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# Effects of repeated sevoflurane and rivastigmine on spatial learning and memory in weanling rats

## Tekrarlı sevofluran ve rivastigminin yavru sıçanların uzaysal öğrenme ve hafıza üzerindeki etkileri

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### Abstract

**Aim:** It has been reported that repeated sevoflurane exposure induces cognitive impairment. On the other hand, there is evidence that rivastigmine can attenuate or antagonize the cognitive dysfunctions caused by anesthetic agents. The aims of this study were to determine the effect of repeated sevoflurane exposure on spatial learning and memory (SLM) in weanling rats and to assess whether rivastigmine provides protection against the neurotoxic effects of sevoflurane at this early developmental stage.

**Methods:** Thirty-two weanling rats were randomly divided into four equal groups: sevoflurane (S: 2% sevoflurane for 2 hours), sevoflurane + rivastigmine (SR: 2% sevoflurane and 2 mg/kg rivastigmine), rivastigmine (R: 2 mg/kg), and control (C: 100% oxygen for 2 hours). Rats were treated four times over 10 days. Four days after the last treatment, the rats were subjected to a Morris water maze test protocol to examine SLM.

**Results:** The escape latencies of all groups gradually decreased day by day during the training trials performed to evaluate spatial learning ( $p < 0.05$ ). Group R showed more improvement than other groups as the rats in this group learned significantly more slowly on the first and second days of the training trials but reached the same levels as Group S and Group SR on the third and last days ( $p < 0.05$ ). In the probe trial to evaluate spatial memory, no significant difference was found among the groups for time spent in the 'platform' quadrant ( $p > 0.05$ ).

**Conclusion:** Sevoflurane negatively affects learning in weanling rat pups but has no detrimental effect on spatial memory. On the other hand, it can be claimed that sevoflurane offsets the memory-sparing effects of rivastigmine.

**Keywords:** Sevoflurane, rivastigmine, spatial learning and memory, rat.

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### Öz

**Amaç:** Literatürde tekrarlı sevofluran uygulamalarının bilişsel işlev bozukluklara neden olduğu bildirilmektedir. Diğer yandan, rivastigminin, anestezi ajanlarının neden olduğu bilişsel işlev bozukluklarını hafifletebileceğine dair bulgular da literatürde yer almaktadır. Çalışmanın amacı tekrarlı sevofluran uygulamasının yavru sıçanların uzaysal öğrenmesine ve hafızasına etkisini ve rivastigmin'in sevofluranın söz konusu nörotoksik etkilerine karşı koruma sağlayıp sağlamadığını araştırmaktır.

**Yöntemler:** Otuz iki yavru sıçan rastgele olarak dört eşit gruba ayrılmıştır: sevofluran grubu (S: 2 saat boyunca % 2 sevofluran), sevofluran ve rivastigmin grubu (SR: % 2 sevofluran ve 2 mg / kg rivastigmin), rivastigmin grubu (R: 2 mg / kg) ve kontrol grubu (C: 2 saat boyunca % 100 oksijen). Sıçanlara, söz konusu ajanlar 10 gün boyunca dört kez verilmiştir. Son tedaviden dört gün sonra, sıçanlar uzaysal öğrenmeyi ve hafızayı incelenmek üzere Morris Su Labirent Testi protokolüne tabi tutulmuştur.

**Bulgular:** Uzaysal öğrenmeyi değerlendirmek için yapılan eğitim denemelerinde tüm grupların labirentten kurtulma süreleri günden güne yavaş yavaş azalmıştır ( $p < 0.05$ ). Uzaysal hafızayı değerlendirmek için yapılan eğitim testlerinde ise platform kadranında geçirilen süre için gruplar arasında anlamlı bir fark bulunamamıştır ( $p > 0.05$ ).

**Sonuç:** Çalışmada sevofluran, emzirme dönemindeki yavru sıçanların uzaysal öğrenmelerini olumsuz yönde etkilemesine karşın uzaysal hafızaları üzerinde zararlı bir etkisi çıkmamıştır. Diğer yandan sevofluranın, rivastigminin sağladığı hafıza koruyucu etkileri dengelediği gözlemlenmiştir.

**Anahtar kelimeler:** Sevofluran, rivastigmin, uzaysal öğrenme ve hafıza, sıçan.

## Introduction

There is growing concern about the side effects of general anesthetics, especially in neonates [1]. Recent studies show that commonly used anesthetic agents such as sevoflurane and desflurane can cause neuronal cell death during neonatal brain development and can bring about developmental disability or cognitive dysfunction in both infants and adults. As a consequence, neurocognitive impairments may arise in children exposed to anesthetic agents [2, 3], and repeated dosing may elevate the risk. Infants can be exposed to repeated inhalational anesthetics for a variety of reasons, including complex surgeries, burn treatments, and interventional or diagnostic radiological procedures [4].

In an animal model, repeated exposure of neonatal rats (between 1 and 2 weeks old) to isoflurane was associated with greater memory impairment compared to single exposure [5]. Similarly, multiple exposures of neonatal rats (beginning at 6 days old) to sevoflurane induced cognitive impairment, whereas no cognitive decline was evident with a single exposure. Moreover, in the same study, adult rats (60 days old) subjected to the same experimental protocol exhibited no cognitive impairment [6]. Another study showed that repeated exposure of pregnant rats to sevoflurane caused a significant increase in apoptosis of neurons in the hippocampus of the offspring [7]. Thus, repeated dosing of these inhalational anesthetics has a demonstrable neurotoxic effect on the developing central nervous system.

In addition, several studies have reported that anesthetic agents have detrimental effects on long-term spatial cognitive functions, but not on short-term spatial cognitive functions [8, 9]. However, another study concluded that sevoflurane or propofol has no effect on both short- and long-term memory in children 7-13 years of age [10]. Controversial results have also emerged in studies of the effects of anesthetic agents on spatial cognitive functions [11, 12].

The detrimental effects of general anesthetics are partially explained by suppression of acetylcholine (ACh) release in the brain [13]. These detrimental effects are more pronounced with the use of volatile inhalation agents such as desflurane and sevoflurane [14]. On the other hand, certain neurodegenerative diseases including Alzheimer's, Parkinson's, and delirium are associated with reduced ACh levels in the brain [15, 16, 17]. Cholinesterase inhibitors are the mainstay in the treatment of these diseases, and their action elevates the cerebral levels of ACh [18]. Efforts to treat the symptoms of such diseases have included the use of acetylcholinesterase inhibitors such as rivastigmine, a semi-synthetic derivative of physostigmine and carbonate [19, 20]. Thus, it can be argued that the reduced ACh level induced by repeated sevoflurane can be offset by using a cholinesterase inhibitor such as rivastigmine. Moreover, rivastigmine can also be used to attenuate or antagonize the cognitive dysfunctions caused by anesthetic agents [13, 21]. Furthermore, some experimental studies have indicated that cognitive impairments induced by ketamine, ethanol, or scopolamine can be similarly reversed by rivastigmine [22, 23, 24].

Although rivastigmine is effective at improving cognitive functions, the dose used is also important. Overdoses of rivastigmine have adverse effects on cognitive functions in rats, whereas doses between 0.1 and 2.5 mg/kg eliminate or reduce the neurotoxic side effects of some anesthetic agents and help ameliorate symptoms in Alzheimer's disease [19, 23-25].

Thus, we sought to investigate the toxicity of sevoflurane to the neonatal brain and its potential amelioration by rivastigmine. The aims of this study were to determine the effect of repeated sevoflurane exposure on spatial learning and memory in weanling rats and to assess whether rivastigmine provides protection against the cognitive impairment induced by sevoflurane.

## Material and methods

This study was conducted at the Istanbul Bagcilar Education and Research Hospital Experimental Research and Ability Development Center (BADABEM®) with the approval of the Istanbul Bagcilar Education and Research Hospital Experimental Animals Ethical Commission (Protocol No: 2014/4, approved February 19, 2014). As the nursing period of neonatal rats can last up to 21 days [26], thirty-two healthy weanling Wistar Hannover male rats (40-50 g) at postnatal day 21 (P21) were used in the experiment. Male rats were chosen because they are less affected by physiological factors [27], and physiological factors such as blood pressure, heart rate, and blood gases were not measured. The rats were housed under standard laboratory conditions from birth (12-hour day/night cycle, 20-22 °C, 50-60% relative humidity). Health reports from the vendor (BADABEM®) indicated that the rats were free of known viral, bacterial, and parasitic pathogens.

Memory and learning, frequently used in assessments of cognitive functions in animal experiments, were also used in this study due to their ease of measurability. In addition to its effects on Ach level, sevoflurane was preferred as a volatile anesthetic agent in this study due to its low side effects and odorless, non-irritating, and soft anesthesia induction. Sevoflurane is also widely used in children because it does not induce airway irritation and does not stimulate the cough reflex, and in this context we decided to use sevoflurane in weanling rats.

Rat pups were randomly divided into four groups, each consisting of eight rats.

*Group S:* Rat pups were exposed to 2% sevoflurane with oxygen for 2 hours, after which 1 mL of saline was injected subcutaneously.

*Group SR:* Rat pups were exposed to 2% sevoflurane with oxygen for 2 hours, after which 2 mg/kg rivastigmine was injected subcutaneously [i.e. 1 mL of an Exelon® 1.5 mg tablet (Novartis) dissolved in 15 mL of saline]. The subcutaneous route was used to prolong the duration of rivastigmine's effect. Rivastigmine doses used in studies in the literature are between 0.1 and 2.5 mg/kg [19, 23-25].

*Group R:* Rat pups were exposed to 100% oxygen for 2 hours, after which 2 mg/kg rivastigmine solution was injected subcutaneously.

*Group C:* Rat pups were exposed to 100% oxygen for 2 hours, after which 1 mL of saline was injected subcutaneously.

For sevoflurane application, each group was put into an induction box of 3000 mL in volume equipped with a gas input and output system. Sevoflurane (Sevorane®, Abbott Lab., Istanbul, Turkey) was fed into the induction box at 2% concentration in oxygen at a rate of 6 L/min with a vaporizer. In accordance with the duration and doses applied in many studies, the rat pups were exposed to 2% sevoflurane for 2 hours [28, 29]. Anesthesia level was monitored by checking respiratory pattern,

speed, and reflexes. Volatile anesthesia application was halted after 2 hours and oxygen was provided with a flow rate of 6 L/min for recovery of the rats. After recovery, the rat pups were returned to their cages.

**Experimental timeline**

Starting on postnatal day 22, Group S and Group SR animals were exposed to 2% sevoflurane on days 22, 25, 28, and 31 for 2 hours each day [5]. Groups R and C were exposed to 100% oxygen for 2 hours on the same days as above. After the exposure, 2 mg/kg rivastigmine was prepared, and 1 mL of this solution was injected subcutaneously into rats of Groups R and SR, whereas 1 mL of saline was injected into Groups C and S.

Beginning on postnatal day 35 and on each of the following three days (days 36, 37, and 38), the rat pups were subjected to training trials using the Morris water maze test (MWMT) [30]. The platform-finding periods of the rats were recorded. One day after the completion of training trials (day 39), probe trials for spatial memory function were carried out. In the training trials, the platform was fixed in the north quadrant, but the rat pups were released into the tank from different quadrants. The experimental timeline is illustrated in Figure 1.

In cognitive studies of experimental animals, long-term spatial cognitive functional tests such as the MWMT are performed after more than one week following administration of the experimental treatment. In short-term studies, tests are performed within 24 hours. In the present study, we selected 4 days (from day 31 to day 35) between the medications and trials to conduct the spatial learning and memory tests.

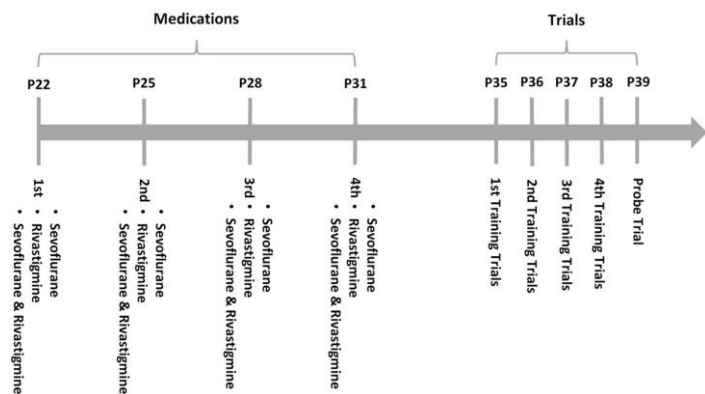


Figure 1. Experimental Timeline.

**Assessment of Spatial Learning and Memory**

Before the MWMT, the weanling rat pups were transferred to the room containing the test apparatus. During the training (learning) trials, the location and shape of the objects and platform were not changed. The experiments were also conducted by the same person. During the experiments, the clothes, hairstyle, jewelry, scent, etc. of the researcher were not changed. Red, black, and white papers were applied to three different sides of the tank so that the rat pups were able to use these visual cues as a means of navigating the maze. All trials were done in a black Plexiglas pool 120 cm in diameter and 80 cm deep. Milk powder (1 kg) was put into the tank to make the water opaque. The tank was divided into four imaginary quadrants: west, east, south, and north. A hidden platform, the same color as the tank and 10 cm in diameter, was put into the north quadrant and submerged 3-4 mm below the water level. The temperature of the water was adjusted to 25±1 °C.

**Training Trials (Learning Function):** In the training trials, the rat pups were released into the water from different quadrants (west, east, south) at 10-min intervals on each of the 4 days (P35, P36, P37, P38). The escape latency, which is the time it takes a rat to find the platform, was noted for each training trial. The average of these escape latencies was calculated for each day and each experimental group. One minute was given to each rat pup to find the hidden platform. If a rat pup could not find the hidden platform within one minute, the researcher helped the rat pup find the platform and it was kept for 30 seconds on this platform. Afterwards, each rat was taken from the platform and dried with paper towels.

**Probe Trial (Memory Function):** A day after the MWMT (day 39), the submerged platform was removed from the apparatus for the memory test. The rat pups were allowed to swim in the tank for 60 seconds. In this experiment, the amount of time the rat spent at the targeted quadrant (the quadrant in which the hidden platform was located in the training sessions) was noted as a percentage of one minute.

**Statistical analysis**

The results were analyzed using IBM SPSS Statistics 20 software. Since each group was subjected to repeated trials in four different directions for each day, the number of observations for each group and each day was over 30. For this reason, it was assumed that the data were normally distributed [31]. One-way and two-way ANOVA were used to analyze differences among the groups. If a group was found to be different from the others, it was then compared bilaterally with post hoc tests. Before the ANOVA, Levene’s test (at 1% significance level) was carried out to test the homogeneity of variances. After the ANOVA, pairwise comparison by a least significant difference (LSD) test was carried out for multiple comparisons of the independent variables in order to find significant differences between two compared group averages. A value of  $p < 0.05$  was considered significant.

**Results**

One rat pup from Group C died (the cause of death could not be identified). It was excluded from the study.

No difference was observed among the groups in terms of average recovery time from anesthesia.

All groups’ escape latencies (measured as the average for each day) gradually decreased day by day during the trials ( $p < 0.05$ ). However, Groups S, SR, and R all had significantly longer escape latencies compared to Group C ( $24.29 \pm 17.31$ ,  $p < 0.01$ ). The escape latencies of Groups S, SR, and R were not significantly different from one another ( $p > 0.05$ ) (Table 1).

A significant difference was found in the average escape latencies of each group between the first ( $44.84 \pm 19.87$ ) and second trial days ( $38.93 \pm 20.89$ ). In addition, highly significant differences were found among the other experimental days ( $p < 0.01$ ) (Table 1).

On the first two experimental days, the escape latencies of Group R ( $55.29 \pm 8.69$ ,  $49.38 \pm 19.06$ ) were longer ( $p < 0.05$ ) than those of Group S ( $42.57 \pm 20.06$ ,  $36.71 \pm 18.09$ ), Group SR ( $43.5 \pm 23.22$ ,  $37.71 \pm 19.39$ ), and Group C ( $38 \pm 21.05$ ,  $31.92 \pm 23.76$ ). Group R showed more improvement than other groups as the rats in this group learned significantly more slowly on the first and second days of the training trials, but reached the same level as Group S and Group SR on the third and last days ( $p < 0.05$ ). On the third experimental day, the escape latency of Group C ( $16.63 \pm 15.04$ ) was shorter ( $p < 0.05$ ) than that of the other groups (Group S:  $30.38 \pm 17.72$ , Group SR:  $29.92 \pm 21.78$ , and Group R:  $28.58 \pm 23.37$ ). On the fourth experimental day, the

escape latency of Group C (10.59±9.39) was highly significantly different (p<0.01) from those of the other groups (Group S: 20.21±17.1, Group SR: 18.88±15.88, and Group R: 19.17±17.21) (Table 1, Figure 2).

Table 1. Escape Latencies (Seconds) in the Training Trials.

Groups	1.Day	2.Day	3.Day	4.Day	Average	
S <sup>‡</sup>	42.57±20.06	36.71±18.09	30.38±17.72	20.21±17.1	32.47±18.24	
ρ values	Group SR	0.867	0.865	0.935	0.543	0.767
	Group R	0.023	0.032	0.753	0.634	0.142
	Group C	0.409	0.413	0.018	0.001	0.001
SR <sup>‡</sup>	43.5±23.22	37.71±19.39	29.92±21.78	18.88±15.88	32.50±20.07	
ρ values	Group S	0.867	0.865	0.935	0.543	0.767
	Group R	0.035	0.048	0.816	0.895	0.078
	Group C	0.321	0.323	0.022	0.001	0.001
R <sup>‡</sup>	55.29±8.69	49.38±19.06	28.58±23.37	19.17±17.21	38.11±17.08	
ρ values	Group S	0.023	0.032	0.753	0.634	0.142
	Group SR	0.035	0.048	0.816	0.895	0.078
	Group C	0.002	0.004	0.039	0.001	0.001
C <sup>‡</sup>	38±21.05	31.92±23.76	16.63±15.04	10.59±9.39	24.29±17.31	
ρ values	Group S	0.409	0.413	0.018	0.001	0.001
	Group SR	0.321	0.323	0.022	0.001	0.001
	Group R	0.002	0.004	0.039	0.001	0.001
Average <sup>‡</sup>	44.84±18.26	38.93±20.08	26.38±19.48	17.21±14.90		
ρ values	1.Day	0.018	0.001	0.001	0.001	
	2.Day	0.018	0.001	0.001	0.001	
	3.Day	0.001	0.001	0.001	0.001	
	4.Day	0.001	0.001	0.001	0.001	

<sup>‡</sup>:mean±standard deviation.

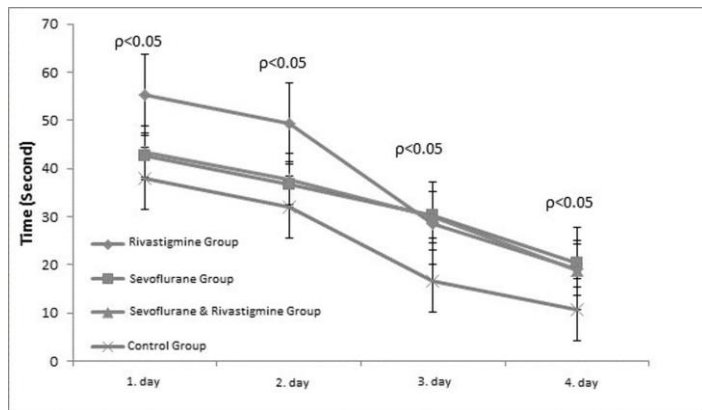


Figure 2. Average learning durations per experimental group.

In the probe trial carried out to evaluate spatial memory, no significant difference was found among the groups in terms of time passed in the north quadrant (p>0.05) (Table 2).

Table 2. Time spent in the north quadrant in probe trial.

Groups	Time Spent in the North Quadrant (Seconds) <sup>‡</sup>
Rivastigmine	43.75±11.76
Sevoflurane+ Rivastigmine	40.13±14.89
Sevoflurane	38.43±12.4
Control	45.17±11.76

<sup>‡</sup>:mean±standard deviation.

## Discussion

Although negative effects have been reported in the vast majority of studies on this subject [1], some studies indicate that sevoflurane has no effect or even a positive effect on spatial cognitive functions [8, 9, 11]. Also, as noted, rivastigmine has been shown to be effective in reversing cognitive impairments, depending on the dose used [19, 23-25]. Within this framework, the effect of repeated sevoflurane exposure on the spatial learning and memory of weanling rats was investigated in this study, as well as whether cognitive impairments induced by sevoflurane can be suppressed by rivastigmine treatment.

We found that repeated sevoflurane exposure impaired spatial learning in weanling rat pups; however, it produced no adverse effect on spatial memory. Considering that sevoflurane has long-term detrimental effects on both learning and memory [5, 8-10], it can be argued, according to the results of the present study, that sevoflurane initially affects learning, and then memory. In other words, the effect of sevoflurane on spatial memory is rather long-term [28], so this effect was not observed in our study. In addition to this, we found that rats of the rivastigmine group showed more improvement than any other group as the rats in this group learned significantly more slowly at the beginning of the training, but reached the same level as the rats in the sevoflurane and sevoflurane + rivastigmine groups at the end.

On the other hand, we found that rats treated with sevoflurane alone (Groups S) had poorer spatial memory numerical values compared to control rats (Group C). In addition, although not statistically significant, the spatial memory values of the sevoflurane and rivastigmine group (Group SR) were better than those of the sevoflurane group (Group S).

The use of weanling rat pups distinguishes this work from other repeated-dose sevoflurane studies, which generally used adult rats or pre-weaned rat pups (most studies on infant rats used postnatal day 7 pups) [6, 7, 26, 28]. Also, the durations between medications and trials are important factors in this study, as well as the doses of sevoflurane or rivastigmine. New studies on the subject could be carried out by increasing the sample sizes, using different durations between medications and trials, using experimental animals of different ages, or changing the doses of sevoflurane and/or rivastigmine.

In conclusion, we found that sevoflurane negatively affects learning in weanling rat pups but has no detrimental effect on spatial memory. On the other hand, it can be claimed that sevoflurane offsets the memory-sparing effects of rivastigmine.

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# Relationship between trochlear morphology and lateral patellar cartilage defect using MR Imaging

## MR görüntüleme kullanarak troklear morfoloji ve lateral patellar kartilaj defekti arasındaki ilişki

Semra Duran<sup>1</sup>, Elif Günaydın<sup>2</sup>

### Abstract

**Aim:** The present study aimed to compare trochlear morphology observed on magnetic resonance imaging (MRI) between patients with lateral patellar cartilage defect and age-matched-pair control patients without cartilage defect.

**Methods:** A total of 75 patients with MRI-verified grade 3/4 lateral patellar cartilage defect were compared with matched-pair control patients without cartilage defects of the patellofemoral joints. Axial sequences were used to detect and evaluate patellar cartilage defects. Trochlear morphology was assessed on the basis of lateral trochlear inclination (LTI), medial trochlear inclination (MTI), sulcus angle (SA), trochlear facet asymmetry (FA), and trochlear width (TW) on axial MR images.

**Results:** SA was higher for both sexes in the cartilage defect group than in the control group ( $p < 0.05$ ). LTI of the cartilage defect group was significantly lower than that of the control group, particularly in females ( $p < 0.05$ ). There were no significant differences in MTI between the two groups for either sex ( $p > 0.05$ ). FA for both sexes was significantly lower in the cartilage defect group than in the control group ( $p < 0.05$ ). TW was significantly higher in the cartilage defect group than in the control group ( $p < 0.05$ ). Finally, TW of females in the cartilage defect group was significantly higher than that of females in the control group ( $p < 0.05$ ).

**Conclusion:** Flattened lateral trochlea is a risk factor for structural damage to the cartilage of the lateral patellofemoral joint, particularly in females.

**Keywords:** patellofemoral joint, chondromalacia patella, magnetic resonance imaging.

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### Öz

**Amaç:** Bu çalışmada lateral patellar kartilaj defektli hastalar ile yaş-taraf eşleştirilmesi yapılmış kartilaj lezyonu olmayan kontrol hastaları arasında manyetik rezonans görüntüleme (MRG) ile gözlenen troklear morfolojiyi karşılaştırmayı amaçladık.

**Yöntem:** MRG ile tanımlanan grade 3/4 lateral patellar kartilaj defektli toplam 75 hasta patellofemoral eklemden kartilaj defekti olmayan yaş-taraf eşleştirilmiş kontrol hastaları ile karşılaştırıldı. Aksiyel kesitler patellar kartilaj defektini saptamada kullanıldı. Troklear morfoloji lateral troklear inklınasyon (LTI), medial troklear inklınasyon (MTI), sulkus açısı (SA), troklear faset asimetrisi (FA) ve troklear genişlik (TG) ile aksiyel kesitlerde değerlendirildi.

**Bulgular:** SA kontrol grubu ile karşılaştırıldığında her iki cinsiyet için defekt grubunda büyüktü ( $p < 0.05$ ). Kartilaj defekt grubunda LTI kontrol grubu ile karşılaştırıldığında, özellikle kadınlarda belirgin olmak üzere, düşüktü ( $p < 0.05$ ). Her iki cinsiyet için her iki grup arasında MTI 'da istatistiksel olarak anlamlı fark bulunmadı ( $p > 0.05$ ). Her iki cinsiyet için kartilaj defekt grubunda FA kontrol grubu ile karşılaştırıldığında düşük idi ( $p < 0.05$ ). TG kontrol grubu ile karşılaştırıldığında defekt grubunda büyüktü ( $p < 0.05$ ). Ayrıca, kartilaj defekt grubundaki kadınlarda TG kontrol grubundan büyüktü ( $p < 0.05$ ).

**Sonuç:** Düzleşmiş lateral troklea özellikle kadınlarda lateral patellofemoral eklemden kartilajda yapısal zedelenme için risk faktörüdür.

**Anahtar kelimeler:** patellofemoral eklem, kondromalazik patella, manyetik rezonans görüntüleme.



## Introduction

Chondromalacia patella is the degeneration of patellar cartilage spanning a wide spectrum of severity ranging from softening and fissuration of the hyaline cartilage to bone erosion and formation of full-thickness articular cartilage defects. Although often observed in adolescents and younger adults, it is more commonly observed in older adults [1-3].

The etiology of chondromalacia patella is multifactorial, with genetic, environmental, or post-traumatic etiologies. Structural abnormalities of the patellofemoral joint may overload the patellar cartilage and subsequently result in cartilage defects [1, 4-7].

Magnetic resonance imaging (MRI) is recognized as an effective, accurate, noninvasive modality for assessing patellar cartilage defects because it offers superior soft tissue contrast, shows multiplanar capability, and allows direct visualization of articular cartilage [4, 8-10]. MRI is used to identify the stage of chondromalacia patella based on the degree of cartilage injury. Severe (grade 3 or 4) cartilage defects can be detected with 84%–87% sensitivity [8-10].

The sulcus angle (SA) and lateral trochlear inclination (LTI) are common measures of trochlear morphology [1, 3-5, 11, 12]. Several studies have investigated the association between patellar cartilage defects and trochlear morphology using MRI. However, these studies combined all patellar cartilage defects (medial, lateral, and central) into a single group. With only few studies analyzing the association between trochlear morphology and lateral patellar cartilage defects (LPCD), this association remains unclear [13-15].

Therefore, this study aimed to identify the morphological measurements of the trochlea associated with LPCD.

## Material and methods

The study was approved by the local ethical committee and conducted according to the principles described in the Declaration of Helsinki. Written informed consent could not be obtained due to the retrospective design of the study.

### Patients

We retrospectively reviewed 180 patients who were diagnosed with grade 3/4 LPCD on the basis of MRI evaluation between January and December 2017. The exclusion criteria were as follows: history of knee surgery or knee trauma (evidence of ligament and tendon tears or bone contusion), any diagnosis of space-occupying knee lesion, age <35 or >55 years, and MR images that were motion degraded or of insufficient quality to accurately assess joint cartilage. Finally, 75 patients with LPCD were included in this study. The control group included 75 age-matched patients with normal cartilage morphology as confirmed by MRI performed for the indication of anterior knee pain. The study was approved by the hospital's institutional review board.

### MRI evaluation

All MRI examinations were performed using a 1.5-T unit (Optima; GE Medical System, Milwaukee, Wisconsin, USA), employing an extremity coil with the patient in the supine position with knee full extension. Axial fat-suppressed proton-density-weighted (PDW) (TR/TE: 2300–2800 /20–40 ms; matrix: 288 × 224; FOV: 18 × 18 cm<sup>2</sup>; and slice thickness: 4 mm) section images were analyzed to evaluate patellar cartilage defects and measure trochlear morphology.

LPCD was diagnosed if irregularity was noted on the cartilage surface with a loss of cartilage thickness in at least two consecutive slices. Severity of the cartilage defect was

determined based on the lesion depth in accordance with the International Cartilage Repair Society Classification System [1, 4, 11] as follows: grade 3, loss of >50% of the cartilage thickness without exposed bone (Figure 1a) and grade 4, full thickness cartilage loss with exposed bone (Figure 1b).

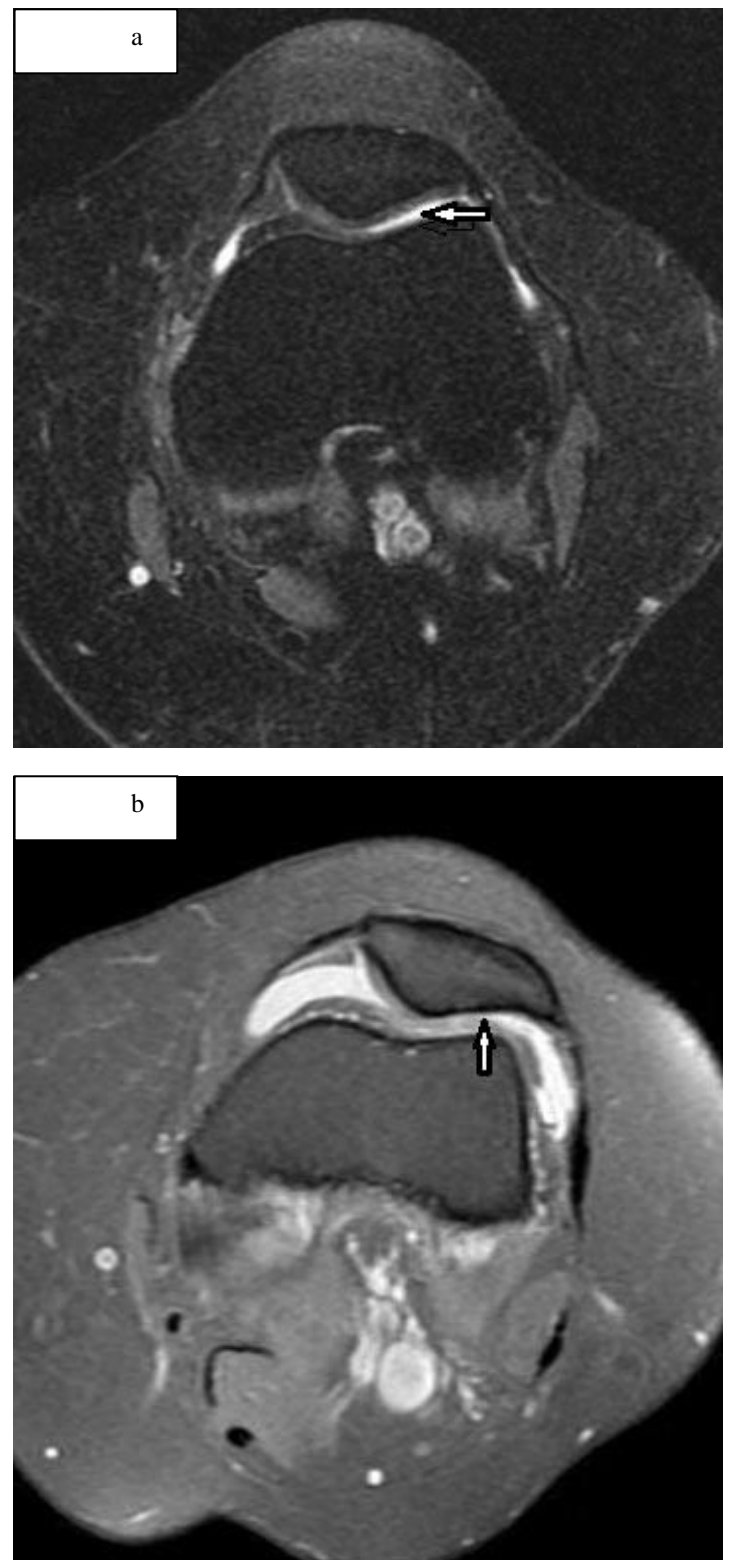


Figure 1. Axial fat-suppressed PDW-MR images of the knee showing (a) grade 3 and (b) grade 4 chondromalacia in the lateral facet of the patella (arrows).

### Trochlear morphology assessment

We evaluated the morphological features of the trochlea using SA, LTI, medial trochlear inclination (MTI), trochlear facet asymmetry (FA), and trochlear width (TW). These

measurements were taken on axial MR images, with the level of the anterior cruciate ligament femoral insertion along the osseous surface [6, 7, 13].

SA was defined as the angle between the medial and lateral trochlear facets (Figure 2a) [1, 4, 15]. The posterior condylar line was drawn along the most posterior surface of the femoral condyles. LTI was defined as the angle between the posterior condylar line and a line drawn along the surface of the lateral trochlear facet (Figure 2b) [1, 15]. MTI was defined as the angle between the posterior condylar line and a line drawn along the surface of the medial trochlear facet (Figure 2c) [1, 15]. FA was defined by the ratio of medial facet to lateral facet length (Figure 2d) [3, 6, 7, 13]. TW was defined as the distance from the line connecting the most anterior parts of the medial and lateral femoral trochlear facets (Figure 2e) [6, 13].

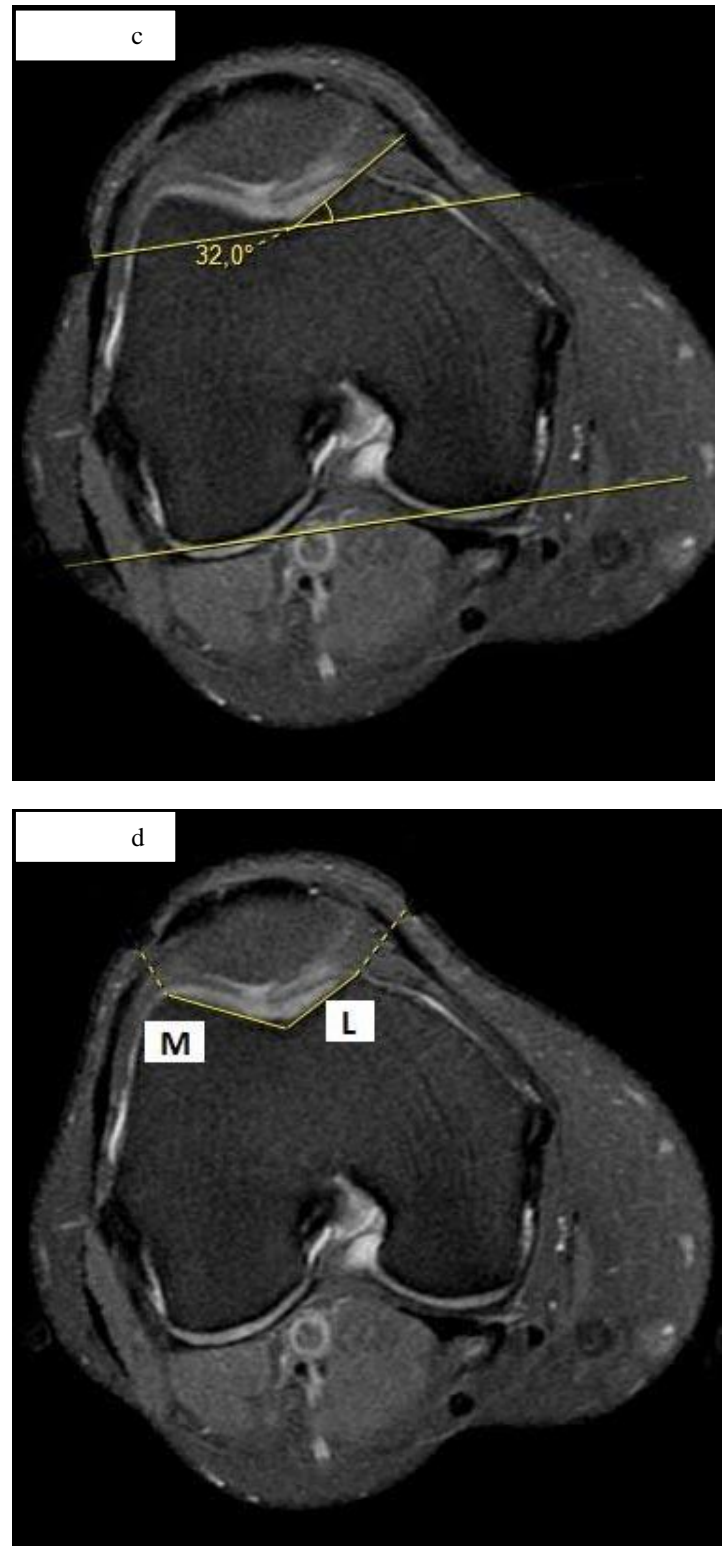
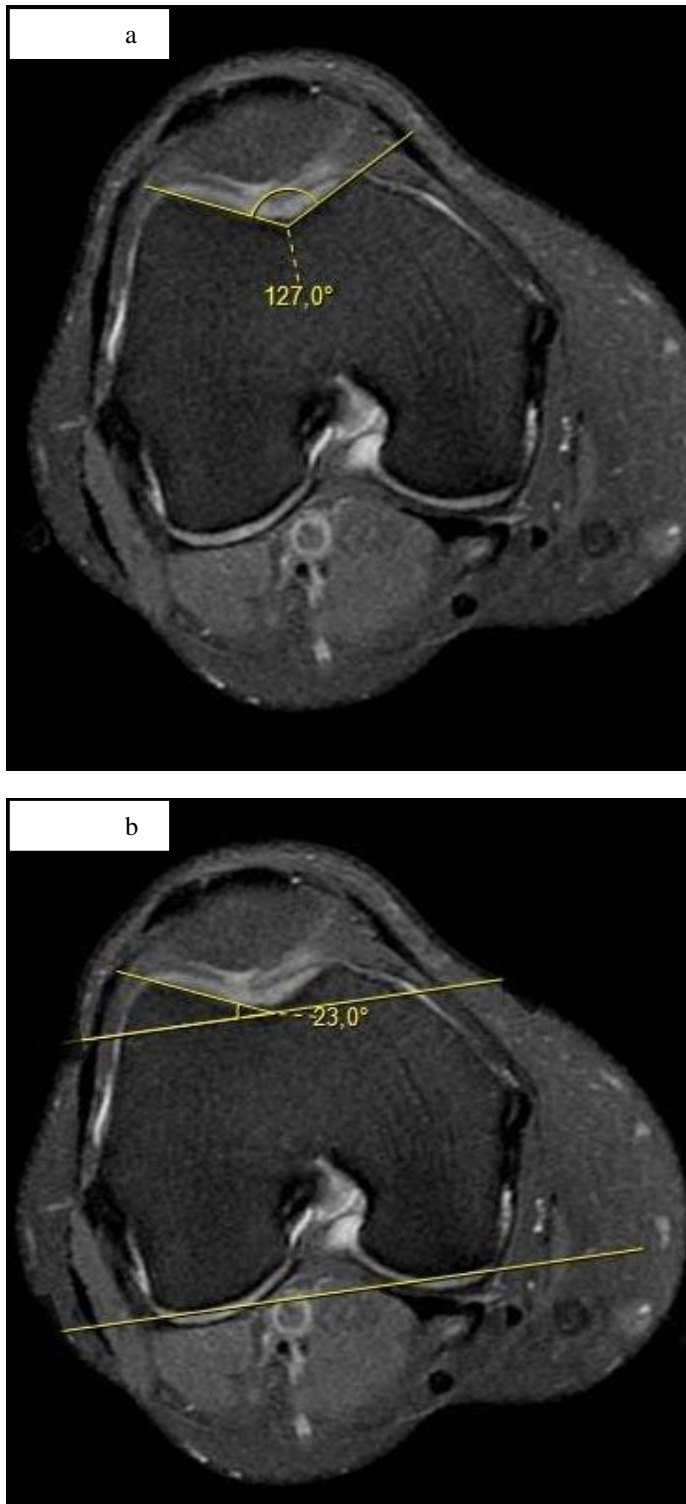


Figure 2 (a-d). Axial fat-suppressed PDW-MR images of the knee showing SA (a), LTI (b), MTI (c), and FA (d).

#### Statistical analysis

Statistical analyses were performed using SPSS (version 20.0; SPSS Inc., Chicago, IL). Normality of data distribution was tested using the Kolmogorov–Smirnov test. Homogeneity of data distribution was determined using Levene’s test. Parametric tests were used for inter-group comparisons. Differences in SA, LTI, MTI, FA, and TW between the LPCD and control groups were analyzed using Student’s t-test. Chi-square test was used to compare groups by sex. A p value of <0.05 was considered statistically significant.

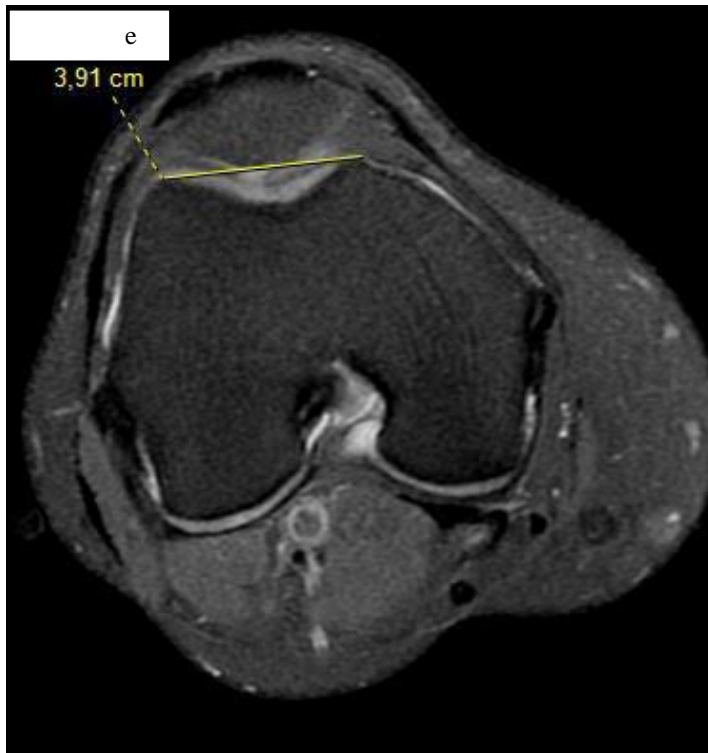


Figure 2 (continued) (e). Axial fat-suppressed PDW-MR images of the knee showing TW measurement.

### Reliability assessment

All measurements of trochlear morphology were performed by one of the authors (SD). To avoid any interobserver errors in trochlear measurements, the parameters mentioned above were measured by the second author (EG) on axial images. To evaluate intraobserver reliability, measurements were performed by the same author (SD) 3 weeks after the first evaluation. Inter- and-intraobserver intraclass correlation coefficients for all measurements were 0.86 and 0.88, respectively.

### Results

The characteristics of the study participants are presented in Table 1.

Table 1. Characteristics of the study population (n = 150)

Patients	Lateral patellar cartilage defect	Control group
Sample size	75	75
Age (year) (mean±SD)	48.7 ± 5.2	48.1 ± 5.4
Gender (female /male)	50/25	50/25
Right/left	39/36	39/36

SD: standard deviation.

SA for both sexes was higher in the LPCD group than in the control group ( $p < 0.05$ ). LTI of the LPCD group was significantly lower than that of the control group ( $p < 0.05$ ). Female patients in the LPCD group, but not in the control group, showed significantly lower LTI than male patients ( $p < 0.05$ ). There were no significant differences in MTI between the two groups for either sex ( $p > 0.05$ ). FA for both sexes was significantly lower in the LPCD group than in the control group ( $p < 0.05$ ). TW was significantly higher in the LPCD group than in the control group ( $p < 0.05$ ). TW in females was higher in the LPCD group than in the control group, whereas no such differences were noted in males ( $p > 0.05$ ).

SA, LTI, MTI, FA, and TW comparisons between groups and sexes are summarized in Table 2.

Table 2. Descriptive analyses of trochlear parameters according to group and sex.

Variable		Lateral patellar cartilage defect (mean±SD)	Control group (mean±SD)	p
SA	All	135.1 ± 7.7	124.9 ± 5.5	0.001
	Female	136.8 ± 7.5	126.6 ± 5.4	0.001
	Male	134.1 ± 7.6	124.3 ± 5.5	0.001
LTI	All	20.2 ± 2.6	25.5 ± 2.3	0.001
	Female	15.3 ± 2.5	25.5 ± 2.4	0.001
	Male	20.4 ± 2.5	25.1 ± 2.3	0.001
MTI	All	28 ± 2.5	29 ± 2.8	0.054
	Female	27.9 ± 2.6	29.1 ± 2.7	0.055
	Male	27.4 ± 2.4	28.8 ± 2.8	0.055
FA	All	0.56 ± 0.09	0.61 ± 0.07	0.001
	Female	0.55 ± 0.08	0.60 ± 0.08	0.001
	Male	0.57 ± 0.09	0.61 ± 0.08	0.001
TW	All	36.4 ± 4	34.8 ± 3.1	0.08
	Female	35.6 ± 3.9	33.6 ± 3.1	0.08
	Male	38.5 ± 3.9	38.5 ± 3.2	0.055

SD: standar deviation, SA:sulcus angle, LTI: lateral trochlear inclination, MTI: medial trochlear inclination, FA: trochlear facet asymmetry, TW: trochlear width.

### Discussion

This study demonstrates that abnormal trochlear morphology may play an important role in LPCD. Structural damage to the lateral patellar cartilage is commonly observed in knees with flattened lateral trochlea, particularly in females.

Patellofemoral pain syndrome is a common orthopedic problem that can cause serious disability, and it usually occurs due to chondromalasia patella. Patellar cartilage defects may develop as a result of patellofemoral morphological variations or anatomical incompatibility. Therefore, evaluation of the morphological properties of the patellofemoral joint plays an important role in the diagnosis of chondromalacia patella [3, 5, 11, 16-19].

Several studies have suggested that patellar cartilage defects are associated with higher SA, indicative of a flattened and shallow trochlea. However, in those studies, all patellar cartilage defects (medial, lateral, and central) were grouped into a single category [1, 3-5, 11, 12]. Few studies have evaluated trochlear morphology in relation to isolated LPCD [13-15]. Noehren et al. [14] reported no difference in SA between the control and LPCD groups. However, Sebro et al. [13] found that higher SA values were associated with the development of lateral patellar osteoarthritis in young patients. Similarly, Stefanik et al. [15] found that lateral patellofemoral osteoarthritic knees with high SA are at a 1.5-fold increased risk of cartilage damage in patients aged  $\geq 50$  years. In the present study, the mean SA was  $135.3^\circ$  in the LPCD group and  $124.9^\circ$  in the control group. These findings are in accordance with the findings of Stefanik et al. [15]. Increased SA is a risk factor for LPCD in both sexes. Shallow femoral trochlea can lead to patellar instability and disproportional load distribution on the articular surface of the patellofemoral joint during knee movement [15, 17, 19].

SA reflects the entire femoral trochlear morphology. In contrast, LTI reflects the lateral trochlear morphology alone. Mehl et al. [3] and Ali et al. [11] found no significant association between LTI and patellar cartilage defects in patients aged  $> 40$  years. However, these studies did not distinguish between medial defects and LPCD. Stefanik et al. [15] demonstrated that knees with low LTI of the lateral patellofemoral joint showed 2.6-fold increased risk of cartilage damage compared with those with high LTI. In the present study, patients with LPCD demonstrated

significantly lower LTI than controls. In contrast to the findings of Stefanik et al. [15], we demonstrated significantly lower LTI in females than in males. Our results indicate that females with flattened lateral trochlea are at an increased risk of LPCD, which is in accordance with the findings of Duran et al [1]. When the lateral facet is flattened, the patella is more likely to be laterally displaced. Thus, the contact between the patella and lateral femoral condyle increases, leading to cartilage damage in the patellofemoral joint [1, 15, 17, 19, 20].

In the present study, we did not find a significant difference in MTI between the LPCD and control groups; these findings are in agreement with those of previous studies [1, 15]. Our results suggest that the geometry of the lateral trochlear facet is more important than that of the medial trochlear facet in the structural damage of the lateral patellar cartilage.

Only few studies in the literature have analyzed the association of FA and TW with patellar cartilage defect. Mehl et al. [3] found that FA was not associated with patellar cartilage defect. However, they did not define cartilage defects in specific anatomic regions of the patellofemoral joint. Moreover, Sebro et al. [13] reported no significant association of FA and TW with lateral patellofemoral osteoarthritis in young patients. Our results suggest that with decrease in FA and increase in TW, there was an associated increase in lateral patellar cartilage structural damage in the knees of adult patients. FA for both sexes was considerably lower and TW in females significantly higher in the LPCD groups than in the control group. We suggest that the morphology of lateral trochlea is an important risk factor for LPCD.

There are some limitations in this study. It is retrospective study, and the diagnosis and grading of patellar cartilage defect were assessed based on MRI findings instead of arthroscopy. Moreover, routine MRI sequences were used for evaluation instead of cartilage-specific three-dimensional MRI for patellar cartilage.

In conclusion, our study demonstrated an association between abnormal trochlear morphology and LPCD of the knee. A flattened lateral trochlea may cause structural damage to the lateral patellar cartilage, particularly in females.

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# Comparison of two plain radiographic and 3D-based measurement methods for posterior malleolar fragment size in trimalleol ankle fractures

## Trimalleoller ayak bileği kırıklarında posterior malleol fragmanın ölçümü için 3D temelli ölçüm metodlarıyla ile iki boyutlu radyoragrafi metodunun karşılaştırılması

Güray Altun <sup>1</sup>, Hatice Çatal Reis <sup>2</sup>, Bülent Bayram <sup>3</sup>, Gürsel Saka <sup>4</sup>

### Abstract

**Aim:** The aim of this study is, to compare the posterior malleolar fragment (PMF) sizing between lateral ankle radiography measurement and computer assisted 3D modelling (CA3DM) methods

**Methods:** Fifty-one patients between January 2015 and November 2018 with posterior malleolar fractures were included in this study. The rate of PMF to the articular surface at the distal end of the tibia was calculated by two different imaging methods by two surgeons. According to posterior fragment size, patients were separated into two groups. Group 1 was consisted of posterior fragment size smaller than 15% and group 2 was bigger than 15% due to CA3DM.

**Results:** The interobserver correlation (IOC) between two observers and CA3DM was 44.3%. Also the IOC between first observer and CA3DM was 35.7% ( $p<0.05$ ), second observer and CA3DM were 46.6% ( $p<0.01$ ) and observers was 51.6% ( $p<0.01$ ). For group 1, IOC between two observers and CA3DM was 41.2% ( $p<0.05$ ), first observer and CA3DM was 30.6% ( $p>0.05$ ), second observer and CA3DM was 51.6% ( $p<0.05$ ) and two observers were 45.8% ( $p<0.05$ ). For group 2, IOC between two observers and CA3DM was 27.9% ( $p>0.05$ ), first and CA3DM was 18.6% ( $p>0.05$ ), second observer and CA3DM was 7.1% ( $p>0.05$ ) and two observers was 49% ( $p<0.05$ ).

**Conclusion:** Our study shows that posterior malleolar fragment size measuring on plain radiography is not a safe method for bigger fragments and CA3DM method may be a more reliable to assess correct fragment size and also to analyze fracture morphology. But for fragments  $\leq 15\%$  CA3DM and plain radiographic measures are not statistically different.

**Key words :** ankle fractures, posterior malleolar fractures, trimalleolar fractures.

### Öz

**Amaç:** Bu çalışmanın amacı, posterior malleol fragman (PMF) boyutunun ayak bileği lateral grafisi üzerinden ölçüm ve bilgisayar destekli 3D modelleme (BD3DM) yöntemleri kullanılarak boyutlarının karşılaştırılmasıdır.

**Yöntemler:** Ocak 2015 ve Kasım 2018 yılları arasında posterior malleol kırığı olan 51 hasta çalışmaya dahil edildi. PMF boyutunun distal tibia eklem yüzeyine oranı iki farklı yöntem ile ve iki cerrah tarafından hesaplandı. Posterior fragman boyutuna göre hastalar iki gruba ayrıldı. BD3DM yöntemine göre group 1 PMF boyutu % 15'ten küçük ve group 2 % 15'ten büyük olanlardan oluşmaktaydı.

**Bulgular:** İki cerrah ve BD3DM arasında interobserver uyum % 44.3, birinci cerrah ve BD3DM % 35.7 ( $p<0.05$ ), ikinci cerrah ve BD3DM % 46.6 ( $p<0.01$ ) ve iki cerrah ile % 51.6 ( $p<0.01$ ) idi. Birinci grupta iki cerrah ve BD3DM arasındaki uyum % 41.2 ( $p<0.05$ ) ve birinci cerrah ile BD3DM arasında ise %30.6 ( $p>0.05$ )' idi. İkinci cerrah ve BD3DM arasındaki uyum % 51.6 ( $p<0.05$ ) ve iki cerrahın kendi aralarındaki uyumu % 45.8 ( $p<0.05$ )' idi. İkinci grupta iki cerrah ve BD3DM arasındaki uyum % 27.9 ( $p>0.05$ ) ve birinci cerrah ile BD3DM arasında ise %18.6 ( $p>0.05$ )' idi. İkinci cerrah ve BD3DM arasındaki uyum %7.1 ( $p>0.05$ ) ve her iki cerrahın kendi aralarındaki uyumu %49 ( $p<0.05$ ) olarak bulundu.

**Sonuç:** Çalışmamız göstermiştir ki posterior malleol fragmanın radyografik yöntemle ölçümü  $>15\%$ 'ten büyük fragmanlar için güvenilir bir yöntem değildir. BD3DM yöntemi ise gerçek fragman boyutunu hesaplamada güvenilir bir yöntemdir. Fakat  $\leq 15\%$ 'ten küçük fragmanlar için iki yöntem arasında istatistiksel fark saptanmamıştır.

**Anahtar Kelimeler:** ayak bileği kırığı, posterior malleol kırığı, trimalleol kırığı.

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## Introduction

Posterior malleolar fractures range from small extra-articular lip fractures to large triangular fragments, extending to the medial and/or lateral malleolus. These fractures generally occur as a result of rotational trauma and account for approximately 7% of all ankle fractures [1]. Less satisfactory outcomes are generally seen in ankle fractures that include a posterior malleolar fragment, compared to uni or bimalleolar fractures [2, 3]. The treatment steps of lateral and medial malleolar fractures are clear but the method to be employed in posterior malleol fractures is still a matter of debate.

A recent study recommended surgical anatomic reduction for PMF of a size > 10% of the articular surface [4]. However, other authors have suggested that only fragments >25-33% and 2 mm displaced fragments should be treated with surgical fixation [5, 6]. There is no consensus in literature regarding the optimal treatment of PMF. The most significant reason for the controversy is the estimation of PMF size from two plain radiographs. A previous radiology-based study suggested that plain radiographs were unreliable for the analysis of PMF size [7]. Recent studies have shown that measuring fragment size on a lateral ankle radiograph was not compatible with computed tomography (CT) images [8, 9]. With CT scans, preoperative fragment sizing is easier and postoperative reduction quality can also be checked. Nevertheless, many surgeons decide which treatment to apply to posterior malleolus fractures without examining CT images. But these studies are independent of the fragment sizes. For bigger sized fragments CT images may be the exact viewing method but for smaller fragments is not clear yet.

The aim of this study is to compare the measurements of posterior malleolar fragment (PMF) size measured on lateral ankle plain radiography and computer-assisted 3D modelling (CA3DM) based on computed tomography (CT) views according to fragment size.

## Material and methods

This research has been approved by the IRB of the authors' affiliated institutions. The study was conducted according to the principles described in the Declaration of Helsinki. Written informed consent was obtained from all study participants.

Retrospective evaluation was made of 75 patients who presented at the Emergency Department with tri-malleolar fractures with PMF, between January 2015 and November 2018. Inclusion criterias were patients with ankle fractures involved posterior malleol fragment, older than 18 years, proper images on radiological database for measurement. Exclusion criterias were ankle osteoarthritis, congenital or acquired ankle deformities, history of ipsilateral ankle surgery. According to posterior fragment size ratio to distal tibia articular surface, patients were separated into two groups. Group 1 was consisted of posterior fragment size smaller than 15% and group 2 was bigger than 15% due to CA3DM. All computer assisted values were compared to two surgeons' manual values for both group.

## Radiological Evaluation

Measurements were evaluated on standard lateral ankle radiographs on which the posterior portion of the distal tibia was superimposed on the distal fibula and both talar domes were superimposed allowing for adequate inspection of the superior articular surface of the talus. All radiographs were obtained on the same digital radiography device (Siemens Axiom Aristos VX, Germany) and CT images were acquired using Siemens

SOMATOM Sensation 16 or 64 scanners at a slice thickness of 4 mm and an average in-plane (x-y) resolution of 0.72 pixels.

## Radiological Measuring Process

The PMF ratio was measured with the 2 plain radiographs of the lateral ankle method by fifteen year experienced two senior orthopedic and trauma surgeons using a/b (a:posterior malleolar fragman articular surface, b:distal tibia articular surface) formula (Figure 1. Ankle CT images were uploaded to 3D-Doctor software (Able Software Corp, Lexington, MA, USA) and the PMF surface ratio was measured by a specialist topographic engineer (Table 1, 2). The medical images were stored in ".dcm (DICOM)" format. In the first step, pre-processing consisted of filtering steps for noise removal, and then in the second step, segmentation was used to differentiate bone tissue from other tissues. A region growing method was applied on the CT images, using Hounsfield Units. The noise was brought about after this interactive method was applied and the results were manually edited. Thus, the results were obtained in the present study and 3D distal tibia articular surface models were then created using 3D-Doctor software. Parts a (posterior fragment area) and b (distal tibia articular area) were saved in separate layers, thereby eliminating textural errors. Finally, biometric measurements were made on the 3D model. The measurement samples are shown in Figure 2.

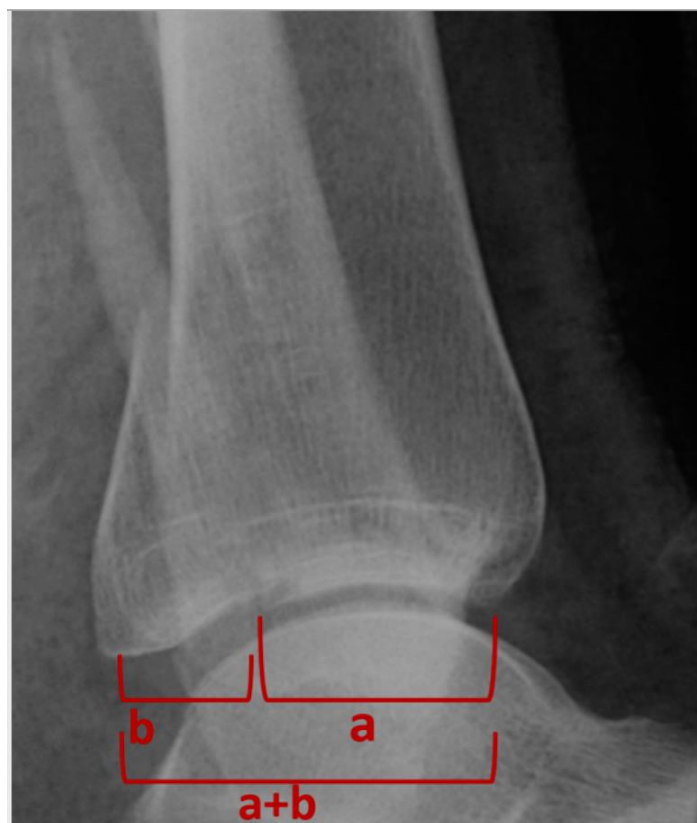


Figure 1: posterior malleolar fragment size measurement on plain radiograph.

a+b: total ankle articular size, b: posterior malleolar fragment articular size.

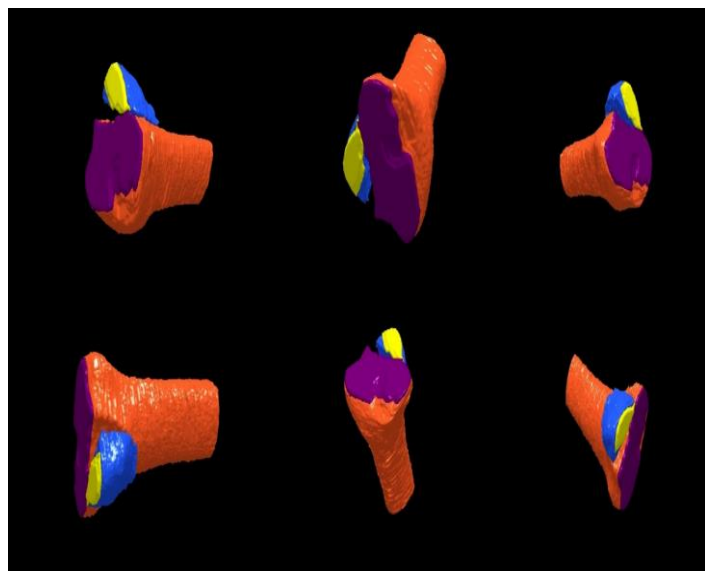


Figure 2: CA3DM images obtained via 3D-Doctor software. yellow: posterior malleolar fracture articular side, purple: ankle articular side, orange: non-articular tibial surface, blue: non-articular fragment surface.

**Statistical Analysis**

Data obtained in the study were analysed statistically using NCSS (Number Cruncher Statistical System) 2007 software (Kaysville, Utah, USA). During the evaluation of the study data, descriptive statistical methods (Mean, Standard Deviation, Median, Minimum, and Maximum) were used. Bland Altman Graphics was used for the in-group comparisons of the variables without normal distribution. Intraclass Correlation Coefficient (ICC) (Table 3) was used for evaluation of pairwise agreement between radiography and CT scan. A value of  $p < 0.05$  was considered statistically significant.

**Results**

A total of 24 patients were excluded: 3 patients with ankle osteoarthritis, 4 with congenital or acquired ankle deformities, 3 with a history of ipsilateral ankle surgery, 3 with poor quality radiographs on which fragment size could not be measured, 6 aged  $< 18$  years and 5 with missing proper CT images. Thus, the study included a final total of 51 patients, comprising 29 males and 22 females with a median age of 42.4 years (range, 19-54 years). Fracture of the right-side extremity was determined in 21 patients and the left in 30. The mechanism of trauma was traffic accident in 9 cases, sports accident in 17, a fall from height in 18 and a bicycle accident in seven.

The IOC between two observers and CA3DM was 44.3% (fair,  $p > 0.05$ ), first observer and CA3DM was 35.7% (poor,  $p < 0.05$ ), second observer and CA3DM was 46.6% (fair,  $p < 0.01$ ) and two observers was 51.6% (fair,  $p < 0.01$ ).

**Group 1 (22 patients)**

The IOC between two observers and CA3DM was 41,2 (fair,  $p > 0.05$ ) first observer and CA3DM was 30.6% (poor,  $p > 0.05$ ) second observer and CA3DM was 51,6% (fair,  $p < 0.05$ ) and between the two observers was 45,8 (fair,  $p < 0.05$ )

**Group 2 (29 patients)**

The IOC between two observer and CA3DM was 27,9%, (poor,  $p > 0.05$ ) for the first observer and CA3DM was 18.6% (poor,  $p > 0.05$ ), for the second observer and CA3DM was 7.1% (poor,  $p > 0.05$ ) two observers was 49% (fair,  $p > 0.05$ ,  $p < 0.05$ ) (Tables 3, 4).

Table 1 : The posterior malleol fragment measurements via 3D computer-assisted program and the measurements of the observers taken on plain radiographs about group 1.

Group 1	a/b# (%)	First observer (%)	Second observer (%)
1	8	8	12
2	9	8	11
3	12	13	10
4	12	11	13
5	12	13	11
6	12	14	10
7	12	11	15
8	12	15	14
9	12	14	14
10	12	12	12
11	13	14	13
12	13	17	13
13	13	14	15
14	13	12	13
15	14	14	14
16	14	14	17
17	14	13	15
18	15	12	15
19	15	11	13
20	15	13	11
21	15	14	15
22	15	10	12

#: measured with CA3DM, a/b: fragment ratio to the ankle articular surface, a: posterior fragment articular area, b: distal tibia articular surface area.

Table 2 : The posterior malleol fragment measurements via 3D computer-assisted program and the measurements of the observers taken on plain radiographs about group 2.

Group 2	a/b# (%)	first observer (%)	second observer (%)
1	16	18	20
2	16	23	24
3	16	19	17
4	17	21	19
5	17	14	15
6	18	18	19
7	18	11	14
8	18	17	22
9	19	17	20
10	19	16	17
11	19	22	24
12	20	24	23
13	20	23	20
14	21	19	21
15	21	24	23
16	22	18	19
17	22	26	28
18	22	2	22
19	22	23	21
20	24	29	30
21	25	22	23
22	25	27	29
23	26	29	30
24	26	19	23
25	26	26	24
26	27	30	29
27	28	24	24
28	28	22	23
29	28	25	22

#: measured with CA3DM, a/b: fragment ratio to the ankle articular surface, a: posterior fragment articular area, b: distal tibia articular surface area.

Table 3: Intraclass correlation coefficient (ICC) table.

ICC	Result
≤ 0.40	Poor
0.40 — 0.59	Fair
0.60 — 0.74	Good
0.75 — 1.00	Excellent

Table 4: The statistical comparisons of the observer measurements and CA3DM results.

		Values		
		total (n=51)	group 1 (n=22)	group 2 (n=29)
3DCAM	Min-Max	0.087-0.289	0.087-0.168	0.175-0.289
	(Median)	(0.168)	(0.14)	(0.225)
	Mean ±SD	0.18±0.05	0.14±0.03	0.22±0.04
Observer 1	Min-Max	0.08-0.37	0.08-0.37	0.08-0.29
	(Median)	(0.16)	(0.14)	(0.19)
	Mean ±SD	0.16±0.07	0.15±0.07	0.18±0.06
Observer 2	Min-Max	0.1-0.3 (0.17)	0.1-0.24	0.13-0.30
	(Median)		(0.14)	(0.19)
	Mean ±SD	0.17±0.05	0.16±0.05	0.20±0.05
	p	0.567	0.819	0.327
	ICC	0.443	0.412	0.279
3DCAM	ICC	0.357	0.306	0.186
Observer 1	p	0.029	0.125	0.262
3DCAM	ICC	0.466	0.516	0.071
Observer 2	p	0.005	0.020	0.404
Observer 1	ICC	0.516	0.458	0.490
Observer 2	p	0.002	0.037	0.038

SD: standard deviation, ICC:Intraclass Correlation Coefficient, p:Friedman test.

## Discussion

The discussion around posterior malleolus treatment is currently a “trend topic“. In cases with trimalleolar fracture, deciding the posterior malleol fragment size with plain radiography alone can lead to misdiagnosis and incorrect treatment mistakes. Nevertheless, measuring fragment size on plain radiographs of the lateral ankle is still the leading option in treatment decisions.

In this study, PMF size was assessed with lateral ankle plain radiograph and the CA3DM technique. The results of these two measurements were incompatible, and the values measured by two surgeons using the 2-dimensional method were also incompatible. The inconsistency between the two methods was higher in group two. This could be attributed to the fact that smaller fragments are more linear resulting in shelf-type fractures, whereas bigger fragments are more irregular. However, interestingly this incompatibility was surgeon independent in respect of fragment size as in larger fragments the congruence between surgeons was 49% and for the smaller fragments, it was 45.8%. But for smaller fragments the size of the posterior malleol was not significantly different between two imaging methods.

Apart from sizing the PMF, Haraguchi (H) and Bartonicek (B) classified these fractures in respect of morphological properties [10, 11]. Although the two classifications of H type 3 and B type 1 are similar, the B classification is more comprehensive as it includes whether or not the fracture line extends to the fibular notch, the PMF height and talar dis/subluxation. In the current series, B type 2 and 3 were determined at higher rates, which was consistent with the findings of Bartonicek's own series.

Fractures with PMF tend to have a poorer prognosis than those without a posterior fragment and thus PMF can be

seen as a negative prognostic factor [2, 12, 13]. This may be due to chondral cellular damage or intra-articular fragments that cannot be determined during surgery [14]. Recent studies have shown that intra-articular impaction and fragmentation seem to be more important than fragment size [15, 16]. This cannot be verified on plain radiographic images and it is difficult to determine the morphology and fracture pattern of PMF for bigger fragments.

Recent studies have shown incompatibility between observers in respect of PMF size on lateral ankle radiographs and this dilemma has forced the surgeons to better understand the accurate sizing and morphology of PMF [7, 17-19]. In the literature, fragment sizing methods are divided into two sections; the assessment of fragment size from plain radiography measurements and CT-based studies [8, 20]. Plain radiography studies have demonstrated poor technique accuracy and interobserver reliability in the visualisation of fragment size. Accurate assessment of articular involvement of the PMF in ankle fractures is mandatory, in addition to comminution and impaction.

Ebrahim Nabil et al. [17] reported that the problem of the distal fibula superimposed on the PF on the ankle lateral radiograph, could be eliminated with 50° external rotation. In a sawbone study by Gonzalez et al. [20], it was shown that a radiograph of the ankle in 20° external rotation would be more helpful in the evaluation of fragment size and displacement. However, the superpositioning of the distal fibula on the lateral radiograph represents the most significant drawback in the evaluation of posterior malleolar fractures. As these fractures show different morphologies according to the mechanism of formation, it is difficult to define a standard position for the taking of radiographs. In contrast, sufficient information may be obtained about both the fragment morphological structure and intra-articular displacement from measurements taken on CT images. All the patients in the current study were also evaluated with ankle CT.

In a study by Evers et al [21], fragments <25% were shown to cause later osteoarthritis in the ankle, as was also reported in the study by Langenhuisen et al [4]. However, as the measurement in the Langerhuisen study [4] had been made with the single plane radiographic method, the interobserver reliability was low, leaving these results open to debate. Although the Julia et al study was supported by CT, the medial malleolar joint surface measurements were not included. In the current study, imaging was performed including all the distal tibia joint surface fracture fragments, because it was thought that not evaluating all the distal tibia joint surfaces in posterior malleolar fractures showing extension to the medial malleolus, such as in B type 3-4 and H type 2, would prevent the accurate calculation of the joint surface area of both the whole ankle joint and the fracture fragments.

This radiology-based study has to be discussed in the light of its strengths and limitations. The primary limitation was the low number of patients, and the results were not supported with patient clinical information. However, strong aspect of the study can be said to be the assessment of fracture size from plain radiographs by two surgeons and that these results were compared with CA3DM. Previous studies have indicated that evaluating PMF size and morphology is more accurate with CT images but the current study is alike in respect of viewing fracture size via the 3D imaging system measurements and taking attention to smaller fragments measurement accuracy using plain radiography.

In conclusion, while evaluating the treatment of trimalleolar fractures, PMF sizing is essential but estimating PMF using plain radiographic images is not a safe method. For



reliable evaluations, CA3DM or similar 3D-based measurement methods should be applied specifically for fragments >15%. But for fragments ≤15%, CT and plain radiographic measures are not significantly different. This study may be helpful in decision-making before discussing treatment choices for ankle fractures with posterior fragment involvement and for smaller fragments complicated imaging methods can be eliminated.

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# Preliminary results of metabolically supported chemotherapy combined with ketogenic diet, hyperthermia and hyperbaric oxygen therapy in stage II-IV rectal cancer

## Evre II-IV rektum kanserinde metabolik destekli kemoterapinin ketojenik diyet, hipertermi ve hiperbarik oksijen tedavisi ile kombinasyonunun ön sonuçları

Mehmet Salih İyikesici<sup>1</sup>

### Abstract

**Aim:** Systemic chemotherapy is a part of multi-modality treatment in patients with stage II-IV rectal cancer. In particular, patients not eligible for curative resection at the time of diagnosis require more efficient approaches to improve outcomes. Metabolically supported chemotherapy (MSCT) is a novel approach targeting dysregulated energy mechanism of the tumor cell. This study aimed to examine the efficacy of MSCT combined with ketogenic diet, hyperthermia and hyperbaric oxygen therapy (HBOT) in patients with stage II-IV rectal cancer not eligible for surgery at baseline.

**Methods:** Twenty-one patients diagnosed with stage II-IV rectal carcinoma who received metabolically supported chemotherapy (MSCT) combined with ketogenic diet, hyperthermia and HBOT were included. First-line chemotherapy regimen was oxaliplatin-based, whereas second line regimen was irinotecan-based. Overall survival and progression-free survival were estimated.

**Results:** Mean duration of follow-up was 33.3±22.0 months. Mean overall survival was 58.6 months (95% CI, 43.3 - 73.9) and corresponding figure for progression-free survival was 45.1 months (95% CI, 28.9-61.2). Mean overall survival for patients with metastatic disease was 35.7 months. Multivariate analysis identified male gender and stage IV disease as independent predictors of worse progression free survival. No other parameter effected survival outcomes.

**Conclusion:** Findings of this study are promising for potential use of this novel combinatorial protocol targeting multiple vulnerabilities of tumor cells in patients with advanced rectal cancer, particularly for patients with metastatic disease, without additional safety concerns. However, long term results are warranted to draw firm conclusion.

**Key words:** rectal cancer, metabolically supported chemotherapy, ketogenic diet, hyperthermia, hyperbaric oxygen therapy, survival

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### Öz

**Amaç:** Evre II-IV rektum kanserinde sistemik kemoterapi multimodalite tedavisinin bir parçasıdır. Özellikle, tanı sırasında küratif rezeksiyona uygun olmayan hastaların sonuçlarını iyileştirmek için daha etkili yaklaşımlara gerek vardır. Metabolik destekli kemoterapi (MDKT) tümör hücresinin bozulmuş enerji metabolizmasını hedef alan yeni bir yaklaşımdır. Bu çalışma, ketojenik diyet, hipertermi ve hiperbarik oksijen tedavisi (HBOT) ile kombine edilmiş MDKT'nin başlangıçta cerrahi için uygun olmayan evre II-IV rektum kanseri hastalarındaki etkinliğini değerlendirmeyi amaçlamıştır.

**Yöntemler:** Metabolik destekli kemoterapi (MDKT) tedavisi ile ketojenik diyet, hipertermi ve HBOT kombinasyonu almış 21 evre II-IV rektum kanseri hastası çalışmaya dahil edilmiştir. Birinci basamak kemoterapi oksaliplatin bazlı, ikinci basamak kemoterapi ise irinotekan bazlıdır. Genel sağkalım ve progresyonsuz sağkalım hesaplanmıştır.

**Bulgular:** Ortalama takip süresi 33.3±22.0 aydır. Ortalama genel sağkalım 58.6 ay (95% CI, 43.3 - 73.9), ortalama progresyonsuz sağkalım 45.1 ay (95% CI, 28.9-61.2) olarak bulunmuştur. Metastatik hastalığı olanlarda ortalama genel sağkalım 35.7 ay olmuştur. Çok değişkenli analiz erkek cinsiyet ve evre IV hastalığın kötü progresyonsuz sağkalım için bağımsız belirteçler olduğunu göstermiştir. Başka hiçbir parametre sağkalım sonuçlarını etkilememiştir.

**Sonuç:** Bu çalışmanın bulguları, tümör hücresinin birçok zayıf noktasını hedef alan bu yeni kombinasyon protokolünün ilerlemiş rektum kanseri hastalarında, özellikle de metastatik hastalığı olanlarda, ek güvenilirlik endişesi oluşturmadan kullanılabileceği yönünde ümit vermektedir. Ancak, daha net çıkarımlara ulaşmak için uzun dönem sonuçlar gereklidir.

**Anahtar Kelimeler:** rektum kanseri, metabolik destekli kemoterapi, ketojenik diyet, hipertermi, hiperbarik oksijen tedavisi, sağkalım

## Introduction

Colorectal cancer represents an important cause of mortality and morbidity. In the US, it is the second leading cause of cancer related deaths and the fourth most frequently diagnosed malignancy [1]. However, last decades witnessed substantial decreases in its incidence and mortality [2-4], most probably owing to screening resulting in early detection and prevention, and advances in treatment modalities.

Recent guidelines recommend combined-modalities consisting of surgery, concurrent fluoropyrimidine-based chemotherapy with ionizing radiation to the pelvis (chemoRT), and chemotherapy for most patients with stage II or III rectal cancer [1]. Several regimens including FOLFOX and FORFIRI have been examined for systemic chemotherapy for metastatic disease [5, 6]. Although encouraging results have been obtained with combination of treatments and advances in modalities, patients who are not eligible for curative resection at the time of diagnosis require more efficient approaches to improve outcome.

Metabolically Supported Chemotherapy (MSCT) is a novel chemotherapy application strategy targeting metabolic vulnerabilities of cancer cells [7-10]. Cancer cells have a dysregulated energy metabolism almost evident in all tumor types [11], which was first recognized by Otto Warburg, in 1924 [12, 13]. Insufficient oxidative phosphorylation is compensated by aerobic fermentation, resulting in glucose dependency and increased lactate production. These abnormalities have been linked to mitochondrial dysfunction and genetic mutations [11, 14, 15]. MSCT targets glucose dependency of tumor cells as well as membrane permeability to chemotherapeutic drugs, which increases by the administration of insulin [16]. It integrates 12-hour fasting and administration of insulin to the usual chemotherapy schedule, thereby reducing the available glucose for the tumor cells. Adopting a ketogenic diet further decreases circulating glucose levels. A high-fat, carbohydrate-restricted ketogenic diet has been shown to slow the progression of cancer [8, 9, 17, 18].

Hyperthermia and hyperbaric oxygen therapy (HBOT) are two modalities that are shown to have additional beneficial contributions to chemotherapy. Hyperthermia causes direct cytotoxicity and acts synergistically with radiotherapy and chemotherapy through sensitizing cancer cells to these therapies [7-10, 19-22]. HBOT on the other hand, results in better oxygenation of tumor cells thus counteracting unfavorable consequences of hypoxia, which has cancer promoting effects and promotes resistance to chemotherapy and radiotherapy [23-27]. To date, several clinical studies demonstrated their benefit when used in combination with chemotherapy and radiotherapy [8-10, 19, 20].

Based on the supporting evidence from the abovementioned studies, MSCT, ketogenic diet, hyperthermia and HBOT have the potential to work together by targeting vulnerabilities of cancer cells and several overlapping metabolic pathways. To date, no study has reported the efficacy of this novel strategy in the treatment of rectal cancer. We hypothesize that this approach would result in encouraging treatment outcomes in rectal cancer patients.

In this study we aimed to evaluate the efficacy of MSCT combined with ketogenic diet, hyperthermia and HBOT in the treatment of stage II-IV rectal cancer patients.

## Material and methods

After approval of the ethical committee, the study was conducted according to the principles described in the Declaration of Helsinki. Written informed consent was obtained from all study participants.

### Study design and patient selection

Twenty-one patients diagnosed with stage II, III, or IV rectal carcinoma between February 2012 and September 2017 who received MSCT together with ketogenic diet, hyperthermia and hyperbaric oxygen therapy were included in this retrospective single-center study. Patients were either not eligible for or refused to have surgical resection at the time of diagnosis. In addition, patients adapted a ketogenic diet and at each chemotherapy session they received hyperthermia application and hyperbaric oxygen therapy. A prospectively maintained institutional database was screened to identify patients from a subset diagnosed with rectal cancer (any class, stage or subtype) and treated at our clinic with this combinatorial protocol during the study period. Inclusion criteria were as follows: biopsy-proven rectal cancer, measurable disease as defined by Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1) [28], radiologically-proven staging of disease, and receiving MSCT together with ketogenic diet, hyperthermia and hyperbaric oxygen therapy.

### Chemotherapy regimen

All patients received a chemotherapy regimen consisting of oxaliplatin 85 mg/m<sup>2</sup> IV over 2 h on day 1 plus leucovorin 400 mg/m<sup>2</sup> IV over 2 h on day 1 plus 5-FU 400 mg/m<sup>2</sup> IV bolus on day 1, then 5-FU 1200 mg/m<sup>2</sup>/day for 2 days (total 2400 mg/m<sup>2</sup> over 46-48 hours) continuous infusion as first-line treatment. This combination treatment was administered in an outpatient setting and repeated every 2 weeks until disease progression. In case of progression, patients were administered a chemotherapy regimen consisting of irinotecan 180 mg/m<sup>2</sup> IV over 30-90 min on day 1 plus leucovorin 400 mg/m<sup>2</sup> IV infusion over 30-90 min on day 1 plus 5-FU 400 mg/m<sup>2</sup> IV bolus on day 1, then 5-FU 1200 mg/m<sup>2</sup>/day for 2 days (total 2400 mg/m<sup>2</sup> over 46-48 hours) continuous infusion repeated every 2 weeks as second-line treatment. Assessment of treatment response was based on radiographic evaluations at the end of each 3-month period and was always done by PET-CT. Patients that achieved complete response (CR), partial response (PR), or stable disease (SD) status continued to receive maintenance therapy with the same regime until death as far as tolerated.

### Administration of metabolically supported chemotherapy

All eligible patients were advised to adapt a ketogenic diet during the study period and they were asked to fast for 12 hours before each chemotherapy session. In addition to the premedication with 45.5 mg pheniramine maleate and 0.25 mg palonosetron HCl, each patient received regular insulin (Humulin® R) in doses ranging between 5-20 IU prior to chemotherapy administration in order to achieve a state of mild hypoglycemia with blood glucose levels around 50-60 mg/dl for normoglycemic patients and in accordance with MSCT protocols [7-10]. Following each MSCT administration, patients received a 60-minute session of hyperthermia and a 60-minute session of

HBOT. Hyperthermia was provided using OncoTherm EHY-3010 HT device (OncoTherm, Troisdorf, Germany). The temperature of the tumoral region was gradually increased to 45°C with a mobile electrode. For HBOT, Quamvis 320 hyperbaric oxygen chamber (OxyHealth, California, US) was used and the patient was subjected to 1.5 atmospheres pressure (ATA).

**Statistical analysis**

IBM SPSS Statistics version 21.0 software (SPSS Inc., Chicago, IL) was used for data analyses. Overall survival was defined as the time between the date of diagnosis and death from any cause. Progression free survival was defined as the time between the date of diagnosis and progression or death from any cause. Patients without event at the last follow up were censored. Survival rates were estimated using Kaplan-Meier analysis, and intergroup comparisons were done with log-rank test. Univariate potential predictors were entered into Cox proportional hazards model to identify independent predictors of survival outcomes. Level of significance was set at <0.05.

**Results**

Table 1 shows demographical and clinical characteristics of the patients. All patients had adenocarcinoma. Mean duration of follow-up was 33.3±22.0 months (median 28.3, range 8.9-84.0 months). During the follow-up period 7 patients died. Mean overall survival was 58.6 months (95% CI, 43.3 - 73.9) and corresponding figure for progression-free survival was 45.1 months (95% CI, 28.9-61.2). Figure 1 shows Kaplan-Meier curves for overall survival and progression free survival. 1-year and 2-year overall survival rates were 95% and 80%, respectively.

Table 1: Demographical and clinical characteristics of the patients.

Characteristic	n=21
Age, year, median (range)	60 (40-82)
Male gender	11 (52.4%)
Stage	
II	5 (23.8%)
III	10 (47.6%)
IV	6 (28.6%)
Histological grade	
2	16 (76.2%)
3	5 (23.8%)
Surgery*	10 (47.6%)
Radiotherapy	18 (85.7%)

Unless otherwise stated, data presented as n (%)

\*These patients received surgical treatment after complete response to chemotherapy.

Table 2 shows overall survival and progression-free survival rates by patient characteristics. None of the patient characteristics have effect on overall survival. However, in univariate analysis, stage IV patients had worse progression-free survival when compared to stage II-III patients (21.2 vs. 51.9

months, p=0.014). In multivariate analysis, patients with stage IV disease (OR, 26.4; 95% CI: 1.5-456.4; p=0.024) and male patients (OR, 33.4; 95% CI: 1.5-744.7; p=0.027) had worse progression-free survival. None of the other patient characteristics effected survival outcomes. Figure 2 shows Kaplan Meier curves for progression free survival by gender and stage.

Table 2: Survival rates by patient characteristics (univariate analysis).

Characteristic	Mean OS (95% CI) (months)	p <sup>a</sup>	Mean PFS (95% CI) (months)	p <sup>a</sup>
All patients (n=21)	58.6 (43.3 - 73.9)		45.1 (28.9-61.2)	
Age				
≤ median (n=12)	63.8 (43.3 - 84.3)	0.392	41.5 (20.6-62.3)	0.355
> median (n=9)	47.5 (28.1-66.9)		50.6 (30.9-70.3)	
Gender				
Male (n=11)	56.2 (38.0-74.3)	0.798	27.7 (16.8-38.6)	0.124
Female (n=10)	56.8 (38.9-74.6)		53.7 (33.7-73.6)	
Stage				
II-III (n=15)	63.3 (46.5-80.2)	0.255	51.9 (33.2-70.7)	0.014
IV (n=6)	35.7 (27.9-43.5)		21.2 (13.1-29.3)	
Histological grade				
2 (n=16)	60.6 (52.3-69.0)	0.333	47.0 (34.0-60.0)	0.356
3 (n=5)	50.3 (22.1-78.5)		36.6 (13.7-59.6)	
Surgery				
Surgery added (n=10)	56.8 (34.8-78.7)	0.897	34.2 (15.1-53.3)	0.201
No surgery(n=11)	53.2 (33.3-73.2)		51.8 (34.4-69.2)	
Radiotherapy				
Radiotherapy added (n=18)	59.6 (9.9-40.3)	0.780	51.6 (31.4-71.7)	0.070
No radiotherapy (n=3)	53.0 (32.6-73.4)		26.7 (12.8-40.7)	

<sup>a</sup>Log-rank test. OS, overall survival; PFS, progression-free survival.

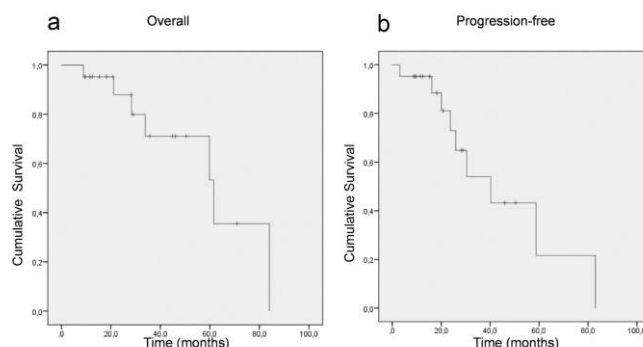


Figure 1: Kaplan Meier curves for overall survival (a) and progression free survival (b) in all patients.

During the study period, no significant or severe problems were encountered due to fasting, hypoglycemia, hyperthermia or hyperbaric oxygen therapy. However, mild

burning sensation at the site of hyperthermia application and mild earache immediately after hyperbaric oxygen therapy were rarely reported by some patients. Both were self-limiting and lasted not more than a couple of hours in all cases.

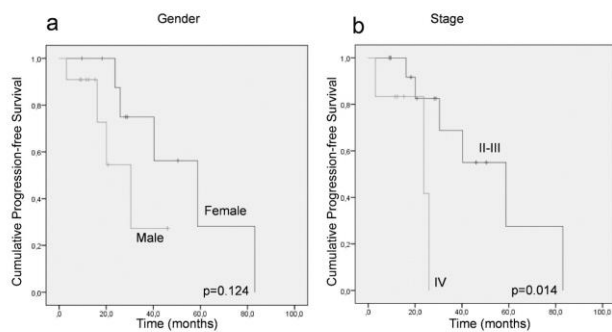


Figure 2: Kaplan Meier curves for progression-free survival by gender (a) and stage (b). P values are calculated with Log rank test.

## Discussion

This is the first study to examine the combination of MSCT with ketogenic diet, hyperthermia and HBOT in rectal cancer, and findings are promising in terms of survival outcomes, particularly for the patients with metastatic disease. This novel combination treatment targets multiple susceptibilities of the tumor cell; at metabolic, cellular and pharmacological levels.

In patients with stage II and III rectal cancer who underwent preoperative chemotherapy, reported long-term overall survival rates are usually over 60% [29-31]. In this study, we administered a novel combination of MSCT with ketogenic diet, hyperthermia and HBOT and achieved promising 1- and 2-year overall survival rates of 95 and 80% in a heterogeneous group of stage II-IV patients who were not eligible for surgery at baseline; however, since the follow-up period is not long enough to reach median survival, it does not seem plausible to give reliable 5- and 10-year overall survival rates. On the other hand, mean overall survival rate among the patients with metastatic disease achieved in this study is 35.7 months, which is relatively long for such group of patients with an unfavorable outlook. For example, a recent study included rectal carcinoma patients with synchronous metastases who received combination of FOLFOX chemotherapy with split-course pelvic chemoradiation (FOLFOX + CRT) and obtained median overall survival of 23 months [32]. Another study compared outcomes of neoadjuvant chemoradiotherapy and postoperative systemic chemotherapy without radiotherapy in metastatic rectal cancer patients and achieved 24 and 27 months of median overall survival, respectively [33].

Clinical evidence on the potential benefit of MSCT comes from clinical studies with pancreatic cancer patients and non-small cell lung cancer patients as well as from two case reports. In a recent study with stage IV ductal pancreatic adenocarcinoma patients, metabolically supported administration of chemotherapy resulted in promising survival outcomes with a reported median survival of 15.8 months [9]. Another recent study reported encouraging results in stage IV non-small cell lung cancer with a mean 42.9 months overall survival rate [10]. In addition, good responses have been reported in an 81-year old patient with locally advanced rectal cancer and a stage IV triple negative breast cancer patient when chemotherapy regimen was administered in a metabolically supported form [7, 8]. Several mechanisms may play role in the favorable contribution of

metabolic support to treatment efficacy. Induced hypoglycemia is the main consequence of metabolic support through fasting and insulin administration. Due to dysregulated energy metabolism, cancer cells are more dependent on circulating glucose, thus scarcity of available glucose will pose an acute metabolic stress on these cells, especially when compared to healthy cells, which will render them more susceptible to the cytotoxic effects of chemotherapy [11-15]. In addition, insulin has the potential to increase membrane fluidity and permeability [34, 35]. Internalization of drug-insulin complexes through receptor mediated endocytosis would ensure rapid transfer into the cell, thereby enhancing cytotoxic effects [36, 37]. The density of insulin and insulin-like growth factor (IGF) receptors is higher on tumor cells when compared to their healthy counterparts [38, 39]. The reaction between insulin and these receptors would extend the S-phase of the cell cycle and render them more susceptible to the cytotoxic effects of chemotherapeutics for longer periods [40]. On the other hand, lower density of these receptors on healthy cells would relatively protect them from the cytotoxic effects of chemotherapeutic drugs, which may translate into less adverse effects.

Ketogenic diet has been used for decades as a treatment for intractable pediatric epilepsy; however, its possible use in cancer therapy has recently been explored. Similar to fasting and insulin administration in conjunction with chemotherapy, adapting a ketogenic diet also targets the increased glucose dependency of the cancer cell. Several studies provided evidence for its potential role in the treatment of cancer [8, 17, 18, 41-45]. Hyperthermia itself is cytotoxic. HBOT also targets the reliance of tumor cells on glycolysis, a major contributor to the upregulation of antioxidant activity responsible for the increased resistance of the tumor to pro-oxidant chemotherapy and radiation therapies [46]. Concomitant use of these therapies and potential synergism between them have been explored in previous studies [8, 11, 18-21, 27, 41, 47, 48]. For example, Ohguri et. al. obtained promising results in NSCLC patients with multiple pulmonary metastases in association with the concomitant administration of carboplatin/paclitaxel chemotherapy regimen, hyperthermia and HBOT [19].

Retrospective design, small sample size, patient heterogeneity and short follow-up are the major limitations of this study. Low sample size might have resulted in low statistical power not sufficient to detect survival differences between risk groups. Due to short follow-up, median survival could not be reached, and long-term survival rates cannot be estimated. Nevertheless, findings of this study underscore the potential benefits of exploring and integrating additional modalities targeting cellular vulnerabilities of the cancer cell to conventional chemotherapy, provided that they are based on biochemical and pharmacological rationale.

In conclusion, our findings suggest that MSCT combined with a ketogenic diet, hyperthermia and HBOT appears to be a feasible approach for the treatment of patients with advanced rectal cancer or in patients that surgical treatment is not feasible. Further research, especially comparative clinical trials with long-term follow-up are warranted to support this protocol.

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# Are routine intraoperative and postoperative leakage tests needed in bariatric surgery?

## Bariatrik cerrahide rutin intraoperatif ve postoperatif kaçak testlerine ihtiyaç var mı?

Erkan Yardımcı<sup>1</sup>, Yunus Yapalak<sup>1</sup>

### Abstract

**Aim:** We aimed to evaluate the efficacy of the results of intraoperative methylene blue test and postoperative upper gastrointestinal contrast graphies for detecting leakage in both primary and revisional bariatric surgery.

**Methods:** Two-hundred-eighty-seven patients, who underwent primary and revision bariatric surgery and routine intraoperative methylene blue test and upper gastrointestinal contrast studies postoperatively for leakage were included in the study. Patients' demographic characteristics, comorbidities, length of hospital stay, operation time, intraoperative, and postoperative complications were analyzed retrospectively.

**Results:** In our study, 256 of 287 (89.1%) patients underwent primary surgery, 221 (75.7%) patients were female, the mean patient age was  $38.4 \pm 11.9$  years, and the mean body mass index was  $44.3 \pm 7.6$  kg/m<sup>2</sup>. The number of patients who had previous abdominal surgery and comorbidity was 108 (37.6%) and 149 (51.9%), respectively. Leakage was detected by a methylene blue test in one (0.3%) patient who underwent one-anastomosis gastric bypass surgery. In one (3.2%) patient who underwent revisional surgery with negative results of methylene blue test, leakage was detected on the first postoperative day due to the clinical findings. There was no leakage detected in any patient with postoperative swallow graphies. There was no statistical difference in leakage between primary and revisional surgery groups ( $p = 0.23$ ). There was no mortality.

**Conclusion:** It could be unnecessary to use postoperative gastrointestinal contrast studies in both primary and revisional bariatric surgery, but the routine use of the intraoperative methylene blue test could be considered useful due to its positive results for the detection of leakage.

**Keywords:** Bariatric surgery, leakage, methylene blue, radio contrast imaging

### Öz

**Amaç:** Primer ve revizyonel bariatrik cerrahide kaçak tanısı için uygulanan intraoperatif metilen mavisi testi ile postoperatif üst gastrointestinal sistem kontrastlı grafilerin etkinliklerini ve sonuçlarını değerlendirmeyi amaçladık.

**Yöntemler:** Primer ve revizyonel cerrahi uygulanan, kaçak tespiti için rutin olarak intraoperatif metilen mavisi testi ile postoperatif üst gastrointestinal kontrastlı grafi yapılan 287 hasta çalışmaya dahil edildi. Hastaların demografik özellikleri, komorbid hastalıkları, hastanede yatış süresi, ameliyat süresi, intraoperatif ve postoperatif komplikasyonlar retrospektif olarak incelendi.

**Bulgular:** Çalışmamızdaki 287 hastanın 256'sına (%89,1) primer cerrahi uygulandı ve hastaların 221'i (%75,7) kadın, ortalama yaş  $38.4 \pm 11.9$  yıl ve ortalama beden kitle indeksi  $44.3 \pm 7.6$  kg /m<sup>2</sup> idi. Daha önce abdominal cerrahi geçiren ve komorbid hastalığı olan hasta sayıları sırasıyla 108 (%37,6) ve 149 (%51,9) idi. Bir (% 0,3) tek-anastomozlu gastrik bypass olgusunda metilen mavisi testinde kaçak tespit edildi. Revizyon cerrahisi uygulanan 1 (%3,2) olguda, metilen mavisi testinde kaçak tespit edilmeyip, postoperatif 1. gün klinik bulgulara göre kaçak tespit edildi. Postoperatif kontrastlı grafi sonuçlarında hiçbir hastada kaçak tespit edilmedi. Primer ve revizyonel cerrahi uygulanan olgular arasında görülen kaçaklarda istatistiksel olarak anlamlı fark tespit edilmedi ( $p=0,230$ ). Mortalite yok idi.

**Sonuç:** Primer ve revizyonel bariatrik cerrahide postoperatif gastrointestinal kontrast çalışmalarının kullanılmasına gerek olmayabilir, ancak kaçak tespitindeki pozitif sonuçları nedeniyle intraoperatif metilen mavisi testinin rutin olarak uygulanması düşünülebilir.

**Anahtar Kelimeler:** Bariatrik cerrahi, kaçak, metilen mavisi, radyo kontrastlı görüntüleme

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## Introduction

The most effective treatment for morbid obesity and obesity-related comorbid conditions is bariatric and metabolic surgery [1]. These surgeries provide long-term weight loss and an increase in life expectancy. The most feared complication after these operations is leakage, and the incidence of leakage has been reported to be between 0.1% and 5.6%, depending on the type of surgery [2]. Various techniques have been described, including larger bougie size in sleeve gastrectomy (SG), reinforcement of staple line with Fibrin Glue, use of absorbable buttressing material, and oversewing staple lines to prevent the leakage [3]. Early diagnosis and treatment of leaks are important to prevent problems such as hemodynamic shock, respiratory distress, and multiorgan failure that might cause mortality [4, 5]. The risk factors that increase the risk of leakage in bariatric and metabolic surgery are: gender (male), age > 50 years, body mass index (BMI) > 50 kg/m<sup>2</sup>, lack of surgical experience, Type 2 diabetes, hypertension, sleep apnea syndrome, and revisional surgery [6-8].

Various methods can be used for intraoperative testing for leaks, including air leak test, methylene blue dye, and endoscopy. The positive results of these tests for leakage are important for early diagnosis, but negative results do not eliminate the possibility of leakage. For these reasons, performing routine intraoperative leak tests is controversial [9, 10]. Postoperative swallow studies are used for the diagnosis of a leak in many centers, but its routine use is controversial as an intraoperative leakage test [11]. In the literature, there is little data for the necessity of routine use of both intraoperative and postoperative leakage tests in bariatric surgery.

This paper aims to evaluate our practice of routine use of both the intraoperative methylene blue test and postoperative gastrointestinal contrast study in our center.

## Material and methods

We retrospectively analyzed the records of the 287 patients who underwent primary and revisional bariatric surgery, which routinely performed intraoperative methylene blue test (IMBT) and postoperative swallow graphy, from January 2018 to November 2019 at Bezmialem Vakif University School of Medicine. The Local Ethics Committee approved the study (2019-18890). The study was conducted according to the principles described in the Declaration of Helsinki. Written informed consent was not obtained from patients due to the retrospective nature of the study. Primary bariatric surgeries included sleeve gastrectomy (SG) and one anastomosis gastric bypass (OAGB); the revisional surgeries were included adjustable gastric band (AGB) to SG/OAGB, SG to OAGB/Roux-en-Y gastric bypass (RYGB), and OAGB to distal RYGB. Patient details were identified from a prospectively maintained database. The inclusion criteria of the patients who underwent primary bariatric and metabolic surgery were: morbidly obese (BMI > 40 kg/m<sup>2</sup>) patients and patients with a BMI of 35 kg/m<sup>2</sup> and had at least one obesity-related comorbidity, such as type 2 diabetes, hypertension, hyperlipidemia, sleep apnea syndrome. The inclusion criteria of the patients who underwent revisional surgery (RS) were: complications (leakage, stenosis, gastroesophageal reflux, twist) that occurred after the primary surgery, the rate of excess weight loss (EWL%) within 2 years postoperatively was less than 50% or weight regain was at least 25% of the total weight loss, and inadequate and/or recurrence of comorbid conditions resolution. Extralumination of methylene blue or contrast agent from the stapler line or gastroenterostomy anastomosis area was evaluated as leakage. For anastomotic or staple line leaks, procedural complications such as esophageal

perforation, massive bleeding due to the insertion of an orogastric tube, and adverse reactions for both methylene blue and contrast agent were evaluated. Visualization of emerging from the staple line would indicate a staple line defect, which can then be immediately repaired or reinforced. Also, operative time, hospital length of stay, re-admission, and overall 30-day complications were examined.

### Techniques of the leakage tests

The orogastric tube was routinely inserted intraoperatively to all patients at the end of the operation by the anesthesiologists. In the SG, the pylor was occluded using a stapler. The jejunum distal to the gastrojejunostomy was occluded with a laparoscopic bowel grasper in OAGB or RYGB by the surgeon. Between 50 and 70 ml of saline solution, which is stained with 3 ml methylene blue, was introduced via the orogastric tube to control the leakage from the staple line or anastomosis (Figure 1). On the second postoperative day, all patients drunk 100 ml water, which included 50 ml Iohexol 350 mg I/ml (GE Healthcare, Ireland), and the presence of the contrast extravasation was evaluated in the swallow grafies (Figure 2).



Figure 1: Intraoperative methylene blue test in SG (a) and OAGB (b).

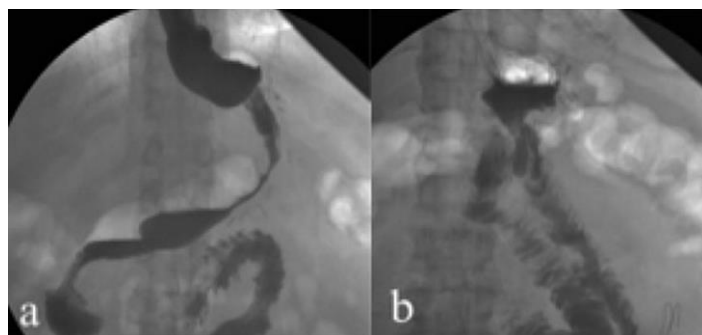


Figure 2: Postoperative swallow grafies in SG (a) and revision of SG to OAGB (b).

### Statistical Analysis

SPSS 23.0 (SPSS Inc., Chicago, IL, USA) software was used for all statistical analyses. Continuous variables were expressed as mean ± standard deviation and as the median value, if necessary. Categorical variables were expressed as frequencies with percentages. The sensitivity and specificity of tests were calculated. An appropriate Chi-square test was used to determine the significance between nominal variables.

## Results

Two-hundred-fifty-six (89.1%) primary bariatric procedures (SG n=236 patients and OAGB n=20 patients) and 31 (10.8%) revisional surgeries (SG to RYGB n=8 patients, SG to distal OAGB n=18 patients, AGB to SG n=3 patients, AGB to OAGB n=1 patient, OAGB to distal RYGB n=1 patient) were done consecutively in the study period at the single institution. The patients' demographic characteristics are shown in Table 1. Synchronous laparoscopic cholecystectomy and hiatal hernia



repair were done in 12 (4.1%) and three (1%) patients, respectively. Two of 31 (6.5%) revisional laparoscopic procedures were converted to laparotomy because of the adhesions resulting from previous abdominal surgery. Three revisional operations were done by open surgery. Overall, the methylene blue test was positive in only 0.3% and demonstrated a sensitivity of only 50%. In this patient, who underwent primary OAGB, leakage was detected by intraoperative methylene blue test at the gastrojejunostomy and the leaking area immediately repaired, and a subsequent confirmatory intraoperative methylene blue test was done. This patient was discharged without any complication on the third postoperative day. In one patient (3.2%), who underwent RS from SG to OAGB, a leak was diagnosed on the postoperative first day by seen bile in an abdominal drain and underwent the second operation without any diagnostic radiological test. Laparoscopy was performed, and the leakage was detected in gastrojejunostomy. The leaking area was oversewn by 2/0 prolene suture and the patient discharged on the fifth postoperative day without any complication. No leakage was diagnosed in the results of the swallow studies, so sensitivity could not be calculated for this test. The sensitivity of the methylene blue test was calculated as 50%. The specificity of both tests was 100%. Subgroup analysis of primary and RS shows no differences in leakage (Fisher exact test,  $p = 0.23$ ). There was no mortality in the postoperative period. There were no intraoperative and/or postoperative complications during the use of leakage tests, such as esophageal perforation and allergic reactions, in our study group.

Table 1: Demographic and clinical characteristics of the study sample.

Characteristic	Primary surgery group	Revisional surgery group
Gender <sup>β</sup>		
Female	193 (75.4)	26 (83.8)
Male	63 (24.6)	5 (16.2)
Age (years) <sup>‡</sup>	37.6 ± 11.9	44.6 ± 10.3
BMI (kg/m <sup>2</sup> ) <sup>‡</sup>	45.4 ± 7.1	35.5 ± 6.3
Previous abdominal surgery <sup>β</sup>	80 (31.2)	31 (100)
Comorbidities <sup>β</sup>		
Type2 DM*	98 (38.2)	10 (32.2)
Hypertension	66 (25.7)	9 (29)
Hyperlipidemia	26 (10.1)	2 (6.4)
Sleep apnea	22 (8.5)	1 (3.2)
Duration of surgery (minute) <sup>‡</sup>	76 ± 23	185 ± 36
Hospital stay (day) <sup>‡</sup>	3.1 ± 0.6	3.4 ± 1.1
30-day morbidity <sup>β</sup>		
Hemorrhage	2 (0.7)	1 (0.3)
Deep vein thrombosis	-	1 (0.3)
Readmission <sup>β</sup>	-	3 (9.7)

<sup>β</sup>: n(%), <sup>‡</sup>: Mean±SD

\*Diabetes Mellitus

## Discussion

Bariatric surgery remains the only proven modality for sustained weight loss with improvement in obesity-related comorbidities in the morbidly obese patient population worldwide. With the increase of the primary bariatric and metabolic surgery numbers, surgeons aim to prevent complications, which can result in prolonged hospital stay and mortality. Parallel to the increase in the rate of primary surgery, the rates of revisional surgeries are increasing. The risk of complications might increase in revision surgery due both to the state of the tissue and the complexity of the procedure itself [4, 12]. Postoperative leakage is the most feared complication after bariatric surgery and the clinical signs of a leak require emergency treatment, such as exploration, endoscopic

procedures, and percutaneous drainage. Surgeons use a variety of techniques (such as staple line reinforcements, methylene blue test, or air insufflation under saline or endoscopic examination of the anastomosis and staple line) to reduce complication rates, especially on leakage, and improve patients' safety. However, a standard technique has not yet been accepted.

The international consensus group on sleeve gastrectomy has not reached a definitive conclusion on whether there is a benefit in the use of routine intraoperative leak tests [10]. On the other hand, some studies found the limited benefit of intraoperative leak tests, but the results of these tests could not predict or prevent leakage in all bariatric procedures [13, 14]. Intraoperative leak tests can only detect the rare leaks due to technical failures, such as stapler misfire or surgeon inexperience [13,15]. In a large multicenter study with 4284 SG patients, 37 (0.9%) postoperative leakages were found. Two of 37 (0.08%) leaks were diagnosed by intraoperative leak test and 21 of 37 patients (0.49%) who had negative intraoperative test findings developed leakage. Leakage occurred in the postoperative period in 14 of 37 patients (32%) who had no intraoperative test. When comparing cases with and without intraoperative leak tests performed, there was no significant difference in leak rates among patients who underwent intraoperative leak tests compared to those that did not (1.0% vs. 0.7%,  $p = 0.41$ ) [16]. In our study, we detected only one positive intraoperative leak test and one postoperative leak had a normal intraoperative methylene blue test. We had a low number of leaks, which might have diminished the value of the leak test. We presume this is due to the experience of the surgeons who perform these operations, as this is known to have a positive impact on outcomes [17]. In our experience, leaks can be avoided by including some important technical points: avoidance of distal stenosis and/or twist, especially in SG; adequate compression time during stapling; gentle tissue handling; avoiding thermal damage at anastomosis and staple line; and staying away from the gastroesophageal junction. We did not identify any major benefit in the routine use of a methylene blue leak test in primary and revisional bariatric surgery.

Many surgeons perform postoperative radiological upper gastrointestinal (UGI) series routinely as screening tests for leakage. However, the role of routine postoperative use of these contrast studies in detecting leaks after bariatric surgery is controversial [18]. The early leaks, which are likely due to technical error or staple line failure, might be detected by routine postoperative UGI contrast studies.

In a study by Sethi et al. [18], routine postoperative UGI contrast studies identified only two of 20 leaks. The patients with both early and delayed leaks demonstrated significant clinical abnormalities, such as fever, abdominal pain, and tachycardia at the time of leak presentation, before the confirmatory radiographic study. The vast majority of leaks returned normal results in the UGI series and presented 2–3 weeks after discharge. These authors concluded that the clinical indicators are the most useful factors to raise concern for leaks and UGI studies and may be preferred in selective patients. Mbadiwe et al. [11] performed a meta-analysis of 10,139 UGI series after bariatric surgery and found the sensitivity and specificity of these tests in detecting leak were 54% and 100%, respectively. Some studies in the literature have reported false negative radiological UGI results [5,19]. Mizrahi et al. [20] reported their large series, and none of the five leaks were detected by routine radiological UGI on the first postoperative day. These authors concluded that routine UGI is not an efficient screening test for the leak after bariatric surgery, and recommended abandoning this practice. We identified only one leak at the first postoperative day without any radiological test, and the results of all UGI studies were

negative. In our study, the specificity of swallow graphy in detecting leak was 100%.

Our study has some limitations: this is a retrospective study, and it includes a small patient group. The occurrence of the leak is low in both primer and revisional surgeries; large numbers are required to detect a significant difference in leak rates.

In conclusion, a negative intraoperative leak test result does not preclude the possibility of a staple line or anastomotic leakage. It is likely unnecessary to use postoperative gastrointestinal contrast studies in both primary and revisional bariatric surgery, but the routine use of the intraoperative methylene blue test could be considered due to its positive results for the detection of leakage.

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# Relation of serum immunoglobulin E level with coronary artery disease and SYNTAX score

## Serum immunglobulin E seviyesi ile koroner arter hastalığı ve SYNTAX skoru arasındaki ilişki

Arif Oğuzhan Çimen<sup>1</sup>, Derya Öztürk<sup>2</sup>

### Abstract

**Aim:** Inflammation has a significant role in the pathogenesis of atherosclerosis and allergic inflammation has also an important impact on atherosclerosis progression. In this study, we investigated whether the serum IgE levels are associated with coronary artery disease (CAD) and SYNTAX score as a parameter for severity of the disease.

**Methods:** A total of 171 patients who were planned coronary angiography were recruited consecutively into this study. The patients who had a diagnosis of asthma, autoimmune diseases, allergic dermatitis, history of allergic diseases, parasitic infections, malignancy, severe renal failure (estimated glomerular filtration rate <30 mL/min), chronic hepatic disease, rheumatic and valvular heart diseases were excluded from the study. The patients were divided into two groups according to the presence of CAD as called CAD and non-CAD groups. CAD was diagnosed according to the presence of more than 50% stenosis at least in one main coronary artery. Two expert cardiologists who were blinded to the patients' clinical and laboratory data reviewed the coronary angiography and evaluated the coronary atherosclerotic lesion severity independently. The SYNTAX score is calculated using the algorithm on the baseline diagnostic angiogram. Serum samples for determining total serum levels of IgE were collected from the patients were at the admission just prior to coronary angiography.

**Results:** Based on the coronary angiography, 88 patients (51.5%) were in the non-CAD and 83 patients (48.5%) were in the CAD group. CAD patients tend to be older (61.9±11.6 years vs 56.7±9.7years, p=0.002) and male (67.5% vs.47.7%, p=0.009) with a higher prevalence of hypertension (80.7% vs 63.6%, p=0.013), and hyperlipidemia (63.9% vs 28.4%, p<0.001) compared to non-CAD patients. The serum IgE levels were significantly higher in the CAD group than those in the non-CAD group (99.05 IU/ml (51-192) vs. 24.25 IU/ml (13.8-55), p<0.001). By multivariate logistic regression analysis, serum IgE levels were found as an independent predictor for CAD (OR 1.003; 95% CI 1.000–1.005; p=0.041). In addition, there was a positive moderate correlation between SYNTAX score and Ig E levels (r:0,483, p<0.001).

**Conclusion:** In this trial, we showed that the serum IgE levels are positively associated with the presence of CAD even after adjusting for traditional cardiovascular risk factors. Furthermore, serum IgE levels are correlated with the SYNTAX score and the complexity of coronary artery disease.

**Key words :** Immunoglobulin E, coronary artery disease, atherosclerosis, SYNTAX score, allergic inflammation

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### Öz

**Amaç:** İnflamasyon aterosklerozun patogeneğinde önemli bir role sahiptir. Alerjik inflamasyonun da aterosklerozun ilerlemesinde önemli bir etkisi mevcuttur. Çalışmamızda IgE düzeyi ile koroner arter hastalığı (KAH) arasında ilişki olup olmadığına baktık. Ayrıca ek olarak IgE ile KAH ciddiyetini gösteren SYNTAX skoru arasındaki ilişkiyi de değerlendirdik.

**Metot:** Çalışmaya ardışık olarak 171 tane koroner angiografi planlanan hasta alındı. Astım, oto immün hastalık, alerjik dermatit, alerji öyküsü, parazit infestasyonu, kanser, ciddi böbrek yetmezliği (glomerüller filtrasyon hızı <30 ml/min), kronik karaciğer hastalığı, romatizmal kalp hastalığı, kalp kapak hastalığı olanlar dışlandı. Hastalar KAH varlığına göre olan ve olmayan diye iki gruba ayrıldı. En az bir ana koroner arterde %50 ve üzeri darlık KAH olarak değerlendirildi. Hastaların klinik ve laboratuvar sonuçlarından bağımsız olarak 2 kardiyoloji uzmanı tarafından KAH ciddiyeti SYNTAX skoru algoritmasına göre değerlendirildi. IgE düzeylerine ilk başvuruda, koroner angiografi öncesinde alınan venöz kan tahlillerinde bakıldı.

**Sonuçlar:** Koroner angiografi sonuçlarına göre 88 hasta (%51,5) KAH olan ve 83 hasta (%48,5) ise KAH olmayan olarak değerlendirildi. KAH olan grupta olmayan gruba göre yaş ortalaması daha yüksek (61,9±11,6 yıl vs 56,7±9,7 yıl, p:0,002), erkek cinsiyet daha fazla (67,5% vs.47,7%, p:0,009), hipertansiyon (80,7% vs 63,6%, p:0,013) ve hiperlipidemi (63,9% vs 28,4%, p<0,001) prevalansları daha fazla idi. Serum IgE düzeyleri KAH olan grupta olmayan gruba göre (99,05 IU/ml (51-192) vs. 24,25 IU/ml (13,8-55), p<0,001) daha yüksek idi. Çoklu logistik regresyon analizine göre IgE düzeyleri KAH için bağımsız bir faktör olarak bulundu (OR 1,003; 95% CI 1,000–1,005; p=0,041). Ek olarak, serum IgE düzeyleri ile SYNTAX skoru arasında orta derecede pozitif bir korelasyon tespit edildi (r:0,483, p<0,001).

**Sonuç:** Çalışmamızda geleneksel risk faktörleri düzenlendikten sonra, serum IgE düzeylerinin KAH varlığı açısından bağımsız pozitif bir faktör olduğu gösterildi. KAH ciddiyetini gösteren SYNTAX skoru ile serum IgE düzeyleri arasında orta derecede pozitif bir korelasyon tespit edildi.

**Anahtar Kelimeler:** Immunoglobulin E, koroner arter hastalığı, aterosklerozis, SYNTAX skoru, alerjik inflamasyon

## Introduction

Atherosclerosis is an inflammatory disease associated with multiple risk factors and a complex pathophysiology. Inflammation has a significant role on pathogenesis of atherosclerosis, initiation and progression of coronary plaque formation [1-2]. In addition, inflammation is associated with coronary plaque instability, stent thrombosis and in stent restenosis after stent implantation [3-4]. Although main classic inflammatory process is mediated by macrophages, neutrophils and Th-1 lymphocytes, allergic inflammation also has an important impact on atherosclerosis progression. Immunoglobulin E (IgE) is a key component of response to allergens/antigens in atopic diseases and systemic anaphylaxis [5]. Biological activity of Ig E occurs via binding to Fc receptors which are present at the surface of mast cells (MCs), basophils, and monocytes. Mast cell accumulation has been shown in the coronary atheromatous lesions, especially at the actual sites of coronary plaque erosion or rupture; therefore, mast cell activation has been accepted as one of the mechanisms related to allergic inflammation with atherosclerotic disease progression [6]. On the recent studies, the relation between IgE level and atherosclerosis has been investigated and elevated Ig E levels were detected in the patients with coronary artery disease. Guo et al. [7] showed the relation between increased total serum IgE levels and coronary artery disease (CAD) and severity of CAD independently of traditional cardiovascular risk factors. Also some studies have evaluated the association between elevated Ig E and coronary artery plaque instabilization, and they observed higher mast cells and IgE levels within coronary artery walls in cardiac deaths from coronary artery thrombosis [5]. However, the relationship between total serum IgE levels and the SYNTAX score remains unclear.

In this study, we investigated whether the serum IgE levels are associated with CAD and SYNTAX score.

## Material and methods

A total of 171 patients who were planned coronary angiography between November 2019 and October 2020 on Fatih Medical Park Hospital were recruited consecutively into this study. The patients who had a diagnosis of asthma, autoimmune diseases, allergic dermatitis, history of allergic diseases, parasites infection, malignancy, severe renal failure (estimated glomerular filtration rate <30 mL/min), chronic hepatic disease, rheumatic and valvular heart diseases were excluded from study. Also, patients admitted to hospital with decompensated heart failure or shock were also excluded from the study. This study was approved by the ethics committee of Bahcesehir University (12/18/2019 and 2019-19/04) and conducted according to the principles described in the Declaration of Helsinki. Written informed consent was obtained from all study participants.

### Coronary angiography and image interpretation

Coronary angiography was performed with standart radial or femoral approach with an angiography unit (Axiom Artis, Siemens). CAD was diagnosed according to the presence of more than 50% stenosis at least in one main coronary arter. Two expert cardiologists who were blinded to the patients' clinical and laboratory data reviewed the coronary angiography and evaluated the coronary atherosclerotic lesion severity independently. SYNTAX score was calculated using the SYNTAX score algorithm on the baseline diagnostic angiogram [8].

### Blood samples and definitions

Hypertension and diabetes were diagnosed according to the current guidelines [9-10]. Blood for determining total serum levels of IgE was collected from the patients at the admission just prior to coronary angiography. After an overnight fast for 12 hours, peripheral venous blood was drawn from all the patients to examine complete blood count and biochemical analysis.

### Variables

Demographic data (age, sex, body mass index), clinical features (coexisting diseases, drug usage and laboratory analysis including white blood cell count (WBC) (K/uL), hemoglobin (gr/dL), hematocit (%), platelet count (K/uL), mean corpuscular volume (MCV) (fl), neutrophil, lymphocyte and eosinophil counts (K/uL), low density lipoprotein-cholesterol (LDL-c) (mg/dL) and creatinine were evaluated and recorded.

### Statistical analysis

Variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov–Smirnov or Shapiro–Wilk's test) to determine whether or not they were normally distributed. Continuous variables with parametric distribution were expressed as mean  $\pm$  standard deviation and with non-parametric distribution were expressed as median and interquartile range. Parametric continuous variables were analyzed using the Student T test and non parametric continuous variables were analyzed with Mann-Whitney U test. Categorical data were expressed as frequencies and their differences were analysed using the Chi-squared test. Immunoglobulin E levels and SYNTAX score results that were anormally distributed, and correlation coefficients and their significance were calculated using the Spearman test. A multiple logistic regression model was used to identify independent predictors of the for the coroner artery disease. Age, gender, hypertension, diabetes mellitus, dyslipidemia, baseline creatinine level, baseline neutrophil count and acetylsalicylic acid (ASA) usage were included in multiple logistic regression analysis. Statistical analyses were performed using SPSS version 21.0 (SPSS Inc, Chicago, Illinois, USA). Statistical significance was taken as  $p < 0.05$ .

## Results

Based on the coronary angiography, 88 patients (51.5%) were in the non-CAD and 83 patients (48.5%) were in the CAD group. The median SYNTAX score of the patients was 8 (IQR 5-14). The baseline clinical characteristics of the patients in the CAD and non-CAD groups are summarized in Table 1. CAD patients tend to be older (61.9 $\pm$ 11.6 years vs 56.7 $\pm$ 9.7 years,  $p=0.002$ ) and male (67.5% vs.47.7%,  $p=0.009$ ) with a higher prevalence of hypertension (80.7% vs 63.6%,  $p=0.013$ ) and hyperlipidemia (63.9% vs 28.4%,  $p<0.001$ ) compared to non-CAD patients. The number of patients on regular antithrombotic and antihyperlipidemic treatments were higher on the CAD group. The serum IgE levels were significantly higher in the CAD group than those in the non-CAD group (99.05 IU/ml (IQR 51-192) vs. 24.25 IU/ml (IQR 13.8-55),  $p<0.001$ ). The CAD patients also had higher creatinine (0.84 mg/dl (IQR 0.78-1) vs. 0.81 mg/dl (IQR 0.69-0.92),  $p=0.007$ ) and neutrophil count (5.3  $\pm$ 1.9 K/uL vs. 4.5  $\pm$ 1.6 K/uL) when compared to non-CAD patients (Table 2). By multivariate logistic regression analysis, serum IgE levels were found as an independent predictor for CAD (OR 1.003; 95% CI 1.000–1.005;  $p=0.041$ ) after adjusting for age, gender, diabetes, hypertension, hyperlipidemia, ASA usage, serum creatinine and neutrophil counts (Table 3). In

addition, there was a positive moderate correlation between SYNTAX score and Ig E levels ( $r=0.483$ ,  $p<0.001$ ) (Figure 1).

Table 1: Demographic and baseline clinical characteristics of the non-cardiac disease (non-CAD) group and cardiac disease (CAD) group.

Variable	non-CAD group (n=88)	CAD group (n=83)	p
Age (years) <sup>β</sup>	56.7±9.7	61.9±11.6	0.002
Male sex <sup>¥</sup>	42 (47.7)	56 (67.5)	0.009
BMI (kg/m <sup>2</sup> ) <sup>β</sup>	29.5±5.5	29.8±7.8	0.841
Hypertension <sup>¥</sup>	56 (63.6)	67 (80.7)	0.013
Hyperlipidemia <sup>¥</sup>	25 (28.4)	53 (63.9)	<0.001
Diabetes mellitus <sup>¥</sup>	21 (23.9)	28 (33.7)	0.154
Chronic kidney disease <sup>¥</sup>	2 (2.3)	3 (3.6)	0.603
Atrial fibrillation <sup>¥</sup>	5 (5.8)	6 (7.4)	0.678
Drugs <sup>¥</sup>			
ASA	34 (38.6)	49 (60.5)	0.005
Beta-bloker	40 (45.5)	49 (60.5)	0.06
Statin	14 (15.9)	37 (45.7)	<0.001
ACE	18 (20.5)	17 (21.0)	0.932
ARB	14 (15.9)	17 (21.0)	0.394
CCB	22 (26.1)	26 (32.1)	0.152
Oral AD	17 (19.3)	23 (28.4)	0.165
Insulin	3 (3.4)	6 (7.4)	0.248

<sup>β</sup>: mean±standard deviation, <sup>¥</sup>: n (%). BMI: Body mass index, ASA: acetyl salicylic acid, ACE: Angioconverting enzyme, ARB: Angiotensin receptor blocker, CCB: Calcium channel blocker, AD: antidiabetics.

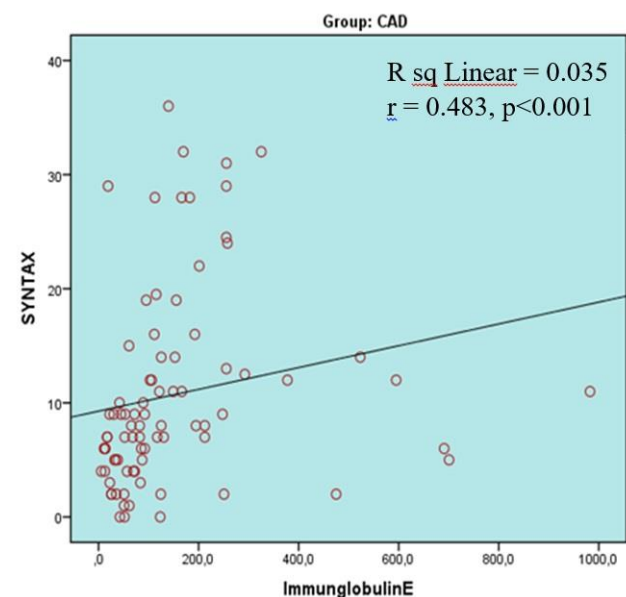


Figure 1: IgE and SYNTAX score correlation curve.

Table 2: Laboratory values of the non-cardiac disease (CAD) group and cardiac disease (CAD) groups.

Variables	non-CAD group (n=88)	CAD group (n=83)	p
WBC (K/uL) <sup>β</sup>	7.6±2.1	8.2±2.3	0.076
HGB (gr/dL) <sup>β</sup>	13.6±1.8	14±1.7	0.106
HCT (%) <sup>β</sup>	41.2±3.8	41.9±4.6	0.283
PLT (K/uL) <sup>β</sup>	260.2±65.8	252.3±61.9	0.365
MCV (fl) <sup>β</sup>	86.1 (81.1-89.3)	85.4 (82.1-88.5)	0.883
NEU (K/uL) <sup>β</sup>	4.5±1.6	5.3±1.9	0.003
Lymphocyte (K/uL) <sup>β</sup>	2.4±0.9	2.1±0.7	0.076
Eosinophil (K/uL) <sup>μ</sup>	0.16 (0.9-0.25)	0.16 (0.11-0.26)	0.686
LDL-c (mg/dL) <sup>β</sup>	129.6±41.7	138.6±49.6	0.204
Creatinine (mg/dL) <sup>μ</sup>	0.81 (0.69-0.92)	0.84 (0.78-1)	0.007
IgE (IU/ml) <sup>μ</sup>	24.25 (13.8-55)	99.05 (51-192)	<0.001

<sup>β</sup>: mean±standard deviation, <sup>μ</sup>: median (interquartile range).

HGB: hemoglobin, HTC: hematocrit, MCV: mean corpuscular volume, WBC: White blood cell, NEU: Neutrophil, LDL-c: Low density lipoprotein cholesterol, IgE: Immunglobulin E.

Table 3 : Independent predictors of coronary artery disease.

Variable	OR (95% Confidence Interval)	p
Age	1.057 (1.016 – 1.10)	0.006
Sex	2.330 (1.018 – 5.336)	0.045
Hypertension	1.016 (0.368- 2.809)	0.975
Diabetes mellitus	0.886 (0.373- 2.104)	0.784
Dyslipidemia	4.366 (1.813 – 10.512)	0.001
Baseline creatinine	0.638 (0.171 – 2.374)	0.502
Baseline neutrophil	1.304 (1.050- 1.619)	0.017
ASA usage	0.802 (0.369- 1.742)	0.802
IgE levels	1.003 (1.000 – 1.005)	0.041

OR: Odds ratio, ASA: acetyl salicylic acid

## Discussion

In this study, we demonstrated a significant association between total serum IgE levels and CAD. Also, we found a positive correlation between SYNTAX score and IgE. In the previous study, the relation between SYNTAX score and serum tryptase level was demonstrated [11]. But according to our knowledge, this is the first study that evaluates the relationship with Ig E and SYNTAX score. Monocytes/macrophages, neutrophils, and T cells, mediated classic inflammatory responses have been found to be involved in the process of atherogenesis. In addition to that, many studies demonstrated that cellular mediators of allergic inflammation also may play a role in coronary artery plaque formation and progression [5]. Ig E has significant role in formation of allergic inflammation via activating mast cells by binding to its high-affinity receptor FcεR1 [5]. Previous studies demonstrated that number of mast cells increase with disease progression in atherosclerotic plaques and activated mast cells have a pro-atherogenic role in the pathogenesis of CAD [12]. Also, mast cells may play a role in

coronary plaque instability via causing intraplaque hemorrhage [13]. Furthermore, IgE can interact with other types of immune cells, such as monocytes and their derivatives, macrophages, which then facilitate the pathogenesis of atherosclerosis by allergy-related mechanisms [14]. In these ways, Ig E may contribute the pathogenesis of CAD. There are numerous investigations have suggested the existence of possible links between atopy/allergic inflammation, high serum IgE, coronary artery atherosclerosis development, and acute coronary syndromes.

Firstly, Criqui et al. [15] investigated the association of serum IgE levels with myocardial infarction, stroke, and noninvasively diagnosed large-vessel peripheral arterial disease. They found a relation between IgE and cardiovascular disease in men but not in women. Szczeklik et al. [16] demonstrated increased IgE levels in the patients that admitted on hospitals with acute myocardial infarction levels. Similarly, in the recent studies had results confirms Szczeklik's results [16] in patients with myocardial infarction and unstable angina pectoris. In our study, we also found that the patients with coronary artery disease have higher serum Ig E levels compared to individuals with normal coronary arteries.

In terms of the relation between severity and Ig E, Guo et al. [7] evaluated the association with Gensini score and Ig E level, and they found a significant linear relation between Gensini score and the serum IgE level. Tryptase, a neutral protease selectively concentrated in the secretory granules of human mast cells, is released by mast cells and it has been used as a marker of mast-cell activation. Morici et al. [17] assessed the association between serum tryptase and the occurrence of major cardiovascular and cerebrovascular events (MACCE) at 2-year follow-up in patients admitted with acute coronary syndrome (ACS). In this trial, showed a significant correlation between serum tryptase level and MACCE and also there was a positive correlation between SYNTAX score and MACCE. However, in this study, no significant correlations between tryptase and SYNTAX score were found.

According to our knowledge, there is not any trial that evaluated the relation between IgE level and SYNTAX score. Our aim in this study to evaluate this relation and we found a statistically significant relation between SYNTAX score and serum Ig E level. So, according to this result Ig E may predict severity and complexity of coronary artery disease.

The main limitations of this study were small number of study population and the SYNTAX score of this study population was low. So, we couldn't divided the study population into groups according to SYNTAX score.

In conclusion, in this trial we showed that the serum IgE levels are positively associated with the presence CAD even after adjusting for traditional cardiovascular risk factors. Furthermore, serum IgE levels are correlated with SYNTAX score and complexity of coronary artery disease.

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# Laparoscopic revision of vertical banded gastroplasty with gastrogastric fistula to sleeve gastrectomy

## Gastrogastrik fistüllü vertikal bantlı gastroplastinin sleeve gastrektomiye laparoskopik revizyonu

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### Abstract

Gastrogastric fistula is one of the complications that can occur after vertical banded gastroplasty surgery for weight loss. Sleeve gastrectomy can be performed successfully in patients with gastrogastric fistula in revision surgery. In this study, we present current treatment for the complication of vertical banded gastroplasty (Mason procedure). Revision of vertical banded gastroplasty to sleeve gastrectomy is a safe and feasible option for patients presenting with gastrogastric fistula.

Key Words: Vertical gastroplasty, gastrogastric fistula, sleeve gastrectomy.

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Informed Consent: The written consent was received from the patient who was presented in this study.

Hasta Onamı: Çalışmada sunulan hastadan yazılı onam alınmıştır.

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

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### Öz

Gastrogastrik fistül, kilo kaybı için yapılan vertikal band gastroplasti ameliyatından sonra ortaya çıkabilecek komplikasyonlardan biridir. Revizyon cerrahisinde gastrogastrik fistülü olan hastalarda sleeve gastrektomi başarıyla yapılabilir. Bu çalışmada, vertikal band gastroplasti (Mason prosedürü) komplikasyonu için güncel tedaviyi sunuyoruz. Gastrogastrik fistül ile başvuran vertikal band gastroplastinin, sleeve gastrektomiye revizyonu güvenli ve uygulanabilir bir seçenektir.

Anahtar Kelimeler: Vertikal gastroplasti, gastrogastrik fistül, sleeve gastrektomi.

## Introduction

Vertical banded gastroplasty a primarily restrictive bariatric surgical procedure was first described by Mason [1]. The procedure was performed via laparotomy and a neo-pylorus was constructed with a Dacron or Marlex mesh. The stomach was stapled but not transected. MacLean revised the procedure and performed it laparoscopically with a complete transection of the stomach [2] (Figure 1a, 1b). Gastrogastric fistula (GGF) is one of the important complications that can occur after vertical gastroplasty (VG) surgery for weight loss. In literature, the incidence of the GGF after VG is 2.5% to 3.5% [3–5].

Although Roux-en-Y gastric bypass (RYGB) is preferred mostly for revisional surgery, sleeve gastrectomy (SG) can also be performed.

In this study, we present the current treatment for the gastrogastric fistula complication of Mason's procedure.

## Case report

A 39-year-old woman underwent VBG with open surgery for the management of morbid obesity 15 years ago. When nausea and vomiting complaints increased after the operation, she was taken to the operation at the same center again and the laparoscopic mesh ring was removed. When the patient admits to our center, she continued to gain weight again over the years and her BMI was 40 kg/m<sup>2</sup>. The physical examination was unremarkable. No comorbidities were present. Routine laboratory investigations did not reveal any specific abnormalities. The patient had a subcostal incision scar belonging to the previous surgery. When examining her during preoperatively, we perform esophagogastroduodenoscopy for the precise anatomy of the former procedure, so we demonstrated the GGF (gastrogastric fistula), just 5 cm below the gastroesophageal junction (Figure 2) Sleeve gastrectomy was selected and performed for revisional surgery due to weight regain. The postoperative course was uneventful, and the patient was discharged on the postoperative day 7.

The written consent was taken from the patient.

The gastrocolic ligament was cut through the angle of His with an energy device, and the diaphragmatic crura were exposed. A laparoscopic stapler was introduced and fired consecutively along the length of the endoscope against the greater curve. The gastric pouch was created by using a linear stapler, with two sequential 4.8/60-mm green load firings for the antrum, followed by three sequential 4.8/60-mm purple cartridges for the remaining gastric corpus and fundus. At the intraoperative endoscopic control, GGF could not be seen anymore (Figure 3). The resected stomach was extracted through the 15-mm port-site. When the specimen was examined macroscopically, it was observed that the previous vertical stapler line was involved and the vertical stapler area was opened, so she had a gastro gastric fistula. (Figure 4a, 4b). A methylene blue dye test was carried out and did not evidence any leak.



Figure 2: Demonstration of the gastrogastric fistula by esophagogastroduodenoscopy.

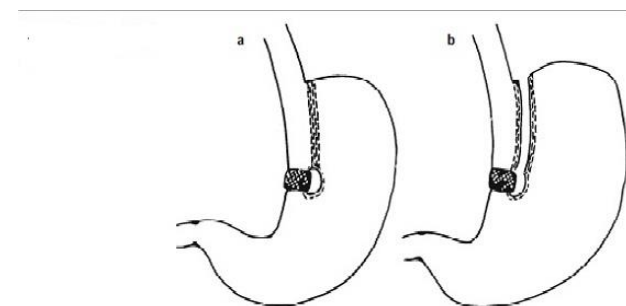


Figure 1: (a) The procedure was performed via laparotomy and a neo-pylorus was constructed with a Dacron or Marlex mesh. The stomach was stapled but not transected (Mason procedure). (b) Complete transection of the stomach (MacLean procedure).

## Surgical Technique

The operation was started by placing five trocars traditionally. The anatomy was defined by lysing the adhesions between gastric pouch, liver, and the remaining stomach. By intraoperative endoscopy, we identified the GGF. The endoscope was passed to the main stomach, finally entering the duodenum. Here was gastro gastric fistula 5 cm below the gastroesophageal

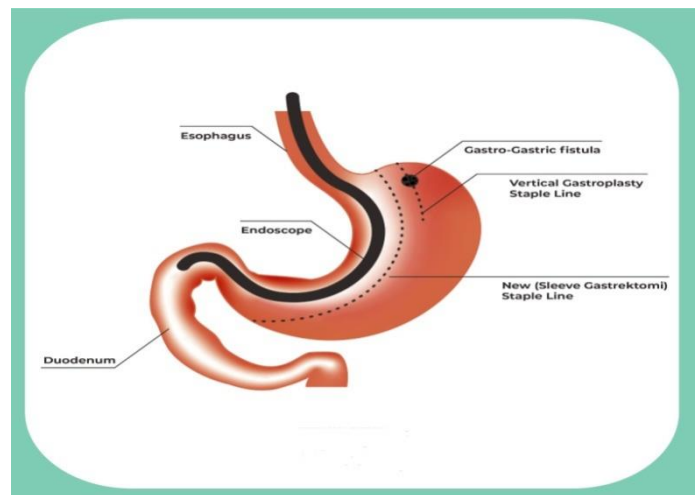


Figure 3: Demonstration for placement of an endoscope before gastric resection.

## Discussion

Vertical band gastroplasty was performed most commonly in 1990th years, although the early results were good, but, there was a return to other surgical procedures in the recent years because of long-term complications and weight regain [6]. Long term complications requiring redo-surgery are common after VBG. Gastric outlet obstruction is a significant complication following VBG, with outlet stenosis rates ranging between 10-20% of VBG patients [7-9]. As a general consequence of this obstruction, patients develop maladaptive eating strategies leading to significant weight regain [7]. The patient underwent a second surgical operation that mesh under



pouch was taken off because of the development of gastric outlet syndrome. However, she continued to gain weight over the years.

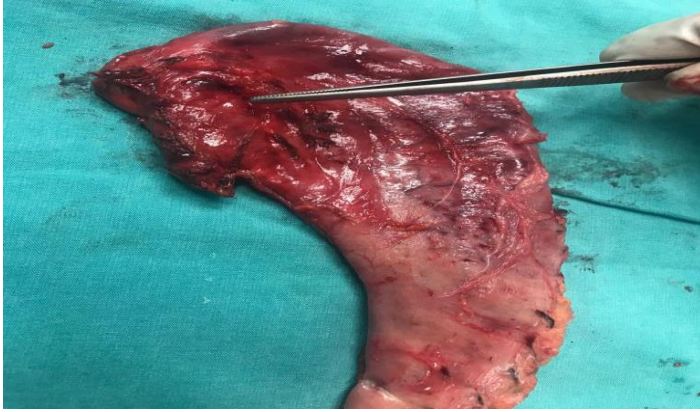


Figure 4: Postoperative anatomy (a) Vertical gastroplasty stapler line (Please note to new sleeve stapler line, 1.5 cm away from previous stapler line).

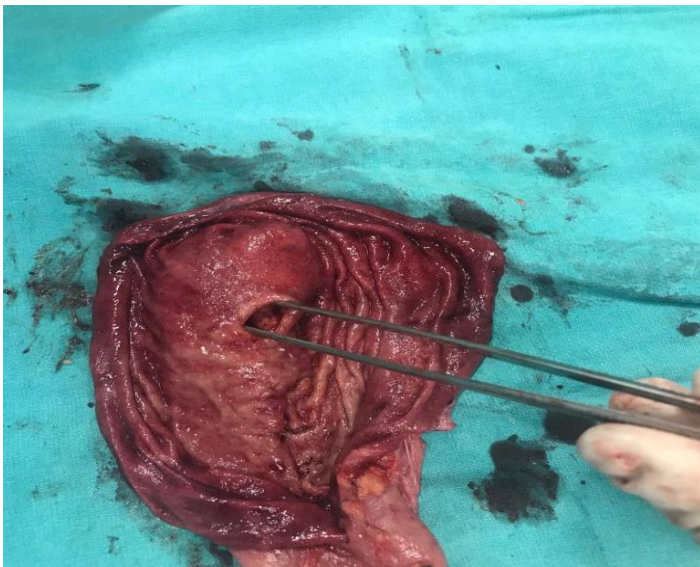


Figure 4 (continued) (b) Opening of the specimen from the greater curvature side and presence of gastrogastric fistula.

Mason procedure was performed via laparotomy and a neo-pylorus was constructed with a Dacron or Marlex mesh. The stomach was stapled but not transected resulting in the long-term 65 % staple line failure rate (gastro-gastric fistula) [10, 11]. MacLean-procedure divided staple lines between the pouch and the remnant stomach. A neo-pylorus was constructed with a small silastic ring. This significantly reduced the risk of the staple line failure and the development of gastro-gastric fistula, which inevitable leads to weight gain [7]. We evaluated that Mason procedure was performed in our patient because of the first surgery operation was 15 years ago and had been gastrogastric fistula.

Sleeve gastrectomy or RYGB could be selected according to the surgeon's experience, and patient's weight status for revisional surgery. Revisional bariatric procedures, in general, are associated with higher complications rate compared to the primary bariatric surgery [12, 13]. RYGB procedures performed as a secondary revisional procedure after other previous failed procedure [14]. But the group of Cadière et al. [15] recently reported gastro-jejunal leaks in 6 of 43 patients (14 %) after conversion of VBG to RYGB. Since our patient had gastrogastric fistula and we were afraid of developing gastrojejunal fistula, our preoperative preparations were in line with sleeve gastrectomy.

Intraoperative endoscopy is important for possible complications and surgical safety when revision surgery is performed in patients with gastrogastric fistula [16].

Although RYGB is preferred mostly, SG can be performed successfully in patients with gastrogastric fistula in revision surgery.

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# Granular cell tumor presenting as an axillary mass: A case report

## Aksiller kitle ile ortaya çıkan granüler hücreli tümör: Bir olgu sunumu

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### Abstract

Granular cell tumor (GCT) is a tumor that can arise at virtually any body site. About 5 to 15% of the cases occur in the breast, and it is relatively rare in the axilla. %1-2 of granular cell tumors are malignant. The clinical symptoms and imaging examinations of breast GCTs are non-specific and can easily be confused with malignant tumors. Most are benign and reportedly malignant cases are rare. We report a 59-year-old male with granular cell tumor in axillary region existed approximately 2 years. The palpable mass was around 15mm in diameter and felt clinically suspicious of malignancy. Tru-cut biopsy of the mass showed granular cell tumor features and surgical excision was performed. Early diagnosis and complete resection of the tumor remains the best treatment method.

Key Words: Granular cell tumor, axilla, breast

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### Öz

Granüler hücreli tumor (GHT), hemen hemen vücudun her bölgesinde ortaya çıkabilen bir tümördür. Vakaların yaklaşık % 5 ila 15'i memede görülür ve aksillada nispeten nadir ortaya çıkarlar. Granüler hücreli tümörlerin % 1-2'si maligndir. Meme GHT'lerinin klinik semptomları ve görüntüleme incelemeleri spesifik değildir ve malign tümörlerle kolayca karışabilir. Çoğu iyi huyludur ve bildirilen malign vakalar nadirdir. Aksiller bölgede yaklaşık 2 yıldır palpabl kitlesi olan ve granüler hücreli tumor tanısı alan 59 yaşında bir erkek hastayı sunuyoruz. Palpabl kitlesi yaklaşık 15 mm çapındaydı ve klinik olarak malignite şüphesi vardı. Kitlenin kalın iğne biyopsisi granüler hücreli tümör özellikleri gösterdi ve cerrahi eksizyon yapıldı. Tümörün erken tanısı ve tam rezeksiyonu en iyi tedavi yöntemi olmaya devam etmektedir.

Anahtar Kelimeler: Granüler hücreli tümör, aksilla, meme

## Introduction

The granular cell tumor, first described by Abrikosoff in 1926, is a relatively rare neoplasm occasionally found in the axilla [1]. Malignant granular cell tumors, which account for %1-2 of granular cell tumors, were first demonstrated by Ravich et al. [2] in 1945. Initially it was thought to be originated by skeletal muscle cells, but due to the similarity of S-100 protein positivity and tumor cells to Schwann cells, investigators indicated that the tumor was caused by Schwann cells between lobular breast tissue [3, 4].

The tongue is the single most common anatomic site involved but granular cell tumor can occur in almost any body site (lower extremity, nuchal region, chest wall, gastrointestinal tract, head and neck but very rarely in breast) and is usually multifocal [5]. Granular cell tumor is seen in the breast in 5-6% of cases [4, 6]. GCT of the breast is generally benign; it rarely shows malignant features [7].

We presented a patient who underwent excisional biopsy of an axillary mass which was diagnosed as granular cell tumor as a result of histopathological examination.

## Case report

59 year old male patient applied to our clinic with a palpable mass just at the lateral border of the right breast that existed for approximately 2 years. In his examination, a mass of 15 mm in diameter with mild tenderness and poor mobility at the lateral border of the right breast was detected. The mammography showed irregular, spiculated, minimally hyperdense lesion at the right axillary region. The USG showed an indefinable, spiculated, hypoechoic lesion of approximately 11x10 mm with indistinctive borders. The USG report suggested that a mass at the lateral border of the right breast is probably a malignant mass or a solid mass secondary to chronic inflammation. Tru-cut biopsy of the mass revealed granular cell tumor. Excisional biopsy was planned to differentiate if the mass was benign or malign. Lumpectomy was performed with adequate margins. Postoperative pathology showed neoplastic infiltration of stroma with cells containing granular eosinophilic cytoplasm, hyperchromatic nucleus and composed of cells arranged in lobules having round to oval vesicular nuclei. Immunohistochemical staining resulted in S-100 and CD 68, PAS and PAS diastase stain positivity (Figure 1a, 1b). All the surgical margins were free of tumor. After 5 years of follow up the patient is doing well without evidence of tumor recurrence.

The written consent was taken from the patient.

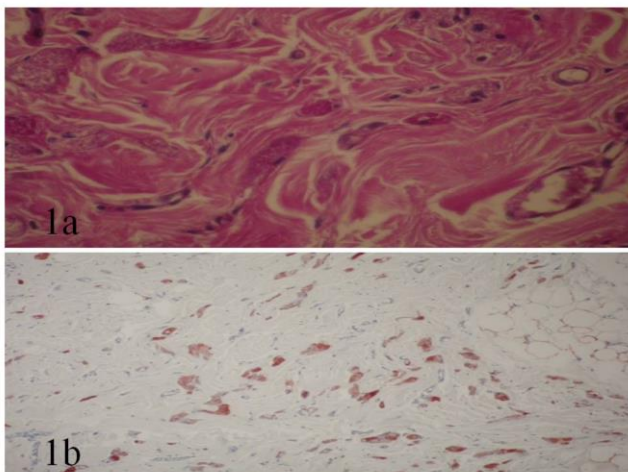


Figure 1a,1b: The immunostaining for S-100 and CD 68, PAS and PAS diastase stain revealed positive results (magnificationx100).

## Discussion

Although granular cell tumors occur throughout the body that contain nerve tissue, these lesions in the breast are 5 to 15% of all cases. About 90% cases present as a solitary tumor but not uncommonly is multifocal and multicentric [6]. These tumors most commonly occur in women between 30 and 50 years of age ranged from 17 to 74 years with a frequency approximately 1 in 1000 breast cancers [8]. Granular cell tumors originate from the intralobular stroma in the breast. Although almost all the lesions are benign neoplasms, malignant lesions are rarely described [9]. Malignant granular cell tumors are differentiated from benign ones with rapid growth, larger size, local recurrence rate and more frequent localization in the lower extremities. They often spread to the lungs, liver, lymph nodes and bones through lymphatic and hematogenous routes.

Granular cell tumor is usually a firm and painless mass that mimics a malignant lesion which may be fixed to the pectoral muscles or the skin [3]. As in our case, the mass is generally well-circumscribed, but few cases with a limited number of poorly-circumscribed masses have been reported in the literature [8]. It shows poor borders at mammography, with minimal microcalcification. It is observed as a poorly circumscribed mass with acoustic shadowing in USG. The ultrasound images of granular cell tumors are not specific. It can be differentiated from breast cancer with homogeneous internal echo and no edema on the edges of the tumor [10]. It is difficult to differentiate granular cell tumors (GCTs) from malignant breast tumors on both ultrasound and mammography. In these tumors, invasive diagnostic tests are required because imaging techniques provide limited information in preoperative diagnosis.

Histopathologically the tumor cells have large granular eosinophilic cytoplasm and uniform nuclei, with no mitotic activity. As a result of histochemical analysis, it confirms whether the granules are diastase resistant and PAS positive. The tumor cells are strongly immunoreactive to S-100 protein and also stain positively for CD68 and vimentin [11, 12]. There is no stain for cytokeratins, epithelial membrane antigen and mucin. This profile is useful for differentiating a granular cell tumor from apocrine carcinoma [13]. Finally, cells are also negative for both estrogen and progesterone receptors [3].

Wide excision is proper for GCT treatment. Local recurrence may be observed in cases without adequate excision. Multifocal cases may also show recurrence and inadequate excision. These patients should be followed annually to rule out late recurrence. Chemotherapy, alone or in association with radiotherapy, is not given unless the tumor is malignant.

As a result, the axillary masses should be approached in a multidisciplinary manner and the treatment should be planned according to the biopsy results. Early diagnosis and adequate resection remains the best treatment for granular cell tumors.

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# Life-threatening citalopram induced hemolytic anemia in a patient with generalized anxiety disorder: A case report

## Yaygın anksiyete bozukluğu olan bir hastada hayatı tehdit eden sitaloprama bağlı hemolitik anemi: Bir olgu sunumu

Mehmet Hamdi Örum <sup>1</sup>

### Abstract

The hemolysis side effect of selective serotonin reuptake inhibitors is potentially lethal, although not much has been reported. We hereby report a 43-year-old male case of hemolytic anemia that developed after use of citalopram 40 mg/day and its improvement with discontinuation. Further studies should be conducted to provide a greater understanding of both its prevalence and etiology.

Keywords: Citalopram, hemolytic anemia, selective serotonin reuptake inhibitor.

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### Öz

Seçici serotonin geri alım inhibitörlerinin hemoliz yan etkisi potansiyel olarak ölümcüldür, ancak çok fazla bildirilmemiştir. Bu yazıda sitalopram 40 mg/gün kullanımı sonrası hemolitik anemi gelişen ve ilacın kesilmesi ile düzelen 43 yaşında erkek bir olguyu sunuyoruz. Hem prevalansı hem de etiyojisi hakkında daha iyi bilgi sahibi olabilmek için daha fazla çalışma yapılmalıdır.

Anahtar Kelimeler: Sitalopram, hemolitik anemi, seçici serotonin geri alım inhibitörü.

## Introduction

Citalopram hydrobromide is a selective serotonin reuptake inhibitor (SSRI) that is used to treat symptoms of depression and anxiety. It has various adverse effects on gastrointestinal, autonomic nervous, central and peripheral nervous, musculoskeletal, urogenital, and cardiovascular system. Although manufacturer has listed hematologic adverse effects such as variants of anemia, no report is available in the literature [1].

Herein, we present a 43-year-old male who developed hemolytic anemia while using citalopram. We discuss the clinical features, etiology and significance of this clinical condition.

## Case report

A 43-year-old male patient was referred to us by internal medicine with the possibility of drug side effects. It was learned that the patient had been treated intermittently for 10 years with anxiety complaints, that he had been using paroxetine 20 mg/day for the last one year but that paroxetine was discontinued due to inefficacy and citalopram 40 mg was started two months ago. Following citalopram 40 mg/day, fatigue, abdominal pain, and chills developed and these complaints increased day by day. Two weeks after citalopram was started, he admitted to internal medicine with these complaints. In the patient whose complete blood count (CBC) values were within normal limits prior to the present psychiatric treatment, some abnormalities were determined: Hemoglobin (HGB) 9.403 g/dL, hematocrit (HCT) 24.56%, red blood cell (RBC) 2.241  $10^6/\mu\text{L}$ , mean corpuscular volume (MCV), 109.6 fL, mean corpuscular hemoglobin (MCH) 41.96 pg, mean corpuscular hemoglobin concentration (MCHC) 38.28 g/dL, red blood cell distribution width-coefficient of variation (RDW-CV) 18.69%. Total bilirubin was 2.3 mg/dl and indirect bilirubin was 1.6 mg/dl. Other CBC parameters, Infection markers, liver, kidney and thyroid functions, electrolytes, vitamin B-12, folate, iron, iron binding capacity, and ferritin were found to be normal. Reticulocyte count and percentage were increased and haptoglobin was decreased. The reticulocyte production index indicated hemolysis with 2.7533. Peripheral smear showed basophilic stippling, polychromatic cells and physical examination revealed splenomegaly. Based on these results, he was diagnosed as acquired hemolytic anemia due to citalopram by internal medicine. Previously used drugs by patient were paroxetine, alprazolam, olanzapine, medazepam, and clomipramine. Family history was unremarkable. Mental status examination revealed an anxious affect. A diagnosis of generalized anxiety disorder was made according to Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5) [2]. Citalopram was discontinued and the patient was started on sertraline 50 mg/day and increased to 100 mg/day. CBC values were regularly monitored by internal medicine during this period. The laboratory findings during the follow-up of the patient are shown in Table 1. Hemoglobin electrophoresis using high pressure liquid chromatography method to exclude beta thalassemia gave normal findings (A2= 3.1%; F= 1.1%). Immunocapture agglutination test to exclude brucellosis, Gruber-Widal test to exclude salmonellosis, direct and indirect coombs tests were negative. Endoscopic biopsy gave normal results. Four months after the drug was discontinued, HGB increased to normal limits, but MCV and MCH reached normal limits at the fifth month. In the sixth week of sertraline treatment, partial regression of anxiety symptoms was observed and the drug was continued at

the same dose. No similar side effects were reported during the follow-up of the patient. The patient and his relatives were warned about anemia due to citalopram use and informed consent was obtained from them for their knowledges. Naranjo Adverse Drug Reaction Probability Scale (NADRPS) score of the patient was 7 [3].

The written consent was taken from the patient.

Table 1. The Patient's Follow-up Laboratory Data

	B	B+ Week 1	B+ Week 5	B+ Week 7	B+ Week 12	B+ Week 16	B+ Week 20
HGB (g/dL)	9.403	9.960	10.83	10.93	11.85	13.24	13.66
HTC (%)	24.56	26.09	29.86	30.07	36.37	37.51	37.17
RBC ( $10^6/\mu\text{L}$ )	2.241	2.367	2.886	3.068	3.255	3.727	3.999
MCV (fL)	109.6	110.2	103.5	98.02	111.7	100.64	92.96
MCH (pg)	41.96	42.07	37.52	35.62	36.39	35.53	33.98
MCHC (g/dL)	38.28	38.18	36.26	36.34	32.58	35.39	35.52
RDW-CV (%)	18.69	16.84	15.11	14.84	18.74	15.43	13.80
Total bilirubin (mg/dl)	2.3	2.1	2.0	2.0	2.5	1.4	1.1
Indirect bilirubin (mg/dl)	1.6	1.4	1.3	1.2	1.7	0.7	0.5
Reticulocyte count ( $10^3/\mu\text{L}$ )	-	-	-	-	161	173	140
Percentage of reticulocyte (%)	-	-	-	-	5.11	4.63	4.28
Haptoglobin (g/L)	-	-	-	-	0.01	0.01	-

B:Beginning (two weeks after starting citalopram); HGB: Hemoglobin, HTC: Hematocrit, RBC: Red blood cell, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RDW-CV: Red blood cell distribution width-coefficient of variation.

## Discussion

This case report was evaluated as a case of hemolytic anemia due to citalopram. Because there was a temporal relationship between them, the side effect began with the addition of the drug and completely cured after discontinuation of the drug. Other causes of anemia, such as beta thalassemia and gastrointestinal system bleeding were excluded. The NADRPS score indicates a probable association between drug use and side effect [3]. World Health Organization (WHO) defines 'probable' as an event or laboratory test abnormality, with reasonable time relationship to drug intake. WHO also says this relationship cannot be explained by disease or other drugs, response to withdrawal clinically reasonable, re-challenge (not necessary) [4].

The mechanism by which citalopram could cause hemolytic anemia has not been fully elucidated. Possible mechanisms in drug-induced hemolytic anemia may be related to RBC coating, drug-membrane interaction true autoimmune. While RBC coating is characterized by extravascular hemolysis, steroid treatment may be required in autoimmune conditions. The mechanism of hemolytic anemia in our patient was associated with drug-membrane interaction. In drug-membrane interaction, drug covalently or non-covalently bond to RBC, creating neo-antigen, and in this type of reaction, fatalities are more common [5]. According to our best knowledge, this is the first case of citalopram-induced hemolytic anemia. The

mechanism by which citalopram causes this side effect is not fully understood. Clinical reports and researches of hemolysis side effects of SSRIs other than citalopram are also insufficient and provide no explanation. Jilani et al. [6] reported in an in vitro study that fluoxetine stimulates eryptosis, the suicidal erythrocyte death, which may result in anemia.

Clinical presentation may be rapid in hemolytic anemia. Pallor, jaundice, fever, tachycardia, splenomegaly are physical findings that can be detected in almost all patients with hemolytic anemia. The increase in indirect bilirubin in patients with hemolytic anemia is a valuable finding since it shows hemocatabolism above the conjugating capacity of the liver. In all hemolytic anemias, reticulocytosis is the most important laboratory finding in the initial evaluation of the case and shows the response of bone marrow to hemolysis. Hemoglobin released in intravenous hemolysis is bound to haptoglobin, an alpha 2 glycoprotein made in the liver, and this complex is destroyed in phagocytic cells in the liver. Therefore, the haptoglobin level in the serum decreases to 0 [7]. Considering all these information, it is clear that the condition in our patient is a hemolysis. The fragmented erythrocytes suggested intravascular hemolysis. Discontinuation of the drug was sufficient in the treatment of case.

As a result, this case report suggests that physicians and relatives should be aware that citalopram may induce hemolytic anemia with a fatality risk, a low quality of life and low compliance. Further systemic research should be conducted with respect to citalopram-associated hemolytic anemia to provide a greater understanding of both its prevalence and etiology.

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