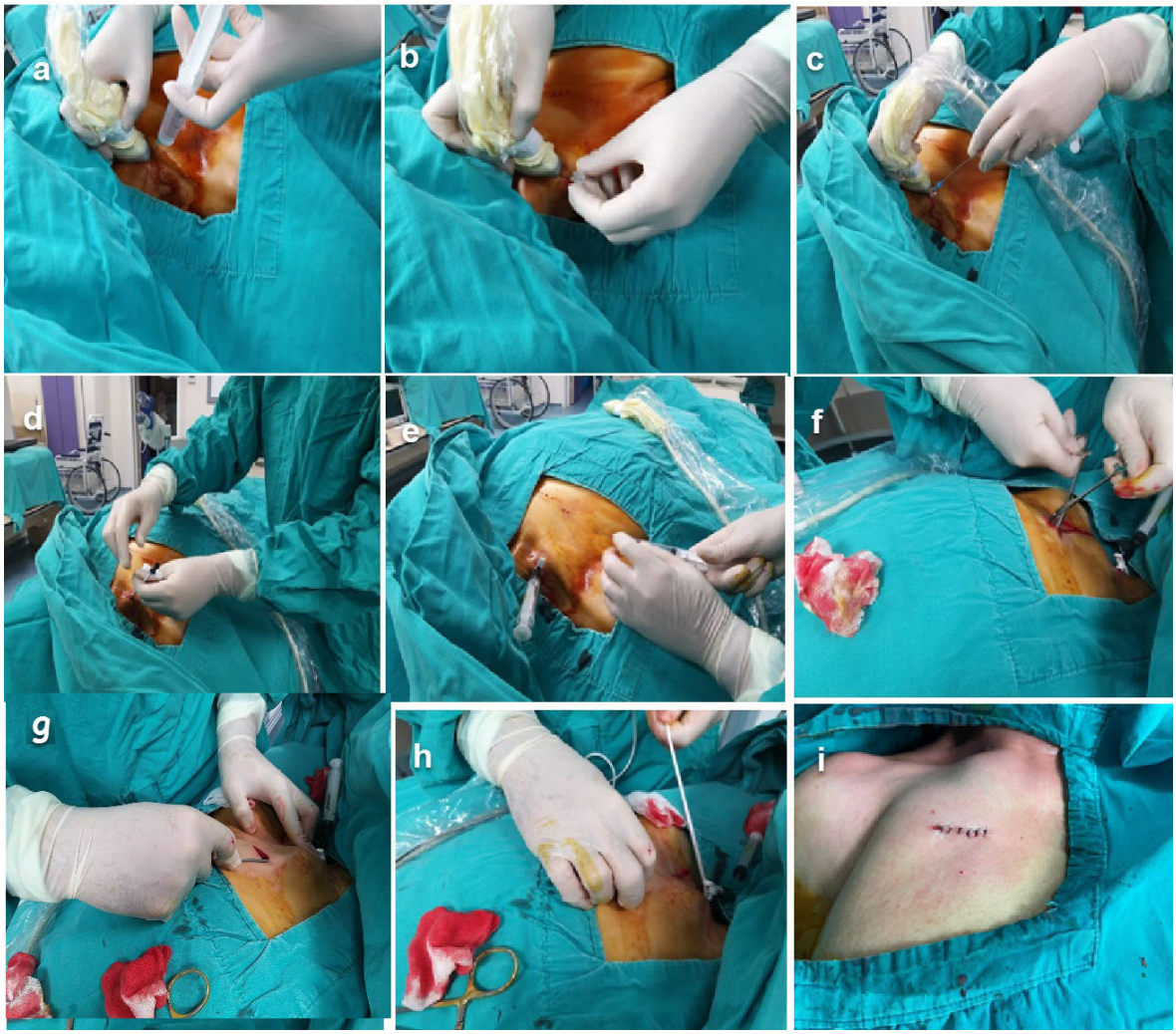

Figure 1. Imaging-assisted port catheter placement steps: a-b) After providing the necessary asepsis conditions, local anesthesia application under ultrasound guidance and needle entry into the vein, c) Insertion of the guide wire through the needle, d) Placement of the peel away sheet over the guide wire, e) Local anesthesia application in the port pocket, f) Opening the port pocket, g) Opening the tunnel with a tunneler, h) Passing the catheter through the tunnel, i) Closing the port pocket with a suture

Complication assessment

Complications were identified from medical records, physician notes, nursing records, and radiological reports. Complications of central venous access were classified into two main groups as early complications (bleeding, haematoma development, catheter malposition, venous perforation, infection, arterial puncture, pneumothorax and air embolism) occurring between catheter placement and first use (<30 days) and late complications (infections, thrombotic and mechanical complications) occurring later (>30 days) (Table 1) [4].

Statistical analysis

Descriptive statistics were used to summarize patient demographics and clinical characteristics. Univariate and multivariate analyses were performed to identify factors associated with the occurrence of IAPC-related complications. Multivariate logistic regression analysis was employed to assess the independent effect of each potential factor on the occurrence of complications, adjusting for confounding variables.

Results

A total of 1247 patients with imaging-guided ports were included in our study. Demographic information, diagnoses and reasons for port catheterization of our patients are given in Table 2. In our patients, it was observed that the right internal jugular vein was most preferred for port catheter placement (93.8%).

There were no major complications such as hemothorax, pneumothorax, arterial injury and nerve injury during port catheter placement. When the data were collected, the port usage period ranged from 7 to 1330 days, the average usage period was 243 days, and the total usage period was 310,503 catheter-days when all ports were taken into account. During follow-up, 213 patients died while their current port was functional. Also, 139 ports were removed for various reasons. The reasons for port removal in these patients are shown in Table 3. Of all complications, 37 were early (<30 days), 70 were late complications (>30 days). Early and late complications, incidence of complications according to 1000-day port catheter stay, and number of ports removed due to complications are shown in Table 4. Infectious complications were the most common complications, with a rate of 4.8% in all patients, while the incidence was significantly higher in hematological malignancies (13.9%). Figure 2 shows examples of venous thrombosis, catheter fracture, catheter misposition, and catheter pinc-off. Figure 3 shows pictures of malposed port catheters in different patients.

Factors affecting the occurrence of complications were assessed through multivariate logistic regression analysis. The complication rate was statistically significantly higher in patients with hematological malignancies (p<0.001). 21 of 62 patients (33.8%) with port infection had hematological malignancy. There was no significant increase in the incidence of complications related to IAPCs in those with head and neck malignancy (p=0.614), breast malignancy (p=0.366) and those without malignancy (p=0.259). In addition, age (p=0.705), gender (p=0.648), insertion of the catheter in the right or left jugular vein (p=0.129) were found to have no statistical significance on the occurrence of complications.

Table 1. Complications after port implantation

Early complications (<30 days)	Late Complications (>30days)
Malposition: intravenous, cardiac	Infection
Arrhythmia	Venous thrombosis, pulmonary embolism
Perforation and bleeding: hemothorax, mediastinal, cardiac tamponade	Venous stenosis
Arterial malpositioning	Catheter pinch-off, fracture and migration
Pneumothorax	Catheter embolization
Thoracic duct injury	Air embolism
Air embolism	

Table 2. The demographic data of the patients and the data regarding the catheters placed

Age (Years) Mean ± SD	59±13 (range 19-95)
Sex (male/female)	
Male	685 (54.9%)
Female	562 (45.1%)
Background disease	
Non-malignant	10 (1%)
Malignant	1237 (99%)
Gastrointestinal malignancies	605
Hematologic malignancies	151
Breast carcinoma	144
Genitourinary system malignancies	94
Head and neck cancer	112
Lung cancer	104
Others	27
Implantation side	
Right	1170
Left	77
Purpose of TIVAP	
Chemotherapy	1214
Nutritional supplementation	25
Both	8

TIVAP, totally implantable vascular access system

Table 3. Results of 1247 port implantations

Duration of catheter stay	For all patients	310503 (catheter-days)
	For a single patient	248 (mean catheter-days)
		7-1330 (range catheter-days)
Follow up	Still in use	895 catheters
	Exitus	213 catheters
	Removal before the end of therapy	82 catheters
	Removal after the end of therapy	57 catheters

Table 4. Complications after port catheter placement

Complication	Patients	Early complications (<30 days)	Late complication (>30 days)	Incidence (%)	Per 1000 catheter days	Port removal
Malposition: intravenous, cardiac	14	10	4	1.12	0.05	6
Arrhythmia	4	4	0	0.32	0.013	0
Perforation and bleeding: hemothorax, mediastinal, cardiac tamponade	0	0	0	0	0	0
Pneumothorax	0	0	0	0	0	0
Thoracic duct injury	0	0	0	0	0	0
Air embolism	0	0	0	0	0	0
Infection	62	8	54	4.97	0.21	59
Venous thrombosis	10	4	6	0.8	0.02	6
Catheter pinch-off, fracture and migration	8	4	4	0.64	0.026	8
Catheter thrombosis	4	2	2	0.32	0.013	1
Hemorrhage, hematoma	3	3	0	0.24	0.01	0
Wound dehiscence	2	2	0	0.16	0.006	2
Total	107	37	70	8.6	0.36	82

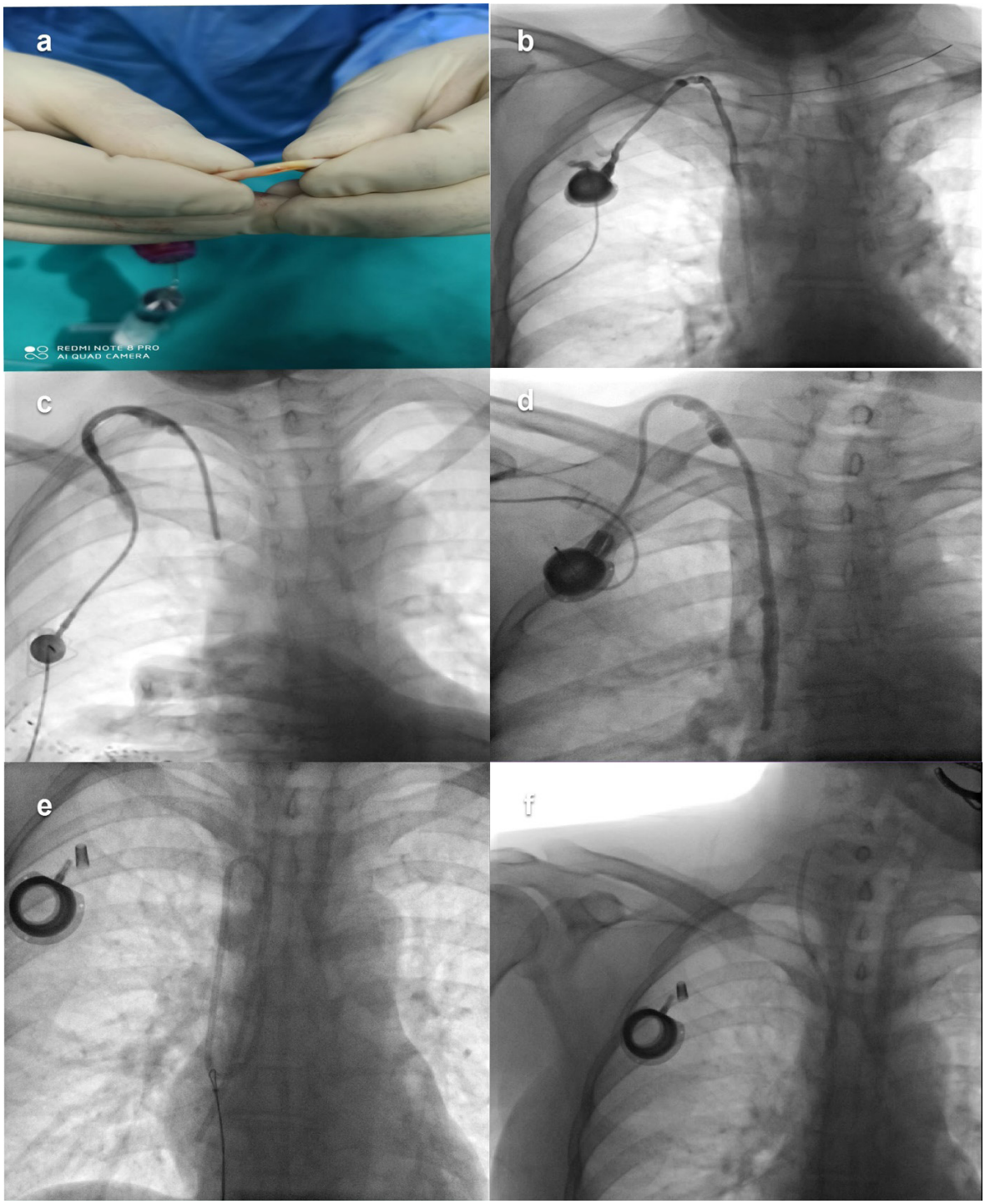

Figure 2. a) Port catheter line fracture, b-d) port catheter thrombosis, e and f) The port catheter line has been separated from the reservoir and has migrated
The port catheter line was removed by catching it with a snare catheter

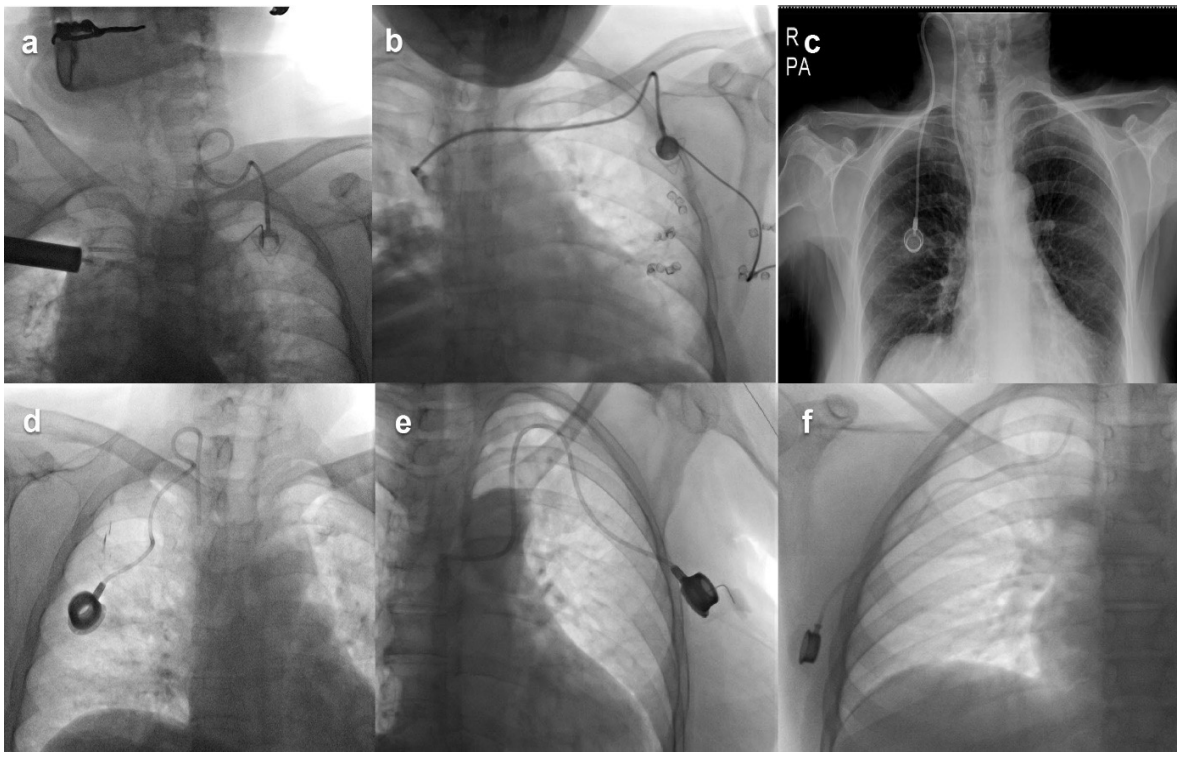

Figure 3. a-f) Pictures of malpositioned port catheters in different patients

Discussion

The present study, exploring the complications associated with IAPCs and their impact on patient outcomes, revealed an overall incidence of 8.6% complications, emphasizing the crucial role of underlying hematologic malignancy as an independent risk factor. The study further highlighted a low complication rate of 0.36 per 1000 catheter days, consistent with recent studies, attributable to the increasing use of image guidance and enhanced procedural expertise. Notably, infectious complications, particularly prevalent in hematologic malignancy patients, were significantly higher (13.9%) compared to other patient groups (3.5%). This underscores the importance of understanding factors influencing complication rates, such as chemotherapy-induced immunosuppression and prolonged neutropenia. The study's findings contribute valuable insights into optimizing the management of IAPCs for long-term intravenous access, emphasizing the need for ongoing research and refinement in this field.

The incidence of complications was significantly higher in hematologic malignancy patients. The higher incidence was strongly related to infectious complications. Hematologic malignancy patients' higher incidence may be attributable to more intense chemotherapy, resulting in prolonged neutropenia, and also to direct impairment of the immune system by the disease itself [5]. Several studies reported higher rates of infection in patients with hematological malignancy [6-9]. It is thought that immunosuppression and prolonged neutropenia increase the risk in these patients [6]. In this study, we found that the rate of infectious complications in patients with hematological malignancies (13.9%) was significantly higher than in other patients (3.5%). We did not see an increased risk of complications with patients with head and neck, and breast cancers. Wang et al. [6] and Bos et al. [10] found an increased risk of complications in head and neck cancers. However, they emphasized that no definite interpretation could be made about the cause and that studies are needed on this subject [6].

In a study conducted with 2713 patients, it was found that only the increase in "number of punctures" increased the risk of complications, while the use of US reduced the risk of complications [11]. While high age was seen as a risk factor in some studies [12, 13], it was not seen as a risk factor in some studies like ours [11, 14]. While body mass index (BMI) was a

risk factor in the study of Nagasawa et al. [15], it was not seen as a risk factor in the study of Bademler et al. [11] and Hourmodzi et al. [16]. We could not analyze BMI as a risk factor because we could not reach sufficient data in our study.

Infections of port catheters include pocket and/or tunnel cellulitis or the more common catheter-related bloodstream infections. Early infections were evaluated due to contamination during the procedure. In late infections, thrombus and fibrin sheath are thought to be biofilms for infection [17]. Therefore, it is thought that there is a relationship between thrombus and infection in catheter infections. In order to prevent infection, procedures such as sterile hand washing, shaving and disinfection of the treatment area are performed. Still, infections are the most common complication after implantation of a venous port system [17, 18]. Moreover, the study of Nezami et al. [8] suggests that a single dose of preprocedural single-dose intravenous prophylactic antibiotic before totally implantable venous access port placement does not prevent short-term procedure-related infections.

In a retrospective study published in 2023, a total of 1406 port catheters were evaluated, revealing a significantly higher prevalence of hematologic malignancies in the infection group compared to the non-infection group. Furthermore, multivariate logistic analysis identified hematoma, preoperative hospital stay, chemotherapy history, and infection history as independent risk factors for infection. Similarly, our study, employing comparable statistical analyses, identified hematologic malignancy as a significant risk factor for port complications [19].

In a study published in 2022, the presence of a history of surgery, inpatient treatment, and hematologic malignancy in patients has been identified as a risk factor for early-stage port catheter infection. Additionally, the inpatient setting has been reported as a risk factor for late-stage port catheter infection [20].

In a 2023 study investigating the predictability of port catheter infections, it is recommended to avoid port implantation, especially in patients under antibiotic treatment or those who have received antibiotic therapy within the last week, particularly in individuals with low serum total protein levels [21].

A strength of this study is the high number of patients in the sample and all procedures were undergone combined US and flouroscopy guided. However, the study is not without limitations. Being a retrospective analysis, it is subject to retrospective collection of data, lack of documents, inherent biases, and limited control over confounding variables. Additionally, the study was conducted at a single institution, potentially limiting the generalizability of the findings to broader patient populations and practice settings. Another limitation is that we could not separately evaluate patients with chronic diseases such as diabetes mellitus, which may cause increased complications.

In conclusion, this research study sheds light on IAPC complications and the factors affecting complications related to IAPC. IAPC has a significant role with low complication rates for long-term intravenous access. The underlying hematologic malignancy was the independent risk factors of complications associated with IAPCs. Further research and ongoing efforts in this field are essential to continually refine and advance the management of imaging-assisted port catheters for the benefit of patients requiring long-term intravenous access.

Conflict of interest: No conflict of interest was declared by the authors.

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Authors' contributions to the article

M.T. and M.A. have constructed the main idea and hypothesis of the study. H.S.A. and H.G.Y. developed the theory and arranged/edited the material and method section. M.T., M.D. and K.A. have done the evaluation of the data in the Results section. Discussion section of the article written by M.T., H.S.A. and B.U.K. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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Effect of anticoagulation on infarct volume and NIHSS score in patients with atrial fibrillation and ischaemic stroke

Atriyal fibrilasyon ve iskemik inme hastalarında antikoagülasyonun infarkt hacmi ve NIHSS skoru üzerine etkisi

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Abstract

Purpose: The aim of this study was to evaluate the use of oral anticoagulation (OAC) in patients with cardioembolic stroke due to non-valvular atrial fibrillation (NVAF). The NIHSS (National Institute of Healt Stroke Scale) score calculated by a neurologist at initial presentation and infarct volume measured semi-automatically in cm3 by Magnetic Resonance Imaging (MRI).

Materials and methods: A total of 101 NVAF patients with acute ischaemic stroke were included in this retrospective study. Patients were divided into 4 groups according to OAC drug use: Non-OAC users (Group 1), subtherapeutic dose warfarin users (under 70 years of age: INR≤2.0; over 70 years of age: INR≤1.6 Group 2), therapeutic dose warfarin users (under 70 years of age: INR≥2.0; over 70 years of age: INR≥1.6 Group 3) and therapeutic dose Non-vitamin K oral anticoagulant (NOAC) users Group 4.

Results: Infarct volume was calculated as 22.20 cm³ median (0.4-235 cm³ lowest-highest) for Group 1; 12.95 cm³ (1.3-129 cm³) for Group 2; 2.25 cm³ (0.3-89 cm³) for Group 3 and 4.40 cm³ (0.2-293 cm³) for Group 4 and the difference was statistically significant (p=0.039). The calculated NIHSS score was 9 (4-23) for the Group 1, 8.5 (3-18) for the Group 2, 6.5 (2-20) for the Group 3, 5 (1-22) for the Group 4 and the effect of anticoagulation use on NIHSS score was statistically significant (p=0.029).

Conclusion: Anticoagulant treatment holds importance in the primary and secondary prevention of stroke and in enhancing the NIHSS score and infarct volumes among stroke patients, as evidenced in the current study.

Keywords: Atrial fibrillation, cardioembolic stroke, ischemic stroke, infarct volume, NIHSS score.

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Öz

Amaç: Bu çalışmada non-valvüler atrial fibrilasyona (NVAF) bağlı kardiyoembolik inme geçiren hastaların oral antikoagülasyon (OAK) kullanımının, ilk başvuruda nöroloji uzmanı tarafından hesaplanan NIHSS (National Institute of Healt Stroke Scale (Ulusal sağlık enstitüleri inme ölçeği) skoru ve çekilen diffüzyon manyetik rezonans görüntüleme (MRG) 'nin cm3 cinsinden semiotomatik olarak ölçülen infarkt volümünün değerlendirilmesi amaçlanmıştır.

Gereç ve yöntem: Akut iskemik inme tanısı alan 101 NVAF hastası retrospektif olarak planlanan bu çalışmaya dahil edildi. Hastalar OAK ilaç kullanımına göre 4 gruba ayrıldı: OAK kullanmayanlar (Grup 1), subterapötik dozda warfarin kullanan (70 yaş altı: INR≤2,0; 70 yaş üzeri: INR≤1,6 Grup 2), terapötik dozda warfarin kullananlar (70 yaş altı: INR≥2,0; 70 yaş üzeri; INR≥1,6 Grup 3) ve terapötik dozda Non-vitamin K oral antikoagülan (NOAK) kullanan Grup 4 olmak üzere 4 gruba ayrıldı.

Bulgular: İnfarkt volümü 1. Grup için 22,20 cm³ medyan: (0,4-235 cm³ en düşük-en yüksek), 2. Grup: 12,95 cm³ (1,3-129 cm³), 3. Grup: 2,25 cm³ 0,3-89 cm³) ve 4. Grup: 4,40 cm³ (0,2-293 cm³) olarak hesaplandı ve aradaki fark istatistiksel olarak anlamlıydı (p=0,039). Hesaplanan NIHSS skoru 1. Grup için 9 (4-23), 2. Grup: 8,5 (3-18), 3. Grup: 6,5 (2-20), 4. Grup 5 (1-22) olarak hesaplandı ve antikoagülasyon kullanımının NIHSS skoru üzerine etkisi istatistiksel olarak anlamlı bulundu (p=0,029).

Sonuç: Antikoagülan tedavi, bu çalışmada gösterildiği gibi inme hastalarında NIHSS skorunu ve enfarktüs hacimlerini iyileştirmenin yanı sıra inmenin birincil ve ikincil önlenmesinde önemlidir.

Anahtar kelimeler: Atrial fibrilasyon, kardioembolik inme, iskemik inme, infarkt volümü, NIHSS skoru.

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Introduction

Cardioembolic stroke is cerebral artery occlusion caused by a cardiac embolism. The rate of ischemic stroke due to cardioembolism is 15-25%, and this rate can reach up to 35% in young patients [1-3]. The presence of atrial fibrillation increases the risk of stroke by approximately five times. At least half of cardioembolic strokes are associated with Atrial fibrilasyon (AF), and NVAF is the most prevalent cardiac arrhythmia [4, 5]. The prevalence of AF increases by aging, and this rate rises to 24% over age of 80. AF is a significant risk factor for strokes in advanced ages [5]. As it is known, OAC agents are used in AF patients due to a possible risk of thromboembolism, and the CHA_2DS_2 (C:Congestive heart failure; H:Hypertension; A_2:Age 75 years or older; D:Diabetes mellitus; S_2:Previous stroke, transient ischemic attack or thromboembolism) and CHA_2DS_2-VAS_C (C:Congestive heart failure; H:Hypertension; A_2:Age 75 years or older; D:Diabetes mellitus; S_2:Previous stroke, transient ischemic attack or thromboembolism; V:Vascular disease; A:Age 65-74 years; S:Sex category (female)) scales are used for thromboembolism risk in patients with NVAF [6-8]. One of the common stroke risk factors is summarized in the CHA_2DS_2-VAS_C score based on the clinical risk factor [9]. Vitamin K antagonists (warfarin) and oral anticoagulants not dependent on vitamin K (NOAC) used as OAC in the treatment highly protect atrial fibrillation patients against stroke [10]. Using warfarin in western countries, the target PT-INR value has been shown as 2.0–3.0 [7, 11]. However, in Japan, which also uses warfarin, it was kept lower in patients over age of 70 years and the ideal PT-INR level was determined as (1.60-2.6). Recently, NOAC have come into use and are now widely used in the treatment of NVAF with strong evidence levels. They have come to the forefront in reducing stroke due to atrial fibrillation in primary prophylaxis with their ability to cause significantly less bleeding compared to warfarin, to prevent stroke at an equal or greater rate, and to be easy to use [12, 13]. In the examination, the NIHSS scale, which evaluates the severity of stroke, is calculated. A high score indicates deteriorating [14].

Measuring the neurological deficit after stroke is very significant to demonstrate the effectiveness of the drug, intervention or surgery used. For this reason, the Modified Rankin Scale (mRS) used is an accepted disability scale [15]. In this context, the objective is to determine the usage of OAC in patients who have had a cardioembolic stroke due to NVAF, with the NIHSS score. The evaluation of infarct volume, measured semi-automatically in cm3 through MRI, is also planned.

Materials and methods

The data of 101 patients with or without OAC (newly diagnosed AF, discontinued or disrupted of using drug) among NVAF patients diagnosed with acute ischemic stroke at a Training and Research Hospital between 2014-2020 were retrospectively analysed. Permission was obtained from Atatürk University Faculty of Medicine Ethics Committee for the study. Patients older than age of 18 years, with diffusion MRI of sufficient quality for volumetric analysis, and with a NIHSS score (calculated by a neurologist) at presentation were included in the study. Exclusion criteria of the study; It was determined as the patient who used OAC for a reason other than AF and had a stroke, but the current neurological examination and death could not be explained by infarction. Within the framework of the current research, demographic data, including age, gender, personal and family medical history of the participants recruited for the study, were collected, vascular risk factors (HT, DM, AF, CAD, CHF, hyperlipidaemia, smoking, etc.), CHA_2DS_2 and CHA_2DS_2-VAS_C scores, systemic and neurological examination findings were analyzed and recorded. Stroke subtypes were determined by Bamford (TACI, PACI, POCI, LACI) classifications [16]. The patients were separated into four groups based on their OAC drug usage. According to this [17].

Group 1: Those who do not use OAC,

Group 2: Patients using subtherapeutic doses of warfarin (INR≤2.0 in patients under age of 70 years, INR≤1.6 in patients over age of 70),

Group 3: Patients using therapeutic doses of warfarin (INR≥2.0 in patients under age of 70 years, INR≥1.6 in patients over age of 70 years),

Group 4: Consisted of patients using NOAC.

An anatomical classification was made by [16, 18] according to the arterial area, in which clinical features were prioritized. This classification was made according to the location of the lesion in terms of treatment; total anterior circulation infarcts (TACI), partial anterior circulation infarcts (PACI), posterior circulation infarcts (POCI), lacunar infarcts (LACI), and developing infarcts according to the usage of OAC status were compared.

The hemorrhagic transformation (HT) may occur as part of the natural history of ischemic stroke or may develop with the using of antiplatelet, anticoagulant or thrombolytic therapy [19], those with HT within the first 24 hours of the patients included in the study were identified and analyzed statistically.

Diffusion MRI imaging

All diffusion magnetic resonance imaging procedures were made by using a 1.5 T MR scanner (Avanto, Siemens Healthcare Services, Erlangen, Germany). For diffusion weight imaging (DWI), echo-planar imaging was made in the transverse plane over the spin-echo sequence, and imaging parameters were used as TR/TE:3100/100; matrix:192x192; NEX:3; section thickness:5 mm; inter-section gap:1.5 mm; examination time:4-5 minutes and FOV:230x230 mm. Diffusion gradients were taken in three planes perpendicular to one another and ADC maps were attained by using 2 different b values (500 and 1.000 sec/mm²).

The semiautomatic technique, which is a quantitative method and takes less time (less than 15 minutes) when compared to the manual technique, was used for the calculation of infarct volumes. Infarct volume in patient groups was evaluated semi-automatically by using DWI on a 3D volume measurement workstation (Figure 1) (Myrian Pro, Intrasense, France).

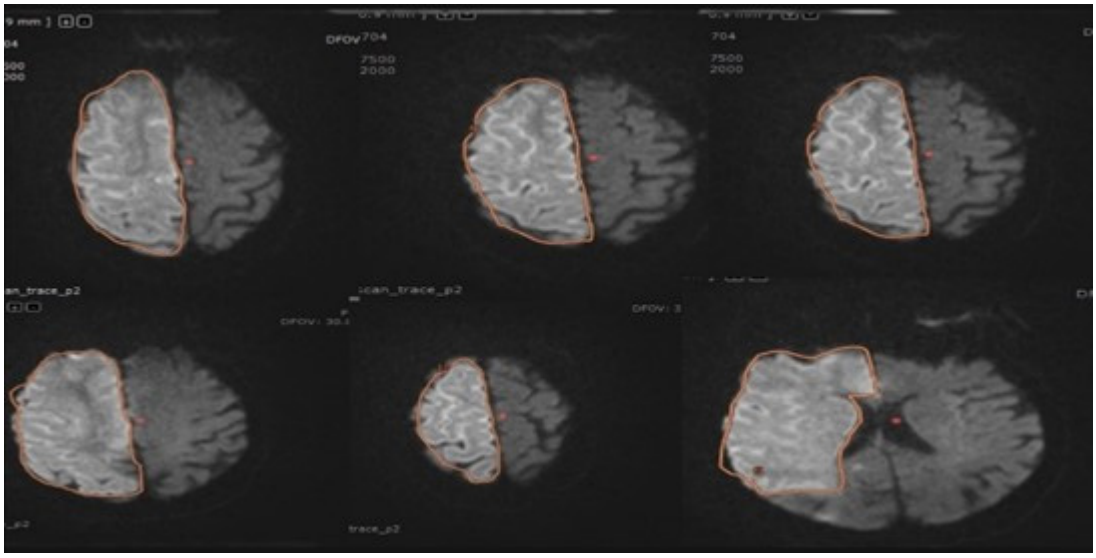

Figure 1. Measuring the infarct volume of a patient with a persistent lesion in the same arterial irrigation area using diffusion-weighted imaging with the Myrian Program

Statistical analysis

Analyses were carried out using IBM SPSS 20 statistical analysis software. Data were presented as mean, standard deviation, median, minimum and maximum percentage. The normal distribution of continuous variables was assessed using the Shapiro-Wilk test when the sample size was less than 50, and with the Kolmogorov-Smirnov test when the sample size exceeded 50. In the comparisons between two independent groups, the Independent Samples t test was used when the normal distribution condition was provided, and the Mann Whitney u test was used if it was not. In the comparison of continuous variables with more than two independent groups, the ANOVA test was used when the normal distribution condition was

provided, and the Kruskal Wallis test was used when it was not. Post-hoc tests after ANOVA test were made by using Tukey test when variances were homogeneous and Tamhane's T2 test when variances were not homogeneous. After Kruskal Wallis test, Kruskal Wallis 1-way ANOVA (k samples) test was used for post-hoc tests. n 2x2 comparisons between categorical variables, the expected value (>5) was calculated by using the Pearson Chi-square test, if the expected value is between (3-5), the Chi-square Yates test, and the expected value (<3) was made by using the Fisher's Exact test. If the comparisons are bigger than 2x2 between categorical variables, Pearson Chi-square test was used when expected value (>5) and Fisher-Freeman-Halton test was used when expected value (<5). Statistical significance level was taken as $p<0.05$.

Results

Within the study's scope, 101 patients who met the inclusion criteria were evaluated and the demographic data such as age and gender of stroke patients with NVAF were examined; 72.92±9.04 years, 64 (63.3%) women, 37 (36.6%) men, median NIHSS score of 8 (min-max 1-23), discharge mRS 3 (0-6.3), the mRS score was measured in third month of discharging 2 (0-6). Generally, 79 (79.7%) patients had AF known, and 22 (22.2%) patients had newly diagnosed AF; The using of VCA was detected in 24 patients (24.2%), of which 5 (25%) were in the therapeutic range, and 55 (55.5%) of them were using NOAC, and 30 (54.5%) of them were using the therapeutic dose. Age, CHA_2DS_2 and CHA_2DS_2-VAS_C score were similar in all four groups. The discharge mRS score did not differ significantly among the groups (p=0.387). In our study group, infarct volumes, measured by basing the reference of 101 NVAF patients and/or MRI imaging mainly with the diffusion, were compared according to the therapeutic status. The median measured infarct volume of patients who did not use OAC was 22.20 cm^3, in patients using subtherapeutic VCA, it was measured at 12.95 cm^3, in patients using VCA at the therapeutic dose, it was measured at 2.25 cm^3, and in those using therapeutic NOAC, it was measured at 4.40 cm^3 (Table1). The infarct volumes measured from patients who did not use OAC were significantly higher than those from other groups (p=0.039, Table 1).

Table 1. Basic and characteristic findings by anticoagulation status

Parameters	Group 1 (n:47)	Group 2 (n:18)	Group 3 (n:6)	NOAC (n:30)	*p* value
Age	73 (49-90)	69 (46-83)	77 (64-86)	71 (53-90)	0.2
Female Gender %	68	50	33	70	0.1
NIHSS score	9 (4-23)	8.5 (3-18)	6.5 (2-20)	5 (1-22)	0.029
Infarct Volume	22 (0.4-235)	12.9 (1.3-129)	2.25 (0.3-89)	4.4 (0.2-293)	0.039
Modified Rankin Scale	3 (0-6)	3 (1-6)	3 (1-6)	2 (0-6)	0.3
INR	1.2 (0.8-2)	1.09 (0.96-1.5)	2.1 (1.76-3.4)	1.3 (1-2.5)	0.00001
Hemorrhagic Transformation %	64.7	17.6	5.8	11.7	0.279
Hypertension %	87	83	83	90	0.8
Diabetes %	38	38	33	35	0.7
Hyperlipidemia %	53	33	50	56	0.4
Coronary Artery %	61	50	33	31	0.5
Chronic Heart Failure %	41	61	50	40	0.4
Carotid Artery Stenosis %	19	5.6	16.7	35.3	0.5
Cigarette %	34	38.9	50	27.8	0.8
Great Vessel Occlusion %	42.6	27.8	33	23	0.3
Disease of White Matter %	45	44	66	56	0.6
CHA_2DS_2 score	3 (1-6)	3 (1-4)	3 (2-4)	3 (1-5)	0.2
CHA_2DS_2-VAS_C score	5 (3-9)	5 (2-7)	4.5 (4-7)	6 (4-8)	0.1

NIHSS score: National Institute of Healt Stroke Scale; INR: Protrombin time
CHA_2DS_2, CHA_2DS_2-VAS_C score: Stroke risk score in patients with atrial fibrillation

Groups 1 and 2 were combined, Groups 3 and 4 were combined and then these two combined groups were compared. Infarct volume of Group 1+2; It was calculated as 16.9 (0.4 -235) cm^3. Group 3+4 infarct volume was measured as 3.5 (0.2-293) cm^3, and the difference between the 2 groups was statistically significant (p=0.005) (Table 2).

Table 2. Basic and characteristic findings by anticoagulation status

Parameters	Group 1+2 (n:65)	Group 3+4 (n:36)	*p* value
Age	74 (46-90)	73 (53-90)	0.503
NIHSS score	9 (3-23)	5 (1-22)	0.004
Infarct Volume	17 (0.4-235)	3.5 (0.2-293)	0.005
Discharging mRS score	3 (0-6)	2 (0-6)	0.122
3rd month mRS score	3 (0-6)	2 (0-6)	0.087

mRS: Modified Rankin Scale, NIHSS score: National Institute of Healt Stroke Scale

The patients in the current study group were classified according to the Bamford system [16] based on Diffusion MRI. The identified risk factors and the using of anticoagulation were compared with the infarct volumes and statistical analysis was performed (Table 3). At the Bamford classification of our patient group, the average age (TACI:74 (54-90), PACI:74 (46-90), POCI:68 (55-81), LACI:72 (53-86) p=0.2) were analyzed as close to each other. In the statistical analysis, CHA_2DS_2 (TACI:3 median (1-5), PACI:3 (1-6), POCI:3.5 (1-5), LACI:3 (1-5)) calculated with the identified risk factors p=0.2) and CHA_2DS_2-VAS_C score (TACI:5 median (3-8), PACI:5 (2-9), POCI:6 (3-8), LACI:5 (2-7) p=0.2) and the involved artery area was not statistically significant. Furthermore, no significant difference was observed between the existing risk factors and the involved artery area. In comparison with the measured infarct volumes; TACI:74 median (0.4-293), PACI:14 (1.7-93), POCI:8.6 (0.2-76), LACI 1.3 (0.2-15), and it was found statistically significant that the infarct volumes measured in TACI infarcts had larger volumes (p=0.0001).

Table 3. Quantitative and qualitative values according to OCSP classification

Group	TACI	PACI	POCI	LACI	*p* value
Group 1 %	30	40	9	21	0.0001
Group 2 %	6	67	6	22	0.6
Group 3 %	17	50	0	33	0.6
Group 4 %	17	30	23	30	0.69
Female %	25	42	9.3	23.4	0.4
Male %	14	43	16	27	0.4
Rate of Mortality %	53.8	30.7	7.6	7.6	0.028
Hemorrhagic Transformation %	47	29.4	17.6	5.8	0.011

TACI: Total anterior circulation infarcts; PACI: Partial anterior circulation infarcts; POCI: Posterior circulation infarcts; LACI: Lacunar infarcts

Group 1 NIHSS score; 9 (4-23) Group 2; 8.5 (3-18), Group 3; 6.5 (2-20), Group 4; (1-22) and the using of OAC showed an improvement in the NIHSS score, and the difference was statistically significant (p=0.029 Tables 1-2). The stroke severity of patients was categorized into three groups based on the NIHSS score: mild (0-7), moderate (7-16), and severe (≥16) [20]. A positive correlation was found between the infarct volume and the NIHSS score, and it was statistically significant (p=0.001) (Table 4). Although the mRS score at the 3rd month after discharge was not statistically significant. Group 1 was found to be higher than the other groups (p=0.239).

Table 4. Qualitative and quantitative values of stroke severity determined by NIHSS score

NIHSS Score	1-7	8-15	≥16	Chi-square	*p* value
Age	74.50 (49-87)	74 (53-86)	74.5 (46-90	1.6	0.4
Infarct Volume	3.40 (0.20-200	14 (0.20-148)	49.4 (0.4-293)	16.4	0.001
PT-INR	1.29 (0.96-3.47)	1.19 (0.76-1.98)	1.08 (0.98-1.76)	8.9	0.001
Discharging mRS Score	2 (0-4)	3 (0-6)	5 (1-6)	14	0.0001
3 rd month mRS Score	2 (0-3)	2 (0-6)	5 (1-6)	14	0.0001
CHA_2DS_2	3 (0-6)	3 (1-5)	3 (1-6	5.1	0.76
CHA_2DS_2-VAS_C	4.50 (1-8)	4 (3-8)	6 (2-8)	5	0.8

NIHSS score: National Institute of Healt Stroke Scale; INR: Protrombin time; mRs: Modified Rankin Scale
CHA_2DS_2, CHA_2DS_2-VAS_C score: Stroke risk score in patients with atrial fibrillation

Statistical analysis was analyzed by considering the anticoagulation status in the Bamford classification: Group 1 TACI was calculated as 30%; PACI:40%; POCI:9%; LACI:21% (*p*=0.0001). TACI and PACI infarction were observed with a high rate in our patient group who did not use anticoagulant agents (Table 3). In female gender, TACI:25%; PACI:42; POCI:9.3; LACI:23.4% were observed, and TACI and PACI infarcts were detected at a high rate in the female group (Table 3 *p*=0.4). When the mortality rate was evaluated, it was observed that TACI:33%; PACI:7.6%; LACI:7.6%. The mortality rate for TACI infarction was determined to be high and statistically significant (*p*=0.0028, Table 3).

Discussion

It has been documented in the literature that AF increases the risk of stroke five times, and this risk varies depending on the presence of certain stroke risk factors [21, 22]. Common stroke risk factors are summarised in the clinical risk factor-based CHA_2DS_2-VAS_C score [9]. The CHA_2DS_2-VAS_C score performs only modestly in predicting high-risk patients in thrombo-embolic events, but low-risk patients (CHA_2DS_2-VAS_C 0 (men) or score 1 (women)) have consistently low rates of ischemic stroke or death (<1%/ year) and do not require prophylactic treatment [23]. In the presence of a non-gender stroke risk factor, women with NVAF have a significantly higher risk of stroke [24, 25].

In this study, the mean age of 101 patients was 72.92±9.04 years, 64 (63.3%) female and 37 (36.6%) male. The fact that a high proportion of the patient population is female showed that female gender is riskier than male gender in the presence of risk factors in AF patients. In the OCSP classification, it was observed that female gender (TACI:25% and PACI:42%, 9.3% *p*=0.469) of the patients who developed POCI. Infarct volume (TACI:median 105 cm^3, PACI:median 14 cm^3, POCI:median 8.6 cm^3) and mortality rate (TACI:53.8%, PACI:30.7%, POCI:7.6% LACI:7.6% *p*=0.028) was analyzed (Table 3). Considering the mortality rate and infarct volume, this rate was found to be high in TACI and PACI infarcts, and it was found to be statistically significant (Table 3). It was observed that TACI and PACI infarcts constituted a large part of the female gender. The mortality and morbidity rates of female patients were high. In another study by [18] which included 1055 patients with ischemic stroke, stroke severity and prognosis at the 3rd and 12th months were evaluated. The findings of this study show that women stay in the hospital longer and have higher CHA_2DS_2 score, NIHSS score, and mRS score. In a study conducted by Giralt Steinhauer et al. [26] on 439 stroke patients with AF, high of CHA_2DS_2-VAS_C scores, female gender and advanced age were found to be associated with increased stroke severity and a poor prognosis [26].

In the EAFT (European Atrial Fibrillation Trial) study conducted in patients with AF for secondary prevention, patients with non-valvular AF who had a stroke or TIA in the last three months were examined. While the annual stroke rate was 10% in the group that used aspirin, it was 4% in the group that received oral anticoagulant therapy [2, 27]. Our study also evaluated 101 NVAF patients with ischaemic stroke according to their use of anticoagulation. The average age of these 4 groups was similar (Group 1:76 (median); Group 2:73; Group 3:78; Group 4:73). The median values of the CHA_2DS_2 score of the 4 groups (Group 1:3

(median); Group 2:3; Group 3:3; Group 4:3) were similar and it was observed that they were a high-risk patient group. The CHA_2DS_2-VAS_C score of the 4 groups was calculated as (Group 1:5 (median); Group 2:5; Group 3:4.5; Group 4:6) and it was observed to be similar.

The NIHSS is used to determine the severity of acute ischaemic stroke [13]. On this scale, a high score indicates deterioration. The NIHSS score is significant in making decisions for treatment or interventional treatment, as well as evaluating the outcome of treatment [14]. When the groups were compared for stroke severity, the NIHSS score (Group 1: 9 (median); Group 2:8.5; Group 3:6.5; Group 4:5; p=0.029), the NIHSS score of the group using OAC was statistically significantly lower. In a study by [28], NVAF patients were grouped according to using of NOAC, and the NIHSS score (10 for those who did not use NOAC, 6; p=0.008 for those who used NOAC) was calculated and found to be statistically significant. The NIHSS score is a clinically significant parameter in measuring stroke severity, and the using of OAC has been shown to improve stroke severity in both studies.

Infarct volumes were measured according to the anticoagulation status of the patients (Group 1: (47) median 22.2 cm^3; Group 2 (18):12.95 cm^3, Group 3 (6):2.25 cm^3, Group 4 (30):4.40 cm^3; p=0.039) and patients using therapeutic dose OAC had significantly lower infarct volumes than the non-using patient population, and the improvement in infarct volume of anticoagulation using was statistically significant. In a similar study by Sakamoto et al. [17]; infarct volumes were compared according to the anticoagulation status and it was shown that the using of OAC resulted in improvements in infarct volume. When analyzed with OCSP classification according to the arterial areas that developed infarct, it was determined that Group 1 TACI 30% and PACI 40% are statistically significant (p=0.00001 Table 3).

In the present study, large vessel occlusion was found to be higher in the group that did not use OAC, which is not statistically significant (Group 1:42.5% p=0.3, Table 1). In a similar study Sakamoto et al. [17], 330 patients were grouped according to using of OAC and it was concluded that major vessel occlusion was observed at a higher rate in the group that did not use OAC. In the present study, the correlation between stroke severity and INR value was examined and the statistics were recorded according to the NIHSS score of the patients (NIHSS score 0-7 INR:1.29 (median), min-max (0.96-3.47); NIHSS score 8-15 between INR:1.19 (median), min-max (0.76-1.98); NIHSS score ≥16 INR:1.08 (median), min-max (0.98-1.76)). It was observed that the severity of stroke decreased as the INR value increased (p=0.012). In a study by [17] it was shown that a high INR value reduces the risk of major vessel occlusion and the severity of stroke (p=0.001).

The mRS score is a disability scale accepted after treatment [12]. In patients with high level stroke according to the NIHSS score, the mRS scores of discharge and of the third month after discharge were found high and statistically significant (p=0.001). There was a significant relationship between NIHSS score and diffusion MRI lesion volume, as seen in previous studies [29, 30], and the results were also similar in our study (infarct volume between NIHSS 0-7:3.4 cm^3 (median); NIHSS Infarct volume between 8-15:14 cm^3 (median); NIHSS ≥16 infarct volume: 49.4 cm^3 (median); p<0.001) and the difference was statistically significant [31].

A number of clinical factors have been associated with HT in stroke patients. Stroke severity and infarct size are the factors that best correlate with HT [31]. To determine the rate of HT in the patient groups in our study, a higher rate of HT developed in Group 1 according to the anticoagulation status (Group 1:64.7%; Group 2:17.6%; Group 3:5.8%; Group 4:11.7%; p=0.161) but the difference was not statistically significant. A study by [20], showed that the use of anticoagulants was effective in reducing the NIHSS score and infarct volume, and that the development of HT was less in the group of patients with an INR≥1.7.

In conclusion, in the management of patients with AF, especially stroke, in the prevention of embolic complications, anticoagulant therapy has a great importance. Anticoagulant therapy is not only important in the primary and secondary prevention of stroke, but also leads to a reduction in stroke severity and infarct volume in patients who have already suffered a stroke, as recent studies have shown. This study showed that stroke severity and infarct volume decreased, and mortality and morbidity rates were improved

under anticoagulant treatment in AF patients presenting with acute ischemic stroke. In conclusion, oral anticoagulant treatment should be perceived as a contemporary necessity in terms of public health, both because it leads to a decrease in the general prevalence of stroke and because the severity of the previous stroke is milder.

Conflict of interest: No conflict of interest was declared by the authors.

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Ethics committee approval: Permission was obtained from Atatürk University Faculty of Medicine Clinical Research Ethics Committee for the study (date: 28.05.2020 and number: B.30.2.ATA.0.01.00/1).

Author contribution

This study is a part of the first author's Ph. D. thesis. G.A. developed the theory and arranged/edited the material and method section, has done the evaluation of the data in the results and Discussion sections. A.E. constructed the main idea and hypothesis of the study, provided editorial advice. All authors reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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Pediatric pineal and tectal region tumors: the use of neuroendoscopy

Pediatrik pineal ve tektal bölge tümörleri: nöroendoskopi kullanımı

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Abstract

Purpose: In this article, we aimed to contribute to the literature by describing our approach to pediatric pineal and tectal masses treated with endoscopic interventions in our clinic.

Materials and methods: We retrospectively reviewed the records of 26 patients with lesions in the pineal and tectal regions who underwent endoscopic procedures between 2012 and 2023 at Gazi University Department of Pediatric Neurosurgery. Demographic data, blood tumor markers (alpha fetoprotein-BETA AFP, alpha fetoprotein-β-hCG), pathological diagnoses, reasons for clinical presentation, presence of hydrocephalus and additional interventions were analyzed.

Results: Of the 26 patients who underwent endoscopic procedure, 6 had lesions in the pineal region and 20 had lesions in the tectal region. The success rate for ETV was 75-80%. The success rate for ETV was 78.3% for patients with tumors in the pineal region and 84% for tectal tumors.

Conclusion: Pineal or tectal region tumors are a rare group of tumors. In addition, these regions are among the most heterogeneous regions in the central nervous system in terms of tumor type and histology. Endoscopic intervention through a single burr hole using rigid endoscopy is a comfortable and safe minimally invasive method for both pathologic sampling and management of hydrocephalus in indicated cases in the same session.

Keywords: Pediatric tumors, pineal tumors, tectal tumors, neuroendoscopy.

Kuzucu P, Borcek AO. Pediatric pineal and tectal region tumors: the use of neuroendoscopy. Pam Med J 2024;17:325-335.

Öz

Amaç: Bu yazıda kliniğimizde endoskopik girişimlerle tedavi edilen pediatrik pineal ve tektal kitlelere yaklaşımımızı anlatarak literatüre katkıda bulunmayı amaçladık.

Gereç ve yöntem: Gazi Üniversitesi Çocuk Nöroşirürji Bilim Dalı'nda 2012-2023 yılları arasında pineal ve tektal bölgede lezyonu olan ve endoskopik girişim uygulanan 26 hastanın kayıtları retrospektif olarak incelendi. Demografik veriler, kan tümör belirteçleri (alfa fetoprotein-BETA AFP, alfa fetoprotein-β-hCG), patolojik tanılar, klinik başvuru nedenleri, hidrosefali varlığı ve ek girişimler analiz edildi.

Bulgular: Endoskopik işlem uygulanan 26 hastanın 6'sında pineal bölgede, 20'sinde ise tektal bölgede lezyon vardı. ETV için başarı oranı %75-80 idi. ETV için başarı oranı pineal bölgede tümörü olan hastalar için %78,3 ve tektal tümörler için %84 idi.

Sonuç: Pineal veya tektal bölge tümörleri nadir görülen bir tümör grubudur. Ayrıca bu bölgeler tümör tipi ve histolojisi açısından santral sinir sistemindeki en heterojen bölgeler arasındadır. Rijit endoskopi kullanılarak tek bir burr deliğinden endoskopik girişim, endikasyonu olan vakalarda aynı seansta hem patolojik örnekleme hem de hidrosefali yönetimi için konforlu ve güvenli bir minimal invaziv yöntemdir.

Anahtar kelimeler: Pediatrik tümörler, pineal tümörler, tektal tümörler, nöroendoskopi.

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Introduction

Pineal or tectal region tumors are relatively rare [1]. Tumors in the pineal region represent 1.5% to 8.5% of pediatric brain tumors and 1.2% of all central nervous system tumors [2, 3]. Brainstem gliomas, on the other hand, account for 10% to 20% of childhood primary brain tumors [4]. Due to the anatomical location of lesions in this region and their mass effect, patients typically present with obstructive hydrocephalus symptoms such as headache, increased intracranial pressure, urinary incontinence, altered consciousness, and seizures [5]. Advances in neuroendoscopic techniques have allowed for the safe and effective management of these region lesions in terms of diagnosis and obstructive hydrocephalus, with the opportunity for cerebrospinal fluid (CSF) sampling and concurrent histopathology [6]. This article aims to contribute to the literature by describing our approach to the pediatric pineal and tectal region masses treated with endoscopic interventions in our clinic.

Material and method

Permission was obtained from the local Ethics Committee of Gazi University. Records of 26 patients with lesions located in the pineal and tectal regions who underwent endoscopic procedures were retrospectively reviewed between the years 2012 and 2023 within the Pediatric Neurosurgery Department at Gazi University. Demographic data of the patients, blood tumor markers (alfa fetoprotein-BETA AFP, alfa fetoprotein-β-hCG), pathological diagnoses, reasons for clinical presentation, presence of hydrocephalus, and additional interventions performed were examined.

The article was scanned by including pediatric-age (<18 years old) human studies published in English through the MEDLINE/ PubMed and EMBASE library electronic databases. The primary search was performed using the keywords “Endoscopy”, “pineal,” “Tectal,” and “Hydrocephalus.” The criteria for inclusion were: 1. Pediatric age group and who underwent endoscopic procedures with pineal and tectal region lesions in the cases 2. reports or case series in which sufficient clinical, histological, and surgical information specific to the patient is available. Studies in which pediatric patients could not be distinguished and mixed age groups were presented were not included.

Surgical procedure

Patients were positioned supine, with the head extended at approximately 20-25 degrees, to center the surgical table under the general anesthesia. The entry point was selected as 1 cm anterior to the coronal suture and 2-3 cm lateral to the sagittal suture on the non-dominant side (Figure 1). After determining the incision site, the periosteum was visualized with a vertical incision of approximately 2-3 cm. When the dura was exposed, a linear incision was made using bipolar cautery. Advancement was achieved with a blunt-tipped trocar through inferoposterior and medial openings, descending into the ventricular cavity. During this process, the trocar’s stylet was removed, allowing for the observation of pressurized CSF flow and the collection of CSF samples for cytological and microbiological analysis. In our clinic, controlled irrigation with Ringer’s lactate or normal saline is used to fill the ventricular cavity, preventing the closure of the field of view. Additionally, this method assists in hemostasis for minor bleeding during the procedure while maintaining a clear field of vision.

Subsequently, a 0° camera is introduced through the trocar to ensure orientation. For orientation purposes, the foramen of Monro, thalamostriate vein, septal region, and choroid plexus are identified, and the advancement is guided such that the foramen of Monro is anterior, the choroid plexus is posterior, the septal vein is medial, and the thalamostriate vein is lateral [7]. Care should be taken not to damage the fornix, which forms the anterior and medial part of the foramen of Monro. The anatomical structures to be identified on the floor in the third ventricle include the bilateral mamillary bodies, basilar artery, dorsum sellae, and infundibular recess (Figure 1) [7]. After achieving orientation, if a biopsy is to be taken, 8-12 biopsy samples are obtained as long as the tumor’s bleeding status allows. Suppose an endoscopic third ventriculostomy (ETV) is performed. In that case, the point farthest from the basilar artery, which is the central point between the two mamillary bodies extending from the infundibulum, dorsum sellae, and closest to the clivus, should be selected for stoma creation. Subsequently, the stoma is dilated with a double-balloon

neuro balloon or a 3F or 4F Fogarty catheter. The structures of the basilar artery and other interpeduncular cistern structures should be visualized in this area, and the presence of any additional membranes must be confirmed. After the procedure, the endoscope is slowly withdrawn, and the ventricular cavity is checked for potential bleeding. After removing the trocar, the cortical opening is closed with Spongostan or Surgicel, and the subcutaneous tissue and skin are sutured to conclude the operation.

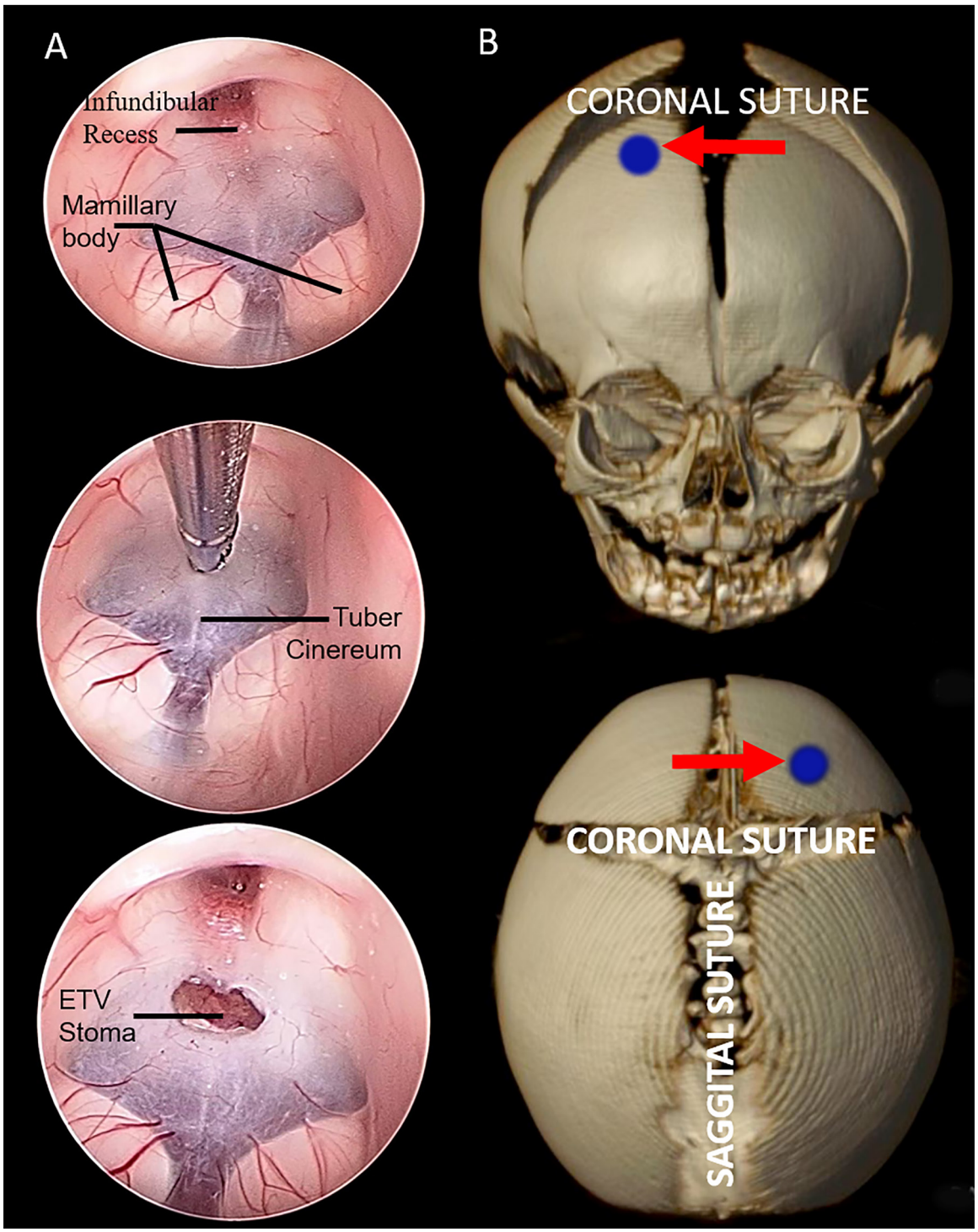

Figure 1. A. Three-Dimensional Computed Tomography (3D CT) scan of the appropriate entry point to be selected in endoscopic surgery, B. The appearance of the surgical area and stoma endoscopically in intraoperative ETV surgery

Results

Among the 26 patients who underwent endoscopic procedures, 6 had lesions in the pineal region, while 20 had lesions in the tectal region. Of the patients, 17 were male and nine were female, with an average age at the time of surgery of 9.5 years (range: 1.1-17.9 years). The most common presenting symptom was headache (46.1% or 12/26), and the most common findings in patients were, in order, unsteadiness 23% (6/26), nausea-vomiting 19.2% (5/26), seizures 11.5% (3/26), visual loss 11.5% (3/26), and spasticity 7.6% (2/26). The follow-up period ranged from a minimum of 15 to a maximum of 126 months, and there were no fatalities among the patients.

All 26 patients had MR findings and clinical symptoms related to hydrocephalus, which led to the performance of ETV. Simultaneous endoscopic biopsies were performed in 6 patients (2 pineal and four tectal). The blood tumor markers of the patients we performed a pineal region-based biopsy were 1-AFP 8.4, β-hCG 2, 2- AFP 182, and β-hCG 135.6. Based on biopsy results, histopathological diagnoses for tectal tumors included Pilocytic Astrocytoma Grade 1 and subependymal astrocytoma, while pineal tumors were diagnosed as germ cell tumors (Figure 2). and pineoblastoma. For two patients with tectal tumors who underwent biopsy, a histopathological diagnosis could not be established. The diagnostic rate for patients who underwent endoscopic biopsy was 66.6% (4/6).

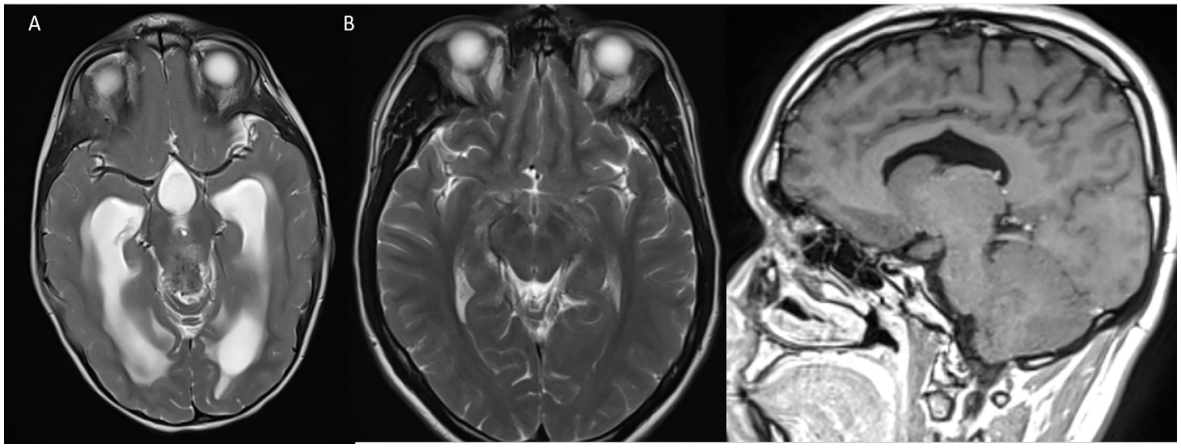

Figure 2. Radiological images of a patient with germ cell tumor pathology ETV+ETB+Adjuvant therapy performed due to pineal mass

A. Preoperative axial T2 MRI image, B. Control axial and sagittal MRI images after eight years postoperative

Among patients who did not undergo simultaneous biopsy and only had ETV (4 pineal and one tectal tumor), subsequent open surgery was performed, resulting in histopathological diagnoses of papillary tumor grade 2-3 in one patient, pilocytic astrocytoma in two patients, oligodendroglioma grade 2 in one patient, and mature cystic teratoma in one patient. ETV was applied to all 26 patients (Figure 3). with 6 (6/11) receiving endoscopic diagnoses and 5 (5/11) undergoing open surgery for diagnosis and treatment. The success rate for ETV in patients was 75-80%. For patients with tumors in the pineal region, the ETV success rate was 78.3%, while it was 84% for tectal tumors. Two patients (2/26) had preexisting shunts before ETV, and two patients (2/26) had VP shunts placed within the first year after ETV (Figure 4). One patient, who initially underwent open tumor resection and did not experience tumor recurrence, developed urinary incontinence three years later, leading to repeat ETV.

No major complications were observed after endoscopic procedures. Two patients had fornix injury, and minor bleeding, which was controlled with irrigation, occurred in six patients after ETV. Long-term follow-up did not reveal radiological progression or clinical deterioration requiring surgical intervention for tumor-related issues.

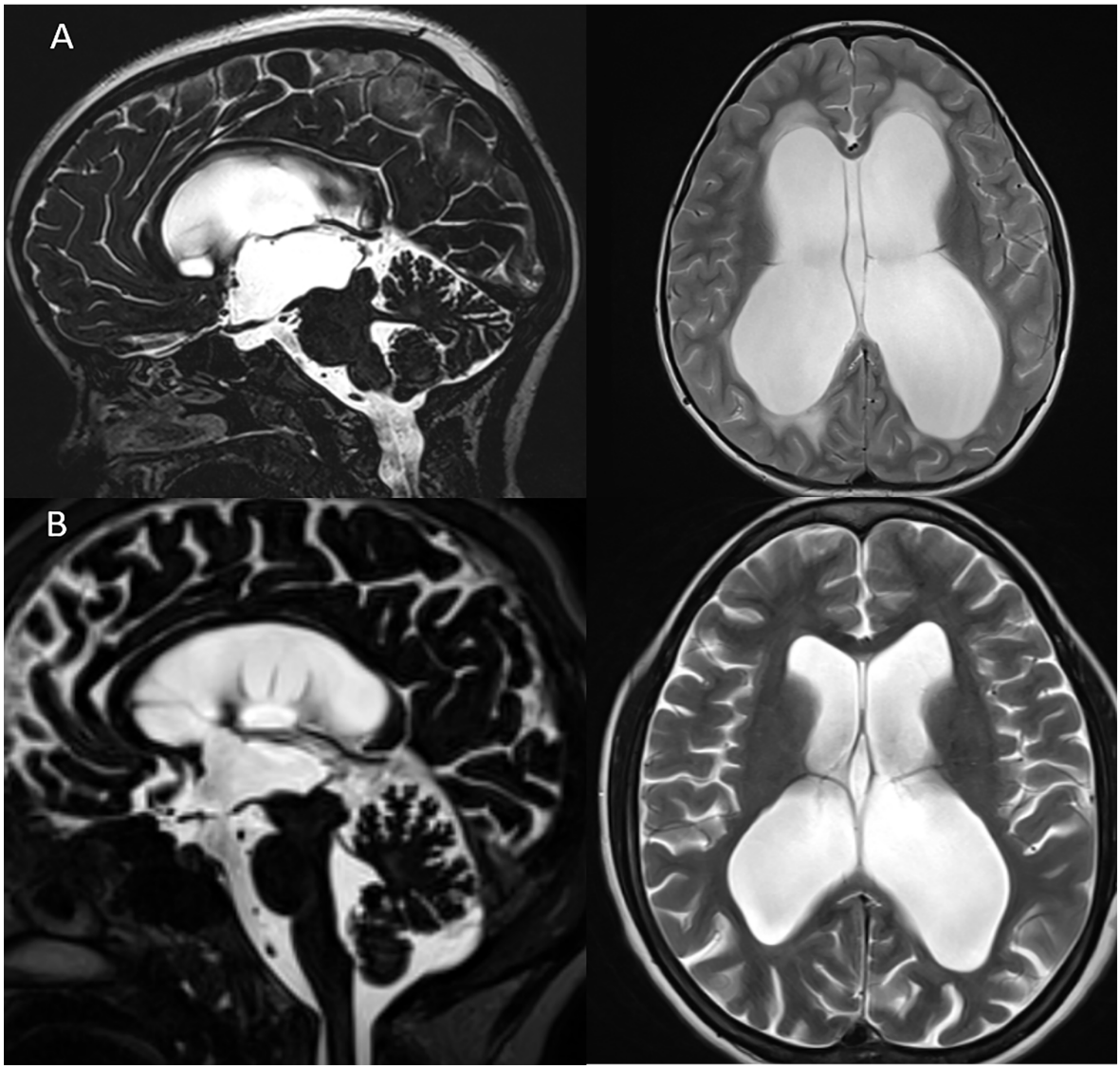

Figure 3. Radiological images of the patient who had only ETV performed due to a tectal tumor

A. Preoperative sagittal and axial MRI, B. Postoperative sagittal and axial MRI

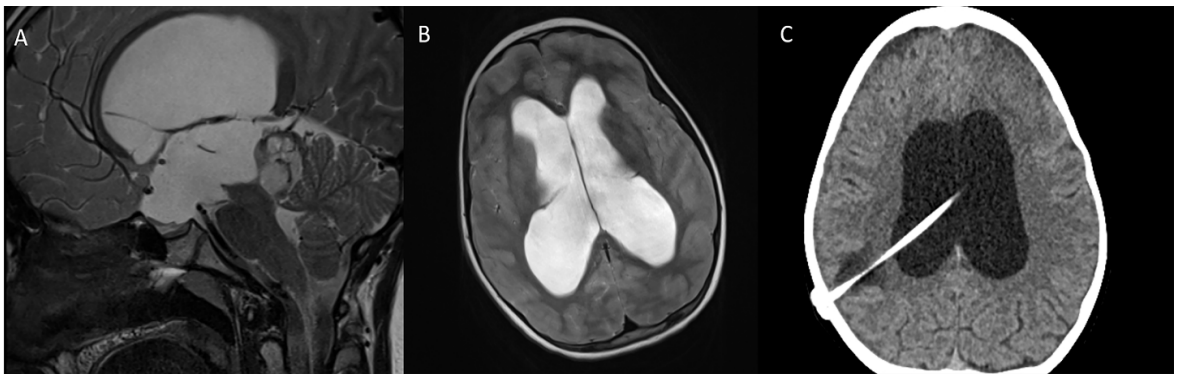

Figure 4. MRI images of a patient with pilocytic astrocytoma pathology with ETB+ETV performed due to a tectal tumor

A. Preoperative Saggital CSF flow MRI and the appearance of tectal-located tumor and hydrocephalus, B. Preoperative Axial T2 MRI image, C. CT image of a VP shunt implanted after ETV in a postoperative patient

Discussion

The pineal and adjacent tectal regions represent the most heterogeneous area in the central nervous system in terms of tumor type and histology [1]. In particular, imaging fails to characterize tumor type accurately in pineal region tumors [8], and appropriate treatment planning depends on establishing a histopathological diagnosis. Additionally, due to the high incidence of concomitant obstructive hydrocephalus in this patient group, urgent treatment is often required. In such cases, minimally invasive surgery using endoscopic techniques, which have evolved parallel to technological advancements since their description in the 1970s [9], can be employed to establish a histological diagnosis and alleviate the symptoms of obstructive hydrocephalus.

However, especially for tectal region tumors, if typical radiological findings such as hyperintensity on T2 and FLAIR sequences, iso/hypointensity on T1 sequences, and the absence of contrast enhancement on contrast-enhanced T1 sequences are present (Figure 5), many studies suggest that these tumors can remain stable for years without surgical debulking or radiotherapy [10, 11]. Tectal gliomas in children are often described as slow-growing, indolent tumors [12, 13]. However, it is emphasized that the management of concomitant hydrocephalus is necessary [14]. Some authors argue that a definitive histological diagnosis is crucial for tectal tumors [15]. In contrast, others suggest that biopsy is not necessary in cases where typical features of low-grade tectal gliomas are seen on magnetic resonance imaging (MRI) [12] (Table 1). Despite numerous publications on these tumors in this region, there is still no universally accepted algorithm [16, 17].

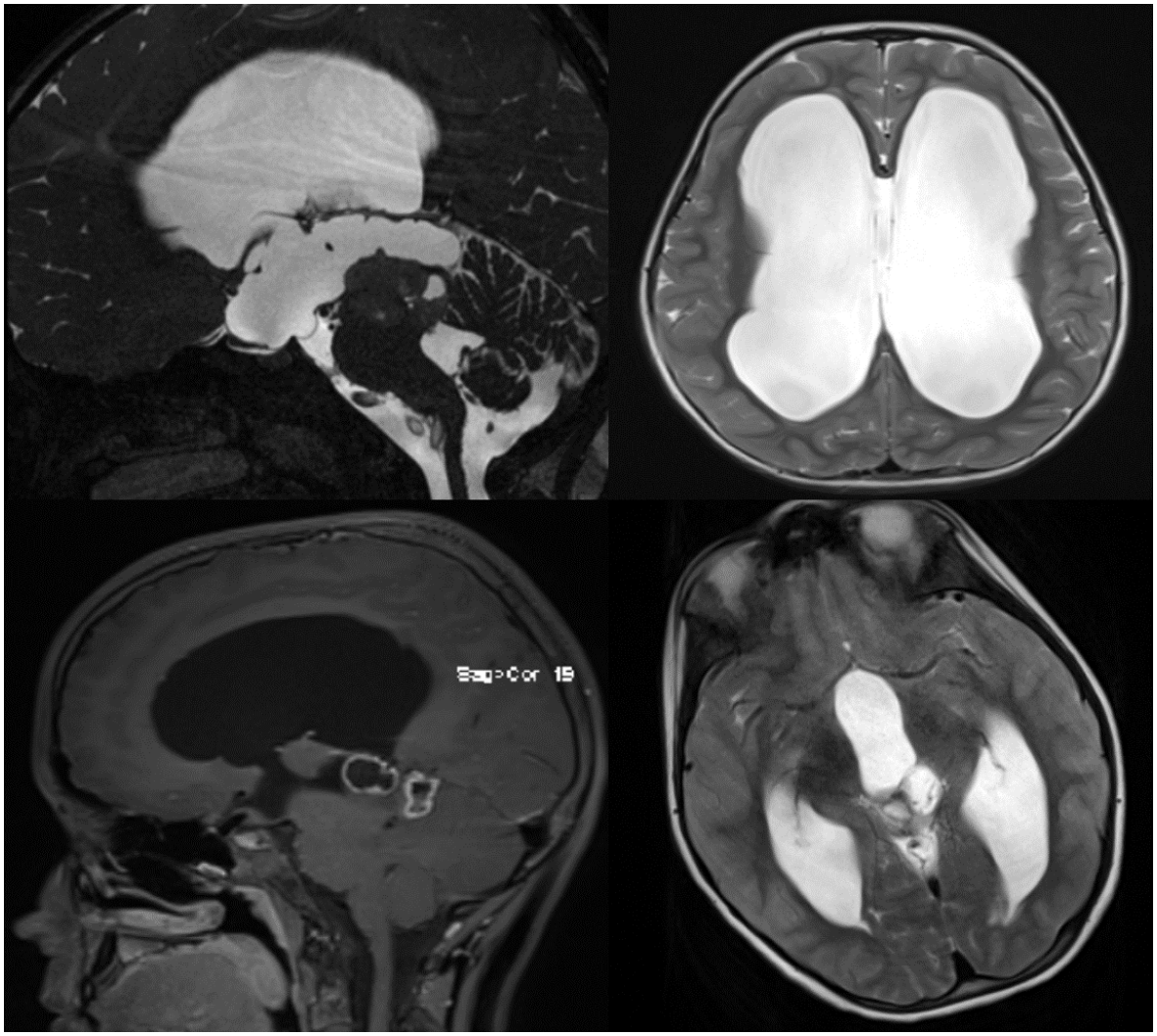

Figure 5. MRI images of a tectal tumor hyperintensity on T2 with hydrocephalus

Table 1. Literature review of ETV and endoscopic tumor biopsy (ETB) studies on tectal tumors

Study	Year	Number Of Patients	Mean Age (in years)	ETB Diagnostic Rate	ETV Success Rate
Al Tamini et al. [27]	2008	8	10	75%	88%
Wong et al. [28]	2011	25	13.5	84%	81%
Herrada Pineda et al. [29]	2015	28	8.5	96%	NA
Schulz et al. [30]	2021	28	12.4	96%	91.7%
Deopujari et al. [15]	2022	22	11	95.45%	85.7%
Present study	2023	4	6.8 (2.4,4.3,9.1,11.7 years-old)	50% (2/4)	82.5 %

In our study on ETV and ETB endoscopic treatment applied simultaneously to pediatric patients only, we found 6 cases series. According to these data, our diagnosis rate by biopsy for tectal regions was found to be low. The ETV success score in all studies was between 80-90%

In our patient follow-up, for pineal region tumors and similar cases, we initially perform blood tumor marker sampling like Deopujari et al. [15]. If the results are negative and there is no hydrocephalus, we proceed with CSF sampling through lumbar puncture. If tumor markers in the CSF are positive, we refer the patients for adjuvant treatment (Figure 6). If the results are negative, we oparete for simultaneous CSF management and CSF tumor marker sampling. Additionally, if the tumor location is suitable and the risk of bleeding is low, we plan an endoscopic biopsy during the same session [15]. Some authors advocate for performing a biopsy before conducting an ETV, particularly due to concerns like potential bleeding [18-20]. However, we support performing these procedures in a single session. Furthermore, we have not encountered scenarios in our cases that warrant an alternative approach, and minor intraoperative bleeding has not negatively affected the feasibility and outcomes of ETV.

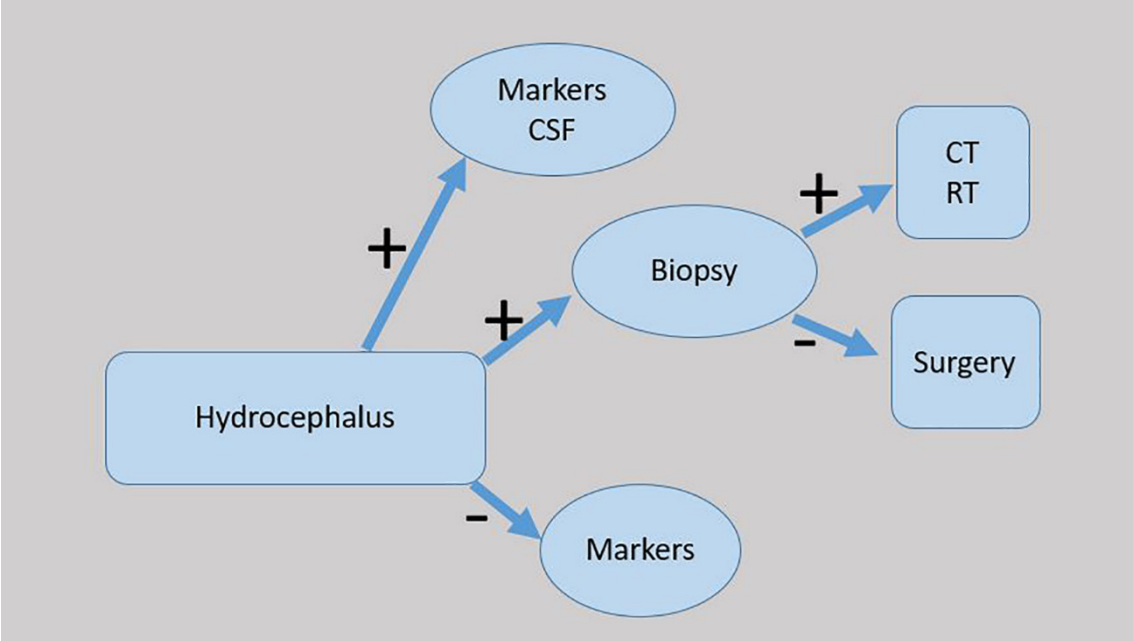

Figure 6. Treatment algorithm for pineal tumors

For pineal region tumors, we perform blood tumor markers AFP and BHCG. If the results are negative and there is no hydrocephalus, we proceed with CSF sampling through lumbar puncture for markers. If tumor markers in the CSF are positive, we refer to adjuvant treatment. If the results are negative, we operate for CSF management and CSF tumor marker sampling. If the tumor location is suitable, we take endoscopic biopsy samples

For tectal region tumors, especially when typical radiological findings are present, we prioritize ETV surgery for hydrocephalus management (Figure 7). However, in cases where unstable conditions such as radiological changes and clinical progression occur during follow-up, we plan for a biopsy. Indeed, in two of our cases, we conducted biopsy surgeries following the development of such conditions after ETV. ETV can be performed using both flexible and rigid endoscopes through one or two burr hole openings. Studies by Morgenstern et al. [21] have shown that there is no significant difference between single and double burr hole approaches. On the other hand, Ahn and Goumnerova found in their study that when flexible endoscopes were used, the biopsy success rate (45.5%) was lower compared to rigid endoscopy (81%) [22]. They attributed this to the limited sampling capacity of biopsy forceps with flexible endoscopes. In our series, we performed all endoscopic surgeries using rigid endoscopes through a single burr hole.

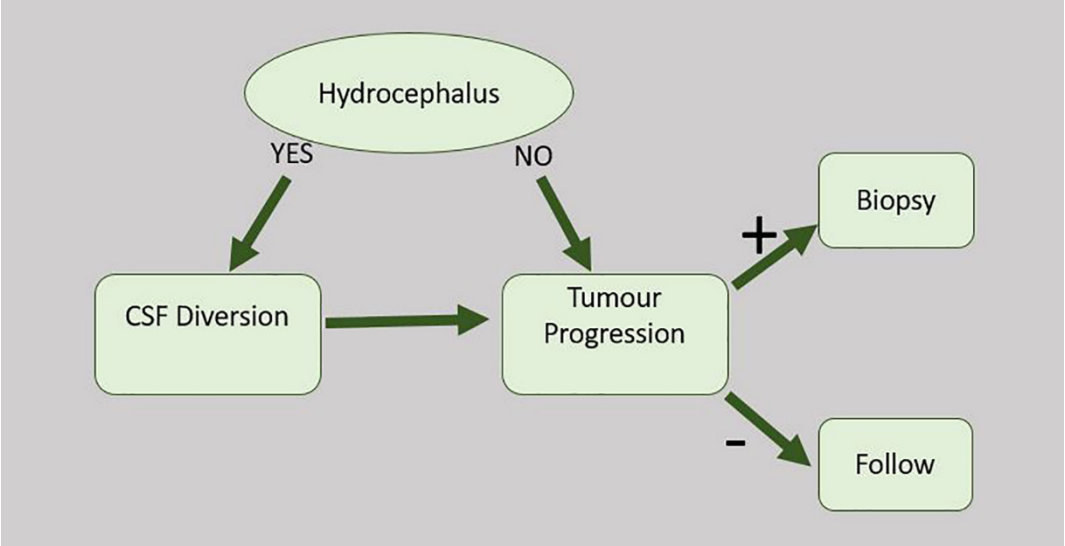

Figure 7. Treatment algorithm of tectal tumors

Tectal region tumors: If there are specific MRI markers and hydrocephalus, we usually perform endoscopic treatment to manage hydrocephalus without biopsying the tumor

In 2010, Abhaya et al. [23] developed the ETV success score (patient's age, cause of hydrocephalus, and VPS history) to predict the prognosis of ETV. According to our cases, the success score for patients with pineal region tumors was 78.3%, while it was 84% for tectal tumors. This suggests that ETV is a suitable option for hydrocephalus management, especially in all cases, including those with tectal lesions.

Yamini et al. [24] mentioned that among 54 patients who underwent ETV and biopsy, a definitive histopathological diagnosis was established in 89% of cases (Table 2). They also reported a 15% shunt placement rate after ETV [24]. Similarly, in a study conducted in 2013 by Mottolese et al. [25], they stated that the accuracy of endoscopic biopsy ranged from 61% to 100%, while the success rate of ETV ranged from 50% to 100%. In our series, the rate of histopathological diagnosis was 66.6%, and the rate of shunt placement after ETV was low, at 7.6%. Therefore, for patients with hydrocephalus due to pineal and tectal region tumors, treatment options include ETV and shunt placement [10].

Table 2. Literature review of ETB studies on pineal tumors

Study	Year	Mean Age (in years)	Number Of Patients	Biopsy Positivity Rate
Robinson et al. [31]	1997	15.7	3	100% (3/3)
Pople et al. [32]	2001	14.5	10	100% (10/10)
Haw et al. [33]	2001	14	1	100% (1/1)
Yamini et al. [24]	2004	8.5	6	66.7% (4/6)
Choi et al. [34]	2007	13	1	100% (1/1)
Al-Tamimi et al. [27]	2008	9.9	8	62.5% (5/8)
Ahn et al. [22]	2010	11.3	20	65% (13/20)
Wong et al. [35]	2011	13.5	21	95.2% (20/21)
Zhu et al. [36]	2012	17	2	100% (2/2)
Ahmed et al. [37]	2015	NA	18	NA
Abbassy et al. [38]	2018	10.7	11	81.8% (9/11)
Liu et al. [39]	2021	10.5	34	100% (34/34)
Schulz et al. [30]	2021	12.4	28	95.8% (23/24)
Cartmill et al. [40]	2000	9	1	0% (0/1)
Present study	2023	4.9 (1.1,8.8)	2	100% (2/2)

In general, ETV is considered to be the first choice for the treatment of hydrocephalus in cases with a tumor of the tectum [26]. Endoscopic aqueductoplasty (EAP) is thought to create a more physiologic state and provide long-term control. However, the long-term results of EAP have not been as successful as expected [27-31]. EAP has been shown to fail frequently. In addition, this procedure has a higher risk of damage to midbrain structures that may lead to neurologic deficits such as oculomotor or trochlear nerve palsy, Parinaud's syndrome, and periaqueductal syndrome [32]. Therefore, ETV, which has a higher long-term success rate and lower risk, is considered a better alternative for the occlusion of the aqueduct.

However, given that ETV is associated with minimal mortality and morbidity [33], we have concluded that neuroendoscopy is important in hydrocephalus management, especially for tumors in this region. Additionally, when a biopsy is indicated, endoscopy is a beneficial intervention that is safer than open microsurgical procedures in this region and facilitates radical resection.

In light of the recommendations seen in our clinical series' long-term results and literature review, as well as our clinical experience, we believe that endoscopic third ventriculostomy with simultaneous biopsy should be the first step in the management algorithm for pineal and tectal region tumors considering mortality and morbidity rates for indicated cases. Endoscopic intervention, performed through a single burr hole using rigid endoscopy, is a comfortable and safe minimally invasive method for both pathological sampling and hydrocephalus management in indicated cases during the same session.

In limitation the validation of this single-center study with a relatively small patient population with a multicenter and more extensive series in the future will be valuable in terms of contribution to the literature.

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

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Authors' contributions to the article

P.K. constructed the main idea and hypothesis of the study. P.K. and A.O.B. developed the theory and arranged/edited the material and method section. The article written by P.K. A.O.B. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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Determining the levels of serum Heat Shock Protein B7 (HSPB7) and tetranectin in patients undergoing hemodialysis

Hemodiyaliz tedavisi alan hastalarda serum Isı Şok Proteini B7 (HSPB7) ve tetranektin düzeylerinin belirlenmesi

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Abstract

Purpose: Heart damage may develop over time in patients with chronic renal disease (CKD), who are undergoing hemodialysis (HD) treatment. Serum levels of heat shock protein B7 (HSPB7) and tetranectin proteins change following damage to the heart muscle. This study aimed to determine HSPB7 and tetranectin levels in patients with CKD undergoing HD treatment.

Materials and methods: The patients aged 30-60 years old, healthy controls (n=30) and HD patients (n=30) participated in the study. Blood samples were taken from healthy subjects who applied to the hospital for check-up and from patients with kidney disease receiving HD treatment. Biochemical parameters were examined from the blood taken. HSPB7 and tetranectin levels from isolated serum samples were determined using measurement kits based on the solid phase sandwich (ELISA) principle.

Results: There was no significant difference between the groups in gender, age, glucose and iron (Fe) values of the subjects (p>0.05). Lymphocyte, platelet counts, hemoglobin and albumin values were found to be lower in patient group compared to the control group (p<0.05). Urea, creatine kinase (CK) and C-reactive protein (CRP) values were found to be higher in patient group than in the control group (p<0.05). A significant increase in HSPB7 levels and a significant decrease in tetranectin levels were detected in patient group compared to control group (p<0.001).

Conclusions: In this study, the changes detected in HSPB7 and tetranectin levels in with CKD undergoing HD treatment. May be early indicators of the possible development of cardiovascular diseases in patients with renal disease.

Keywords: Hemodialysis, chronic renal disease (CKD), HSPB7, tetranectin.

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Öz

Amaç: Kronik böbrek hastalığı olan ve hemodiyaliz (HD) tedavisi alan hastalarda zamanla kalp hasarı gelişebilmektedir. Isı şok proteini B7 (HSPB7) ve tetranektin proteinlerinin serum düzeyleri kalp kasında gelişen hasarı takiben değişmektedir. Bu çalışmada, HD tedavisi alan KBH hastalarında HSPB7 ve tetranektin düzeylerinin araştırılması amaçlanmıştır.

Gereç ve yöntem: Çalışmaya 30-60 yaş, sağlıklı ve HD hastası denekler katılmış olup iki grup oluşturulmuştur (n=60): Kontrol grubu (n=30) ve Hasta grubu (n=30). Hastaneye check-up için başvuran sağlıklı deneklerden ve böbrek hastalığı olup HD tedavisi alan hastalardan kan örnekleri alınmıştır. Alınan kanlardan biyokimyasal parametreler incelenmiştir. İzole edilen serum örneklerinden HSPB7 ve tetranektin düzeyleri solid faz sandwich (ELİSA) prensibine dayanan ölçüm kitleri kullanılarak belirlenmiştir.

Bulgular: Deneklerin cinsiyet, yaş, glukoz ve Demir (Fe) değerlerinde gruplar arasında anlamlı fark saptanmamıştır (p>0,05). Lenfosit, trombosit sayıları, hemoglobin ve albümin değerleri diyaliz hastalarında kontrol grubuna kıyasla düşük bulunmuştur (p<0,05). Diyaliz hastalarında üre, kreatin kinaz ve CRP değerleri sağlıklı kontrol grubuna göre yüksek saptanmıştır (p<0,05). Hastalarda sağlıklı deneklere kıyasla, HSPB7 düzeylerinde anlamlı artış ve tetranektin düzeylerinde ise anlamlı düşüş tespit edilmiştir (p<0,001).

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Sonuç: Bu çalışmada, diyaliz tedavisi alan KBH hastalarında HSPB7 ve tetranektin düzeylerinde tespit edilen değişimler, böbrek yetmezliği olan hastalarda olası gelişebilecek kardiyovasküler hastalıkların gelişiminin erken göstergesi olabilir.

Anahtar kelimeler: Hemodiyaliz, kronik böbrek hastalığı (KBH), HSPB7, tetranektin.

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Introduction

Chronic kidney disease (CKD) is a pathology that arises from the irreversible loss of kidney functions affecting many organ systems [1]. In CKD, chronic and progressive deterioration is observed in the kidney's fluid-solute balance and metabolic-endocrine functions due to the decrease in glomerular filtration rate (GFR). When the GFR value decreases to 5-15 ml/min/1.73m^2, it is referred to end-stage kidney failure (ESKF), and patients require renal replacement therapies such as dialysis or renal transplantation [2]. The life expectancy of patients has begun to increase, and their quality of life has started to improve with Dialysis technology. Hemodialysis (HD) is preferred in the treatment of ESKF, which is resistant to medical treatment, and when the GFR value falls below 10 ml/min [3]. HD is a therapeutic procedure where blood taken from the patient is filtered through a semi-permeable membrane using a device, homogenized with dialysate coming from the opposite direction, and then returned to the patient, regulating acid-base, electrolyte, and toxic substances [4].

Patients with CKD are at high risk for cardiovascular disease (CVD) even in the early stages of the disease. The risk of CVD in patients with ESKF is reported to be 10-20 times higher than the general population [5]. Clinically, in these patients, the risk of developing CVD, and early death increases with decreased kidney functions and the presence of albuminuria. Studies have shown a relationship between the decrease in GFR and CVD (congestive heart failure, myocardial infarction (MI), stroke, peripheral vascular disease, etc.). Moreover, a positive correlation between the progression of CKD and cardiovascular risk has been identified [6]. Therefore, more than 50% of HD patients develop CVD, increasing the risk of death [7, 8]. Thus, the development of CVD in patients with ESKF undergoing HD treatment becomes one of the main causes of morbidity and mortality [9].

Heat shock protein beta-7 (HSPB7) is a cardiovascular heat shock protein preserved α-crystalline in its C-terminal region [10]. The HSPB7 protein interacts with filamin C, playing a significant role in the formation of actinin in the cell skeleton and anchoring actin filaments to the cell membrane. In the absence of this protein, filamin C separates from its location in the cell, leading to structural damage in the cell membrane [11]. HSPB7 primarily functions as ATP-independent molecular chaperones that assist in the formation of the cell skeleton, suppress the accumulation of stress-resistant and denatured proteins [12]. It binds denatured proteins and facilitates their proteolytic degradation by transporting them to other heat shock proteins with ATPase activity, proteasomes, or autophagosomes [13]. The highest protein level of HSPB7 has been detected in heart tissue, and its mutations have been associated with heart diseases [14]. Therefore, serum HSPB7 levels increase when released from cardiomyocytes as a result of myocardial damage [15, 16].

Tetranectin, with the gene name CLEC3B, is a calcium-binding homotrimeric protein from the C-type lectin protein family [17]. It interacts with a range of cellular factors, including plasminogen, apolipoprotein A1, and heparin. It has a protective function in muscle, bone, and circulatory systems. Additionally, it is observed in serum and extracellular matrix (ECM) during tissue regeneration [18]. Tetranectin plays significant roles in binding ECM components (fibrin, plasminogen), stimulating the proteolytic activation of proteases and growth factors, and regulating ECM proteolysis during tissue reshaping [19]. A negative correlation has been shown between serum tetranectin levels and the development of CVD [20].

Although in the literature, there are various studies that demonstrated the association of HSPB7 and tetranectin with CVDs, there is no study in the literature evaluating the relationship between HSPB7 and tetranectin levels in patients with CKD that are at high risk of developing CVD. In this context, this study aims to determine the levels of HSPB7 and tetranectin in patients with CKD undergoing HD treatment.

Material and methods

Formation of experimental groups

Sample

A total of 60 subjects aged between 30-60 years participated in the study and were divided into two groups: control group (n=30) and patient group (n=30). The study included healthy volunteers with normal check-up results who visited the nephrology department of Pamukkale University Hospital for health screening purposes and patients diagnosed with CKD receiving hemodialysis treatment. Patients were selected from those routinely undergoing HD treatment at Pamukkale University Hospital, while patients receiving peritoneal dialysis or kidney transplant recipients were excluded from the study. In addition to these, patients have not been diagnosed with MI in the last 6 months, severe valve disease, chronic heart failure, coronary artery disease, atrial fibrillation, coronary bypass, and malignancy excluded from study. The control group for the study included subjects without any additional diseases and with CKD (GFR>60 ml/min/1.73 m^2).

Collection and storage of blood samples from participants

In the study, serum samples of patients and healthy volunteers who visited the hospital simultaneously were collected at the time of hospital admission following a 12-hour fasting period. In both groups, parameters such as hemoglobin (Hb), glucose, urea, creatine kinase (CK). C-reactive protein (CRP), albumin, iron (Fe), leukocyte count, neutrophil count, lymphocyte count, neutrophil lymphocyte ratio (NLR), and platelet lymphocyte ratio (PLR) were requested from blood test results. A blood sample taken in a 2 ml biochemistry tube was used for the determination of serum tetranectin and HSPB7 levels. Within 15 minutes, it was centrifuged at 3500 rpm for 10 minutes at room temperature, and the obtained serums were stored at -80°C until the day of analysis.

Determination of serum tetranectin and HSPB7 levels by ELISA method

After allowing the serum samples to reach room temperature, serum tetranectin (E6262Hu, BT lab) and HSPB7 (E5380Hu, BT lab) levels were determined by comparing with reference samples using commercial kits and the ELISA method.

Statistical evaluation

The effect size obtained from reference study was found to be quite strong (d=0.957) [21]. Assuming that we could obtain a lower level of power, a power analysis was conducted, and it was calculated that when at least 60 individuals (at least 30 for each group) were included in the study for an effect size of d=0.8, a power of 80% could be achieved at a 95% confidence level. All calculations for power analysis were performed using the GPower package program (version 3.1.9.2. HeinrichHeine-Universitat, Dusseldorf, Germany).

For statistical comparison of demographic and laboratory parameters from patient and control groups. Shapiro wilk test were used for determination of normal distribution. If parametric test conditions were satisfied. Independent samples t test was used for comparisons among groups. If parametric test conditions were not satisfied, Mann Whitney U-Test was used and associations between categorical variables were evaluated using the chi-square test (χ^2 test). All analyses were performed using SPSS 24 software for statistical analysis. Results were presented as mean ± standard deviations and $p \leq 0.05$ was considered as statistically significant. Ethical approval was obtained from the Pamukkale University Medical Ethics Committee.

Results

Evaluation of demographic and laboratory parameters of the participants

When the gender and age values of the participants were compared, no statistically significant differences were found between the control group and the dialysis patient group

(p>0.05). There was no statistically significant difference between the patient and control groups in Fe values (p>0.05). However, Hb (p=0.001) and albumin (p=0.003) values were lower, while glucose (p=0.003), urea (p=0.001), CK (p=0.001), and CRP (p=0.029) values were found to be higher in patient group compared to the control group (Table 1).

Table 1. Demographic and laboratory parameters of the subjects and control

	Control (n=30)	Patient (n=30)	Test Statistics	
			Test value	*p* value
Men (%)	44.8%	55.2%	χ^2=0.739	0.39
Age	44.76±1.83	44.54±0.87	t=0.106	0.916
Hemoglobin (Hb) (g/dL)	13.88±.47	11.14±0.42	t=4.340	**0.001****
Glucose (mmol/L)	92.12±2.58	155.29±20.76	t=-3.080	**0.001****
Urea (mg/dL)	25.72±1.52	125.83±6.16	t=-16.076	**0.001****
Creatine Kinase (CK) (U/L)	0.80±0.033	8.27±0.49	t=-15.483	**0.001****
C-reactive protein (CRP) (mg/L)	2.98±0.58	13.60±4.78	t=-2.249	**0.029***
Albumin (g/L)	44.86±0.64	37.52±2.17	t=3.298	**0.003***
Iron (Fe) (µg/dL)	77.06±6.13	69.54±5.29	t=0.926	0.359

Results are given as mean ± standard deviation, n=30, t: Independent-samples *t* test, χ^2: Chi-Square test statistics
*: p<0.05 indicates a significant difference from the control group, **: p<0.01 indicates a significant difference from the control group
Hgb: Hemoglobin, CK: Creatine Kinase, CRP: C-reactive protein, Fe: Iron

Biochemical findings

Table 2 shows the biochemical parameters of the control and patient group. No statistically significant differences were detected between the groups in NLR, PLR, neutrophil, and leukocyte counts (p>0.05). Lymphocyte and platelet counts were statistically significantly lower in dialysis patients group compared to the control group (p=0.009 and p=0.001, respectively).

Table 2. Biochemical parameters of control and dialysis patients

	Control (n=30)	Patient (n=30)	Test Statistics	
			Test value	*p* value
Neutrophil count (K/µL)	4.28±0.21	3.92±0.30	t=0.972	0.336
Lymphocyte count (K/µL)	2.31±0.09	1.89±0.12	t=2.713	**0.009****
NLR: Neutrophil/Lymphocyte Ratio	1.89±0.090	2.29±0.21	t=-1.736	0.089
PLR: Platelet/Lymphocyte Ratio	126.30±8.054	116.06±12.37	t=0.699	0.488
Leukocyte count (K/µL)	7.22±0.273	6.98±0.37	t=0.498	0.621
Platelet count (K/µL)	281.24±14.99	195.12±11.38	t=4.547	**0.001****

Results are given as mean ± standard deviation; n=30, t: Independent-samples *t* test , **: p<0.01 difference from the control group
NLR: Neutrophil/Lymphocyte Ratio, PLR: Platelet/Lymphocyte Ratio

Determination of serum tetranectin and HSPB7 concentrations

The serum tetranectin level was determined using an ELISA kit in ng/mL. The serum tetranectin level was found to be statistically significantly lower in the patient group (63.50±24.21) compared to the control group (84.17±42.45) (z=-2.764, p=0.006) (Figure 1). The serum HSPB7 level was determined using an ELISA kit in ng/mL. The serum HSPB7 level was found to be statistically significantly higher in the patient group (10.50±5.20) compared to the control group (5.90±1.39) (z=4.558, p=0.001) (Figure 2).

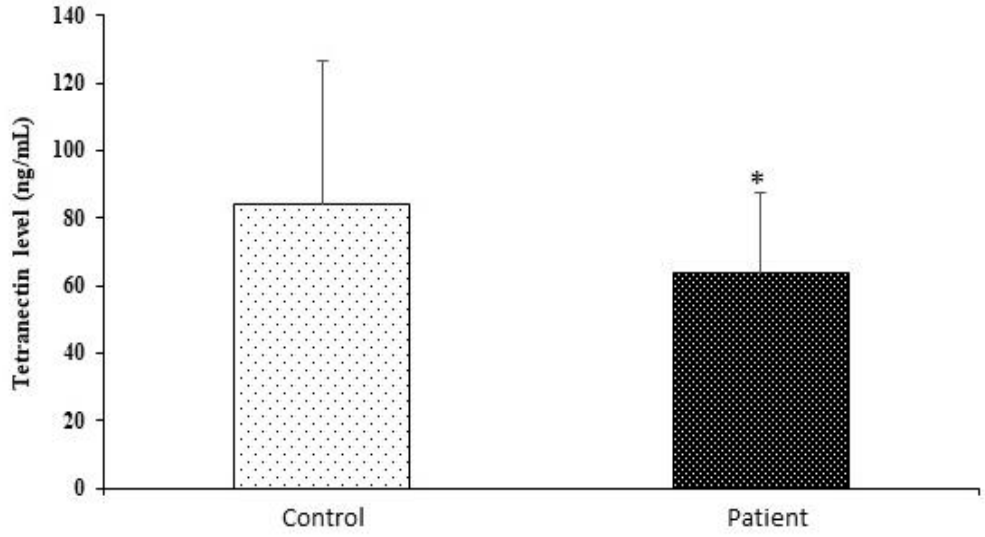

Figure 1. Serum tetranectin levels in control group and dialysis patients (ng/mL)

Mean ± standard deviation; n=30, Mann Whitney U-Test was used
*: Significant difference from the control group at $p<0.01$ level

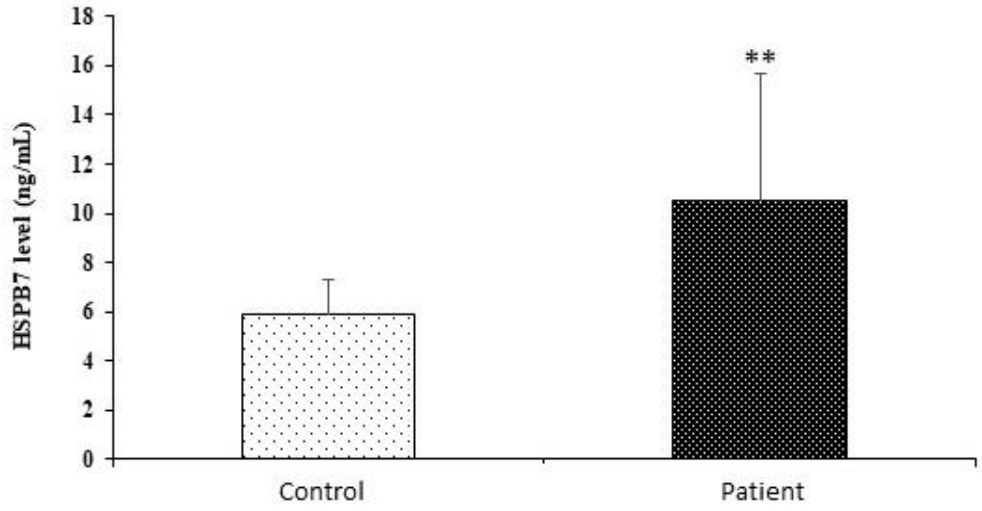

Figure 2. Serum HSPB7 levels in control group and dialysis patients (ng/mL)

Mean ± standard deviation; n=30, Mann Whitney U-Test was used
*: Significant difference from the control group at $p<0.001$ level

Discussion

In this study, our aim was to demonstrate the changes in the serum level of HSPB7 and tetranectin, cardiac-spefic proteins, in CKD patients undergoing HD. We found that HSPB7 protein level of CKD patients undergoing HD was high in the serum, while the tetranectin protein was low compared to healthy individuals. Additionally, we reported that laboratory parameters as Hb, albumin, lymphocyte, and platelet levels were decreased, and glucose, urea, CK, CRP values were increased in the HD patients.

Patients with CKD undergoing HD have a significantly higher risk of CVD than the general population, and approximately 50% of the causes of death are due to CVDs. Progressive destruction of renal parenchyma and loss of

functional nephrons are characteristic of CKD pathology [2]. The most important indicator of CKD is the irreversible decrease in GFR over time. In today's diagnosis of CKD, many laboratory parameters, especially GFR levels in the blood, are important [22].

It is known that inflammation plays a significant role in the mortality of HD patients and HD treatment itself increases the production of inflammatory mediators in CKD patients [23]. In recent years, due to the advantage of easily obtaining routine hemogram and laboratory parameters without adding an economic burden, they have become the focus of researchers as indicators of inflammation. Among these parameters, CRP is a parameter that indicates inflammation in patients undergoing HD treatment, and a positive correlation has been detected between CRP levels and the development of CVD [24].

In studies conducted, it has been shown that in patients undergoing HD treatment CRP increases and albumin decreases, and Alanli et al. [24] have suggested that these parameters can be used to evaluate the risk of mortality in HD patients [25]. In this study, in line with the literature, it was found that CRP levels were high and albumin levels were low in CKD patients undergoing HD treatment compared to controls.

Another laboratory finding is that CK isoenzyme and urea levels are high in patients with CKD undergoing HD treatment [26]. In this study, in line with the literature. CK and urea levels were found to be statistically significantly higher in CKD patients undergoing HD treatment compared to controls.

Platelets, one of the routine hemogram parameters, secrete inflammatory mediators and growth factors taking part in hemostasis, inflammation, and tissue repair [27]. A decrease in lymphocyte count is observed in many inflammatory diseases. NLR is a hemogram parameter obtained by dividing the number of neutrophils by lymphocytes. In previous studies, it has been reported that NLR has a negative correlation with GFR, a positive correlation with the stage of CKD, and thrombocytopenia is observed in patients undergoing HD treatment [28]. In this study, in line with the literature, it was found that lymphocyte and platelet levels were statistically significantly lower and NLR levels increased in CKD patients undergoing HD treatment compared to controls.

Studies have shown that CVDs can develop in CKD patients undergoing HD treatment. Ng et al. [8] reported a significant increase in cardiac mortality in patients undergoing HD treatment with CKD. In patients with CKD undergoing HD treatment, it is thought that HD treatment can lead to the development of CVD due to a decrease in perfusion of vital organs (heart, intestines, kidneys, and brain) and can also be considered as a reason for progressive dysfunction in the cardiovascular system due to an increase in arterial pressure and total blood volume [29, 30].

HSPs, which constitute approximately 10% of all cellular proteins, have the main function of preventing the accumulation of denatured proteins and controlling protein homeostasis [31]. It has been found that they increase not only in response to increased temperature but also to many adverse conditions such as toxins, inflammation, ischemia, and hypoxia [32]. The expression of HSPB7 increases under different adverse stress conditions. HSPB7 can participate in the selective degradation of denatured proteins in autophagosomes, control the redox state, and assemble and protect the cell skeleton [33]. Data obtained from studies have characterized HSPB7 as a protein mainly expressed in the heart and named it as a cardiac heat shock protein. It has been concluded that HSPB7 affects cardiac morphogenesis and can accompany congenital and acquired heart diseases [34]. HSPB7 stabilizes by binding to sarcomeric proteins and maintains contraction integrity [14]. In addition, it is necessary for the growth of ventricular cardiomyocytes to maintain the number and size of cells [34]. Loss of HSPB7 protein from cardiac tissue leads to irregularities in myofibrils and disruption of the sarcolemma structure. When HSPB7 is released from muscle tissue, its levels increase in the serum. On the other hand, the expression of HSPB7 protein in muscle tissue prevents the apoptosis of myocardial cells by providing reoxygenation and protects the heart from the harmful effects of ischemia-reperfusion [35]. In the study conducted by Liao et al. [36], they found that the dislocation of HSPB7 resulted in the disruption of the gap-junction complex and intercalated disc structures in cardiomyocytes.

This led to a decrease in the expression of connexin 43 and mislocalization of N-cadherin and desmoplakin proteins, inducing arrhythmic sudden death. In this study, it has been demonstrated that serum HSPB7 levels in CKD patients undergoing HD treatment have significantly increased compared to the control group. This finding suggests that the increase in the HSPB7 protein in muscle tissue may be due to potential damage that could have developed in cardiomyocytes as a result of HD treatment.

Tetranectin is a type C lectin that specifically binds to the plasminogen kringle-4 domain, thereby enhancing plasminogen activation, making it an endogenous ligand [37]. It primarily plays a role in tissue remodeling and development [38]. Tetranectin released from platelets possesses anti-thrombotic properties (through increased plasminogen activation) and anti-proliferative characteristics (associated with endothelium) [39]. One of the most significant effects of tetranectin is to support better blood circulation in the heart muscles. In a study conducted by Yin et al. [39], it was found that tetranectin levels were inversely proportional to the risk of cardiovascular events (CVD), and Chen et al. [40] found lower serum tetranectin levels in relation to the prognosis of coronary artery disease. In this study, it was demonstrated that serum tetranectin levels in CKD patients undergoing HD treatment have significantly decreased compared to the control group. This finding suggests that the decrease in this heart-specific protein may result from potential damage that could occur in cardiomyocytes as a result of HD treatment.

In conclusion, despite some limitations in this study, it was determined for the first time that serum HSPB7 levels increased and tetranectin levels decreased in CKD patients undergoing HD treatment. As a result, our findings suggest that changes in these proteins specific to the heart muscle may serve as indicators for potential CVD developments that are important in terms of morbidity and mortality in CKD patients receiving HD treatment.

There are certain limitations to our study. The study was conducted based on a single center, and patients from other centers were not included. Although a larger sample could not be examined, power analysis was performed, and an adequate number of patients were included accordingly. In future studies, monthly follow-ups can be conducted during hemodialysis treatment in CKD patients, allowing the observation of changes in these proteins over time, and confirming the ELISA results with RT-PCR.

Conflict of interest: No conflict of interest was declared by the authors.

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Authors' contributions to the article: Conceptualization: O.K.E. and G.G. Literature Review: O.K.E., G.G. and D.S. Design: O.K.E., G.G. and M.B.K. Data Collection: D.A. and M.A. Analysis and Interpretation: O.K.E. and G.G. Manuscript Writing: O.K.E., G.G., D.A., D.S., M.A. and M.B.K. Critical Review: D.A., G.G. and M.B.K.

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Effect of quercetin on perirenal adipose tissue adiponectin and resistin levels in rats with metabolic syndrome induced by high fructose-diet

Yüksek fruktozlu diyet ile metabolik sendrom oluşturulmuş sıçanlarda quercetinin perirenal yağ dokusu, adiponektin ve resistin düzeyleri üzerine etkisi

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Abstract

Purpose: Metabolic syndrome (MetS) is a cluster of risk factors for developing heart disease, stroke, and type 2 diabetes. Visceral adiposity and insulin resistance are crucial mechanisms of MetS. The increase in adipose tissue observed in MetS causes proinflammatory and anti-inflammatory cytokine imbalance. Accumulating evidence on quercetin, one of the antioxidants frequently used in MetS treatment, suggests that quercetin has significant anti-obesity and lipid-lowering effects simultaneously. Our aim is to investigate the effects of quercetin supplementation on MetS parameters and adipose tissue adipokine levels in rats fed high fructose.

Materials and methods: Sprague Dawley rats, 8-10 weeks of age, were divided into 4 groups, including a control group (C), high fructose (HF) group, quercetin (Q) group, and high fructose+quercetin (HF+Q) group. Fructose was administered to HF groups as a 20% solution in drinking water for 10 weeks. The rats in the Q groups were given 50 mg quercetin per kg BW by gavage for the last 4 weeks of experiment. The body weight, triglyceride (TG), high density lipoprotein (HDL), fasting insulin, fasting glucose, and HOMA-IR were determined in rats. Adiponectin and resistin levels were determined by ELISA assay from perirenal adipose tissue homogenates.

Results: We showed that quercetin acts to improve TG, fasting glucose and insulin resistance in high fructose-fed rats. In this study, we found no effect of quercetin on perirenal adipose tissue adiponectin and resistin levels.

Conclusion: These results showed that high fructose could induce MetS in rats, while quercetin could favorably affect these parameters.

Keywords: Metabolic syndrome, quercetin, perirenal adipose tissue, adiponectin, resistin.

Kilic Toprak E, Tunc Ata M. Effect of quercetin on perirenal adipose tissue adiponectin and resistin levels in rats with metabolic syndrome induced by high fructose-diet. Pam Med J 2024;17:347-357.

Öz

Amaç: Metabolik sendrom (MetS) kalp hastalığı, inme ve tip 2 diyabet gelişimi için bir risk faktörleri kümesidir. Visseral adipozite ve insülin direnci MetS'in önemli mekanizmalarıdır. MetS'de gözlenen yağ dokusu artışı proinflamatuar ve antiinflamatuar sitokin dengesizliğine neden olur. MetS tedavisinde sıklıkla kullanılan antioksidanlardan biri olan quercetin hakkındaki kanıtlar, quercetin'in önemli anti-obezite ve lipid düşürücü etkilerinin eş zamanlı olduğu gösterilmiştir. Amacımız, yüksek fruktozla beslenen sıçanlarda quercetin takviyesinin MetS parametreleri ve yağ doku adipokin düzeyleri üzerindeki etkilerini araştırmaktır.

Gereç ve yöntem: 8-10 haftalık Sprague Dawley sıçanlar, kontrol grubu (C), yüksek fruktoz (HF) grubu, quercetin (Q) grubu ve yüksek fruktoz+quercetin (HF+Q) grubu olmak üzere 4 gruba ayrıldı. Fruktoz, HF grup sıçanlarına içme suyunda %20'lik çözelti halinde 10 hafta süreyle uygulandı. Quercetin grubundaki sıçanlara deneyin son 4 haftasında vücut ağırlığı kg başına 50 mg quercetin gavaj yoluyla verildi. Sıçanlarda vücut ağırlığı, trigliserit (TG), yüksk dansiteli lipoprotein (HDL), açlık insülini, açlık glukozu ve HOMA-IR belirlendi. Adiponektin ve resistin seviyeleri perirenal yağ dokusu homojenatlarından ELISA yöntemi ile belirlendi.

Bulgular: Yüksek fruktozla beslenen sıçanlarda quercetin'in TG'yi, açlık glikozunu ve insülin direncini iyileştirdiğini gösterdik. Bu çalışmada quercetin'in, perirenal yağ dokusu adiponektin ve resistin düzeylerine etkisi bulunmadı.

Sonuç: Bu sonuçlar, yüksek fruktozun sıçanlarda MetS'i indükleyebileceğini, quercetin'in ise bu parametreleri olumlu yönde etkileyebileceğini göstermektedir.

Anahtar kelimeler: Metabolik sendrom, quercetin, perirenal adipoz doku, adiponektin, resistin.

Kılıç Toprak E, Tunç Ata M. Yüksek fruktozlu diyet ile metabolik sendrom oluşturulmuş sıçanlarda quercetinin perirenal yağ dokusu, adiponektin ve resistin düzeyleri üzerine etkisi. Pam Tıp Derg 2024;17:347-357.

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Introduction

Metabolic syndrome (MetS) is a combination of interrelated conditions that often occur together, including obesity, insulin resistance, glucose intolerance, hypertension, and dyslipidemia [1]. MetS is diagnosed as the presence of at least three of the following five characteristics: insulin resistance, high blood pressure, elevated blood sugar level, increased blood lipids [2]. Central obesity is the main cause of the etiological cascade of MetS. Abnormal fat distribution, rather than adiposity itself, is a more important risk factor for obesity-related disorders [3, 4].

Bioactive substances called adipocytokines which include visfatin, resistin, adiponectin (ADP), tumor necrosis factor alpha (TNF-α) and leptin are secreted by adipocytes. Numerous essential physiological processes, including as inflammation, insulin sensitivity, energy metabolism, and cardiovascular function, are regulated by them [5]. Adipose tissue has a high expression of ADP, which causes the body to become instantly sensitive to insulin [6]. In obese, insulin-resistant mouse models, ADP expression is decreased in contrast to the expression of adipokines that cause insulin resistance, such as resistin and TNF-α [7]. Furthermore, resistin has been shown to preventing insulin-mediated glucose absorption into cells, raise hepatic glucose synthesis, and reduce glucose tolerance, all of which contribute to the development of insulin resistance and the inhibition of adipogenesis in obesity [8].

These adipokines released into the blood attach to specific receptors on the target cells surface to affect the metabolism of tissues and organs. Adipokines can reduce the insulin sensitivity of tissues, leading to inflammation and the development of chronic complications [9]. Consequently, systemic inflammation and ultimately metabolic disorders arise from deregulation of metabolism and adipokine release in adipose tissue [10].

Treatment methods for the control of all these pathologies have not yet provided a complete solution to obesity. Therefore, there is a need for new approaches in the treatment of diseases leading to morbidity caused by MetS. Flavanoids are phytochemicals and have anti-oxidant, anti-inflammatory and anti-diabetic effects that are known protective against obesity-related diseases. An essential flavonoid, quercetin is present in human food and forms combinations with other flavonoids like rutin, hesperidin, and naringenin. Animal and human studies have reported different pharmacological effects of quercetin as attenuation of blood pressure [11], cardiovascular protection [12], loosing of weight [13], improvement of hyperglycemia [13], and hypolipidemic effects [14]. The studies have indicated that quercetin may also help regulate metabolic diseases through a variety of processes, including raising ADP, lowering leptin, antioxidant activity, reducing insulin resistance, raising insulin levels, and inhibiting calcium channels [15].

The aim of this study was to investigate the effect of quercetin on adipocytokine activity from perirenal adipose tissue in MetS rats induced by a high fructose diet, to obtain information about the underlying processes and to identify new therapeutic targets for the treatment of MetS.

Material and methods

Animals

The Pamukkale University Experiments Animal Research Ethics Committee approved all experimental protocols used in our work. Animals were housed in stainless-steel cages in standard conditions (24±2°C and 50±5% humidity) with a 12-h light-dark cycle.

Experiment design

For this study, 24 *Sprague-Dawley male rats* (8-10 weeks old, 135-200 gr) were used. The animals were divided into two groups and were randomly assigned to one of the two following groups: high fructose (HF) (n=12) and control group (C) (n=12). HF had a D-fructose-enriched drink for 10 weeks, while the other group C had tap water for this study. At the end of 6 weeks, the HF and C rats were were randomly separated into four experimental groups as follows:

I: Control (C, n=6) group

II: Quercetin (Q, n=6) group

III: High fructose (HF, n=6) group

IV: High fructose+quercetin (HF+Q, n=6) group

On the other hand, Q and HF+Q group 50 mg/kg/day quercetin was administered by oral gavage. The experimental period is 10 weeks in total (Figure 1).

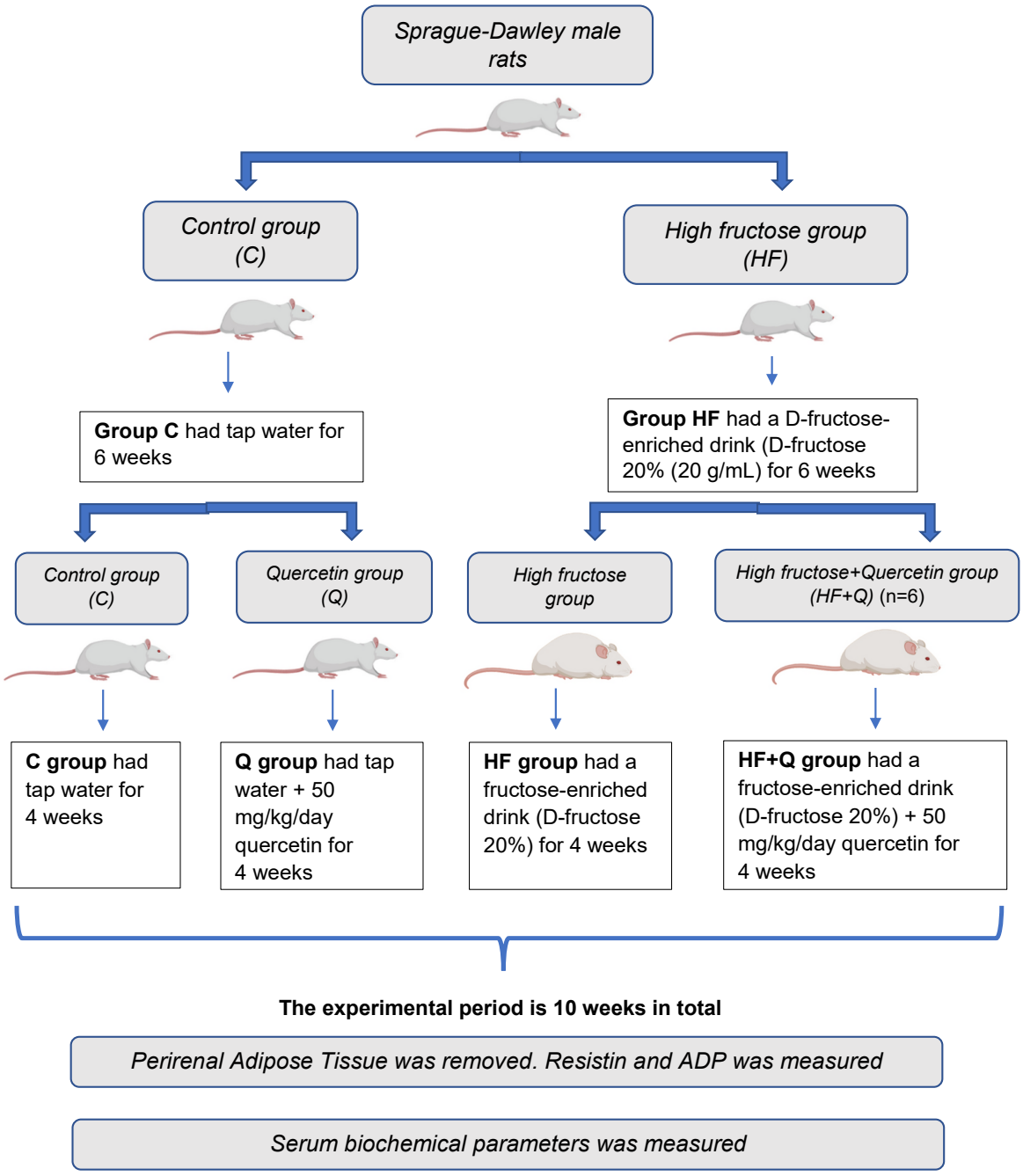

Figure 1. Study design

Preparation of fructose drinking water

The D-Fructose >99% was used in this study to develop a rat model of HF. Rats from groups HF and HF+Q were fed with fructose-enriched drink (D-fructose 20%, Biomatik, CAS:57-48-7, MW: 180.16) (20 g/mL, orally) for 10 weeks. Fructose-enriched drinks were prepared fresh every other day, water bottles were sterilized every week. 20 g of fructose were diluted in 100 mL of tap water to make 20% of the fructose-enriched drinks [16]. For 10 weeks, the rats were given the HF beverages on an ad libitum basis every day. Rats in group C were given tap water.

Quercetin application

Quercetin (Lot: SLCC9071, 10G SIGMA) in powder form was dissolved in 1 mL ethanol+4 mL 0.9% serum physiological and administered to Q and HF+Q rats by oral gastric gavage method at a dose of 50 mg/kg/day for last 4 weeks of the study.

Serum biochemical parameters

At the end of experimental period, animals were fasted for 12h. An abdominal cavity incision of 25 mm was made along the ventral midline. The blood samples were obtained from the abdominal aorta of anesthetized rats and were centrifuged to obtain serum. On the experimental day, fasting insulin (E-EL-R3034), triglyceride (TG) (E0249Ra), high density lipoprotein (HDL) levels (AD1756Ra) were measured from serum by ELISA method.

Adipose tissue homogenate

Perirenal adipose tissue was extracted from the abdominal cavity. The perirenal adipose tissue were accessible by retracting the intestines to the side. The samples were immediately frozen in liquid nitrogen and stored at −80^{0}C for later analysis. This fat tissue was resistin (AD3196Ra) and ADP (AD3187Ra) were measured from perirenal adipose tissue homogenates by ELISA method.

Body weight and HOMA-IR measurement

Body weight (BW) was measured, and the results were recorded. Blood samples were collected from the tail of each animal after 12 hr fasting. The tail was cleaned with alcohol and about 1 mm of its end was cut, and a drop of blood was used for the blood glucose test using a handheldglucometer (ACCU-CHEK Performa Nano).

The following formula was used to determine the Homeostatic Model Assessment of Insulin Resistance. (HOMA-IR) index: Fasting glucose (mmol/L) × fasting insulin (mIU/L) / 22.5 is the formula for HOMA-IR.

Statistical analysis

The data were analyzed with the software package SPSS 25.0. Continuous variables are expressed as the mean±standard error (SEM). Shapiro-Wilk method was used to determine whether the data were normally distributed. For parametric tests we used one-way analysis of variance (Tukey test for pairwise examinations). For non-parametric tests we used Kruskal-Wallis variance analysis (Bonferroni-corrected Mann-Whitney U test for pairwise examinations). In all analyses, $p \leq 0.05$ was considered statistically significant.

Results

At the end of the 10-week experimental period; body weight and fasting insulin level increased in the HF group due to HF diet, body weight of HF+Q group significantly higher compared to the C+Q group (p=0.012), but no significant difference was observed between the groups for fasting insulin level (p=0.103).

According to the lipid profile results, HDL level did not differ between the groups (p=0.08), whereas TG level increased significantly in the HF group compared to the C group (p=0.009) and HF+Q group TG level significantly lower compared to the HF group (p=0.009). The results showed that this increment in the HF group was normalized by quercetin.

Fasting glucose levels were high in animals given a HF diet. HF and HF+Q group fasting glucose level was significantly higher than the C group (p=0.0001, p=0.011, respectively; C+Q group fasting glucose level was significantly lower than the HF group (p=0.011); HF+Q group fasting glucose level was significantly higher than the C+Q group (p=0.001). Quercetin was observed to have a positive effect on the increased fasting glucose level.

According to HOMA-IR results, HF group results was significantly higher than the C group (p=0.012); C+Q group level was significantly lower than the HF group (p=0.013); HF+Q group level was significantly lower than the HF group (p=0.015); it was observed that insulin resistance occurred in the HF group due to HF. Although quercetin had the effect of reducing insulin resistance in the HF group, this reduction was not significant (Table 1).

When ADP and resistin levels between the groups were analyzed, it was observed that there was no significant difference between the 4 groups (Figure 2, 3).

Table 1. General characteristics of C, HF, Q and HF+Q group rats

Parameters	C (n=6)	HF (n=6)	C+Q (n=6)	HF+Q (n=6)	*p*
Body weight (g)	359.4±14.1	395.5±12.3	355.2±7.4	398.1±7.7$^{\$}$	**0.012**
TG (mmol/L)	4960.6 (4192.0-5026.3)	5945.6 (5856.4-6695.6)*	5566.5 (4630.6-5858.3)	4715.3 (4660.7-5352.8)$^{\#}$	**0.029**
HDL (pg/mL)	237.1 (216.4 - 248.6)	106.9 (100.8-145.2)	295.2 (123.8-396.3)	261.6 (218.7-307.5)	0.08
Fasting insulin (ng/mL)	50.9±4.8	72.3±8.3	54.5±7.4	52.9±1.7	0.103
Fasting glucose (mg/dL)	125.1±9.2	255.1±17.5*	143±10.1$^{\#}$	216±17$^{*\$}$	**0.0001**
HOMA-IR	485.0 (401.1-662.0)	976.7 (913.9-1341.8)*	611.51 (166.3-724.7)$^{\#}$	507.8 (467.1-610.6)$^{\#}$	**0.031**

Body weight, fasting insulin, fasting glucose were described as mean ± standard error (SEM), and determined by One Way Analysis of Variance
Other parameters were described as median and interquartile range and determined by Kruskal Wallis Variance Analysis
$p \leq 0.05$ is considered statistically significant. Results having statistical significance are represented by symbols
*: Groups that differ significantly from group C
$^{\#}$: Groups that differ significantly from group HF
$^{\$}$: Groups that differ significantly from group C+Q
C: Control group, HF: High fructose group, C+Q: Control+Quercetin group, HF+Q: High fructose+Quercetin group
TG: Triglyceride, HDL: high-density lipoprotein, HOMA-IR: Homeostatic model assessment of insulin resistance

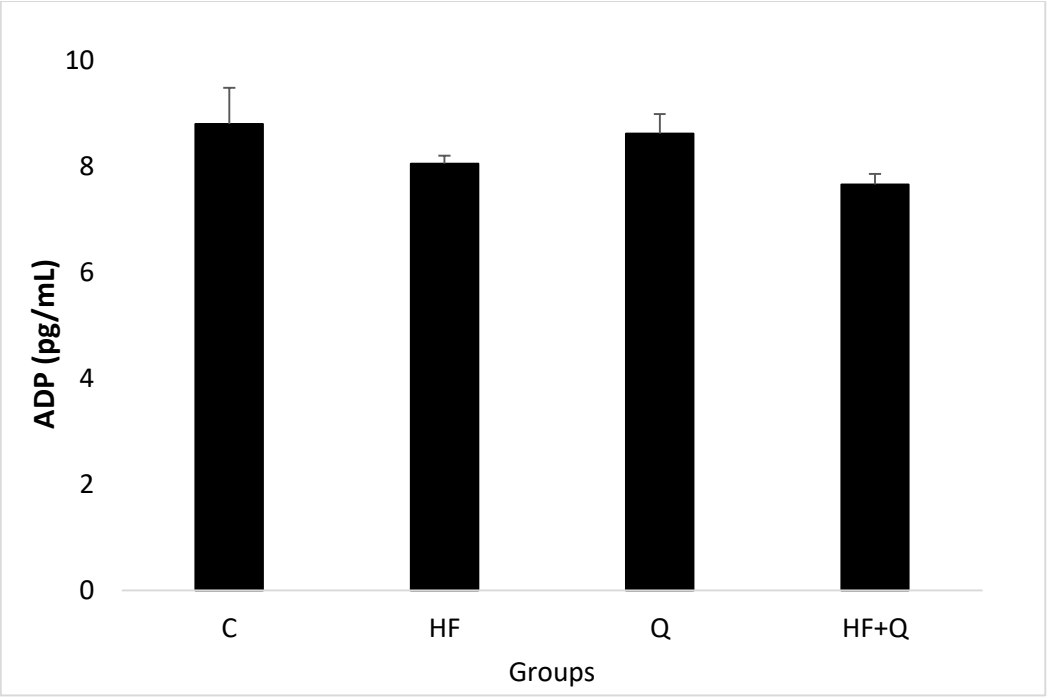

Figure 2. 10th week ADP data of 4 groups. Arithmetic mean and the standard error is used to express the results ($p \leq 0.05$). One-way ANOVA were used to analyze the data

C: control, (n=6); HF: high fructose, (n=6); Q: quercetin, (n=6); HF+Q: high fructose+quercetin, (n=6)

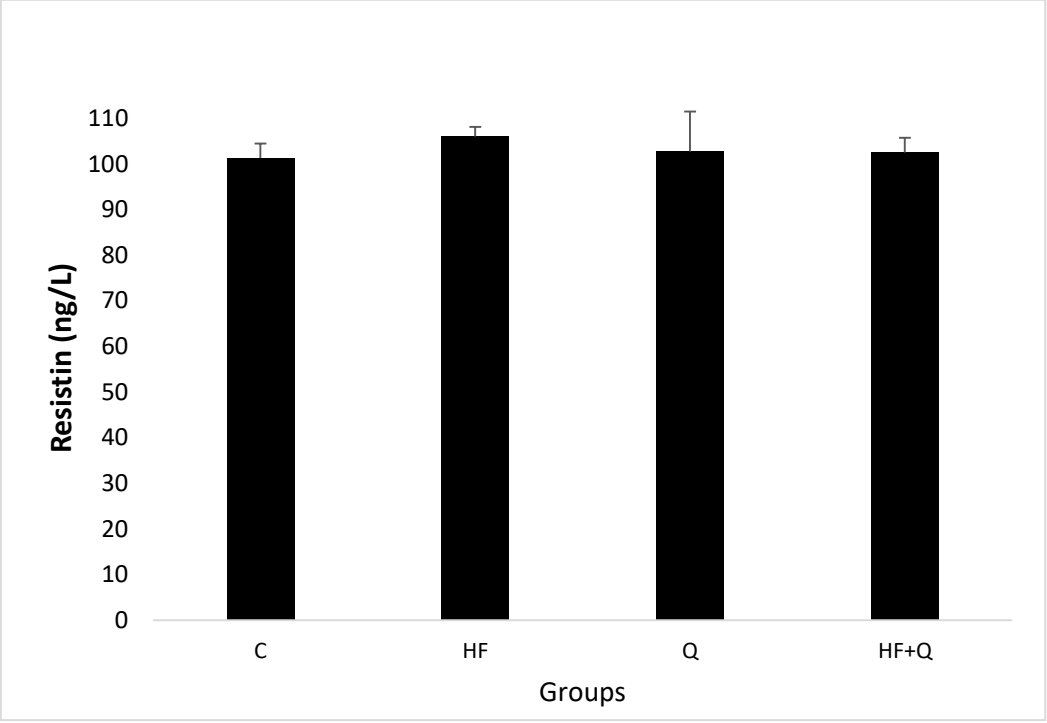

Figure 3. 10th week resistin data of 4 groups. Arithmetic mean and the standard error is used to express the results ($p \leq 0.05$). Kruskal-Wallis test were used to analyze the data

C: control, (n=6); HF: high fructose, (n=6); Q: quercetin, (n=6); HF+Q: high fructose+quercetin, (n=6)

Discussion

MetS is considered an epidemic and is a complex and heterogeneous disease. Clinical signs of a complicated illness include hypertension, insulin resistance, dyslipidemia, abdominal obesity, and hyperglycemia [2]. Various therapeutic strategies used to control MetS include lifestyle changes (such as increased physical activity and calorie restriction), pharmacological agents, and natural compounds [17]. Nevertheless, pharmaceutical drugs have occasionally been linked to cytotoxic effects. Because of this, accumulating evidence showed that several natural polyphenols (resveratrol, quercetin, procyanidins, curcumin, etc.) were beneficial to obesity and other metabolic disorders [17, 18].

As a significant flavonoid, quercetin offers a number of benefits, including lowering blood pressure, preventing hyperlipidemia and hyperglycemia, and having anti-oxidant, antiviral, anticancer, anti-inflammatory, antimicrobial, neuroprotective, and cardioprotective qualities [16]. In the present work, we analyzed the effects of quercetin, on body fat, serum insulin, glucose, TG, HDL parameters; ADP and resistin levels of perirenal adipose tissue in rats fed a high fructose-diet. The current study demonstrated TG, fasting glucose level and HOMA-IR increased in HF group compared to the C group, and these levels were normalized by quercetin treatment, while HDL, ADP and resistin levels were found to be similar all groups. Evaluation of this effect may help us to provide a better understanding of the pathophysiology and treatment of MetS. In recent years, there has been an increasing emphasis on the study of adipocyte biology due to the rising prevalence of adipose tissue-related health issues on a global scale.

Over the past few years, there has been an increase in the body of research demonstrating the health advantages of polyphenols. Several of these polyphenols have demonstrated a definite anti-obesity effect in animal models in the field of MetS. The well-known polyphenolic flavonoid molecule quercetin (3,5,7,3’,4’-pe ntahydroxyflavone) is found in berries, onions, broccoli, tomatoes, and apples. It has hepatoprotective, anti-inflammatory, and anti-oxidant qualities [16]. Numerous studies have examined its advantageous impacts on obesity, insulin resistance, and MetS [19, 20]. A lot of investigations have exhibited the advantageous impacts of quercetin in decreasing body fat and enhancing insulin sensitivity [21]. According to earlier research by Vazquez Prieto et al. [22], adipose tissue mass was significantly reduced after 6 weeks of treatment with a combination of resveratrol and quercetin at doses that did not significantly reduce body fat when given separately (15 mg/kg/day and 30 mg/kg/day, respectively). The other study, Panchal et al. [23] showed that rats given a high-fat diet supplemented with 0.08% quercetin for eight weeks had reduced abdominal obesity (-37%). On the other hand, Wein et al. [24] demonstrated that quercetin feeding over 4 weeks did not affect body weight gain, body composition, or plasma leptin levels compared to other groups (high-fat diet and low-fat diet without quercetin). Similar to these results, Aranaz et al. [25] showed that no effects were observed on body weight during quercetin supplementation for 8 weeks. In our study, consistent with Wein et al. [24] and Aranaz et al. [25], when the effect of quercetin on body weight was analyzed, these parameters were increased in the HF group, but there was no significant change between the groups. Our results are supported by research conducted in the literature on the impact of the effects of polyphenols, which are dependent on the dose and the time of treatment. These variable results of quercetin on body weight may probably be due to the differences in the models used.

There are several studies in the literature showing alterations in lipid profile in rats with a high-fructose diet [25-27]. Fructose consumption for 6 weeks led to the development of metabolic alterations characteristic of MetS. In our study, according to the lipid profile results, TG level increased significantly in the HF group compared to the C group. The results showed that this increment in the HF group was normalized by quercetin. HDL level did not significantly differ between the groups, whereas HDL level was decreased in the HF group compared to the other groups; Q and HF+Q levels were higher than the HF group, but these changes were not significant. According to several studies, quercetin prevented mice fed a high-fat and high-sucrose diet from accumulating hepatic fat [25, 28]. Similarly, Jung et al. [26] showed that mice given a diet supplemented with 0.025%

quercetin for 9 weeks showed significant reductions in serum TG, TC, and epididymal adipose tissue weight. In another investigation, Peredo Escárcega et al. [29] found that, in comparison to control animals, MetS rats developed dyslipidemia with lower levels of HDL-C and elevated levels of non-HDL-C and triglycerides. Conversely, in the MetS group, the maximum dose of resveratrol+quercetin was effective in lowering triglycerides and non-HDL-C, but in the control group, only the highest dose was able to lower the concentration of non-HDL-C. In line with previous investigations, Wang et al. [30] demonstrated that the percentage of abdominal fat was considerably reduced when quercetin intake increased, with the greatest outcome occurring at 0.06% dietary quercetin supplementation when compared to the control group. Concurrently, quercetin at concentrations of 0.04% and 0.06% dramatically lowered serum levels of TG, TC, and LDL. Taken together, our data show that quercetin may improve lipid profile, and these different results of quercetin on lipid profile, may probably be due to the distinct dose and the time of treatment.

Our investigation found that animals given a HF diet had high fasting glucose levels. Quercetin was observed to have a positive effect on the increased fasting glucose level. Studies have shown quercetin to boost ADP expression and secretion while lowering blood levels of insulin, TG, cholesterol, and glucose [30]. As previously noted, obesity increases the mass and functionality of adipose tissue, which can result in elevated levels of free fatty acids in the blood, which can obstruct the transmission of insulin signals and induce glucose intolerance. Because of this, we set out to find out if quercetin supplementation may enhance the MetS rats' insulin sensitivity and glucose tolerance. According to our study's HOMA-IR results, it was observed that insulin resistance occurred in the HF group due to HF diet. Like our result, Henagan et al. [31] have demonstrated that quercetin supplementation at low levels (50 mg/day for 8 weeks) had positive effects on body fat and diet-induced insulin resistance in rats, but not at higher dosages (600 mg/day). Similarly, Vazquez Prieto et al. [22] showed that rats given high fructose for six weeks experienced lower plasma ADP, dyslipidemia, insulin resistance, obesity, and inflammation of the adipose tissue. All of these indicators were enhanced by dietary supplementation with 20 mg/kg/d of quercetin. In line with these findings, we recently observed that a 50 mg/kg dose of quercetin supplementation dramatically lowered the HOMA-IR score, pointing to a possible function in preventing diet-induced insulin resistance. Although quercetin had the effect of reducing insulin resistance in the HF group, this reduction was not significant.

Adipokines related to inflammation, lipid and glucose homeostasis, and adipocyte synthesis have all been demonstrated to occur in adipose tissue. It is proposed that these compounds could be useful therapeutic targets. Adipose tissue in good health is essential to human health. In addition to its other functions, it helps maintain energy homeostasis and isolates interior organs. A metabolically active tissue is the perirenal adipose tissue (PAT), which is a part of visceral adipose tissue. This is because PAT can create a panel of adipokines or cytokines that control renal activity through endocrine or paracrine mechanisms [32, 33]. Thus, PAT is considered to be a very useful cell source in therapeutic aspects. To the best of our knowledge, this is the first study to investigate the effects of quercetin on ADP and resistin levels from perirenal adipose tissue in rats fed HF.

In animal studies showed that obese animals had lower levels of ADP, but calorie restriction increased ADP levels [34]. Reports on changes in ADP levels with quercetin are controversial. Some studies report that there is no change, while others report an increase or decrease. Leptin and ADP concentrations were found to be significantly higher in MetS rats compared to control rats, according to Peredo Escárcega et al. [29]. The MetS rats' ADP levels were only slightly decreased (13% by resveratrol 50 + quercetin 0.95), and leptin concentration was not affected by supplementation with resveratrol + quercetin. Furthermore, it has been demonstrated that supplementing mice with a high-fat diet to create obesity led to decreased levels of glucose, insulin, TG, and cholesterol but increased ADP secretion [35]. Kim et al. [36] found that quercetin-rich onion peel extract supplementation increased ADP mRNA levels in mesenteric adipose tissue but found no differences in ADP mRNA levels from

in the perirenal adipose tissue of rats with diet-induced obesity. In our study, when the effect of quercetin on adipokines was analyzed, there was no significant change in ADP levels between the groups.

Our data revealed that ADP and resistin levels didn't show a significant difference between the groups. The multiple administration periods that were used could be the cause of this disparity with other studies. It is possible that adipokine levels would alter if the duration of the polyphenol treatment interval was extended. Insulin resistance is promoted by elevated levels of resistin, a circulating hormone unique to adipocytes in rodents that is elevated in obesity [35, 37]. Although conflicting data in animal models suggests that resistin levels are low in obesity, it is commonly believed that resistin levels are elevated in obesity [38, 39]. Iqbal et al. [40] found no relationship between the degree of obesity and resistin levels in 71 obese patients. In our study, similar to this result, no significant increase in resistin level was observed in the Q and HF+Q groups compared to the control group. The dosage and timing of treatment have an impact on the effects of polyphenols. More research on the biological role and control of quercetin is necessary because the implications of these discoveries are not entirely obvious.

In conclusion, we effectively created a high-fructose diet rat model by administering 20% fructose and by providing adequate criteria. Administration of quercetin alone improved fasting glucose and insulin resistance. Co-administration of quercetin with fructose normalized TG levels. Quercetin administration did not affect ADP and resistin levels in perirenal fat of high fructose-treated rats. Our study's findings imply that 50 mg/kg of quercetin given for four weeks may be advantageous for fructose-mediated lipid and carbohydrate metabolism. Thus, it will be guiding us to evaluate the antiobesity activity of quercetin and its potential to be developed as a drug in the fight against MetS.

Conflict of interest: The authors declare that they have no conflicts of interest.

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Ethics committee approval: The Pamukkale University Experiments Animal Research Ethics Committee approved all experimental protocols used in our work (the registry number: 60758568-020-441007; date: 19.10.2023). This study was planned as an additional study from the study in which animal tissues were removed and ethical approval was obtained previously (the registry number: e-60758568-020-197540; date: 05.04.2022).

Authors' contributions to the article

E.K.T. and M.T.A. have constructed the main idea and hypothesis of the study. E.K.T. and M.T.A. they developed the theory and arranged/edited the material and method section. E.K.T. and M.T.A. have done the evaluation of the data in the results section. Discussion section of the article. Written by E.K.T. and M.T.A., E.K.T. and M.T.A. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

Research Article

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Descriptive characteristics of spinal traumas in the Eastern Anatolia region of Türkiye: a 3-year retrospective analysis

Türkiye'de Doğu Anadolu bölgesinde spinal travmaların tanımlayıcı özellikleri: 3 yıllık retrospektif analiz

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Abstract

Purpose: Understanding the descriptive characteristics of traumatic spinal injuries such as etiology, epidemiology, mortality and their associations with mortality may facilitate the diagnosis and management of spinal traumas. Moreover, its incidence can be reduced through eliminating preventable causes. The present study aimed to assess the descriptive characteristics of spinal traumas and the conditions associated with mortality in our country.

Materials and methods: Our study was conducted retrospectively in the emergency service of a tertiary care hospital. Data of patients visiting to our hospital's emergency room between 2020 and 2023 with spinal trauma, were obtained from the hospital information management system by scanning electronic patient records for inclusion in the study.

Results: A total of 1835 patients were included in our study, of which 427 (23.3%) were female and 1408 (76.7%) were male. The most common complaint of the included patients who presented to the emergency room was observed as falls (n=1112). 52.8% (n=968) of those patients with special traumas had other concomitant injury. 3.7% (n=68) of the included patients resulted in death.

Conclusion: Falls are the most common cause of special traumas in our country and concomitant head trauma and thoracic trauma are closely associated with mortality. While men are more frequently presented to the emergency room with special trauma, there has been no difference between both sexes in terms of mortality rate. Moreover, the most common vertebral fractures after spinal trauma occur in the parts of the corpus and spinous processes of the lumbar vertebrae.

Keywords: Vertebra fracture, spinal trauma, etiology, epidemiology, Eastern Anatolia.

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Öz

Amaç: Spinal travmaların etiyoloji, epidemiyoloji, mortalite, mortalite ile ilişkili tanımlayıcı özelliklerinin bilinmesi spinal travmaların tanı, tedavisini kolaylaştırabilir. Ayrıca önlenebilir nedenlerin ortadan kaldırılması ile görülme sıklığı azaltılabilir. Bu çalışmada da ülkemizde spinal travmaların tanımlayıcı özellikleri ve mortalite ile ilişkili durumların değerlendirilmesi amaçlandı.

Gereç ve yöntem: Çalışmamız üçüncü düzey bir hastanenin acil servisinde retrospektif olarak yapılmıştır. 2020-2023 yılları arasında hastanemiz acil servisine omurga travması nedeniyle başvuran hastaların verileri, çalışmaya dahil edilmek üzere hastane bilgi yönetim sisteminden elektronik hasta kayıtları taranarak elde edildi.

Bulgular: Çalışmamıza toplamda 1835 hasta dahil edilmiştir. Bu hastalardan 427 tanesi kadındı. Çalışmaya dahil edilen hastaların en sık acile başvuru şikâyeti düşme (n=1112) olarak gözlendi. Çalışmaya alınan hastalardan %52,8'inde (n=968) spinal travmaya eşlik eden başka bir yaralanma daha mevcuttu. Çalışmaya dahil edilen hastalardan %3,7'si (n=68) exitus olarak sonlanmıştır.

Sonuç: Ülkemizde en sık spinal travma nedeni düşmeler olup eşlik eden kafa travması ve toraks travması olması mortalite ile yakından ilişkilidir. Spinal travma ile erkekler daha sık acil servise başvururken mortalite oranı açısından her iki cinsiyet arasında bir fark bulunmamamıştır. Ayrıca spinal travma sonrasında en sık görülen vertebra fraktürü lomber vertebranın corpus ve processus spinosus kısımlarındadır.

Anahtar kelimeler: Vertebra fraktürü, spinal travma, etiyoloji, epidemiyoloji, Doğu Anadolu.

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Introduction

Physicians dealing with the trauma frequently encounter spinal traumas (ST) and vertebral fractures (VFr). The incidence of VFr has been reported as 24-90 cases per 100.000 people [1]. Despite current treatment modalities, prolonged rehabilitation, prolonged absence from work or permanent disability may occur. Consequently, VFr is often associated with a significant impact on daily life activities, leading to a considerable primary and secondary socioeconomic cost burden.

Today, changing living conditions have led to changes in the socio-demographic characteristics and trauma mechanisms of patients impacted by ST. Increased life expectancy has also increased the risk of VFr development at advanced ages [2]. The mechanism of trauma can result from various causes such as falls, traffic accidents, gunshot wounds, battery, sports injuries, etc. Currently, it has been reported that ST and VFr-related injuries frequently occur as a result of falls in elderly patients and motor vehicle accidents in young patients [3]. Different results were obtained in similar studies. This may vary with factors such as geographical structure, level of development of the country and differences in driving legislation [4].

Concomitant injuries in patients with spinal trauma vary depending on factors such as the patient's presence of comorbid diseases, treatment options (the need for surgical repair) and gender in the studies [4, 5]. A study analyzed concomitant injuries based on the three spinal regions: cervical, thoracic, and lumbar. That study demonstrated that cranial injuries were the most common in patients with cervical ST, lung injuries were the most common in patients with thoracic ST, and intra-abdominal organ injuries were the most common in patients with lumbar ST [6]. Another study found that patients with lumbar ST had a higher risk for concurrent thoracic and retroperitoneal cavity visceral injuries [7].

We consider that the data we will obtain from the center where our study was conducted will be descriptive for the Eastern Anatolia region of our country, due to frequent admission of patients from rural centers and other provinces in the Eastern Anatolia region. Given the different outcomes of ST in different countries and regions, in this study, we aimed to assess the demographic variations in ST in our region, the most common vertebra and part of the vertebra where a fracture occurs if VFr occurs, the mechanism of injury, treatment options (outpatient, inpatient), the presence of comorbid conditions and concomitant injuries.

Material and method

Our study was conducted retrospectively in the emergency room of a tertiary care hospital. Data of patients visiting to our hospital's emergency room from January 1, 2020 to January 1, 2023 with ST and suspected with Vfr, were obtained from the hospital information management system by scanning electronic patient records for inclusion in the study.

Ethical approval for this study was obtained from Ataturk University Faculty of Medicine Clinical Research Ethics Committee.

Study population

From January 1, 2020 to January 1, 2023, 8956 patients presenting to the emergency room with suspected VFr who had ST were identified from the hospital information management system. No age range was determined for the patients included in our study. Patients for whom we could access all data and patient records from the electronic system were included in the study. Patients who were found to have only vertebral radiographs in the electronic patient files were excluded from the study. Also, patients who were seen to be pregnant and who received spinal magnetic resonance imaging for spinal imaging were excluded from the study. In addition, since the study evaluated patients who were first presented to the emergency room, patients who received spinal tomography other than at the time of presentation to the emergency room or whose image quality was not suitable for spinal evaluation were also excluded from the study. Patients who were planned to be included in the study but whose data were incomplete were also excluded. Patients who requested discharge during clinical observation were also excluded from the study because we could not follow their final status. When all inclusion and exclusion criteria were applied, 1835 patients were included in the study (Figure 1).

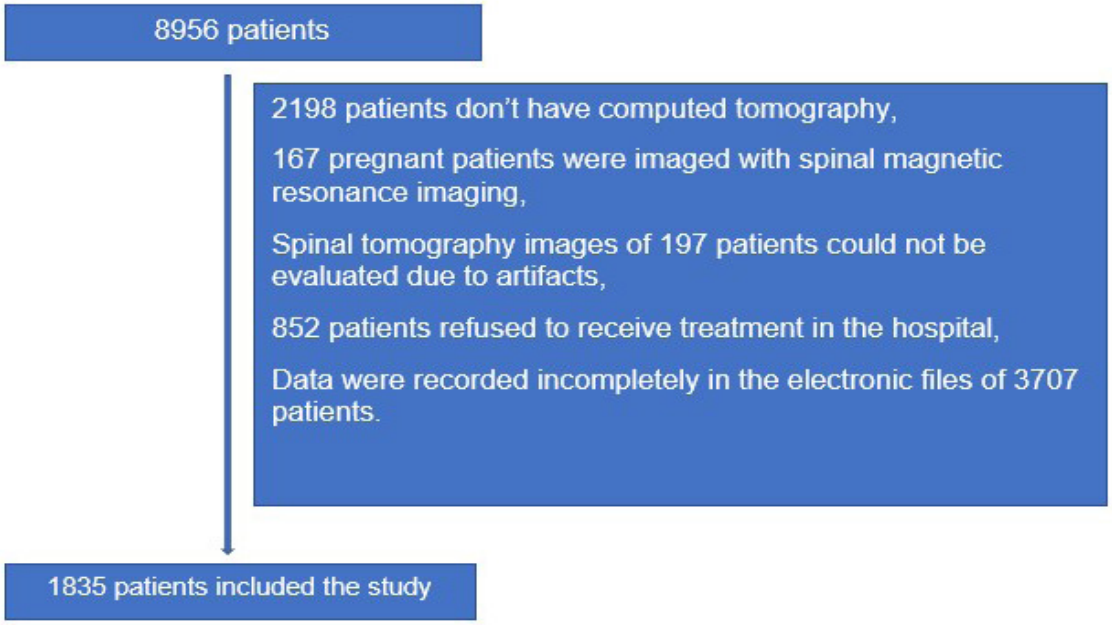

Figure 1. Flow chart showing the patients included in the study

Acquisition of data

The electronic health records of patients with suspected VFr who had ST in the emergency room including age, gender, trauma mechanism, presence of accompanying comorbid conditions (such as diabetes mellitus, hypertension, coronary artery disease, chronic endocrine disorders requiring chronic medication use), treatment modality (outpatient, inpatient treatments), presence of VFr, if VFr is present, which part of the vertebral column is affected (cervical, thoracic, lumbar), which part of the vertebra is affected (corpus, transverse process, spinous process), the presence of concomitant injury, which part of the vertebra is affected if concomitant injury is present (brain, thorax, abdomen, genitourinary, pelvis, extremity, neck, maxillofacial), the clinical outcomes of the patients (death or discharge) and vertebral tomography images were obtained from the electronic patient records.

The CT scans for the patients at the time of their presentation to the emergency department were re-evaluated by two radiologists with 5-10 years of professional experience. Patients' vertebral and other systemic pathologies were recorded.

Statistical analysis

Statistical analyses were performed with the IBM SPSS 20 statistical analysis program. Data are presented as percentages and numbers. Comparisons between categorical variables were made using the Pearson Chi-square test or Fisher's Exact test. The statistical significance level was taken as $p<0.05$.

Results

A total of 1835 patients were included in our study, of which 427 (23.3%) were female and 1408 (76.7%) were male. The most common complaint of the included patients who presented to the emergency room was observed as falls (n=1112). 442 patients were evaluated in the emergency room due to ST caused by traffic accidents. VFr was present in 833 of the patients included in our study. The most commonly injured vertebrae were lumbar vertebrae (n=568). The socio-demographic characteristics of the patients included in the study and the descriptive characteristics of their trauma are summarized in Table 1.

Table 1. Socio-demographic characteristics of the patients and descriptive characteristics of their trauma

Variable	**Mean±SD (min-max)**	
Age	36.8±21.5 (0-93)	
Variable	**n**	**%**
Gender		
Female	427	23.3
Male	1408	76.7
Cause of trauma		
Falls	1112	61
Traffic accident	442	24
Battery	83	4.5
Animal attack	50	2.7
Gunshot wound	8	0.4
Sharp object injuries	16	0.8
Electric shock	36	2
Vaccines	24	1.2
Trapped under rubble or Injured by falling object	64	3.4
Type of Medical Care		
Hospitalization	1108	60
Discharged from emergency	727	40
Receiving spinal surgical treatment	311	16.9
Presence of fracture in any vertebra	833	45
Fractured cervical vertebra	63	3.4
Fractured thoracic vertebra	202	11
Fractured lumbar vertebrae	568	30.6
Vertebral corpus fracture	309	16.5
Spinous process fracture	395	21.5
Transverse process fracture	112	6.1
Spondylolisthesis	13	0.7
Odontoid process	4	0.2

Of the patients included in the study, 52.8% (n=968) had another concomitant injury associated with ST. The most common concomitant injury was thoracic trauma (n=357). The rate of consultation to neurosurgery clinic in patients with ST was 29.9% (n=548). Of the patients included in the study, 5.3% (n=97) had comorbid diseases such as diabetes, hypertension, ischemic heart disease, and heart failure. The rate of death in patients was 3.7% (8n=68). Other concomitant injuries associated with ST and consultation information are summarized in Table 2.

Table 2. Other concomitant injuries in patients with spinal trauma and their consultation information

Variable	n	%
Presence of other concomitant injuries associated with spinal trauma	968	52.8
Thoracic injury	357	19.5
Extremity injury	336	18.3
Head trauma	269	14.6
Maxillofacial injury	227	12.4
Abdominal injury	126	6.9
Pelvic injury	120	6.5
Neck injury	9	0.5
Genitourinary injury	3	0.2
Patients requested for consultation	1080	58.9
Number of patients for whom neurosurgical consultation was requested	548	29.9
Number of patients requested for orthopedic consultation	407	22.2
Number of patients requested for thoracic surgery consultation	355	19.4
Number of patients requested for general surgery consultation	140	7.6
Number of patients requested for otolaryngology consultation	136	7.4
Number of patients requested for ophthalmology consultation	107	5.8
Number of patients requested for urology consultation	83	4.5
Number of patients requiring maxillofacial surgery consultation	79	4.3
Number of patients requested for cardiovascular surgery consultation	47	2.6
Number of patients requested for pediatric surgery consultation	45	2.5
Number of patients for whom plastic and reconstructive surgery consultation was requested	39	2.1
Patient's outcome		
Death	68	3.7
Discharge	1767	96.3
The presence of chronic disease		
Having a chronic disease	97	5.3
No chronic disease	1738	94.7

Of the patients included in the study, 3.7% (n=68) resulted in death. An examination of the association between the outcome of these patients and the presence of traumas other than ST, indicated that the presence of concomitant injury with ST was associated with the patient's outcome (p<0.001). The association of gender, concomitant injury and consultations with patient's outcome is summarized in Table 3.

Table 3. Association of gender, concomitant injury and consultations with patient's outcome

	Patient's outcome				
	Death		Discharged		*P* value (%)
Variable	**Number of (n)**	**Percentage (%)**	**Number (n)**	**Percentage (%)**	
Gender					
Female	20	29.4%	407	23%	0.222
Male	48	70.6%	1360	77%	
Concomitant injury	63	92.6%	905	51.2%	**<0.001**
No concomitant injury	5	7.4%	862	48.8%	
Concomitant head injury	40	58.8%	229	13%	**<0.001**
No concomitant head injury	28	41.2%	1538	87%	
Concomitant thorax injury	40	58.8%	317	17.9%	**<0.001**
No concomitant thoracic injury	28	41.2%	1450	82.1%	
Concomitant abdominal injury	15	22.1%	111	6.3%	**<0.001**
No concomitant abdominal injury	53	77.9%	1656	93.7%	
Concomitant pelvic injury	13	19.1%	107	6.1%	**<0.001**
No concomitant pelvic injury	55	80.9%	1660	93.9%	
Concomitant extremity injury	18	26.5%	318	18%	0.076
No concomitant extremity injury	50	73.5%	1449	82%	
Concomitant neck injury	1	1.5%	8	0.5%	0.289
No concomitant neck injury	67	98.5%	1759	99.5%	
Concomitant maxillofacial injury	12	17.6%	215	12.2%	0.178
No concomitant maxillofacial injury	56	82.4%	1552	87.8%	
Concomitant genitourinary injury	0	0%	3	0.2%	1
No concomitant genitourinary injury	68	100%	1764	99.8%	
Consultation requested	68	100%	1012	57.3%	**<0.001**
Consultation not requested	0	0%	755	42.7%	

Table 3. Association of gender, concomitant injury and consultations with patient's outcome (continued)

	Patient's outcome				
	Death		Discharged		*P* value (%)
Variable	**Number of (n)**	**Percentage (%)**	**Number (n)**	**Percentage (%)**	
Neurosurgery consultation requested	58	85.3%	490	27.7%	**<0.001**
Not requested	10	14.7%	1277	72.3%	
Thoracic surgery consultation requested	39	57.4%	316	17.9%	**<0.001**
Not requested	29	42.6%	1450	82.1%	
General surgery consultation requested	20	29.4%	120	6.8%	**<0.001**
Not requested	48	70.6%	1647	93.2%	
Orthopedic consultation requested	22	32.4%	385	21.8%	**0.04**
Not requested	46	67.6%	1382	78.2%	
Urology consultation requested	10	14.7%	73	4.1%	**<0.001**
Not requested	58	85.3%	1693	95.9%	
Ophthalmology consultation requested	5	7.4%	102	5.8%	0.779
Not requested	63	92.6%	1664	94.2%	
Otolaryngology consultation requested	8	11.8%	128	7.2%	0.163
Not requested	60	88.2%	1639	92.8%	
Maxillofacial surgery consultation requested	4	5.9%	75	4.2%	0.534
Not requested	64	94.1%	1692	95.8%	
Pediatric surgery consultation requested	3	4.4%	42	2.4%	0.231
Not requested	65	95.6%	1725	97.6%	
Cardiovascular surgery consultation requested	2	2.9%	45	2.5%	0.692
Not requested	66	97.1%	1722	97.5%	
Plastic-reconstructive surgery consultation requested	5	7.4%	34	1.9%	**0.013**
Not requested	63	92.6%	1733	98.1%	
Patient has a comorbidity	52	76.5%	1686	95.4%	**<0.001**
Patient has no comorbidity	16	23.5%	81	4.6%	

Discussion

As a result of our study, we observed that patients with ST who were evaluated at our hospital over the past three years had a mortality rate of 3.7%, which was independent of gender. While the incidence of ST was observed more frequently in male patients in our study consistent with the literature, no significant difference was observed between both genders in terms of mortality, which aligns with the findings of the study of Barbiellini Amidei et al. [8]. This may be linked with patient-dependent factors such as concomitant comorbid diseases, age at exposure to trauma, social engagement or the type of trauma.

The etiology of ST may differ depending on the country and region of residence. Some previous studies have shown that falls are the primary etiology of ST in developing countries, while motor vehicle accidents are the typical primary etiology in developed countries [9]. Alshahri and Alshehri [10] identified motor vehicle accidents as the most common cause of ST in their country. A study conducted in our country in 2013 found that falls were the most common etiologic cause of ST, consistent with our findings [11]. The decrease in motor vehicle accidents in various countries can be attributed to different factors [9]. The fact that the vehicles produced in today's technology are more protective, increased compliance with traffic rules, and legal regulations, potentially leading to less severe motor vehicle accidents.

Our study showed that the most common vertebrae fractured as a result of ST were lumbar vertebrae. In the study by Mahmud et al. [12] however, cervical vertebral fractures were observed most frequently. However, the most common etiology in the study by Mahmud et al. [12] was motor vehicle accidents, while the most common etiology of ST in our study was falls. Hence, the level of fractured vertebra may have changed depending on the mechanism of trauma. Furthermore, the fact that lumbar vertebrae are less protected and more flexible may increase the frequency of lumbar vertebral fractures. Shahriari et al. [13] also identified fractures in the corpus and transverse process of the lumbar vertebrae most frequently in patients with ST resulting from a fall. Yet, when the same study was evaluated in detail, it was observed that spinous process fractures in ST caused by falls were higher than spinous process fractures in motor vehicle accidents, the second most common cause of ST [13]. In our study, corpus and spinous process fractures were frequently observed in the lumbar vertebrae. Falls on the spinous process during falls or hyper-extension movement to avoid falls may have increased the injuries in the posterior vertebrae.

Injuries in other systems are likely to be present in patients with ST. The likelihood of concurrent injuries with ST was observed in 63% of patients with cervical spine injury, 79% of patients with thoracic spine injury, and 71% of patients with lumbar spine injury [6]. Of the patients included in our study, 52.8% had another concurrent injury with ST. These injuries may affect the brain, thorax, abdomen, genitourinary system or extremities. Studies are showing that abdominal injury is frequent, especially in patients with lumbar VFr [13]. In contrast to this, in our study, thoracic injuries were observed to be the most common concurrent injury in patients with VFr. Similarly, Anandasivam et al. [6] emphasized that the most common concurrent injury in patients with lumbar VFr was in the thoracic region.

The mortality rate in patients with ST is between 4-18% [14]. Our study found a mortality rate of 3.7% which is consistent with the literature. In patients with ST, mortality was most commonly observed in patients with cervical VFr [8]. In our study, the association of mortality with the injured vertebral region was not evaluated. Nevertheless, the association of mortality with concurrent injuries of the patients was evaluated. Mortality will increase as concurrent injuries increase in patients with ST. In our study, 63 of the 68 patients who resulted in death had concurrent injuries. Our study showed that head traumas, and thoracic traumas in particular, were more prevalent among other injuries in patients resulting in death. When trauma patients are evaluated overall in the literature, brain and thorax injuries have a high rate among the leading causes of premature death. Since the first visits of ST patients to the hospital were evaluated in this study, it is not surprising that brain and thorax injuries were frequently observed in our patients resulting in death. Also, our work on this situation showed

that the most frequent consultation requested, apart from the neurosurgery clinic, was for the thoracic surgery clinic in patients resulting in death.

Our study has several limitations. Since our study was retrospective, our data were collected by scanning electronic patient records. This has resulted in missing data. Furthermore, we were unable to obtain outcome information for some patients who requested to seek treatment at other centers. We had to exclude these patients from the study, which resulted in missing data. The association of mortality with the level of VFr of our patients was not evaluated. Moreover, when evaluating the etiologies of ST in patients, the fall group was not divided into falls from their height level or falls from a height. Therefore, the difference between these two groups could not be evaluated.

In conclusion, our study is significant for highlighting the descriptive characteristics of STs in the recent period in Türkiye, particularly in the Eastern region. While ST is a more common cause for the male population presenting to the emergency room, there is no difference between both sexes in terms of mortality. ST is more common in young and middle-aged individuals who are active in daily life. Despite variations in etiology, considering the frequency of ST in the group who are active in daily life, the advancing technology, and living conditions today, falls are the most common cause of ST. Although it depends on the etiology of ST, VFr is most commonly seen in the corpus and spinous process region in the lumbar region. Concomitant injuries are common. However, head trauma and thoracic trauma are the most common concomitant injuries and are also the most frequently associated with mortality.

Conflict of interest: No conflict of interest was declared by the authors.

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Authors' contributions

M.C. constructed the main idea and hypothesis of the study. M.C., F.T. and K.K. developed the theory and arranged/edited the material and method section. M.C., F.T. and K.K. have evaluated the data in the results section. The discussion section of the article was written by M.C. and F.T.. K.K. reviewed, corrected, and approved. In addition, all authors discussed the entire study and approved the final version.

Research Article

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Effects of boric acid on oxidant-antioxidant, proinflammatory cytokine levels, and biochemical parameters in aged rats

Yaşlı sıçanlarda borik asidin oksidan-antioksidan, proinflamatuar sitokin seviyeleri ve biyokimyasal parametreler üzerine etkisi

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Abstract

Purpose: As a result of the literature studies, it was seen that boric acid was the subject of many studies, and its effects on living things were investigated and examined. This study aimed to investigate the effects of oral boric acid supplementation at pharmacologic doses on physiological and biochemical systems in aged rats.

Material and methods: A total of 32 Wistar Albino male-aged rats were randomly and equally divided into the following four groups: 1st; Control=1 ml saline; 2nd; Low-dose boric acid (L-BA)=10 mg/kg; 3rd; Medium-dose boric acid (M-BA)=20 mg/kg; 4th; High-dose boric acid (H-BA)=40 mg/kg. Boric acid was given orally to aged rats for 28 days. Blood, liver, and kidney samples of rats were collected on day 29 to be analyzed for oxidants, antioxidants, proinflammatory cytokines, and biochemical changes.

Result: Boric acid significantly increased albumin, total protein, calcium levels equally in all boric acid groups compared to the control group (p<0.05), increased cholesterol parameter only in H-BA group (p<0.05), increased phosphor level in M-BA and H-BA groups compared to control and L-BA groups (p<0.05), total bilirubin level was increased only in L-BA group (p<0.05), blood urea nitrogen level was increased in L-BA and M-BA groups (p<0.05), alanine aminotransferase level was increased only in M-BA group (p<0.05), creatine kinase and glucose levels were increased boric acid in all groups compared to control group (p<0.05). However, boric acid did not affect globulin, creatine, alkaline phosphatase, and amylase levels in a dose-dependent manner (p>0.05). Boric acid significantly decreased MDA levels (p<0.05) and increased GSH, SOD, and CAT enzyme activities (p<0.05) in liver and kidney tissues in a dose-dependent manner. In addition, boric acid decreased plasma IL-6 and TNF-α proinflammatory cytokine levels (p<0.05).

Conclusion: This study demonstrated that boric acid supplementation has ameliorative effects in a dose-dependent manner on lipid peroxidation, immunomodulation, and regulation of many blood biochemical parameters in aged rats.

Keywords: Age, antioxidant, boric acid, oxidant, proinflammatory cytokine.

Basegmez M, Dogan MF. Effects of boric acid on oxidant-antioxidant, proinflammatory cytokine levels, and biochemical parameters in aged rats. Pam Med J 2024;17:369-379.

Öz

Amaç: Yapılan literatür çalışmaları neticesinde borik asit ’in birçok çalışmaya konu olduğu ve canlıya olan etkilerinin araştırılarak incelendiği görülmüştür. Bu çalışmada yaşlı sıçanlarda farmakolojik dozlarda uygulanan oral borik asit takviyesinin fizyolojik ve biyokimyasal sistemlerdeki etkilerinin araştırılması amaçlanmıştır.

Gereç ve yöntem: Toplam 32 adet Wistar Albino erkek yaşlı sıçan rastgele ve eşit olarak aşağıdaki dört gruba ayrılmıştır: 1.; Kontrol=1ml salin, 2.; Düşük-doz borik asit (L-BA)=10 mg/kg, 3.; Orta-doz borik asit (M-BA)=20 mg/kg, 4.; Yüksek-doz borik asit (H-BA)=40 mg/kg. Yaşlı sıçanlara 28 gün boyunca borik asit oral olarak verilmiştir. Sıçanların kanı, karaciğer ve böbrek örnekleri oksidan, antioksidan, proinflamatuar sitokinler ve biyokimyasal değişiklikler açısından analiz edilmek üzere 29. günde toplanmıştır.

Bulgular: Borik asit, albümin, total protein ve kalsiyum düzeylerini kontrol grubuna kıyasla tüm borik asit gruplarında eşit oranda artırırken (p<0,05), kolesterol parametresini sadece H-BA grubunda artırmıştır (p<0,05). Borik asit fosfor düzeyini kontrol ve L-BA gruplarına kıyasla M-BA ve H-BA gruplarında artırırken (p<0,05), total bilirubin düzeyini sadece L-BA grubunda (p<0,05), kan üre nitrojen düzeyini L-BA ve M-BA gruplarında artırmıştır (p<0,05). Ayrıca alanin aminotransferaz düzeyini sadece M-BA grubunda artırırken (p<0,05), kreatin kinaz ve glukoz düzeylerini kontrol grubuna kıyasla tüm gruplarda artırdığı tespit edilmiştir (p<0,05). Ancak borik asidin globulin, kreatin, alkalin fosfataz, amilaz düzeyleri üzerinde doza bağlı bir etkisi olmamıştır (p>0,05). Borik asit karaciğer ve böbrek dokularında MDA düzeylerini önemli ölçüde azaltmış (p<0,05) ve GSH, SOD ve CAT enzim aktivitelerini artırmıştır (p<0,05). Ayrıca, borik asit plazma IL-6 ve TNF-α proinflamatuar sitokin düzeylerini azaltmıştır (p<0,05).

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Sonuç: Bu çalışma borik asit takviyesinin yaşlı sıçanlarda lipid peroksidasyonu, immünomodülasyon ve birçok kan biyokimyasal parametresinin düzenlenmesi üzerinde doza bağlı olarak iyileştirici etkileri olduğunu göstermiştir.

Anahtar kelimeler: Yaş, antioksidan, borik asit, oksidan, proinflamatuar sitokin.

Başeğmez M, Doğan MF. Yaşlı sıçanlarda borik asidin oksidan-antioksidan, proinflamatuar sitokin seviyeleri ve biyokimyasal parametreler üzerine etkisi. Pam Tıp Derg 2024;17:369-379.

Introduction

The aging process is one of the most significant examples of homeostasis disruption, which affects physiological systems, especially the immune, nervous, and endocrine systems, including oxidative stress and inflammation [1]. Thus, aging can be characterized as a progressive and widespread decline in the organism's functioning that results in a reduced capacity to adapt to changes and maintain homeostasis [2].

Boric acid, which is widely used in both industrial and consumer products, exists in nature as a mineral substance [3]. It has been proven in various experimental studies that boron and its compounds are beneficial for humans and animals [4]. Boric acid has been shown to play a significant function in preserving health through nutritional and pharmacological intakes [5]. The effects of boric acid on enzyme, mineral, and hormone metabolism have been previously demonstrated [5]. Indeed, boron supplements in diets have been demonstrated to play key roles in bone development by reducing calcium excretion and increasing plasma-ionized calcium and phosphorus levels [6]. It is known that boron plays an important role in the inflammatory response by suppressing the activities of some enzymes, such as 6-phosphogluconate [4]. There are also a few studies showing the importance of boron on antioxidant activity by destroying reactive oxygen species [7]. It has been reported that oral boric acid administration increases GSH and CAT antioxidant enzyme levels in the body and prevents oxidative damage by inhibiting reactive oxygen species [8]. In a previous study, boric acid and borax supplementation were shown to reduce lipid peroxidation and increase antioxidant activity by decreasing blood MDA levels and increasing blood GSH levels [9]. Boric acid has been suggested to be better than borax in combating heavy metal toxicity [10]. Furthermore, boron compounds, particularly boric acid and borax, have been demonstrated to decrease oxidative stress by boosting the antioxidant system in several disease and toxicity models [3].

The most important sources of boron and its derivatives are plants and drinking water. A report by the World Health Organization (WHO) reports that nearly 1.2 mg of boron is consumed daily through nutrients, with 0.2-0.6 mg ingested with drinking water. It has been reported that the safe dosage range for individuals is 1-13 mg/day and that a daily intake of less than 1 mg is inadequate [11]. Oral boric acid supplements have been observed to be easily absorbed from the gastrointestinal system in humans and animals and quickly dispersed throughout the body. Regardless of the mode of administration, boric acid has been observed to be quickly excreted unchanged in the urine in less than 24 hours in both people and animals [12].

Considering previous studies, the importance of boric acid, a boron compound, for homeostasis is emphasized. In this study, different doses of oral boric acid supplementation were administrated to aged rats. It investigated how oral boric acid supplementation influenced biochemical parameters in serum, as well as oxidant and antioxidant markers in kidney and liver tissue.

Materials and methods

Chemical

Boric acid (H_3BO_3) (Code number: V55901), purchased from Chemistry Lab Istanbul, Türkiye, was used as a test compound.

Animals and experimental model

This study began following the approval of the Pamukkale University Animal Experiments Ethics Committee. In this study, thirty-two male-aged rats (aged between 24-28 months) were purchased from Pamukkale University Experimental Surgery Application and Research

Center, Denizli, Türkiye. The rats were checked daily at regular intervals under the supervision of a veterinarian. Rats were housed in cages in the experimental animal unit at a temperature of 22±1°C, 50% humidity, 12 hours of light/darkness, and regular ventilation. To feed the rats, standard rat food and fresh drinking water were given ad libitum every day. The control group of rats received 1 ml of physiological saline orally by gastric gavage daily for 28 days. Boric acid was applied by oral gastric gavage for 28 days at the dose ranges specified in Table 1.

Table 1. The procedure process and experimental groups

Groups	28. days	29. days
Control group	1mL oral saline was given for twenty-eight days	Rats in all groups will be sacrificed on day 29 of the experiment
Boric acid 10 mg/kg group (L-BA)	10 mg/kg of boric acid was given for twenty-eight days	
Boric acid 20 mg/kg group (M-BA)	20 mg/kg of boric acid was given for twenty-eight days	
Boric acid 40 mg/kg group (H-BA)	40 mg/kg of boric acid was given for twenty-eight days	

After the application period, which lasted a total of 28 days, the animals were sacrificed by anesthetizing with a combination of xylazine HCl and ketamine HCl after a 24-hour fast following the last application. Samples of blood were extracted from the abdominal aorta of each rat into lithium heparin and serum separator tubes. After centrifugation of blood samples at 3000 rpm for 10 minutes at 4°C, plasma and serum samples were obtained.

Determination of TNF-α and IL-6

Plasma TNF-α (Cat. No. E0764Ra) and IL-6 (Cat. No. E0135Ra) levels were determined using a commercial rat ELISA kit from Bioassay Technology Laboratory, according to the manufacturer's instructions.

Biochemical evaluation

Albumin (ALB), total protein (TP), globulin (GLO), calcium (CA), creatine (CRE), phosphorus (P), total bilirubin (TBIL), blood urea nitrogen (BUN), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatine kinase (CK), amylase (AMY), glucose (GLU), and cholesterol (CHOL) were measured from serum samples using the MNCHIP Veterinary Chemistry Analyzer Pointcare v3 device. To determine oxidant and antioxidant markers in the liver and kidney tissues of rats, the tissues were first washed with 0.9% NaCl. Subsequently, tissues were homogenized at a 1:40 w/v ratio in 0.1 M phosphate buffer (PH=7.4). The tissue homogenates were then centrifuged at 4°C and 3500 rpm for 10 minutes to obtain the supernatant [13]. The supernatants that were collected were used to test the activities of glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA). The MDA concentration in tissue supernatants, an indication of lipid peroxidation, was measured using Ohkawa et al. [14] methods. The GSH concentration was determined in tissue supernatants using the method presented by Beutler et al. [15]. For SOD enzyme activity, Sun et al. [16] and for CAT enzyme activity, using the method described by Aebi [17] were used.

Statistical analysis

The results obtained from the study were analyzed in SPSS 28.0 (IBM SPSS Statistics 28 software (Armonk, NY: IBM Corp.)). GraphPad Prism 9.5 (GraphPad Software, San Diego, CA, USA) was used for graphical presentations. All results are expressed in mean±standard error of the mean (SEM). The normal distribution of the data was performed by the Shapiro-Wilk test, and data normality was found to be greater than 0.05. In addition, the assumption of homogeneity of the variance of the data was examined by Levene's statistical test, and a value greater than 0.05 was obtained. When the parametric test assumptions were met, one-way analysis of variance was used to compare independent group means, and the level of statistical significance between the means was determined by the Duncan test. In all analyses, $p<0.05$ was considered statistically significant.

Results

In the study, the effects of oral boric acid supplements administered at pharmacological doses [3] on aged rats on serum biochemical parameters are given in Table 2. While oral boric acid supplementation did not affect GLO, AMY, ALP, and CRE levels (p>0.05), it was observed to significantly increase ALB, TP, and CA levels without any dose-related statistical effect (p<0.05). The GLU and CK levels significantly increased in the boric acid group compared with the control group (p<0.05). Also, the M-BA and H-BA groups demonstrated a similar statistical rate of increase in GLU and CK levels (p>0.05).

The P level was not different between the control and L-BA groups (p>0.05), but considerably increased in the M-BA and H-BA groups compared to the control and L-BA groups (p<0.05). The H-BA group demonstrated a significant reduction in CHOL levels compared to the control and boric acid groups (p<0.05). BUN levels were statistically similar in control and H-BA doses, whereas L-BA and M-BA doses significantly increased BUN levels (p<0.05). Significant increases in ALT and TBIL levels were observed in the M-BA and L-BA groups, respectively (p<0.05).

Table 2. Effects of boric acid supplementation on serum biochemical parameters (n=8)

Parameters	Control	L-BA	M-BA	H-BA
ALB (g/dL)	2.56±0.14^{b}	3.20±0.12^{a}	3.30±0.08^{a}	3.31±0.06^{a}
TP (g/dL)	6.03±0.13^{b}	6.74±0.09^{a}	6.81±0.16^{a}	6.82±0.08^{a}
GLO (g/dL)	3.61±0.09	3.61±0.11	3.43±0.10	3.51±0.12
CA (mg/dL)	8.08+0.10^{b}	9.48±0.13^{a}	9.60±0.11^{a}	9.64±0.06^{a}
CRE (mg/dL)	0.48±0.06	0.52±0.02	0.54±0.05	0.49±0.03
P (mg/dl)	6.08±0.20^{b}	6.56±0.26^{b}	7.50±0.15^{a}	7.66±0.19^{a}
TBIL (mg/dL)	0.18±0.02^{b}	0.24±0.02^{a}	0.22±0.01^{ab}	0.19±0.02^{ab}
BUN (mg/dL)	15.54±0.46^{c}	16.95±0.41^{b}	18.08±0.25^{a}	16.08±0.26^{bc}
ALT (U/L)	48.00±1.83^{b}	46.25±1.92^{b}	55.25±2.09^{a}	48.37±1.44^{b}
ALP (U/L)	152.75±9.28	128.00±10.59	130.75±6.30	135.50±7.76
CK (U/L)	587.25±15.99^{c}	710.37±22.80^{b}	771.56±25.98^{a}	885.12±25.87^{a}
AMY (U/L)	522.25±29.40	527.25±14.13	559.88±21.64	526.63±15.86
GLU (mg/dL)	175.12±4.82^{c}	201.50±6.05^{b}	229.37±7.31^{a}	240.75±6.03^{a}
CHOL(mg/dL)	115.38±6.04^{a}	119.50±7.56^{a}	118.00±4.85^{a}	91.50±5.55^{b}

The values were expressed as means ± SEM
^{a,b,c} In the same line values with different letters show statistically significant differences in serum (p<0.05)
ALB: Albumin, TP: Total Protein, GLO: Globulin, CA: Calcium, CRE: Creatine, P: Phosphor TBIL: Total bilirubin, BUN: Blood Urea Nitrogen
ALT: Alanine Aminotransferase, ALP: Alkaline Phosphatase, CK: Creatinine Kinase, AMY: Amylase, GLU: Glucose
CHOL: Cholesterol.L-BA: Boric Acid 10mg/kg, M-BA: Boric Acid 20mg/kg, H-BA: Boric Acid 40mg/kg

In the study, the effects of oral boric acid supplements administered at pharmacological doses to aged rats on liver MDA, GSH, SOD, and CAT activities are given in Table 3. Boric acid did not statistically affect MDA levels in the L-BA group (p>0.05) and significantly reduced MDA levels in the M-BA and H-BA groups (p<0.05). Boric acid significantly increased dose-dependent GSH levels compared to the control group (p<0.05). Boric acid did not statistically affect SOD levels in the L-BA group (p>0.05) and increased SOD levels in the M-BA and H-BA groups (p<0.05). Boric acid did not statistically affect CAT levels in the L-BA group and dose-dependently increased CAT levels in the M-BA and H-BA groups.

Table 3. Effects of boric acid supplementation on MDA, GSH, SOD, and CAT activities in the liver tissue of rats (n=8)

Groups	Liver MDA (nmol/g tissue)	Liver GSH (nmol/g tissue)	Liver SOD (nmol/g tissue)	Liver CAT (nmol/g tissue)
Control	4.51±0.52^{a}	19.52±0.64^{c}	3.24±0.16^{c}	1.48±0.11^{c}
L-BA	4.35±0.32^{a}	23.82±0.74^{b}	3.69±0.13^{c}	1.78±0.09^{c}
M-BA	3.13±0.38^{b}	25.06±1.29^{b}	4.30±0.72^{b}	2.18±0.10^{b}
H-BA	1.57±0.12^{c}	31.44±1.25^{a}	5.19±0.23^{a}	2.51±0.12^{a}

The values were expressed as means ± SEM, ^{a,b,c} Different letters in the same column represent statistically significant differences (p<0.05)
MDA: Malondialdehyde, GSH: Glutathione, SOD: Superoxide Dismutase, CAT: Catalase, L-BA: Boric Acid 10mg/kg
M-BA: Boric Acid 20mg/kg, H-BA: Boric Acid 40mg/kg

In the study, the effects of oral boric acid supplements administered at pharmacological doses to aged rats on kidney MDA, GSH, SOD, and CAT activities are given in Table 4. Boric acid did not statistically affect MDA levels in the L-BA group (p>0.05) and significantly reduced MDA levels in the M-BA and H-BA groups (p<0.05). Boric acid significantly increased dose-dependent GSH levels compared to the control group (p<0.05). Boric acid did not statistically affect SOD levels in the L-BA group (p>0.05) and increased SOD levels in the M-BA and H-BA groups (p<0.05). Boric acid did not statistically affect CAT levels in the L-BA group and increased CAT levels in the M-BA and H-BA groups (p<0.05).

Table 4. Effects of boric acid supplementation on MDA, GSH, SOD, and CAT activities in the kidney tissue of rats (n=8)

Groups	Kidney MDA (nmol/g tissue)	Kidney GSH (nmol/g tissue)	Kidney SOD (nmol/g tissue)	Kidney CAT (nmol/g tissue)
Control	7.15±0.63^{a}	19.86±0.55^{c}	3.98±0.21^{c}	0.44±0.02^{b}
L-BA	6.69±0.43^{ab}	25.36±0.50^{b}	4.31±0.27^{bc}	0.54±0.03^{b}
M-BA	5.21±0.61^{bc}	30.40±0.77^{a}	4.83±0.23^{ab}	0.73±0.04^{a}
H-BA	4.83±0.34^{c}	32.28±1.30^{a}	5.06±0.19^{a}	0.89±0.04^{a}

The values were expressed as means ± SEM, ^{a,b,c} Different letters in the same column represent statistically significant differences (p<0.05)
MDA: Malondialdehyde, GSH: Glutathione, SOD: Superoxide dismutase, CAT: catalase, L-BA: Boric Acid 10mg/kg
M-BA: Boric Acid 20mg/kg, H-BA: Boric Acid 40mg/kg

In the study, the effects of oral boric acid supplements administered at pharmacological doses to aged rats on plasma TNF-α levels are given in Figure 1. As shown in Figure 1, boric acid caused a significant dose-dependent decrease in TNF-a levels in the other study groups compared to the control group (p<0.05).

In the study, the effects of oral boric acid supplements administered at pharmacological doses to aged rats on plasma IL-6 levels are given in Figure 2. As demonstrated in Figure 2, L-BA decreased IL-6 levels in comparison to the control group, but the difference was not statistically significant. Boric acid significantly reduced IL-6 levels in the M-BA and H-BA groups in a dose-dependent manner compared to the control group (p<0.05).

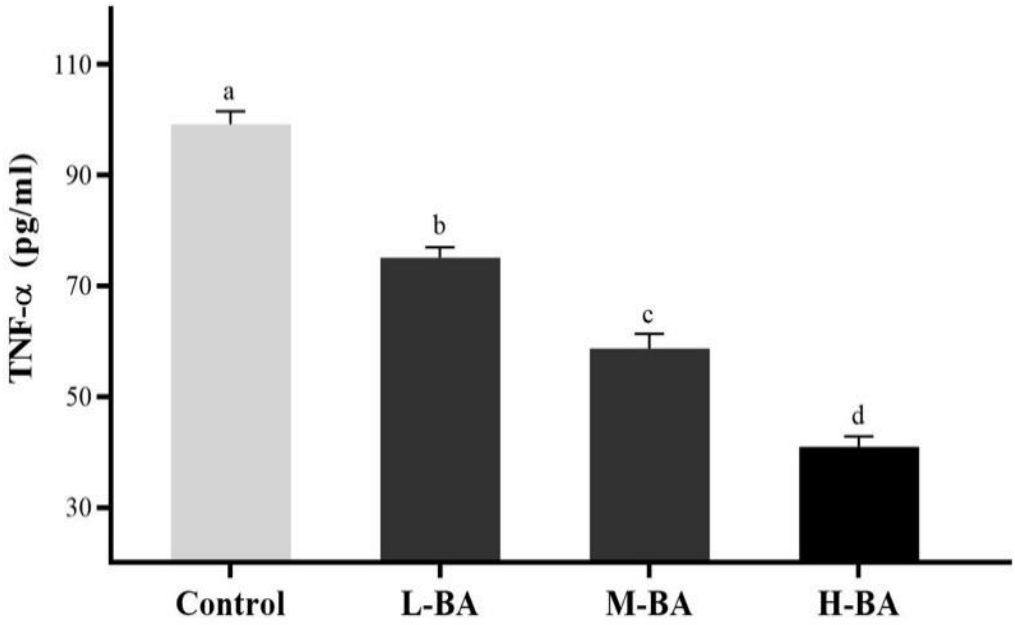

Figure 1. Plasma TNF-α levels in boric acid-treated rats with pharmacological dose

Each value represents the mean±SEM (n=8). The letters (a, b, c, d) indicate statistically significant differences between the groups, $p<0.05$
L-BA: Boric Acid 10mg/kg, M-BA: Boric Acid 20mg/kg, H-BA: Boric Acid 40mg/kg

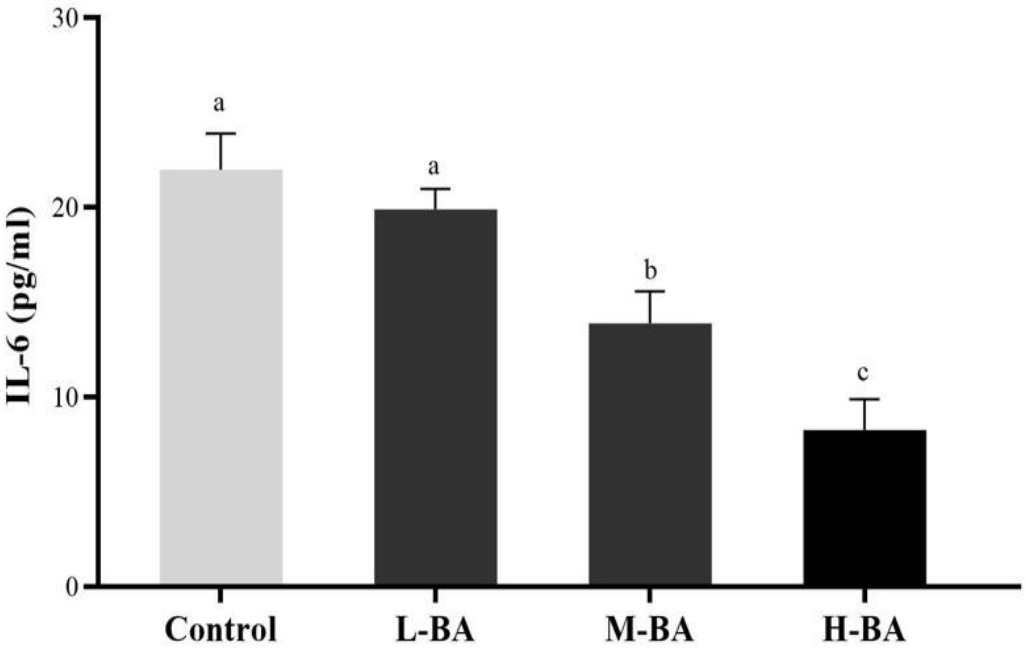

Figure 2. Plasma IL-6 levels in boric acid-treated rats with pharmacological dose

Each value represents the mean±SEM (n=8). The letters (a, b, c) indicate statistically significant differences between the groups, $p<0.05$
L-BA: Boric Acid 10mg/kg, M-BA: Boric Acid 20mg/kg, H-BA: Boric Acid 40mg/kg

Discussion

The study investigated how the pharmacological dose of boric acid supplementation affects serum biochemical parameters, liver and kidney tissue oxidant and antioxidant enzyme levels, and IL-6 and TNF-α proinflammatory cytokine levels, which are important in immune system regulation.

The liver is the primary source of serum proteins and produces albumin, globulin, fibrinogen, and the majority of other clotting factors [18]. In the study, oral boric acid supplementation increased serum TP and ALB levels equally in all doses compared to the control group and did not affect GLO levels. Similarly, previous studies have shown no effect on GLO levels when boric acid is added to broiler diets [19] and when rabbits were administered boric acid dose-dependently [20]. In addition, Kan and Kucukkurt [21] showed that 10 and 20 mg/kg of boric acid supplementation did not change the TP level in rats compared to the control group. In contrast, TP and ALB levels in aged rats increased in a dose-independent manner in the boric acid groups compared to the control group. This finding suggests that different animal species do not respond in the same way to boric acid supplementation. This could be due to the study used aged rats and administered boric acid orally.

Considering the reports that dietary or supplemental boric acid affects many biochemical parameters [22, 23], it is important to reveal the effects of dose-dependent boric acid supplementation on biochemical parameters in aged rats. In the study, serum calcium concentrations increased in rats supplemented with oral boric acid for 28 days. This study is consistent with reports that the boron diet prevents prenatal metabolic disorders by increasing serum calcium levels and dietary boron intake increases calcium content in the femur bones of rats [24] and chickens [25]. In addition, the increase in fracture strength in the lumbar vertebrae of rats [26], femur bones [6] and layers [27] of rabbits with the addition of boron to diets supports this study. Also, it has been reported that boron species, which have an essential function in bone development and mineralization, ensure the incorporation of calcium into the bone, joints, and cartilage, leading to a significant improvement in bone development observed in 95% of the patients [28]. The findings of the study suggest that oral boric acid intake may be a useful tool for increasing serum calcium levels and that dose-dependent supplementation may not provide additional benefits.

In this study, creatine did not change depending on the dose of oral boric acid. This finding is similar to reports showing that serum creatinine levels were unchanged when boron and boric acid were added to the diets of rabbits [20] and rats [21]. In addition, the dose-dependent effect of boron was reported to cause an increase in creatinine levels in rats exposed to acrylamide [29]. When the impact of boric acid on creatinine levels is examined, different results emerge. In most of the studies, creatinine levels were not affected by boric acid supplementation in a dose-dependent manner, as in this study. We also analyzed that creatine kinase enzyme activity was significantly higher in all boric acid groups compared to the control group. This finding was consistent with previous research [19] showing boron supplementation in broiler diets at levels of 750 and 1000 mg/kg increased creatine kinase enzyme activity compared to the control group.

During the aging period, several cellular and physiological changes occur in the liver tissue, which has a remarkable ability to renew and maintain its function throughout life [30]. It has been observed that serum AST, ALT, ALP, and bilirubin activities are commonly used to assess liver structural integrity [31]. Kan and Kucukkurt [21] reported that the different doses of boron supplementation had no statistically significant effect on serum ALP activity levels in rats compared to the control group. In the present study, serum ALP levels did not change in the L-BA and H-BA groups but increased only in the M-BA group. This is in line with the previous report that there was no effect on ALT levels in rats given boric acid at a dose of 10 mg/kg [21]. In addition, when oral borax decahydrate was given to rabbits at a dose of 10 mg/kg, it did not affect ALT levels [32]. In this study, TBIL levels increased only in the L-BA group. Indeed, these results show that BA produces different effects on the liver depending on the dose.

Previous reports have indicated that boron supplements can alter the amounts of nitrogen metabolites in blood and urine by affecting the

consumption of certain amino acids or proteins [22]. When compared with the control group, the BUN level was statistically higher in the group given L-BA and M-BA boric acid. Similarly, it was reported that boron given with acrylamide caused an increase in urea nitrogen level [29]. In addition, similar to our study, boron has been shown to increase serum urea levels in laying hens [33]. However, it was reported that increased serum BUN levels in gentamicin-treated rats decreased in dose-dependent boron groups (5, 10, and 20 mg/kg) [34]. The main reason for the different results of the studies on the effect of boric acid on serum BUN activity may be due to differences between doses and administration methods. Therefore, further studies with different doses and administration methods should be performed to clarify the issue in aged rats.

The increased blood GLU levels of boric acid administered orally in the study support the previous report [35] on increased blood GLU levels when rats were given feed enriched with boric acid at doses of 250, 500, and 1000 ppm. Some studies have shown that boron does not affect increasing or decreasing blood glucose levels [21]. This may be due to differences in the dose, route of administration, absorption, distribution, and catabolism of boric acid with age, sex, and race differences between living organisms.

In a recent study, it was concluded that boron supplements affect lipid metabolism as a result of the relationship between total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride levels in rats fed a high-fat diet [36]. In our study, except for an important decrease in the H-BA group, the other study groups did not affect serum CHOL levels in aged rats. This finding is in agreement with the report [37] that dietary boron supplementation in Japanese quail at a dose of 1000 mg/kg compared to 500 mg/kg and 750 mg/kg boric acid supplementation decreased total cholesterol levels. In this case, boron-containing drugs could have a reducing effect on serum cholesterol levels.

Free oxygen radicals are highly reactive and can cause damage to cell membranes and organelles through lipid peroxidation [9]. In our study, MDA levels, which indicate lipid peroxidation, decreased significantly in the liver and kidney tissues of aged rats given oral boric acid supplementation at M-BA and H-BA dosages. We also found that oral boric acid supplementation dose-dependently increased GSH levels, SOD, and CAT antioxidant enzyme activities in aged rats. Consistent with our results, oral boric acid supplementation acted as a scavenger of superoxide, hydroxyl radical, and singlet molecular oxygen [38]. Similarly, Kar et al. [39] found a substantial decrease in MDA levels in the group given boric acid after ischemic reperfusion injury of kidney tissue, while an increase in SOD, CAT, and GSH levels was observed. However, low doses of boric acid supplementation (15-50 mg/L) did not alter MDA and CAT concentrations in human peripheral blood cultures [40]. It was also reported that boric acid supplementation in rats does not change the MDA level in liver and kidney tissues, while SOD and CAT antioxidant enzyme parameter levels decrease [9]. The reason for the difference may be the difference in the dose of boric acid used in the study, the method of administration, the duration, and the age of the rats used. Indeed, this suggests that oral boric acid supplementation may play a significant role in maintaining homeostasis by increasing the activity of these enzymes in a dose-dependent manner.

It is known that boric acid affects the function of the immune system [41]. The need for boric acid can vary greatly due to infection, increased inflammation, and metabolic disorders, and this can be particularly pronounced for malnourished individuals. In particular, diet boron is known to help control the normal inflammatory process by serving as a signaling suppressor that down-regulates certain enzymatic activities typically elevated at the site of inflammation [42]. In the present study, a decrease in IL-6 and TNF-α cytokine levels was observed in aged rats due to the pharmacologic dose of boric acid. It has been similarly shown that boron supplementation decreased proinflammatory cytokine levels such as IL-6 and TNF-α [43]. This study was also consistent with the report [44] that boric acid supplementation significantly decreased TNF-α proinflammatory cytokine levels, which play an important role in the pathophysiology of the knee osteoarthritis model in rats. In the previous study, oral boric acid supplementation

similarly reversed BPA-induced TNF-α, IL-6, and IL-1β expression levels [45] as well as acrylamide-induced TNF-α expression levels [29]. These results show that giving aged rats a boric acid supplement in pharmacologic doses lowers their elevated levels of IL-6 and TNF-α.

In summary, this study demonstrated that dose-dependent supplementation of boric acid has ameliorative effects on lipid peroxidation, immunomodulation, and regulation of many blood biochemical parameters in aged rats. The study found that boric acid supplementation reduced systemic inflammation markers such as IL-6 and TNF-α. This suggests that boric acid may help reduce the risk of inflammatory diseases in the elderly. Boric acid contributes to the maintenance of antioxidant capacity, which is an important approach in the treatment of aging-related disorders. Furthermore, oral boric acid supplementation for humans and animals would be useful to determine its nutritional value. In the future, our study will provide perspectives that will contribute to new studies to compare the effects of different doses and routes of administration of boric acid in different age and gender groups.

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Author contributions

M.B. and M.F.D. designed the study and provided the relevant materials and analytical tools for experiments. M.B. and M.F.D. performed the experiments. M.B. and M.F.D. analyzed the data. M.B. wrote the manuscript.

Research Article

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Repair of vesicovaginal fistula with transvaginal and abdominal technique: Pamukkale University Urology Clinic's results

Vezikovajinal fistülün transvajinal ve abdominal teknik ile onarımı: Pamukkale Üniversitesi Üroloji Kliniği sonuçları

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Abstract

Purpose: Vesicovaginal fistula (VVF) is a pathological condition that causes urinary incontinence from a tract between the bladder and the vagina, negatively affecting the quality of life, social life, and patient health. The preferred method for VVF repair depends on the patient's characteristics, the features of the fistula, and the surgeon's experience. There is no definitive guideline for choosing between vaginal and abdominal VVF repair. This study aims to retrospectively evaluate VVF cases operated on in our clinic, comparing and interpreting patient characteristics and outcomes with the literature.

Materials and methods: The data of 35 patients who underwent vaginal and abdominal VVF repair in our clinic were evaluated retrospectively.

Results: Transvaginal repair was preferred in 23 (65.7%) of the patients and abdominal repair was preferred in 12 (34.3%). The success rate of VVF surgery performed in our clinic was determined to be 88.6%. Success rates were similar; 91.4% in vaginal repair and 83.4% in abdominal repair. Recurrence was observed in 2 of 23 patients (8.6%) who underwent transvaginal repair and in 2 of 12 patients (16.6%) who underwent abdominal repair.

Conclusion: In vesicovaginal fistula surgery, patient characteristics and fistula characteristics guide the preferred surgery. However, the surgeon's experience also plays a big role. Vaginal and abdominal VVF surgeries are performed with similar high success rates.

Keywords: Vesicovaginal fistula, vaginal vesicovaginal fistula repair, abdominal vesicovaginal fistula repair.

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Öz

Amaç: Vezikovajinal fistül (VVF), mesane ve vajina arasındaki bir trakttan idrar kaçırmaya sebep olan, yaşam kalitesini, sosyal hayatı ve hasta sağlığını olumsuz etkileyen patolojik bir durumdur. VVF onarımında tercih edilen yöntem hastanın, fistülün özelliklerine göre ve cerrahın deneyimine göre yapılmaktadır. Vajinal ve abdominal VVF onarımı tercihinde kesinleşmiş kılavuz bilgileri bulunmamaktadır. Bu çalışmada, kliniğimizde opere edilen VVF vakaları retrospektif olarak değerlendirilerek hasta özelliklerinin ve sonuçlarının literatür ile karşılaştırılarak yorumlanması amaçlanmaktadır.

Gereç ve yöntem: Bu çalışmada kliniğimizde vajinal ve abdominal VVF onarımı yapılan 35 hastanın verileri retrospektif olarak değerlendirildi.

Bulgular: Hastaların 12'sinde (%34,3) abdominal yöntem tercih edilirken 23 (%65,7) hastada transvajinal yöntem tercih edildi. Kliniğimizde uygulanan VVF cerrahisinin başarı oranı %88,6 olarak tespit edilmiştir. Vajinal onarımda %91,4, abdominal onarımda ise %83,4 olarak birbirine yakın başarı oranları görüldü. Transvajinal teknik tercih edilen 23 hastanın 2'sinde (%8,6) ve abdominal teknik tercih edilen 12 hastanın ise 2'sinde (%16,6) nüks izlendi.

Sonuç: Vezikovajinal fistül cerrahisinde hastanın ve fistülün özellikleri tercih edilecek cerrahiye yön gösterse de özellikle cerrahın deneyimi büyük rol oynamaktadır. Vajinal ve abdominal VVF cerrahisi birbirine benzer ve yüksek başarı oranları ile uygulanmaktadır.

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Anahtar kelimeler: Vezikovajinal fistül, vajinal vezikovajinal fistül onarımı, abdominal vezikovajinal fistül onarımı.

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Introduction

Vesicovaginal fistula (VVF) is a pathological condition that causes urinary incontinence from a tract between the bladder and the vagina, negatively affecting the quality of life, social life and patient health [1]. Common causes in developed countries include bladder injury during gynecological pelvic surgery and pelvic radiotherapy. In developing countries, the most common cause is vaginal birth [2]. Vaginal necrosis and bladder necrosis, especially during difficult labor due to cephalopelvic discordance, increase the frequency of VVF [2]. The incidence of VVF in the population can reach 2% [3]. Although there may be variations depending on the hysterectomy technique, it has been generally demonstrated that VVF associated with hysterectomy occurs in the range of 0.02% to 0.22% [1, 4].

Vesicovaginal fistulas can be classified in various ways. Generally, small fistulas (≤0.5 cm), without exposure to radiation and occurring in isolation, are termed simple fistulas. On the other hand, large-sized fistulas (≥2.5 cm), with radiation exposure and unsuccessful fistula repairs, are defined as complex fistulas. Fistulas developed after radiation exposure are often multiple and challenging to treat due to poor tissue viability. Medium-sized fistulas (between 0.5 and 2.5 cm) are commonly considered complex fistulas by most authors [5]. Surgical inflammatory reaction reduction in the fistula area is necessary for vesicovaginal fistula operation, and therefore, the operation is planned 3-6 months after the onset of symptoms [6]. During this period, the patient is catheterized, and a decrease in postoperative inflammation and edema is anticipated. This conservative approach may result in spontaneous recovery in about 15-20% of cases with simple fistulas [5].

Small and uncomplicated cases can be attempted to be treated conservatively and with minimal invasive methods, but surgical treatment is the primary method of repair [7]. Factors such as the location and size of the fistula, previous surgeries, history of VVF operations, the need for simultaneous abdominal surgery, history of radiotherapy, patient preference, and the surgeon's experience are considered in the selection of the surgical technique [5]. There are both transvaginal and abdominal repair techniques available for VVF surgery. However, due to lower operative complications, shorter hospital stays, less blood loss, and lower postoperative pain, and additionally similar success rates with abdominal VVF repair, the vaginal approach is more commonly preferred [8]. The abdominal technique is chosen when vaginal repair is not possible. When success rates are generally examined, the transvaginal technique varies between 86-91%, and the abdominal technique ranges from 84-100% [3]. There are limited randomized controlled studies in the literature comparing abdominal and vaginal approaches [3].

In this study, a retrospective evaluation of VVF cases operated on in our clinic is conducted. The aim is to compare and assess patient characteristics and outcomes with the existing literature and interpret the success of the current practices in our clinic.

Material and method

In the study, 35 patients who underwent VVF surgery in our clinic between January 1, 2012 and November 1, 2022 were evaluated retrospectively. During the postoperative period, patients were monitored for sexual function, urinary function, complications, and recurrence. For the purposes of this study, women with recto-vaginal fistula or other causes of urinary incontinence were excluded. Permission was obtained from Pamukkale University Non-Interventional Clinical Research Ethics Committee for the study.

As a transvaginal technique, the optically guided transvaginal repair method applied in our clinic was used [9]. The patients underwent a cystoscopy before fistula repair. Retrograde pyelography (RGP) was conducted on both ureters to rule out ureteral injury. A thin Foley catheter was inserted into the fistula to provide

traction. After fistula catheterization, surgical dissection and suturing techniques were performed under 5 mm, 30 degree optical lens vision. The fistula was released from the surrounding tissues to the healthy tissues, and first the bladder mucosa, then the layers containing the detrusor and serosa were closed separately in two layers. After that, the fistula was repaired in 3 layers in total, with the vagina perpendicular to this line. The urethral foley was removed 14 days later.

Before the repair with the abdominal technique, a cystoscopy was performed on the patients and fistula characteristics were determined. RGP was performed on both ureters, ureteral damage was evaluated, and a ureteral catheter was placed in the ureters in the necessary patients, depending on the location of the fistula. After the subumbilical incision, the peritoneum was opened and the bladder was found. The bladder was opened with a vertical incision. The fistula tract was reached by visualizing the fistula mouth. The bladder and vaginal wall were dissected with sharp dissections. The vaginal opening was sutured. Fixation sutures were placed to create an omental flap between the bladder and vagina. The bladder was repaired as double-layer waterproof, and a leak test showed no leakage. The omental flap was brought between the vagina and bladder and fixed with fixation sutures. Urethral foley was removed 14 days later.

Statistics and analysis

The data was analyzed using SPSS 25.0 statistical software package. Number, percentage, average and minimum-maximum expressions were used for descriptive statistics.

Results

The average age of the patients was calculated as 47.17±7.09. The general characteristics of the patients are presented in Table 1. All of the patients included in the study had a history of gynecological surgery; 32 (91.4%) had a history of hysterectomy, 2 had a myemectomy, and 1 had an oophorectomy.

Table 1. Patient characteristics

	Transvaginal Repair	Abdominal Repair	Total
Number of patients	23 (65.7%)	12 (34.3%)	35
VVF surgery history	-	9 (75%)	9
Simultaneous abdominopelvic surgery	-	2 (16.6%)	2
History of pelvic radiotherapy	-	1 (8.3%)	1

While the abdominal method was preferred in 12 (34.3%) of the patients, the transvaginal method was preferred in 23 (65.7%) patients. It was noteworthy that 9 of the patients who underwent abdominal technique had a history of VVF surgery and 1 patient had a history of radiotherapy. Recurrence was observed in 2 of 23 patients (8.6%) for whom the transvaginal technique was preferred and in 2 of 12 patients (16.6%) for whom the abdominal technique was preferred, and no significant difference was found between the two methods in terms of recurrence.

After transvaginal surgical treatment, recurrence was observed in one patient, where a single fistula was localized on the opposite wall, and in another patient, three millimeter-sized fistulas were identified. Among the patients who underwent abdominal technique and experienced recurrence, one had a large fistula tract (4 cm), while the other exhibited adhesions and poorly healed tissues due to a previous VVF operation. Following the recurrence after abdominal VVF repair, two patients underwent a second abdominal VVF repair. For the two patients with recurrence after vaginal VVF surgery, a repeat vaginal VVF repair was performed, and in the follow-up of both groups, no further recurrences were observed. During the follow-up of the patients participating in the study, 1 patient developed

pain during sexual intercourse (dyspareunia) and 1 patient developed stress incontinence. Two of these patients were in the abdominal repair group. Additionally, in two patients who underwent abdominal VVF repair, simultaneous ureteroneocystostomy was performed.

The absence of recurrence was considered a success criterion, and the success rate was found to be 91.4% for the transvaginal technique, 83.4% for the abdominal technique. Overall, the success rate was found to be 88.6% (Table 2). Fistula characteristics are presented in Table 3.

Table 2. Recurrence and success rates of surgical techniques

	Transvaginal Repair (n:23)	Abdominal Repair (n:12)	Total (n:35)
Recurrence	2 (8.6%)	2 (16.6%)	4 (11.4%)
Success	21 (91.4%)	10 (83.4%)	31 (88.6%)

Table 3. Fistula characteristics

	Transvaginal Repair (n:23)		Abdominal Repair (n:12)		Total (n:35)	
Fistula locations within the bladder	Base	20 (87%)	Base	5 (41.7%)	Base	25 (71.4%)
	Anterior Wall	2 (8.7%)	Anterior Wall	3 (25%)	Anterior Wall	5 (14.3%)
	Trigonal Area	1 (4.3%)	Trigonal Area	4 (33.3%)	Trigonal Area	5 (14.3%)
Average Fistula Size (mm)	9 mm (5-20)		21 mm (15-40)		12 mm (5-40)	
Average Number of fistulas	1.13 (1-3)		1.17 (1-2)		1.14 (1-3)	

Discussion

Most vesicovaginal fistula repairs are performed by a small number of vesicovaginal fistula surgeons, even in regions with high prevalence [10]. In our clinic, VVF surgery is performed with a success rate of 88.6%. It was observed that vaginal (91.4%) and abdominal (83.4%) VVF surgery had similar success rates. The success rates of VVF repair with the optically guided transvaginal approach applied in our clinic are in line with the literature [3].

In the etiology of vesicovaginal fistula, a history of hysterectomy is frequently observed, accounting for 80% of cases [1]. In our clinic, 91% of patients who underwent VVF repair had a history of hysterectomy. Hysterectomy emerged as a prominent etiological factor in this patient group as well. It is noteworthy that all VVF patients presenting to our clinic had a history of gynecological surgery as the etiology, with none having a history of obstetric surgery.

Pelvic gynecological surgeries should be approached with caution in the presence of adhesions. In cases where bladder injury is suspected, it is essential to examine the bladder with cystoscopy. Fistulas developed after hysterectomy performed for malignant reasons should be considered to potentially involve malignant tissue [11].

It is necessary to comply with certain principles in vesicovaginal fistula repair, as in other operations. There must be adequate vascular support tissue in the surgical field, and no necrosis, inflammation, or malignancy should be present. A multilayered suture line should be created with absorbable sutures that is tension-free, waterproof, and avoids overlapping sutures. Continuous bladder drainage should be ensured after surgery [6, 12]. The first operation has the highest success rate in vesicovaginal fistula repair. Although the cause, location, size and onset time of the fistula

are taken into consideration when choosing a surgical technique, the method chosen may generally depend on the training and experience of the surgeon. The best method is probably the one with which the surgeon is most familiar. In the reports given by surgeons who prefer vaginal and abdominal methods, they make the choice of method by adopting their individual preferences and reviewing their experiences in their own institutions [13].

The advantages of the vaginal approach are short surgery time, short hospital stay, and less blood loss [14]. As a surgical technique, the transvaginal method is preferred in rates ranging from 42-81% [3]. In our clinic, we preferred the vaginal method more frequently (65.7%).

It is known that some factors are effective in the choice of abdominal technique. Short vaginal length for sexual intercourse, high location of the fistula, complicated fistulas, recurrence after VVF surgery, scarred fistula, history of pelvic radiotherapy, additional abdominal intervention and the need for ureter reimplantation are the prominent situations in choosing the abdominal method [14]. History of fistula surgery, history of pelvic radiotherapy, and the need for simultaneous abdominal intervention were also decisive in the choice of abdominal technique in our clinic. In our clinic, we preferred abdominal repair with a rate of 34.3%. In the 10-year meta-analysis reviewed by Shrestha et al. [3], it was reported that abdominal VVF repair was performed at a rate of 49.5%.

Recurrence was observed in two patients who were operated on via the abdominal approach. There was no feature other than a history of VVF operation in one of the patients. In the other patient, we operated on a 4 cm fistula. We performed VVF surgery again with the abdominal method on these two patients and did not observe any recurrence during follow-up. Opinions have been reported in the literature that previous VVF surgery either increases or does not change the risk of recurrence [15]. In a study examining the success factor, it was stated that one of the main determinants was the fistula size, and even the presence of >3 cm fistula was at high risk for recurrence [15]. In our study, recurrence was observed in patients with the profiles predicted in the literature.

In this study, recurrence was observed in 2 (8.6%) of our patients to whom we preferred the vaginal approach. We repaired both of these patients using the vaginal method again due to the location of the fistula. Kapoor et al. [16] reported recurrence in 1 patient (3.1%) in their 32-patient vaginal VVF repair series. The absolute contraindication for vaginal repair of vesicovaginal fistula is the coexistence of fistulas with other abdomino-pelvic organs such as ureters, small and large intestine [5]. Situations such as radiation exposure, scarred, and recurrent fistulas increase the failure rate, and these are important factors that the surgeon should consider when choosing the operation based on personal experience. These should not be considered as absolute contraindications [5], but some authors view these conditions as contraindications for VVF vaginal repair [16].

Pushkar et al. [17] evaluated the results of the vaginal approach for 210 patients who developed radiation-induced VVF. The success rate of the first repair was reported as 48.1%. They stated that the cumulative success rate was 80.4% after 3 recurrent surgeries in patients with relapse. They reported that subsequent repairs did not reduce the chance of recovery but cumulatively increased recovery rates. They emphasized that failed treatments may be due to tissue reaction to radiation exposure and recommended that re-surgery of failed fistula repair should be considered as the first surgery. In another study examining 30 patients who developed VVF after gynecological pelvic surgery, 23 patients underwent abdominal repair, 7 underwent vaginal repair, and recurrence was observed in 3 patients within 1 month. They found the overall success rate of VVF surgery to be 90%. Additionally, radiation-induced VVF patients were examined as a separate group, but a high success rate was not achieved. Urinary diversion has been emphasized as a more preferable method for this group [18]. In our study, there was only 1 patient with radiation exposure and no recurrence was observed after the first repair.

Angioli et al. [5] reviewed the studies on VVF repair. In this study, they reported that the success rate of VVF surgery in patients without radiation exposure varied between 70% and

100%, and the average success rate was 92%. Similar success rates of 91% and 96% have been reported for vaginal and abdominal repair, respectively. Another study showed that the success rate of transvaginal repair (90.8%) was higher than transabdominal repair (83.9%) [10]. In a series including 52 VVF patients, 32 patients with simple fistulas underwent vaginal repair, and 20 patients with complex fistulas underwent abdominal repair. Recurrence occurred in 1 patient who underwent vaginal repair and in 2 patients who underwent abdominal repair. They achieved more successful results in VVFs with vaginal repair [16]. In our study, the absence of recurrence was considered as the success criterion, and we determined the overall success rate to be 88.6%. The success rate was calculated as 91.4% for the vaginal approach and 83.4% for the abdominal approach. The results demonstrate success rates similar to the literature [3, 5].

Abdominal VVF repair can also be performed using laparoscopic and robot-assisted laparoscopic approaches. The success rate in large series can reach up to 86%. While minimal invasive methods are not yet common in VVF surgery, they have been successfully used in surgery performed in a single region. Despite the many advantages of minimally invasive surgery, the most successful approach is the one the surgeon is most familiar with [19].

The limitations of our study are that the patient group in our study was evaluated retrospectively and only the absence of recurrence was taken as the success criterion. As a result, in vesicovaginal fistula surgery, the characteristics of the patient and the fistula affect the success rate and direct the surgery to be preferred. However, it should not be forgotten that the surgeon's experience also plays a big role. In our clinic, vaginal and abdominal VVF surgery are performed with similar high success rates.

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Authors' contributions to the article

K.K. constructed the main idea and hypothesis of the study. A.S. developed the theory and arranged/edited the material and method section. Discussion section of the article written by M.B.D., Y.O. and S.C. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

Case Report

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Transient neonatal diabetes mellitus: a case report

Geçici neonatal diyabetes mellitus: bir olgu sunumu

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Abstract

Neonatal diabetes mellitus (NDM) is defined as hyperglycemia in the first six months of life for at least two weeks and requiring insulin therapy. Although its incidence is 1 in 90000-160000 live births, its frequency rises to 1/30000 in societies such as our country, where consanguineous marriages are common. NDM may be seen as transient (50-60%), permanent or a part of syndromes. Clinical manifestations of NDM are intrauterine growth retardation, growth cessation, excessive urination, dehydration, and ketoacidosis.

Since hyperglycemia may develop due to many different reasons in premature or low birth weight babies, difficulties and delays may occur in diagnosis. If the high blood glucose level lasts longer than 7-10 days after excluding other causes of transient hyperglycemia, it is recommended to suspect NDM and perform genetic examination, especially in infants with blood glucose above 250 mg/dL.

Herein, a newborn, referred to our neonatal unit due to persistent hyperglycemia on the postnatal 15th day and diagnosed as transient NDM after excluding other causes of hyperglycemia, is presented due to the rarity of the disease.

Keywords: Congenital, neonatal diabetes mellitus, transient, diagnosis.

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Öz

Neonatal diyabetes mellitus (NDM), yaşamın ilk altı ayında en az iki hafta süren ve insülin tedavisi gerektiren hiperglisemi olarak tanımlanır. Görülme sıklığı 90000-160000 canlı doğumda bir olmasına rağmen ülkemiz gibi akraba evliliklerinin yaygın olduğu toplumlarda görülme sıklığı 1/30000'e kadar çıkmaktadır. NDM geçici (%50-60), kalıcı veya sendromların bir parçası olarak görülebilir. NDM'nin klinik belirtileri intrauterin büyüme geriliği, büyümenin durması, aşırı idrara çıkma, dehidratasyon ve ketoasidozdur.

Prematüre veya düşük doğum ağırlıklı bebeklerde birçok farklı nedene bağlı olarak hiperglisemi gelişebileceğinden tanıda zorluklar ve gecikmeler yaşanabilmektedir. Geçici hipergliseminin diğer nedenleri dışlandıktan sonra kan şekeri yüksekliği 7-10 günden uzun sürüyorsa özellikle kan şekeri 250 mg/dL'nin üzerinde olan bebeklerde NDM'den şüphelenilmesi ve genetik inceleme yapılması önerilir.

Burada postnatal 15. günde inatçı hiperglisemi nedeniyle yenidoğan ünitemize başvuran ve diğer hiperglisemi nedenleri dışlandıktan sonra geçici NDM tanısı alan bir yenidoğan, hastalığın nadir görülmesi nedeniyle sunulmaktadır.

Anahtar kelimeler: Konjenital, neonatal diyabet, geçici, tanı.

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Introduction

Neonatal diabetes mellitus (NDM) is defined as hyperglycemia in the first six months of life, persisting for at least 15 days, and requiring insulin therapy [1-3]. A blood glucose level of >150 mg/dL in newborns is defined as hyperglycemia. Hyperglycemia is common, especially in premature and low-birth-weight infants, due to stress, sepsis, and the use of drugs such as steroids and beta-adrenergic agents [1, 4]. After excluding the other causes of transient hyperglycemia, NDM should be suspected in infants whose blood glucose level is still high, especially in infants whose blood glucose level is above 250 mg/dL [1, 4, 5]. Although the frequency of NDM is reported as one in every 90000-160000 live births, its frequency increases in societies where consanguineous marriages are common [1, 6]. In a study covering 5 provinces in the Southeastern Anatolia region of our country, the incidence of NDM was found to be at least 1/30000 [7].

Clinical findings of neonatal diabetes are failure to gain weight, growth retardation, excessive urination, and secondary dehydration. If the diagnosis is delayed, severe ketoacidosis may also be added to these symptoms [8]. The birth weight of babies is generally low due to intrauterine insulin deficiency [9]. NDM can be transient, permanent or a part of syndromes. Transient NDM is present in 50% to 60% of cases with neonatal diabetes [1, 8]. Transient NDM goes into remission in the first 3-6 months of life, but recurrence may be seen in 40-50% of cases, especially during puberty, so it is recommended not to exclude infants from clinical follow-up. Clinically, it is very difficult to distinguish between transient and permanent diabetes. More than 20 genetic causes have been reported for transient or permanent NDM [1, 6]. Insulin is the essential therapy for neonatal diabetes, and an infusion pump is often used in insulin therapy because of the low-dose insulin requirement in newborns [1, 10]. Although rare, oral sulfonylurea therapy is also used in some types of NDM [11, 12].

In this article, a newborn, referred to our neonatal unit due to persistent hyperglycemia on the postnatal 15th day and diagnosed as transient NDM after excluding other causes of hyperglycemia, is presented because of its rarity.

Case report

The patient, born at 37 weeks' gestation to a 26-year-old mother, had respiratory distress and was admitted to the neonatal intensive care unit with the preliminary diagnosis of transient tachypnea of the newborn. After the therapies of appropriate parenteral fluid (60 mL/kg/day, 10% dextrose, 4.2 mg/kg/minute glucose infusion) and nasal continuous positive airway pressure, the respiratory symptoms of the patient were resolved completely on the second day. Enteral nutrition was started with breast milk on the first postnatal day.

According to the patient history, hyperglycemia (825 mg/dL) was detected on postnatal first day, and also it was observed that hyperglycemia (735 mg/dL) persisted despite appropriate fluid therapy (80 mL/kg parenteral 5% dextrose, 2.4 mg/kg/minute, and enteral nutrition). In addition, although the patient had normal blood gases, negative keton in the urine, and reduction of glucose infusion, its blood glucose level was higher than 250 mg/dL. Therefore, it was decided to start the insulin infusion at a dose of 0.01 U/kg/h; afterwards, the insulin dose was increased to 0.07 U/kg/h. The patient, whose glucose levels were regulated after insulin therapy and whose hyperglycemia recurred when insulin therapy was interrupted (>250 mg/dL), was admitted to our neonatal intensive care unit (NICU) with a prediagnosis of neonatal diabetes for further examination and therapy.

It was learned from the patient's history that her parents were first-degree cousins, and they did not have a known disease such as diabetes. In addition, the patient's mother did not use any medication during pregnancy. On physical examination, the patient's body weight was 1945 grams (<3 percentile), head circumference was 33 cm (10 percentile), and height was 47 cm (3-10 percentile). Her general condition was good; she was active, and her system examinations were normal. She was externally female and did not have a syndromic facial appearance. In the laboratory evaluation, electrolytes and kidney

and liver function tests were normal; acute phase reactants were negative. The patient, who was considered small for gestational age (SGA), had negative TORCH serology. Blood glucose level was 462 mg/dL, C- peptide was 0.08 ug/L (N:0.9-7.1 ug/L), insulin was 2.3 mU/L (N:2.6-24.9 mU/ L), urine ketone was negative, urine glucose was 2 positive, anti-insulin antibody, anti-glutamate decarboxylase (anti-GAD) and islet cell antibodies were negative. The patient's stool steactocrit value was 0.014%, and pancreas was seen to be structurally normal on magnetic resonance imaging. In consultation with the genetics department, an examination was performed for neonatal diabetes genes, but no known mutations were detected.

A sensor-assisted insulin pump was fitted to the patient on the second day of hospitalization in the NICU. Basal insulin was determined to be 0.025 u/h (0.012 u/kg/h), the insulin sensitivity factor was 300 mg/dL, and the carbohydrate/ insulin ratio was 45/1. She was fed orally with breast milk every three hours. If the pre-feeding blood glucose level was above 150 mg/dL, a bolus wizard was used, and if it was below, a manual bolus was administered (0.15U for 100-150 mg/dL, 0.10U for <100 mg/dL). If the blood glucose level was below 60 mg/dL, no insulin was administered. Postprandial blood glucose was measured 1.5 hours after feeding, and a correction insulin dose was administered with a bolus wizard at values of blood glucose above 300 mg/dL.

On postnatal day 57, it was observed that the patient's insulin requirement started to decrase. On postnatal day 59, the patient no longer needed insulin, and her blood glucose level was in the normal range (between 70-100 mg/dl). The patient, whose blood glucose was regulated and did not require insulin, was discharged on postnatal day 62 with a diagnosis of transient NDM, and clinical follow-up was planned.

Discussion

Neonatal diabetes can be recognized in the first few days of life, and it may be difficult to diagnose, especially in premature or low-birth-weight babies, as hyperglycemia may develop due to many different reasons [9]. The prevalence of hyperglycemia in premature infants is 25-75%. Hyperglycemia is often seen in the first days of life and usually returns to normal levels after a few days [13]. There are many causes of hyperglycemia in the neonatal period, such as parenteral glucose administration, sepsis, increased regulatory hormones due to stress, and drugs like steroids [2-4]. Diabetes should be suspected in a newborn if hyperglycemia lasts longer than 7-10 days, and genetic examination should also be recommended if it exceeds 2-3 weeks [1, 5]. In the examinations of our patient for the causes of transient hyperglycemia, acute phase reactants were negative, and blood and urine cultures showed no growth. On the 15th day, she was followed up with the diagnosis of NDM because her hyperglycemia continued and her insulin need continued.

Neonatal diabetes mellitus may be presented with growth retardation, dehydration, and ketoacidosis, which may be fatal if the diagnosis is delayed [5, 6, 14]. Severe hyperglycemia (blood glucose >360 mg/dl) may cause significant osmotic changes leading to intraventricular bleeding [5]. It is very important to measure serum glucose, urine ketone, serum c- peptide, and insulin levels in the initial evaluation of infants with suspected of having this disease. Radiological evaluation of the pancreas should be recommended for the presence or absence of the pancreas, because this will guide the diagnosis and treatment [5, 10, 15]. Our patient had a low birth weight, but normal growth was achieved by insulin therapy, and laboratory values were also compatible with NDM. No structural anomaly or agenesis was detected in the pancreas radiologically with an MRI.

Neonatal diabetes is detected frequently in the first 6 months of life, but rarely until the 12th month of life, and it is highly likely to be caused by a monogenic defect [6, 16]. Genetic mutations cause NDM development by disrupting pancreatic beta cell functions, leading to decreased insulin secretion, pancreatic hypoplasia/agenesis or beta cell damage [15, 17]. Therefore, genetic examination is recommended in all NDM cases [6, 17]. In a large international cohort study including patients clinically diagnosed with neonatal diabetes; it was reported that 80% of the patients had an underlying genetic defect. It has also been reported that genetic analysis should be performed regardless of time in patients presenting with acute severe hyperglycemia

(serum glucose higher than 1000 mg/dL) [1, 5, 14]. So, we analyzed the genetic mutations of the patient for NMD by next generation sequence analysis; however, no known mutations could be detected.

Neonatal diabetes may be divided into transient, permanent, and syndromic forms according to its phenotypic characteristics [1]. Duplication of genes (ZAC, HYMAI) in the 6q24 region is responsible for the majority of the transient forms. Mutations of *the KCNJ11* and *ABCC8 genes,* which encode the potassium channel subunit of the beta cell responsible for insulin secretion, are in the second frequency range. In transient NDM, remission is usually between 13-18 weeks; however, in about 40% of the cases, hyperglycemia may recur in later life. Mutations of *KCNJ11, ABCC8*, and insulin *(INS) genes* are responsible for the permanent form of NDM. The genotype-phenotype relationship is weak, and it is difficult to distinguish the transient/permanent form clinically [1, 16]. Remission was developed in our patient between 8-9 weeks, and no genetic mutation was detected.

Early diagnosis and initiation of insulin therapy are very important in terms of the metabolic effects of ketoacidosis and preventing the development of chronic and irreversible complications of diabetes [1]. If the blood glucose level is higher than 180-200 mg/dL and there is +2 glucosuria in a newborn, insulin therapy should be needed [18]. The prognosis and treatment options for monogenic forms of neonatal diabetes vary greatly depending on which gene is affected. 40% of neonatal diabetes patients have mutations in KCNJ11 and ABCC8 that affect the pancreatic beta cell K-ATP channel [19]. Patients with these mutations can be treated with oral sulfonylureas. Studies show that, unlike insulin, early sulfonylurea therapy may improve neurodevelopmental outcomes in sulfonylurea-responsive patients [11, 12, 20]; however, without genetic conclusions, empirical sulfonylurea therapy is not recommended. Insulin therapy was started for our patient. Insulin therapy was administered with an infusion pump, as it provided ease of administration at very low doses. While insulin therapy is administered with an infusion pump, a blood glucose sensor is simultaneously placed subcutaneously, and continuous blood glucose is monitored. No severe symptomatic hypoglycemia was experienced in the clinical follow-up. The presented patient's blood glucose was regulated between 8 and 9 weeks; she did not need insulin therapy on the 62nd day, so she was discharged with the consideration of transient NDM and followed up clinically.

In conclusion, if hyperglycemia in the newborn exceeds one week, neonatal diabetes should be suspected; if it exceeds two weeks, molecular genetic studies should be started; and it should not be forgotten that early diagnosis and treatment have very important effects on the clinical course of these infants with NDM.

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Consent to participate: The authors certify that they obtained all the appropriate patient consent forms. The patient's mother and father provided their consent for the patient's images and other clinical information to be reported in this journal. The patient's mother and father understand that the patient's name and initials will not be published, and that although due effort has been made to conceal the patient's identity, anonymity cannot be guaranteed.

Authors' contributions to the article

S.A.A., O.M. and A.O. constructed the main idea and hypothesis of the study. D.Y. and M.O. developed the theory and arranged/edited the material and method section. G.O.C. and H.E. have done the evaluation of the data in the results section. Discussion section of the article was written by G.S.D., O.M.A.O. and G.S.D. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

Case Report

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Malignancy smell in the air: widespread eroded, hemorrhagic, and lichenified plaques in an older man

Havada malignite kokusu var: yaşlı erkekte yaygın erode, hemorajik ve likenifiye plaklar

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Abstract

Paraneoplastic pemphigus (PNP) is a rare autoimmune mucocutaneous disease associated with underlying neoplasia. It is typically characterized by painful mucosal erosions and dark, patchy skin eruptions. A 66-year-old male was admitted to the internal medicine outpatient clinic with complaints of suddenly started rashes, loss of appetite, dyspepsia, weakness, and unexplained gross weight loss. The patient was cachectic, and physical examination revealed widespread eroded, erythematous, thick-middle, yellow-pitted, lichenified plaques on bilateral arms and legs and the hands and feet dorsum. Scattered seborrheic keratosis lesions on the trunk were also detected. He had microcytic anemia with elevated CA 19-9 measures. Abdomen computed tomography showed a malignant mass in the antrum. An endoscopic biopsy of the gastric mass revealed poorly differentiated adenocarcinoma composed of discohesive signet ring cells, and the skin punch biopsy was compitable compatible with paraneoplastic pemphigus.

In patients with rapidly developing skin lesions with constitutional symptoms, underlying malignancies should be kept in mind.

Keywords: Paraneoplastic pemphigus, Leser Trelat, gastric adenocarcinoma.

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Öz

Paraneoplastik pemfigus (PNP), altta yatan neoplazi ile ilişkili nadir bir otoimmün mukokutanöz hastalıktır. Tipik olarak ağrılı mukozal erozyonlar ve koyu yamasal döküntülerle ile karakterizedir. Altmış altı yaşında erkek hasta ani başlayan kızarıklık, iştahsızlık, hazımsızlık, halsizlik ve açıklanamayan aşırı kilo kaybı şikayetleri ile dahiliye polikliniğine başvurdu. Kaşektik olan hastanın fizik muayenesinde her iki kol ve bacakta, el ve ayak sırtında yaygın aşınmış, eritematöz, ortası kalın, sarı-çekirdekli, likenifiye plaklar saptandı. Gövdede dağınık seboreik keratoz lezyonları da olan hastanın yüksek CA 19-9 düzeyi ile mikrositer anemisi vardı. Karın bilgisayarlı tomografisinde antrumda malign bir kitle görüldü. Mide kitlesinin endoskopik biyopsisinde diskohezif taşlı yüzük hücrelerinden oluşan az diferansiye adenokarsinom saptandı ve cilt biyopsisi paraneoplastik pemfigus ile uyumlu izlendi.

Yapısal semptomlarla birlikte hızla gelişen cilt lezyonları olan hastalarda altta yatan maligniteler akılda tutulmalıdır.

Anahtar kelimeler: Paraneoplastik pemfigus, Leser Trelat, gastrik adenokarsinom.

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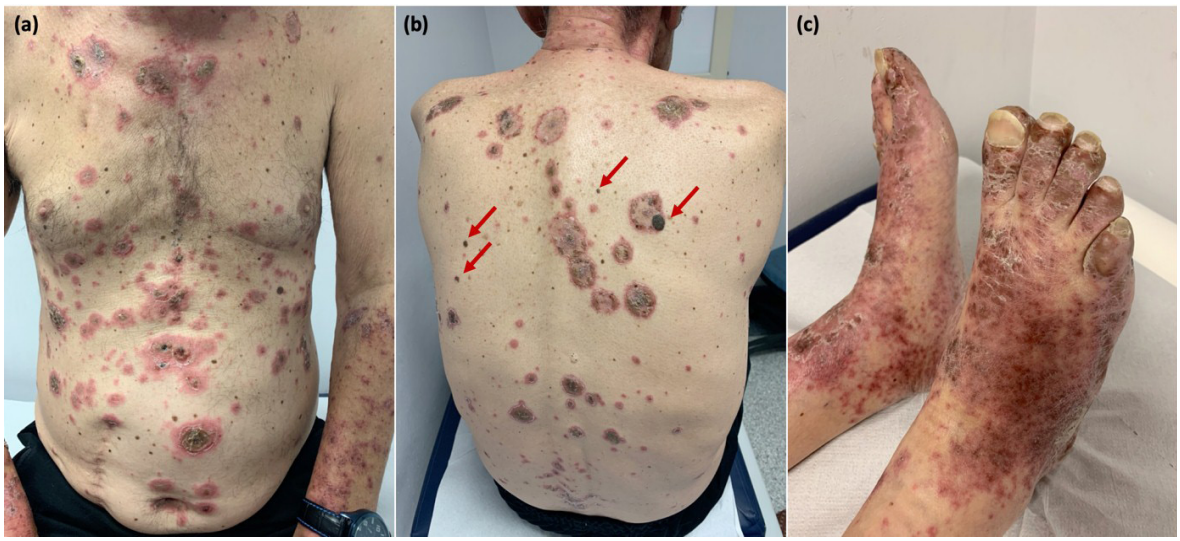
Introduction

Some malignancies may trigger several cutaneous manifestations. Although recognition of some typical paraneoplastic dermatoses may lead to early diagnosis of a neoplasm, some may progress with atypical involvements [1]. Sometimes, patients may apply to the physician for the first time not only with symptoms related to the primary diagnosis but also with cutaneous manifestations that seem unrelated.

Here, we present a rare case of metastatic gastric adenocarcinoma applying to the general internal medicine outpatient clinic with complaints of constitutional symptoms and widespread cutaneous manifestations.

Case report

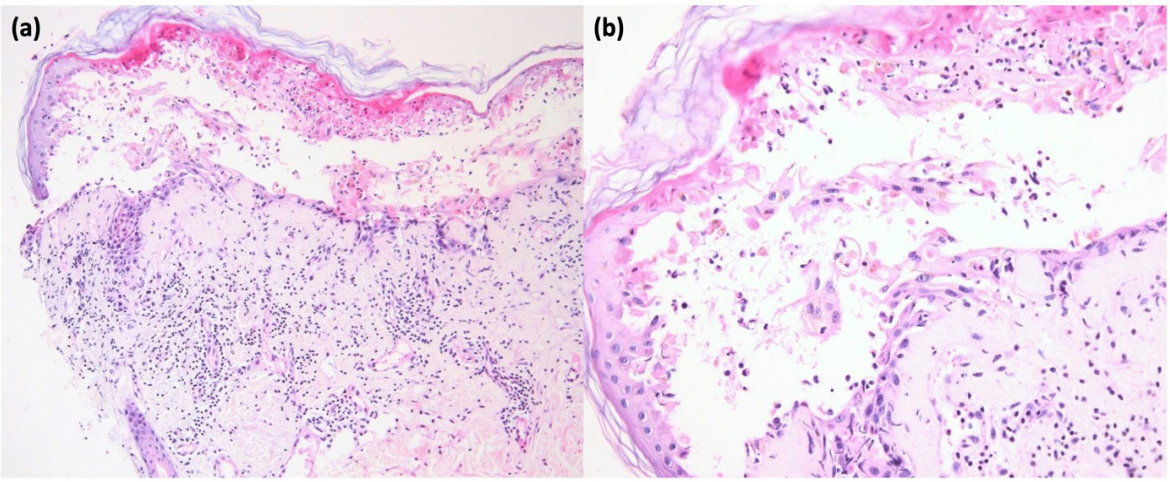
A 66-year-old male patient with type 2 diabetes, hypertension, and coronary artery disease was admitted to the general internal medicine outpatient clinic with complaints of rashes that started on the hands and spread to the trunk and feet 2 months ago. It was accompanied by loss of appetite, dyspepsia, weakness, and unexplained gross weight loss (15 kg per 2 months). The patient was cachectic, and physical examination revealed widespread eroded, erythematous, thick-middle, yellow-pitted, lichenified plaques on bilateral arms and legs and dorsum of the hands and feet. Scattered seborrheic keratosis lesions on the trunk were also detected (Picture 1). He was on metformin, dapagliflozin, telmisartan, metoprolol, and clopidogrel treatment for 4 years and had a 20-pack-year smoking history. Laboratory studies revealed microcytic anemia with a hemoglobin level of 8.3 g/dL, leukocyte count of $5.6x10^3$/ml, platelet count of $321x10^3$/ml, creatinine level of 1.5 mg/dL, serum sodium 126 mEq/L, potassium 3.58 mEq/L, chlorine 94 mEq/L, hemoglobin A1C 6%, normal liver functions, and negative acute phase reactants. He was admitted to the hospital ward, and intravenous fluid resuscitation and topical 0.05% clobetasol propionate were administered. Antinuclear antibody, anti-neutrophil cytoplasmic antibody, rheumatoid factor, indirect immunofluorescence on rat bladder epithelium, prostate-specific antigen, carcinoembryonic antigen, and alpha-fetoprotein were negative, while CA 19-9 was 65.7 U/mL (normal range: 0-35). Subsequently, abdomen computed tomography showed a malignant mass in the antrum, 12 mm thickening in the right adrenal gland, three heterogeneous contrasted lesions, the largest of which is 34x24 mm in the left adrenal gland, and multiple paraaortic, paracaval, and portal hilar lymphadenopathies up to 36x46 mm in size. Upper endoscopy revealed an ulcerovegetative antral mass surrounding the pylorus.



Picture 1. (a) and (b) Widespread eroded, erythematous, thick-middle, yellow-pitted, lichenified plaques on the trunk, (b) arrow signs; scattered seborrheic keratosis lesions on the trunk, (c) dorsum of the feet

An endoscopic biopsy of the gastric mass revealed poorly differentiated adenocarcinoma composed of discohesive signet ring cells. A skin punch biopsy showed intense dyskeratosis, suprabasilar clefting, lymphocyte exocytosis, and acantholysis in the epidermis (Picture 2). Immunofluorescence examination of tissue from the perilesional skin showed weak intraepidermal positivity with C3. IgG, IgA, and IgM were negative. The findings were compatible with Paraneoplastic pemphigus.

Written informed consent for the publication of their details was obtained from the relatives of the patient.



Picture 2. Suprabasal splitting is observed in the lower epidermis, and dense dyskeratosis is observed in the upper epidermis (H&E 40x)

(a) Larger magnification shows acantholytic cells in the splitting cavity with diffuse dyskeratosis and residual basal layer cells due to suprabasal detachment (H&E, 100x) (b)

Discussion

Paraneoplastic pemphigus (PNP) is a rare autoimmune mucocutaneous disease associated with underlying neoplasia. It is typically characterized by painful mucosal erosions and dark, patchy skin eruptions. Widespread epidermal loss can lead to severe dehydration, protein loss, and an increased risk of infection. In at least 80% of patients, the underlying malignancy is lymphoproliferative diseases, primarily non-Hodgkin lymphomas. Despite its association with many solid organ malignancies, only 4 cases of gastric cancer have been published to date [2, 3]

At first glance, the lesions on the trunk suggested Leser-Trelat sign, which is considered to be a fairly rare paraneoplastic cutaneous marker of internal malignancy (gastric adenocarcinoma being the overall most common malignancy, followed by breast cancer and lymphoproliferative disorders/lymphoma), with the hallmark finding being an abrupt eruption of multiple seborrheic keratoses [4]. However, the biopsy was not taken from these seborrheic keratoses lesions; PNP turned out to be a more accurate diagnosis.

Unfortunately, there is no current diagnostic guide or treatment protocol for PNP [5]. The treatment of patients with PNP consists of suppressing of the disease manifestations and managing patient symptoms. Treatment of the underlying malignancy is beneficial in some cases. We consulted our patient with the oncology department for the treatment of gastric adenocarcinoma.

It should be kept in mind that some malignancies may trigger cutaneous manifestations. Underlying malignancies should not be forgotten in patients with rapidly developing skin lesions with constitutional symptoms.

Conflict of interest: No conflict of interest was declared by the authors.

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Informed consent: Written informed consent for publication of their details was obtained from the relatives of the patient.

Authors' contributions to the article

R.I. constructed the main idea and hypothesis of the study. O.A.U. developed the theory and edited the material and method section. B.K. and M.O. have done the evaluation of the data in the results section. Discussion section of the article written by R.I. and O.A.U.

D.A.O. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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Traumatic knee dislocation “about”

Travmatik diz çıkıkları “hakkında”

Uğur Ertem

Posted date:03.01.2024 Acceptance date:15.01.2024

Dear Editor,

I would like to share my opinions on the article “Traumatic knee dislocation” [1]. First of all, I would like to thank the authors for contributions to the science of a subject that is of great concern to physicians interested in the musculoskeletal system and whose treatment and rehabilitation is very difficult. I will try to contribute to such an important issue as a physical medicine and rehabilitation physician.

Traumatic knee dislocation is an extremely rare injury, with a prevalence of less than 0.2% of all orthopedic injuries. However, because it is frequently accompanied by meniscus, ligament, bone and soft tissue injuries, it is a difficult pathology to treat for orthopedics, physical medicine and rehabilitation physicians [2, 3]. Apart from this, knee dislocation can be accompanied by various vascular and neurological injuries, and if timely diagnosis and treatment is not performed, it may lead to consequences such as limb loss [4]. Today, there is still no consensus on the best treatment strategy for the optimal surgical treatment of traumatic knee dislocations [4, 5]. Bektas et al. [1] point out in their article the importance of early and appropriate patient-based intervention by experienced orthopedic surgeons and high awareness of neurovascular injuries.

Post-operative rehabilitation is as important as the surgical treatment of traumatic knee dislocations in terms of preventing morbidity in patients. Treatment of traumatic knee dislocations is surgically difficult and there is no standard treatment protocol, and the situation is similar in the field of rehabilitation. It can generally occur with poor functional results in these patients. In such injuries, combined instability is observed in many planes of the knee [6]. Owens et al. [7] stated that the treatment results of patients who did not receive a modern rehabilitation program following early surgery were unsuccessful. Postoperative protection for multiple ligament injuries is important for healing of surgically repaired or reconstructed tissues, but major injuries and complex surgeries can develop stiffness and loss of motion in the knee joint [8, 9]. Postoperative protection is as important for the recovery of the surgical field as it is for the improvement of functional status in rehabilitation. In a review, Mook et al. [10] found that late knee reconstruction of severe multiligament knee injuries may provide similar outcomes for stability compared to acute surgery. But, it was concluded that early initiation of mobility in patients is better than immobilization.

Rehabilitation of multiple ligament injuries is different from isolated ligament injuries. In such injuries, a more protective rehabilitation program is planned. Surgical treatment is generally essential in the presence of multiple ligament injuries, especially traumatic knee dislocations. Arthrofibrosis is the most common complication of these surgeries. Early rehabilitation is of great importance in preventing complications. After multiple ligament injury surgery, partial load should be given for 6-8 weeks. Return to sports should not occur before 1 year [11]. Therefore, a comprehensive rehabilitation program should be organized after knee dislocation surgery, including strengthening exercises, range of motion exercises, mobilization exercises, walking and balance exercises.

As a result, the rehabilitation program is as important as the success of the surgical treatment in terms of successful functional results in traumatic knee dislocations. Surgery and postoperative rehabilitation program are two integral elements for successful results in such injuries.

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Traumatic knee dislocation “author's response”

Travmatik diz çıkıkları “yazarın yanıtı”

Mert Bektaş, Harun Reşit Güngör, Kadir Gem

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Dear Editor,

We would like to thank authors for their invaluable comments on our article. These types of complicated injuries are challenging traumas that can be overcome with the cooperation of orthopaedics and traumatology and physical therapy and rehabilitation units [1, 2]. Physical therapy is at least as important as surgery in restoring the patient's previous movement function, especially in the postoperative period [3, 4]. With this awareness, we aimed to restore the pre-injury functions of the patients by receiving the necessary support from our physical therapy and rehabilitation unit in the patients we included in our study. We have seen that early stage physical therapy and rehabilitation, especially after surgery, reduces the limitation of function. In addition, we concluded that physical therapy and rehabilitation specific to the patient and the type of injury will be more effective.

In conclusion, as you mentioned in your article, such injuries with high morbidity are injuries that many medical departments can overcome by working together. Physical therapy and rehabilitation is the most important building block of the postoperative process.

Conflict of interest: No conflict of interest was declared by the authors.

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