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Editorial

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Effect of apelin-13 on anxiety like behaviour in male rats

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Abstract

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Aim: It is known that apelin-13 is one of the major neuropeptides with a clear and crucial role in the circuits that are involved in mood disorders. In the development and/or maintenance of pathological anxiety, abnormalities of the hippocampus and amygdala play an important role. Here, we assessed the potential anxiolytic effect of apelin-13 on anxiety-like behaviors in male rats.

Materials and Methods: A total of 48 male Wistar rats were divided into 4 groups (n=12). Control (C), Social Isolation (SI), Apelin-13 (A), and Social Isolation + Apelin-13 (SI+A). In the C and A groups, four animals in each cage for 8 weeks. In the SI and SI+A groups, each animal was housed individually for 8 weeks. After that apelin-13 administration was applied by osmotic pump. Anxiety/depression-related behaviors were evaluated using the Elevated Plus Maze (EPM), Open Field Test (OFT), and Light-Dark boxes (LDB). We also measured the expression of Apelin-13, Apelin receptor (APJ), Brain Derived Neurotrophic Factor (BDNF), Mammalian Atonal Homolog 1 (MASH1), Nestin, Doublecortin (DCX) and Neuritin in the hippocampus. These are important markers indicating the anxiety mechanism in the hippocampus.

Results: The results of our study showed that apelin-13 administration reduced anxiety behaviors. Open arm entires and time spent were higher in the A group. In the open field test, grooming and rearing were lower in the SI group. Moreover, apelin-13 and APJ gene expression was higher in the A group.

Conclusion: The results of the study indicate that apelin-13 infusion may lead to a decrease in anxiety-related behaviors in male rats.



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Introduction

Over the last decade, our understanding of mood disorders has expanded rapidly. Stressful experiences in early life can have long-lasting detrimental effects on mood and cognitive function, raising the risk of mental illnesses like anxiety. [1]. Anxiety disorders are among the most prevalent pathologies in clinical psychiatry, accounting for approximately 30% of all psychiatric disorders [2]. The mechanisms underlying the pathogenesis of anxiety are not entirely understood, as multiple factors contribute to the development of anxiety and depression. Notably, stress is a critical inductive factor [3].

Anxiety disorders often manifest in childhood and adolescence, with individuals who experience early life trauma being particularly vulnerable [4]. Recent research has demonstrated that the hippocampus and amygdala are highly susceptible to stress [5]. Chronic stress, including models of SI, leads to neurochemical changes and depression-like behaviors [6]. To investigate these effects, researchers commonly use maternal separation and early deprivation models [7]. For instance, depriving mice of maternal care from 21 to 42 days, post-partum results in anxiety and fear behaviors in adulthood [8]. Similarly, SI during early development in humans contributes to the onset of anxiety and is frequently employed as a measure of mood disorders [9].

The apelinergic system consists of the peptide apelin-13

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and its receptor, APJ. APJ is a G-protein coupled receptor primarily found in various brain regions of rats, notably the hippocampus and hypothalamus. These areas are critical for stress and emotional responses [10,11]. Research indicates that apelin-13 plays a significant role in numerous physiological and pathological processes, including memory modulation, cardiovascular effects, insulin secretion, fluid homeostasis, and the regulation of anxiety and depression [12,13].

Mood disorders like anxiety and depression can be caused by changes in how the brain works because of things in our environment. These changes affect chemicals that can affect pathways. One of these, apelin, is controversial. Some studies say apelin-13 makes people more anxious, while others say it has no effect. This study will look at whether apelin affects anxiety or depression in young male rats that are isolated. It will also look at the role of BDNF and APJ in these effects. Studies have shown that apelin-13 may have anxiogenic [14] or anxiolytic effects [15,16]. However, the precise impact of apelin-13 on mood disorders remains largely unexplored.

Materials and Methods

Test animals Male Wistar rats weighing 230-260 g were purchased from KONÜDAM. The animals were maintained in a 12-hour light/dark cycle and the ambient temperature was set at 21 ± 2 °C. They have open access to food and water and are housed in stainless steel cages. Rats were divided into two groups, SI and C. The isolation group was housed alone and the C group consisted of eleven animals. Tests were conducted during the light cycle (09:00 to 12:00). All experimental procedures were carried out according to the guidelines of KONÜDAM's Ethics Committee. The aim was to minimise animal suffering and distress. The animal experiments were approved by the local ethics committee with decision number 2018-011, dated 23.02.2018, and all procedures in the study were performed according to the ethics committee protocol.

Experimental protocol

Rats were taken from their mothers on their 28th day and randomly divided into four groups, as the C (4 animals/cage), A (4 animals/cage) SI stress (1 animal/cage), the SI+A (1 animal/cage) groups and reared for 8 weeks.

C (n = 12): Reared with littermates (4 animals/cage)

A (n = 12): The group without SI and last 2 weeks infused apelin-13 (3.5 g/kg) (4 animals/cage)

SI (n = 12): Animals were administered SI (1 animal/cage)

SI+A (n = 12): After SI applied for 6 weeks, the apelin-13 (3.5 g/kg) was infused subcutaneously for 2 weeks. SI was also applied during the infusion of apelin-13.

Apelin-13 administered by osmotic pump and first dose of apelin-13 infusion started at week 6, same dose repeated up to 14 days. Rats were decapitated and brain tissue stored at -80°C for examination at the end of the experiment. Behavioural tests were used to determine whether apelin-13 had an anxiolytic or anxiogenic effect in both groups after injection, and whether the SI group experienced higher levels of anxiety after SI modelling than the C group. To

make these assessments, anxiety-related behaviours were evaluated by OFT, EPM and LDB two different times, after SI and apelin-13 application. Apelin-13, APJ, neurtin, MASH1, nestin, BDNF, and DCX gene expression levels were analyzed to look into alterations in the hippocampus and amygdala during the onset of anxiety.

Drug treatment

Osmotic pumps had an ISV infusion of 200 µg reservoir volume, 5 µl / hour / week. Before apelin-13 application, rats were anesthetized using combination of Xylazine (10mg/kg) and ketamine (mg/kg). Apelin-13 infusion was carried out with osmotic pumps prepared for animals in the A group (C and SI) for 15 days. Osmotic pump was placed subcutaneously and controlled drug release applied.. Rats were decapitated and brain tissues were removed and analyzes were started.

Behavioral tests

EPM, OFT and LDB were used to evaluate the anxiety behaviors and locomotor activities of animals. All tests were done in the same room under the same lighting. All behavior was recorded on video during testing and scored by an observer who was blind to group and testing conditions.

EPM

The EPM mechanism has been introduced to the software program (Ethovision Video Tracking System XT11, Netherlands) and regions are marked as open arms, closed arms, center. The animal was always left in the same direction with the head facing the open area and tested for 5 minutes. The number of times the arms were open and closed, and the time spent with the arms open and closed, were recorded during this period. At the end of each experiment, the apparatus was wiped with 10% ethyl alcohol.

OFT

The animal was introduced to the software program (Ethovision Video Monitoring System XT11, The Netherlands), marked, the whole experiment was recorded by the program. Each animal was left to the apparatus from the same edge at the beginning of the 5-minute process, and during this period, time spent in center, speed, mobility and immobility time were calculated. At the same time, rearing, grooming and defecation scores were calculated by observing during the experiment. The tests were carried out between 09:00 and 12:00 during the day, taking into account the physiological conditions of the animals. At the end of each experiment, the apparatus was cleaned with 10% ethyl alcohol solution.

LDB

The animals were introduced to the software program specially used for LDB (Ethovision Video Monitoring System XT11, The Netherlands), marked with a red dot, the entire experiment was recorded through the program. Each animal was left to the apparatus from the same edge at the beginning of the 5-minute process, and the time spent in the light and dark box were calculated.

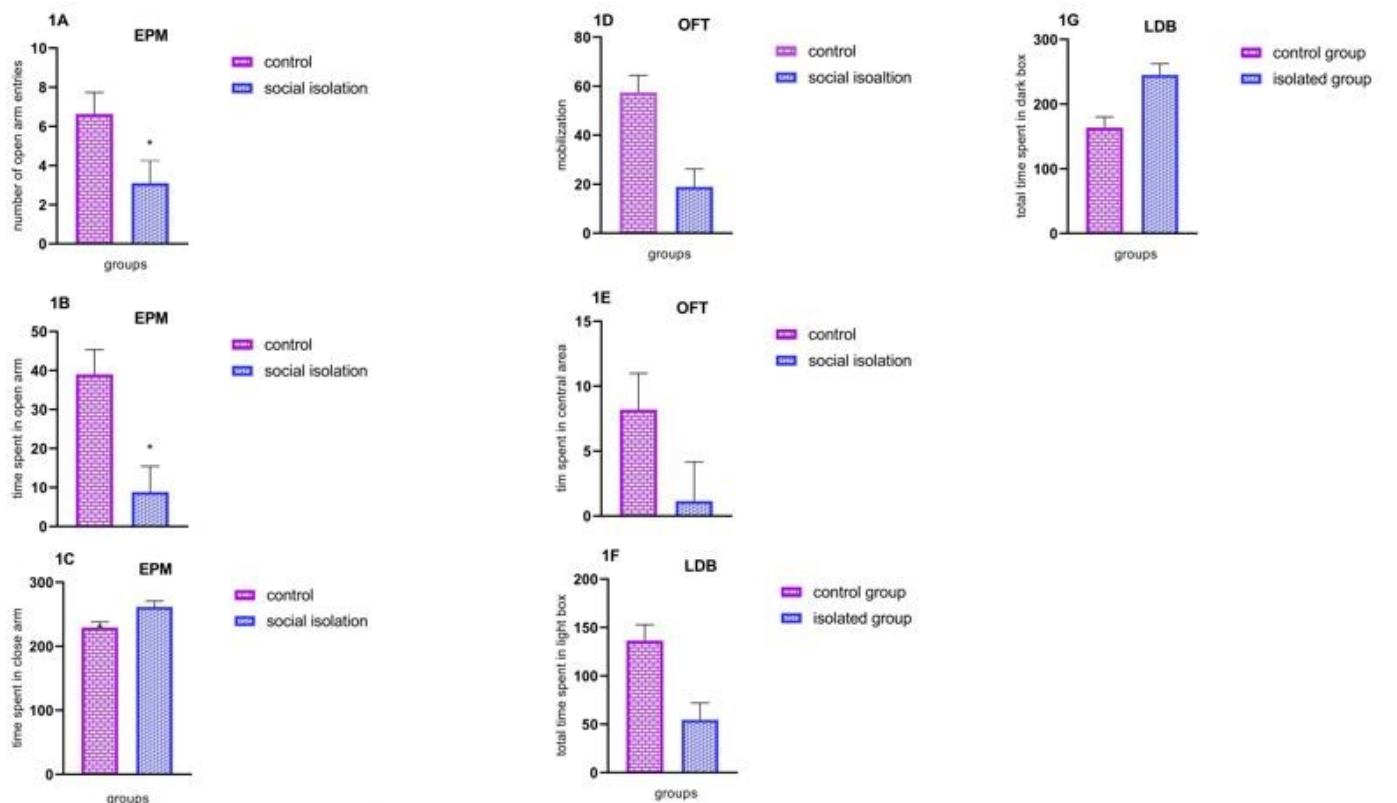


Figure 1. Effect of SI on behavioural tests: EPM, OFT and LDB. A: Open arm entries, B: Time spent in open arm, C: Time spent in close arm, D: Mobilization, E: Time spent in central area, F: Time spent in light box, G: Time spent in dark box. The statistics are presented with mean±SEM (n=12 per group). One-way ANOVA with Tukey HSD for multiple comparisons. Abbreviations: EPM, Elevated Plus Maze; OFT, Open Field Test; LDB, Light-Dark Box. All data were expressed as mean ± SE values and analysed by one-way factorial ANOVA with Tukey post-hoc test. *p<0.05 is considered significant.

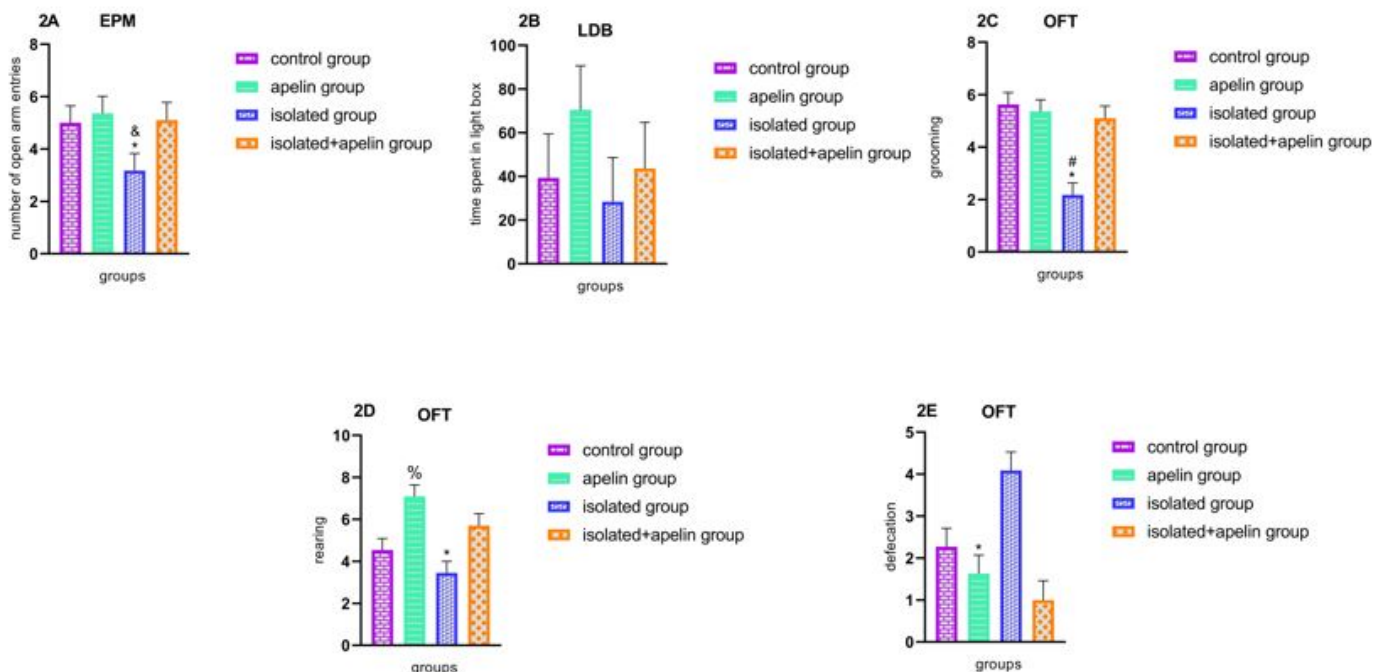


Figure 2. Effect of apelin administration on behavioural tests: EPM, OFT and LDB. A: Open arm entries, B: Time spent in light box, C: Grooming, D: Rearing, E: Defecation. The statistics are presented with mean±SEM (n=12 per group). One-way ANOVA with Tukey HSD for multiple comparisons. Abbreviations: EPM, Elevated Plus Maze; OFT, Open Field Test; LDB, Light-Dark Box. All data were expressed as mean ± SE values and analysed by one-way factorial ANOVA with Tukey post-hoc test. *p<0.05 is considered significant.

Gene expression analysis

For gene expression assessment, RNA was isolated from hippocampal and amygdala tissue using the TRIzol

method. The concentration and quality of the total RNA samples were checked by spectrophotometric and agarose



Figure 3. Effect of apelin administration on gene expression levels. A: Gene expression levels of Apelin, APJ, BDNF, Neuritin, Nestin, DCX and MASH1 in the hippocampus. After the SI, B: Gene expression levels of Apelin, APJ, BDNF, Neuritin, Nestin, DCX and MASH1 in the hippocampus. After the apelin administration. All data were expressed as mean $\pm$ SE values and analyzed by one-way factorial ANOVA with Tukey post-hoc test. There is no significant difference between the groups ($p>0.05$).

gel electrophoresis methods. mRNA-level expression of the candidate and reference genes was detected by qRZR. PZR products of all genes were observed in the agarose gel (2%) electrophoresis.

Statistical analysis

In accordance with earlier research, a minimum sample size of $n=12$ per group was determined using a mean difference of 0.4 and a standard deviation of 0.1, which yielded 90% power at a 95% confidence level with reference to.

To analyze the gene expression data, all genes' Ct values were compared to the reference genes' Ct values (PGK1, RPL13A, and GAPDH), from which Δ Ct values were obtained. Differences in gene expression among the groups were reported as mean $\pm$ SE values and analyzed using one-way factorial ANOVA followed by the Tukey post-hoc test. A significance level of $*p<0.05$ was applied.

Results

SI causes anxiety-like behavior in rats

The EPM, OFT and LDB tests were used to assess the effects of SI on anxiety-like behaviour in rats. In the EPM test, the SI group spent significantly less time in the open arms than the C group, and they also entered the open arms significantly less frequently than the C group. In the EPM test, entries into the open arms ($p<0.05$, Figure 1A-B). However, time spent in closed arms did not change significantly ($p>0.05$, Figure 1C). No significant differences were found between the groups in the OFT and LDB tests ($p>0.05$, Figure 1D-F).

Apelin-13 administration may reduce anxiety-like behaviors in rats

In order to assess the effects of apelin-13 on behaviour, the EPM test was carried out. Open arm entries were significantly lower in the SI group compared to both the C and A groups ($p<0.05$, Figure 2A). However, in terms of time spent in open arms, there were no significant differences between the groups ($p>0.05$, Figure 2B).

In the OFT, the SI group showed fewer grooming and defecation behaviors compared to the C and A groups. Conversely, rearing in group A was significantly higher than the others ($p<0.05$, Figure 2C-E).

No significant differences between groups were observed for any parameter in the LDB test ($p>0.05$).

SI stress may have varying effects on different regions of the brain

According to the results of real-time PCR analysis, there was a significant decrease in the expression of the MASH1 gene in the hippocampal tissue of the SI group compared to the C group ($p<0.05$, Figure 3A). In rats that received apelin-13 treatment following SI, there were no significant differences in gene expression in the hippocampus and amygdala tissues ($p>0.05$, Figure 3B).

Discussion

The mechanisms underlying the psychopathological effect of stress remain largely unknown. Several stress methods used in animal modeling are important in terms of exposing this mechanism and have shown that there is a relationship among behavior and brain areas such as hypothalamus, hippocampus, and amygdala. It has been shown that there is apelin-13 and apj gene expression in these brain regions [17] which are considered to be central points for stress and emotional responses [18]. The existence of apelin-13 and APJ in these regions suggests that apelin-13 may have a potential role in the behavioural process. Moreover, different special biomarkers are expressed at the stages that take place in the neurogenesis process. Through biomarkers, the neurogenesis process and the path of newborn cells can be observed in detail and clearly [19,20]. The present investigation demonstrates activation of the BDNF, Nestin, Neuritin, MASH1 and DCX expression levels in hippocampus and amygdala following apelin-13 infusion in the rat. After creating anxiety with the SI model in rats, apelin-13 was injected as subcutaneous for 14 days. To find out how apelin-13 affected anxiety, behavioral tests and the expression levels of multiple genes in the hippocampus and amygdala were analyzed.

In the OFT, the scores for the time spent in the central area were significantly higher in the A group than in the B group. The decreasing of time spent in the central part of the square is considered as an indicator of anxiety, anxiolytic agents increase research behavior and prolong this time [21,22]. In the current study, the SI group had a significant decrease in time in the centre after application, while the A group had an increase in time in the centre after application.

Another parameter is the exploratory behavior of the rats to investigate and obtain information. The frequency of this behavior decreases anxiety [23,24]. We also determined that the scores of rearing and grooming were significantly lower in the SI group in the OFT. It is seen that the increase in the rearing after application of the A group supports the anxiolytic effect of the apelin-13.

The distance parameter measured at OFT gives information about the locomotor activity of rats. The study's findings indicate that the rats in the SI group were less distant than those in the C group, but that this difference was reversed following the administration of apelin-13. According to the results of this study, rats in the SI group decreased. Defecations increases in anxiety due to activation of the autonomic system [25]. Considering the C group, there has been significant increase defecations in the SI group. Apelin-13 application significantly reduced defecations. However, both views are available in grooming behavior [26]. Decrease in grooming activity can be attributed to an increase in anxiety behaviour however, anxiolytic drugs can reverse this by increasing grooming activity [27]. Grooming decreased in the SI group, while apelin-13 increased in the group applied. According to the current findings, considering that anxiety decreases after apelin-13 application, the increase in grooming behavior after apelin-13 application can be considered as a decrease in anxiety.

Open arm entries and time spent in open arms are the main parameters considered in the EPM [28]. In a study conducted by [29] they investigated the effect of fluoxetine, an effective antidepressant treatment, on anxiety and they reported that the duration of the animals receiving fluoxetine increased in the open arm. We determined that the open arm entries and time spent in open arm. We observed an increase in both groups of apelin-13 may have occurred due to anxiety reduction.

LDB scored the time spent in the light box and the time spent in the dark box. An indicator of anxiety may be the prolonged time spent in the dark box in the SI group. Anxiogenic agents increase the number of passes to the bright box and the residence time, while anxiolytic substances increase the number of passes to the dark box and the length of stay in this region [30]. A potential correlation between hippocampal neurogenesis and anxiety-related behaviors has been proposed. The inhibition of neurogenesis has been linked to an increase in certain types of behaviour associated with anxiety. According to our findings, it was determined that the number of passes from dark box to bright box decreased in the SI group, and this transition behavior increased with the application of the apelin-13.

Apelin-13 gene expression has been shown to decrease in brain regions as a result of neurodegenerative diseases [31].

Injection of intrahippocampal apelin-13 has been reported to have a modulatory effect on mood disorders such as anxiety and depression by modulating various cellular signalling pathways such as ERK and PI3K [32]. In our study, apelin-13 gene expression levels of rats investigated in the hippocampus and amygdala tissues, but no significant changes were found.

APJ is a receptor found in many tissues such as hippocampus, hypothalamus, amygdala in addition to peripheral tissues in rats [33]. The increase of APJ in the hippocampus, where the stress response occurs, can be effective in maintaining moods by increasing the apelin-13 binding capacity. Recently, it was reported that apelin-13 regulates stress response by increasing the expression of the APJ found in the hippocampus [34]. We did not detect a significant change on the APJ expression level in SI group.

Neuritin is a protein that regulates neuronal plasticity [35] and increases the level of BDNF in the hippocampus in a similar way to antidepressants [36]. Researchers have demonstrated that long-term stress lowers the expression of neuritin in the rat hippocampal CA1 and CA3 regions [37]. In agreement with the recent experimental data indicating increased expression of neuritin by chronic antidepressant treatment in the rat brain [38], we also observed that the expression level of neuritin in the A group increased compared to the C group but not statistically significant. This may be due to the removal of the total hippocampus tissue, not specifically the CA1 and CA3 sections.

DCX is used to measure the degree of neurogenesis and is only expressed in neonatal neurons [39]. It was reported that the level of DCX expression decreased in anxiety group. They applied Tamoxifen injection to suppress neurogenesis [40]. We also determined that the level of DCX expression was lower in the SI group. Decreased in DCX expression level may be considered as postpartum depression [41]. It has been reported that maternal separation reduces hippocampal cell proliferation but does not have a mitigating effect on DCX expression [42]. According to our findings, Decrease in DCX expression in the SI group, but increase in DCX expression after apelin-13 injection. These results may give clues that neurogenesis is suppressed because of anxiety and the injection of apelin-13 has a positive effect on neurogenesis.

Many areas of the brain, including the amygdala and hippocampal regions, express BDNF and as hippocampal neurogenesis increases, there is also an increase in BDNF gene expression [43]. It has been noted that apelin-13 plays a healing role in memory loss by increasing the level of hippocampal BDNF [44]. According to our findings, anxiety has reduced the level of BDNF gene expression in hippocampus tissue. Gray et al. demonstrated that stress is a factor that causes a decrease in BDNF levels in the CA1 and CA3 regions of the hippocampus, which is consistent with our findings [45].

Nerve stem cells divide and differentiate in the neurogenesis phase, forming the precursor cells. Nestin is a precursor biomarker [46]. Recently a study noted that the level of the positive nesting cell increased with anxiety treatment [47]. In our experiment, apelin-13 infusion decreased the level of nestin gene expression in hippocampus tissue. This may

be due to the extraction of the entire hippocampus tissue or insufficient infusion time. In amygdala tissue, it has been observed that apelin-13 increased the level of nestin gene expression. It may support the idea that apelin-13 has a positive effect on neurogenesis by increasing neural precursor cells in different parts of the brain.

MASH1 is an important transcription factor that regulates differentiation processes during neurogenesis and differentiates neural progenitor cells [48]. Research has demonstrated that adult DC ischaemia is associated with elevated MASH1 cell production and MASH1 mRNA expression. This implies that neurogenesis is significantly influenced by the growth of these cells [49]. Therefore, we focused on MASH1 protein expression in the hippocampus and investigated its physiological effects. According to the findings of our study, MASH1 gene expression was significantly decreased in hippocampus tissue in both SI and A groups. This may be due to the activation of neural precursor cells as a result of negatively affected neurogenesis.

Conclusion

In conclusion, when all these findings are evaluated, it is likely that hippocampal neurogenesis may play a role as an intermediary mechanism under the anxiolytic effect of apelin-13. Apelin-13 may have increased hippocampal neurogenesis, so it causes a decline in anxiety behaviours. The present findings indicate that SI has increased anxiety-like behaviours and have a suppressive role in hippocampal neurogenesis. Behavioural experiments have demonstrated that apelin-13 has inhibition of anxiety.

Ethical approval

Ethical approval was obtained for this study from Necmettin Erbakan University KONÜDAM Experimental Medicine Application and Research Center Animal Experiments Local Ethics Committee (Decision number: 2018-011).

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Relationship between lactate albumin ratio and mortality in patients with ischemia and non-obstructive coronary artery disease (INOCA)

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Abstract

Aim: Ischemia with non-obstructive coronary artery disease (INOCA) is when there is myocardial ischemia without occlusive coronary artery disease. Over time, this could result in a reduced quality of life, frequent hospital visits, and a higher risk of cardiovascular-related deaths. Diagnosis, management, and prognosis of INOCA pose challenges. Lactate-albumin ratio (LAR) has been associated with mortality from many cardiovascular diseases. The study examined the link between LAR and mortality in patients with INOCA.

Materials and Methods: The study analyzed data from 987 patients diagnosed with ischemia through myocardial perfusion imaging using single photon emission computed tomography (MPI-SPECT) at our center between 2017 and 2023. After applying the exclusion criteria, we included 279 patients in the study. Medical histories, laboratory parameters, and patient death information were recorded.

Results: The mean follow-up time was 20547 days. Mortality occurred in 5% of patients (14 patients). Patients were divided into two groups: mortality and non-mortality. The LAR was 0.65 ± 0.26 in the non-mortality group and 1.05 ± 0.32 in the mortality group, indicating a meaningful disparity between the two groups ($p=0.017$). Cox regression analysis was conducted to determine mortality predictors. In INOCA patients, mortality was independently predicted by age and LAR ($p=0.03$, and $p=0.005$, respectively). To evaluate the efficacy of LAR in predicting mortality, we executed the Receiver Operating Characteristic (ROC) analysis. The examination revealed an area under the curve (AUC) of 0.689 (0.519-0.858), a cut-off of 0.656, a sensitivity of 57.1%, and a specificity of 52.8% ($p=0.017$).

Conclusion: Our study found that LAR performs as an independent predictor of mortality in patients with INOCA.



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Introduction

Ischemia with non-obstructive coronary artery disease (INOCA) is described as the presence of myocardial ischemia without occlusive coronary artery disease [1]. INOCA has been considered a chronic coronary syndrome. Ischemia in patients with INOCA can be detected through stress electrocardiogram, abnormal cardiac stress imaging, and elevated troponin levels. This specific patient population can experience long-term complications like heart failure with preserved ejection fraction, myocardial infarction, decreased quality of life, repeated hospitalizations, and increased cardiovascular mortality [2,3]. Although INOCA causes symptoms more frequently in women, it can also

be seen in men and even has a worse prognosis in men [4]. The pathophysiology of INOCA is not clear, but it is evident that most patients experience coronary vascular dysfunction [5]. INOCA can be ignored due to the absence of obstructive coronary lesions. However, it can be a significant cause of cardiovascular morbidity and mortality. It is one of the diseases that is difficult to diagnose and predict prognosis because it has a heterogeneous patient group [6]. Therefore, new tools to predict INOCA prognosis are needed.

Lactate is an end product of anaerobic glycolysis. Although the increase in lactate levels may be due to various factors such as decreased excretion due to liver and kidney disease and acceleration of anaerobic glycolysis, in clinical practice, it is thought that the lactate level increases due to tissue perfusion disorder [7,8]. Recent studies have sug-

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gested that high serum lactate levels may be associated with short- and long-term mortality from cardiovascular diseases like acute myocardial infarction and heart failure [9].

Albumin levels have proven to be a reliable indicator in clinical practice, effectively reflecting nutrition, liver function, and inflammation status for many years. Hypoalbuminemia is strongly linked to a poor prognosis, especially in diseases where inflammation is involved in their pathophysiology [10]. Similarly, hypoalbuminemia indicates a poor prognosis in many cardiovascular diseases in which inflammation has been implicated [11].

The Lactate-Albumin Ratio (LAR) is calculated by dividing the lactate level by the albumin level. This ratio was first employed to evaluate the prognosis of individuals with sepsis [12]. Recent studies have linked it to a poor prognosis for cardiovascular diseases like heart failure and acute myocardial infarction [13,14].

INOCA mortality has become a growing problem in recent years. Identification of mortality predictors may provide insight into prognosis. Therefore, the study investigated the association between mortality and LAR in patients with INOCA.

Materials and Methods

Study design and patient selection

This study was a retrospective, single-center, and observational study. The evaluation began by examining the data of patients who presented to the cardiology outpatient clinic with chest pain, had a medium-high probability

of coronary artery disease before the test, and had myocardial ischemia detected on myocardial perfusion imaging (MPI) with single photon emission computed tomography (SPECT). Data from 987 patients diagnosed with ischemia using MPI with SPECT and who underwent coronary angiography at our center between 2017 and 2023 were examined for the study. Two cardiologists monitored the patients' coronary angiography. If they could not agree on the percentage of coronary lesions, a third cardiologist viewed the images. Patients with stenosis greater than $\geq 50\%$ in at least one epicardial coronary artery were excluded from the study because they did not meet the INOCA criteria. When the coronary angiography records of 987 patients were reviewed, 638 patients were eliminated from the trial due to the presence of a lesion of 50% or greater in at least one epicardial coronary artery. The medical history and laboratory parameters of the remaining 349 patients were thoroughly analyzed. Six patients were excluded from the study due to severe heart valve disease. One patient was eliminated from the trial due to pericardial effusion. Three participants were eliminated from the research due to diabetic ketoacidosis. Seven patients were eliminated from the trial due to severe liver and kidney disease. Sixteen participants were eliminated from the research because of active malignancy. Five participants were eliminated from the research because of chronic inflammatory disease. Three participants were excluded from the trial due to a diagnosis of malnutrition. Nineteen patients were eliminated from the trial because of missing data. In addition, the sample size required for G power analysis is 184, according to a 5% error and 95% confidence level. Also, considering a 10% loss, it was planned to include 200 patients. Nevertheless, the sample size was found to be greater, and a total of 279 participants participated in the study. Figure 1 presents the flow chart. Medical history, hemogram, biochemistry, and venous blood gas information were recorded. Patient death information was obtained from the hospital registration system and the Ministry of Health's death notification system. The local ethics committee approved the study and adhered to the Declaration of Helsinki's principles.

Collection of blood samples

Blood samples were collected within the 24 hours after patients were admitted to our clinic for coronary angiography. Samples were drawn from either the left or right antecubital vein following the application of mild venous stasis using a tourniquet on the upper arm. The samples were collected in potassium EDTA tubes for a complete blood count. We measured hemoglobin, hematocrit, platelet, and white blood cell counts using the Beckman Coulter LH 780 with the electrical impedance method. Standard laboratory methods were used to assess the biochemical parameters. The blood gas test was examined using an ABL 800 FLEX blood gas analyzer (Radiometer).

Definitions

According to the 2021 American Heart Association, INOCA is defined as the demonstration of coronary dysfunction by invasive means or the demonstration of ischemia by non-invasive methods in addition to $<50\%$ stenosis in the

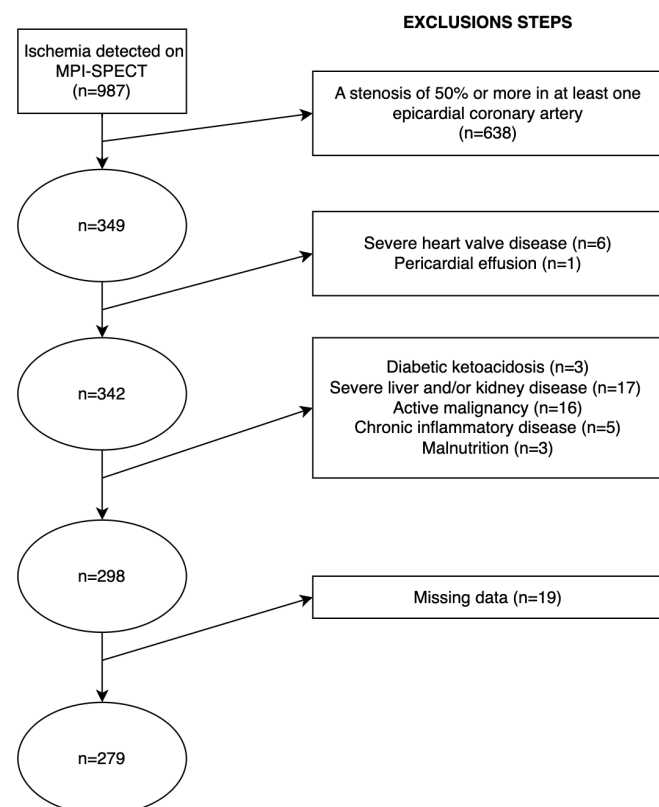


Figure 1. Flow chart of exclusion steps.

epicardial coronary arteries. Stress cardiac magnetic resonance imaging (CMR) and MPI-SPECT are non-invasive examinations [15].

Hypertension (HT) is represented as having an arterial blood pressure of 140/90 mmHg or higher or using at least one antihypertensive drug [16].

Diabetes mellitus (DM) is diagnosed when the glycated hemoglobin is 6.5% or higher and/or the fasting blood glucose level is ≥ 126 mg/dL after an 8-hour fast [17].

Statistical analysis

We employed numbers and percentages to effectively represent categorical data, providing a clear and concise visualization of our findings. For normal distributions, continuous variables were recorded as mean $\pm$ standard deviation (SD); for non-normal distributions, they were recorded as median (interquartile range [IQR]). Nonparametric data was analyzed using the χ^2 test. Before conducting significance tests, we assessed the normality of all variables using the Kolmogorov–Smirnov test and scrutinized the homogeneity of variances with the Levene test. T-tests for independent groups were confidently utilized for data exhibiting a normal distribution and homogeneity. In cases where parameters did not adhere to a normal distribution between two groups, we relied on the Mann–Whitney U test. Univariable and multivariable Cox regression analyses were conducted to thoroughly examine risk factors for mortality and decide independent risk factors. All analyses were executed using the SPSS statistical package version 23.0 (IBM Corp., NY, USA), with a significance level of $p < 0.05$ for all statistical assessments. We assessed LAR's effectiveness in predicting mortality among patients with INOCA. This was accomplished by carefully examining receiver operating characteristic (ROC) curves and determining the area under the curve (AUC) value.

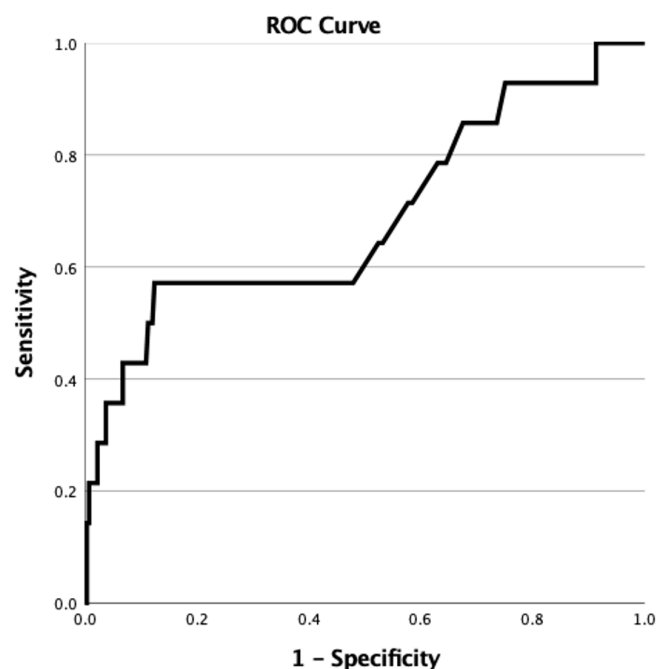


Figure 2. ROC-AUCs for LAR's prediction of mortality development in INOCA.

Table 1. Baseline demographic characteristics.

Variables	Mortality (-) (n=265)	Mortality (+) (n=14)	P value
Gender, male (%)	104 (39.2)	8 (57.1)	0.184
Age (years)	62.49 $\pm$ 12.87	74.59 $\pm$ 9.02	<0.001
HT, n (%)	174 (65.7)	6 (42.9)	0.083
DM, n (%)	48 (18.1)	5 (35.7)	0.102
CAD, n (%)	232 (87.5)	10 (71.4)	0.084
CVE, n (%)	3 (1.1)	0 (0)	0.689
LVEF (%)	56.3 $\pm$ 2.1	55.6 $\pm$ 1.8	0.726
LAd (mm)	35.7 $\pm$ 4.8	36 $\pm$ 4.6	0.402
RAd (mm)	34.8 $\pm$ 4.3	34.9 $\pm$ 4.5	0.776
pH	7.39 $\pm$ 0.83	7.41 $\pm$ 0.39	0.089
HCO ₃ (mmol/L)	23.61 $\pm$ 4.05	19.37 $\pm$ 6.95	0.044
PCO ₂ (mmHg)	43.6 $\pm$ 9.4	52.4 $\pm$ 13.6	0.070
Lactate (mmol/L)	2.1 (1.5-3.1)	5.8 (3.6-8.0)	0.005
Albumin (g/dL)	4.30 $\pm$ 0.42	4.21 $\pm$ 0.60	0.992
WBC (10 ³ /μL)	8.6 (6.7-10.9)	10.9 (7.2-12.5)	0.190
Hemoglobin (g/dL)	14.2 $\pm$ 2.1	13.9 $\pm$ 2.1	0.782
Platelet (10 ³ /μL)	252 (209-298)	228 (157-258)	0.085
CRP (mg/L)	1.9 (0.43-6.65)	8.6 (4.9-15.3)	0.188
Glucose (mg/dL)	104 (90-130)	103 (98-157)	0.401
ALT (U/L)	23 (17.5-32)	16 (14-27)	0.040
Total Cholesterol (mg/dL)	170.8 $\pm$ 42.1	151.8 $\pm$ 48.9	0.197
HDL (mg/dL)	39.7 $\pm$ 12.2	37.2 $\pm$ 11.8	0.358
LDL (mg/dL)	118.5 $\pm$ 39.7	98.7 $\pm$ 25.6	0.049
Triglyceride (mg/dL)	146 (103-222)	120 (97-230)	0.793
Creatinine (mg/dL)	0.84 $\pm$ 0.27	0.89 $\pm$ 0.26	0.450
Sodium (mg/dL)	139.3 $\pm$ 4.3	138.8 $\pm$ 2.6	0.357
Potassium (mg/dL)	4.22 $\pm$ 0.49	4.23 $\pm$ 0.30	0.703
LAR	0.65 $\pm$ 0.26	1.05 $\pm$ 0.32	0.017

Abbreviations: HT: Hypertension, DM: Diabetes mellitus, CAD: Coronary artery disease, CVE: Cerebrovascular event, LVEF: left ventricular ejection fraction, LAd: left atrial diameter, RAd: right atrial diameter, HCO₃: Bicarbonate, PCO₂: Partial carbon dioxide, WBC: White blood cells, CRP: C-reactive protein, ALT: Alanine aminotransferase, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, LAR: Lactate-albumin ratio.

Results

Our research involved a total of 279 patients diagnosed with INOCA. The mean follow-up time was 205 $\pm$ 47 days. Mortality was observed in 5% (14 patients) of the patients. Patients were split into two groups: mortality and non-mortality. The age difference between the mortality and non-mortality groups was 74.59 $\pm$ 9.02 and 62.49 $\pm$ 12.87 years, respectively. The age in the mortality group was statistically significantly older ($p < 0.001$). HT, DM, and coronary artery disease (CAD) appeared statistically similar in the two groups. The pH was comparable in both groups, but the HCO₃ levels were statistically significant, measuring 19.37 $\pm$ 6.95 in the mortality group ($p = 0.044$). While lactate level was 2.1 mmol/L in the non-mortality group, it was 5.8 mmol/L in the mortality group. The two groups had a statistically significant difference in lactate levels ($p = 0.005$). Alanine aminotransferase (ALT) and low-density lipoprotein (LDL) levels were considerably reduced in the mortality group ($p = 0.04$ and $p = 0.049$, respectively). While LAR was 0.65 $\pm$ 0.26 in the non-mortality group, it was 1.05 $\pm$ 0.32 in the mortality group, indicating a meaningful significant disparity between the two groups

Table 2. Univariable and Multivariable Cox Regression Analysis.

Variables	Univariable HR (%95 CI)	P value	Multivariable HR (%95 CI)	P value
Age	1.089 (1.036-1.144)	<0.001	1.074 (1.006-1.146)	0.032
ALT	0.970 (0.923-1.020)	0.238	0.986 (0.935-1.040)	0.605
LDL	0.986 (0.970-1.001)	0.067	0.987 (0.966-1.003)	0.074
LAR	4.069 (1.946-8.510)	<0.001	3.148 (1.417-6.992)	0.005

Abbreviations: ALT: Alanine aminotransferase, LDL: Low-density lipoprotein, LAR: Lactate- albumin ratio, HR: Hazard ratio, CI: Confidence interval.

($p=0.017$). Please refer to Table 1 for the details.

To identify mortality factors, Cox regression studies, both univariable and multivariable, were conducted. Age and LAR emerged as independent predictors of mortality in INOCA patients ($p=0.03$ and $p=0.005$, respectively). The outcomes of the regression analysis are presented in Table 2.

We conducted a ROC analysis to consider LAR's capability to predict mortality. The analysis yielded an AUC of 0.689 (0.519-858) with a cut-off value of 0.656. The sensitivity was established at 57.1%, while the specificity was recorded at 52.8% ($p=0.017$) (Figure 2).

Discussion

Our study found a considerable connection between LAR and mortality in patients with INOCA. Our study is the first to investigate the association between INOCA mortality and LAR, according to our knowledge.

INOCA is a chronic coronary syndrome diagnosed by the presence of ischemia detected by invasive or non-invasive methods in addition to non-obstructive epicardial coronary arteries. Studies have found that INOCA is associated with deterioration in quality of life, recurrent hospitalization, and increased cardiovascular mortality [18,19]. Diagnosis, patient management, and prediction of prognosis are challenging due to the absence of obstructive lesions in the coronary arteries and patient heterogeneity [3]. The pathophysiology of patients with INOCA is believed to be epicardial coronary artery dysfunction or coronary microvascular dysfunction, which coronary spasms can sometimes accompany. Although the pathophysiology is not clearly understood, increased inflammation and oxidative stress, in addition to cardiometabolic risk factors may cause epicardial coronary and coronary microvascular dysfunction. In addition, recurrent angina, even in the absence of coronary obstruction, can linked to cardiac autonomic dysfunction [20]. It is estimated that approximately 3-4 million people worldwide have angina attacks each year, even though they do not have obstructive coronary artery disease [21]. Until recently, the medical community widely believed that INOCA had a favorable prognosis. Recent studies have revealed that patients with INOCA face a raised risk of significant unfavorable cardiac events, stroke, and cardiovascular mortality [22]. In addition, patients with single-vessel obstructive coronary artery disease and those without it demonstrate comparable one-year mortality and myocardial infarction rates [18]. Advanced age, HT, DM, and smoking history are associated with increased mortality in INOCA patients [3]. In our study, advanced age predicted INOCA mortality

similarly to the literature. However, our study did not detect HT and DM as a mortality predictor. Additionally, we did not have information about the smoking history of the patients in our study.

Myocardial ischemia is caused by an imbalance in heart oxygen supply. As a result, cardiac myocytes show decreased mitochondrial oxidative phosphorylation and a shift toward the glycolytic pathway. In this case, an increase in lactate level is observed due to an increase in the glycolytic pathway due to impaired oxidative phosphorylation [7]. Lactate is the end product of the anaerobic glycolytic pathway. Its level may increase in cases where oxygenation is impaired at the cellular level. Increased serum lactate levels have been linked to numerous cardiovascular diseases, such as cardiac arrest, acute myocardial infarction, heart failure, atrial fibrillation, atherosclerosis, and silent myocardial ischemia [23-28]. Lactate also has significant predictive value for mortality in many cardiovascular diseases [9]. In our study, similar to the literature, serum lactate levels were statistically significantly higher in INOCA patients with mortality.

Hypoalbuminemia has long been recognized as a negative acute-phase reactant. It has been used to determine the prognosis of acute diseases, especially sepsis [29]. Significant liver disorders, inflammatory conditions, and malnutrition may affect serum albumin levels [30]. Recent studies have shown that hypoalbuminemia may be associated with mortality from cardiovascular diseases such as heart failure and acute myocardial infarction [31,32]. In our study, albumin level was lower in the mortality group. However, no statistically significant change was seen. This may be because INOCA does not present with acute and severe signs such as acute myocardial infarction and heart failure.

LAR was initially utilized to assess the prognosis of patients with sepsis, and it is believed to offer superior predictive value compared to the sole measurement of serum lactate level [33]. Since both high serum lactate level and hypoalbuminemia indicate poor prognosis in cardiovascular diseases such as heart failure and coronary artery disease, LAR has been used to predict poor prognosis in cardiovascular diseases [14,32]. Patients with INOCA commonly experience coronary vascular dysfunction. The resulting oxygen-supplying imbalance may trigger hypoxia, increased inflammation, and oxidative stress at the cellular level. At the cellular level, hypoxia may cause cardiomyocytes to shift to anaerobic glycolysis rather than oxidative phosphorylation, increasing serum lactate levels [9]. Hypoalbuminemia may also be a sign of increased inflammation [10]. Inflammation and oxidative stress un-

deniably play a pivotal role as pathophysiological causes of atherosclerotic heart diseases [34]. Considering all these mechanisms, it may not be surprising that a practical, easily applicable, and inexpensive parameter such as LAR stands out in predicting the prognosis of a chronic coronary syndrome such as INOCA.

We faced several limitations in our study. First, our study was limited by a relatively small sample size and by being a retrospective, single-center study. Secondly, our limitations were that we did not use an invasive method to detect coronary dysfunction, and patients' medical treatment data and smoking information could not be accessed. Finally, another limitation was that only the initial value of LAR was included in the study, and dynamic change was not monitored.

Conclusion

Our research suggests that LAR could potentially be a key factor in predicting mortality among patients with INOCA. INOCA still presents significant challenges in diagnosis, prognosis, and treatment. Using a cheap and easily calculable parameter such as LAR to estimate prognosis in INOCA patients may provide clinical benefits.

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Declaration of conflicting interests

Regarding this paper's research, writing, and publication, the authors declare they have no conflicts of interest.

Ethical approval

The Health Sciences University, Erzurum City Hospital, Ethical Committee approved this study (Ethics Committee Decision Date: 11/09/2024, Ethics Committee Decision Number: 2024/09-169).

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Analysis of coronary artery anomalies and variants in cardiac risk groups through coronary computed tomography angiography

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Abstract

Aim: The purpose of this study is to investigate prevalence of coronary artery anomalies and variants in a cardiac risk group by using coronary computed tomography (CT) angiography.

Materials and Methods: Coronary CT angiography scans were retrieved for 527 consecutive adult patients (265 male, 262 female) with a preliminary diagnosis or suspicion of coronary artery disease between 2021 and 2023. Coronary artery anomalies and vascular variants were retrospectively reviewed.

Results: The study revealed that 85.97% had right dominant coronary circulation, 22.39% had myocardial bridging, and 9.3% having a ramus intermedius artery. Among the identified coronary anomalies, anomalies of origination and course were the leading causes (3.6 %), except for hypoplasia, which had a prevalence of 6.07 %. Overall, no significant relationships were found between gender or coronary artery disease and the frequency of coronary artery anomalies.

Conclusion: Coronary artery anomalies and variants, which are mostly asymptomatic, represent a broad spectrum of entities that can be accurately diagnosed by coronary CT angiography.



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Introduction

The coronary arteries are the main vessels supplying blood to the myocardium of the heart [1]. The left main coronary artery (LMCA) typically arises from the left coronary sinus, while the right coronary artery (RCA) regularly originates from the right coronary sinus and courses through the right atrioventricular groove [1,2]. The LMCA bifurcates into the left anterior descending (LAD) artery and left circumflex (LCx) artery [2,3]. While the LAD gives rise to diagonal branches, the LCx gives rise to obtuse marginal branches, and the RCA gives rise to the conus artery, acute marginal branches and the posterior descending artery (PDA) in right dominant coronary circulation [1-3]. Coronary arteries, which usually follow a specific pattern, may sometimes exhibit variations or anomalies [4]. Coronary artery variations are often incidental findings during imaging and considered benign. In contrast, coronary artery anomalies, though rarer, they can cause decreased blood flow to the myocardium and lead to angina, arrhythmias, and even sudden cardiac arrest [5]. Coronary artery varia-

tions and anomalies can be diagnosed using various imaging methods such as conventional coronary angiography, cardiac computed tomography (CT), and magnetic resonance imaging (MRI) [6]. Whether treatment is required depends on the type of anomaly, the patient's symptoms, and clinical evaluations. In some cases, surgical intervention may be necessary, while in other cases, only monitoring and symptom management may be required [7-9]. In this study, we aimed to demonstrate the coronary artery anomalies and variants in cardiac risk groups (defined as the presence of one or more cardiovascular risk factors, including high blood pressure, high cholesterol, smoking, diabetes mellitus, obesity, or a family history of cardiovascular disease) using cardiac CT imaging.

Materials and Methods

This retrospective study is approved by the Institutional Review Board of the Bolu Abant İzzet Baysal University (Project decision number: 2023/332). Given the retrospective setting, informed consent was waived by the committee. This research study followed the Declaration of Helsinki and adhered to the STROBE guidelines for reporting [10].

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Study design and population

Based on the reported prevalence of coronary artery anomalies and variations, the sample size was calculated to be 185 patients, using a significance level (α) of 0.05 and a confidence interval of 95%. Cardiac CT examinations of 627 consecutive adult patients with a preliminary diagnosis or suspicion of coronary artery disease from January 2021 to December 2023 were retrospectively reviewed. Patients aged 18 years or older, with at least one cardiovascular risk factor (including high blood pressure, high cholesterol, smoking, diabetes mellitus, obesity, or a family history of cardiovascular disease), and who underwent coronary CT angiography for clinical indications during the study period were included. Patients with high heart rates resulting in nondiagnostic images (N=38), high coronary artery calcium score (Agatston score greater than 400, N=34), those who had only non-enhanced calcium score images (N=22), undergone prior coronary revascularization (N=6) were excluded from the study. A history of allergic reactions to iodine contrast medium, renal failure, or pregnancy was considered contraindications for cardiac contrast enhanced computed tomography. Figure 1 presents a flowchart summarizing the data collection procedure. The main indications for cardiac CT were typical or atypical chest pain, screening for coronary artery disease and determination of the patency of stents. One of the patients was referred from the emergency department, one from interventional radiology, one from neurology, one from internal medicine, two from the coronary intensive care unit, six from the cardiovascular surgery outpatient clinic, and 515 from the cardiology outpatient clinic for cardiac CT imaging.

The primary endpoint of this study was the prevalence of coronary artery anomalies and variants identified through coronary computed tomography (CT) angiography.

Patient preparation

Patients are asked not to eat four hours prior to scan and they should avoid caffeinated drinks and smoking on the day of examination. Administering a beta-blocker (up to a total dose of 20 mg metoprolol) as premedication on the study day may help lower the heart rate and reduce waiting times. An intravenous (IV) line should be inserted in the right antecubital vein or forearm using an 18-20 gauge catheter.

Cardiac CT imaging protocol and image acquisition

All CT images were acquired using a 64-slice scanner (Revolution Evo, GE Healthcare, Milwaukee, Wisconsin, United States). The patient is kept in supine position, electrocardiography (ECG) electrodes are placed on the anterior chest wall, and moved into the scanner feet first with legs flat and both arms above their head on the table. When starting the imaging, first a scout image is obtained to determine the boundaries of the heart. In order to perform calcium scoring in patients between the ages of 40-70, non-contrast images are acquired from the level of tracheal bifurcation to the inferior border of the heart [11]. Depending on the patient's heart rate, prospective

axial (if the patient's heart rate less than 70) or retrospective helical scanning was done with single breath hold and image acquisition starting from the tracheal bifurcation till 1 cm below diaphragm. A biphasic injection protocol with bolus tracking was used, starting with 70-90 mL of non-ionic contrast medium (iohexol 350 mgI/mL) at 5-6 mL/s, followed by a 30 mL saline chaser at the same flow rate. Saline chaser bolus was used to flush the contrast from the right side of the heart. The threshold for initiating image acquisition is set at 200 HU in the descending aorta. The procedure approximately takes 15-20 minutes.

Image reconstruction and interpretation

Cardiac CT scans were processed using multiplanar reconstruction (MPR) and 3-dimensional volume rendering (3D VR) techniques on a workstation (cardIQ Xpress software, Advantage Workstation 4.3, GE Healthcare, Milwaukee, WI, USA). Two experienced cardiovascular radiologists independently assessed the scans, and the final diagnoses were determined by consensus. Coronary artery anomalies are classified based on a system modified from the Angelini classification as follows: 1. Anomalies of origination and course, 2. Intrinsic anomalies; 3. Anomalies of termination [7,12,13].

Statistical analysis

All statistical analyses were performed using SPSS 24.0 software (IBM Corp., Armonk, NY, USA). The Kolmogorov-Smirnov test was used to test normality of data. Categorical variables were expressed as number and percentage, while numerical variables were expressed by mean $\pm$ SD, or median and interquartile range according to distribution of data. The relationships between gender and the frequency of coronary artery anomalies, as well as between coronary artery disease and the frequency of coronary artery anomalies, were evaluated using the chi-square test. An independent samples t-test was used to compare the calcium scores of individuals with and without a ramus intermedius (RIM). A p-value less than 0.05 was considered statistically significant.

Results

This study was conducted on eligible 527 patients in the period from January 2021 to December 2023. The mean age of the participants was 49.36 years with a standard

Table 1. Baseline demographics and clinical characteristics of the study population.

Characteristic, n=527	
Mean age (years)	49.36 $\pm$ 11.98
Age min-max (years)	19-85
Male, n (%)	265 (50.3%)
Female, n (%)	262 (49.7%)
Patients with calcium score scans, n (%)	293 (55.6%)
Calcium score, median (IQR)	0 (47)
Calcium score, min-max	0-393
Min: minimum, max: maximum, n: number, IQR: Interquartile range.	

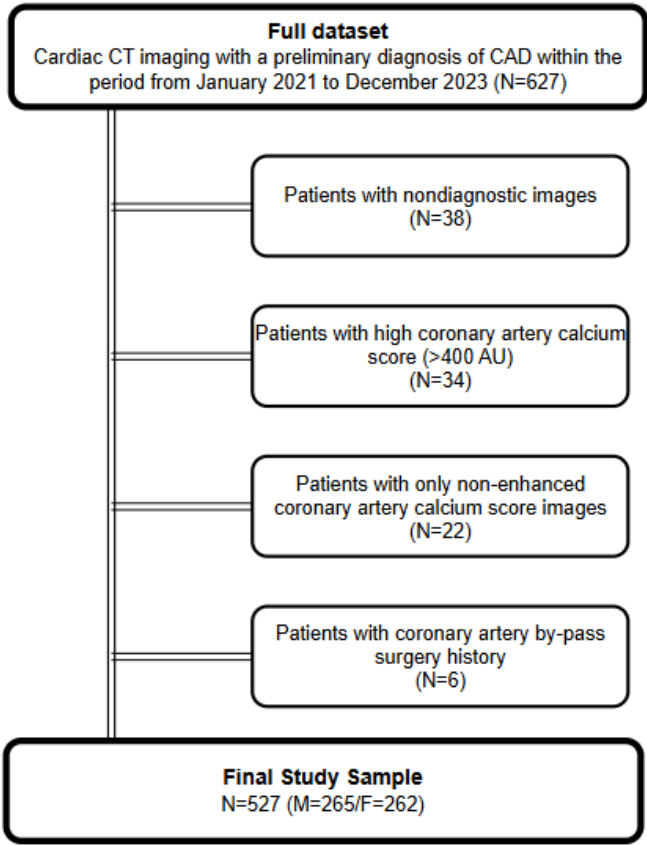


Figure 1. Flowchart of the study.

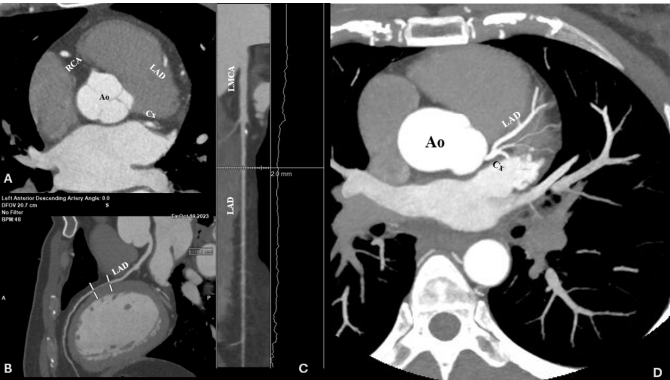


Figure 2. 49 year-old male patient with superficial myocardial bridging of left anterior descending (LAD). Axial computed tomography slice (A), and multiplanar reformat (MPR) images are presented (B, C). A 43-year-old female patient has a ramus intermedius branch (RIM) (D). Maximum intensity projection (MIP) images from the axial computed tomography scan are provided. Ao: Aorta, LMCA: Left main coronary artery, LAD: Left anterior descending artery, RCA: Right coronary artery, RIM: Ramus intermedius, LCx: Left circumflex artery.

deviation of 11.98 years, ranging from 19 to 85 years. The study population consisted of 265 males (50.3%), and 262 females (49.7%). Only 293 participants (55.6%) also had coronary artery calcium score scans, and the median calcium score value was 0 and interquartile range was 47. The baseline demographics and clinical characteristics of the study population are summarized in Table 1. The chi-square test revealed no statistically significant relationship between gender and the frequency of coronary artery anomalies ($p = 0.869$), nor between coronary artery dis-

ease and the frequency of coronary artery anomalies ($p = 0.87$). Additionally, no statistically significant difference was observed between patients with and without a ramus intermedius in terms of coronary calcium score ($p=0.472$).

Normal variants

Of the coronary artery circulation, 453 cases (85.97%) were right dominant, 71 cases (13.47%) were left dominant, and 3 cases (0.57%) were co-dominant. Myocardial bridging

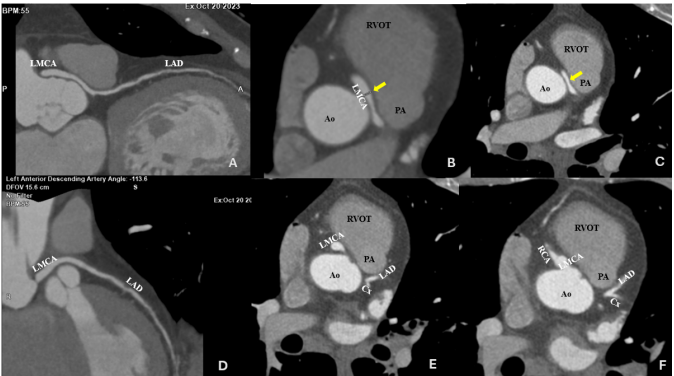


Figure 3. A 41-year-old male patient has an interarterial course of the left main coronary artery (LMCA), which originates from the right coronary sinus of Valsalva. Curved multiplanar reformat (MPR) images (A, D) and axial computed tomography images (B, C, E, and F) are presented. Ao: Aorta, LMCA: Left main coronary artery, LAD: Left anterior descending artery, Cx: Circumflex artery, RCA: Right coronary artery, PA: Pulmonary artery, RVOT: Right ventricular outflow tract.

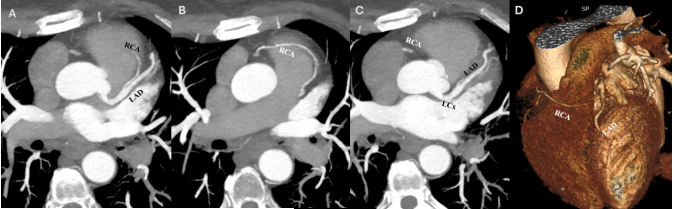


Figure 4. A 70-year-old female patient with a Lipton-Yamanaka class LIIA-V2 single coronary artery is shown in maximum intensity projection images (A, B, and C) and a three-dimensional virtual reality (3D VR) image (D). All coronary arteries arise from the left coronary sinus, while the right coronary artery exhibits a prepulmonic course. LAD: Left anterior descending artery, LCx: Left circumflex artery, RCA: Right coronary artery.

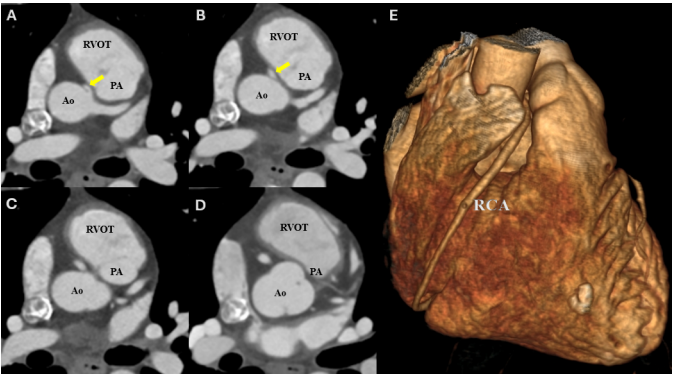


Figure 5. A 19-year-old male patient has a malignant interarterial course of the right coronary artery. Images A, B, C, and D are axial CT slices, while image E is a three-dimensional virtual reality (3D VR) image. Ao: Aorta, RCA: Right coronary artery, PA: Pulmonary artery, RVOT: Right ventricular outflow tract.

Table 2. The prevalence of coronary artery variants.

Dominancy	
Right coronary dominancy, n(%)	453 (85.97%)
Left coronary dominancy, n(%)	71 (13.47%)
Co-dominant coronary circulation, n(%)	3 (0.57%)
Myocardial bridging	
Deep myocardial bridging, n(%)	2 (0.4%)
Superficial myocardial bridging, n(%)	116 (22.01%)
Ramus intermedius artery, n(%)	49 (9.3%)
Short LMCA	11 (2.09%)
Acute take-off coronary arteries	8 (1.5%)
Separate origin of conus branch	6 (1.14 %)

LMCA: left main coronary artery, n: number.

was observed in 118 cases (22.39%), with 2 (0.4%) classified as deep (≥ 2 mm depth of overlying myocardium) and 116 (22.01%) as superficial (less than 2 mm depth of overlying myocardium) (Figure 2A,B,C). A ramus intermedius artery resulting from trifurcation of left main coronary artery (LMCA) was identified in 49 (9.3%) participants (Figure 2D). In 11 cases (2.09%), a short LMCA (less than 5 mm) was detected. Additionally, acute take-off of coronary arteries was noted in eight cases (1.5%), with two cases (0.38%) involving the right coronary artery (RCA) and six cases (1.14%) involving the LMCA. Separate origins of the conus branch were observed in six cases (1.14%), with the conus artery arising directly from the aorta in four cases (0.76%), and from the left circumflex artery (LCx) in two cases (0.38%) (See Table 2).

Coronary anomalies

Anomalies of origination and course

Ectopic coronary origin from the contralateral coronary sinus

We identified the left coronary artery originating from the right coronary sinus in one case (0.19%) (Figure 3). A circumflex artery originating from the right coronary sinus was found in two cases (0.38%). There was one case (0.19%) of a single coronary artery (Figure 4). The RCA originating from the LCx was detected in one case (0.19%). We also observed one case (0.19%) where the circumflex artery originated from the RCA. Finally, a circumflex artery originating from the diagonal branch of LAD was noted in one case (0.19%). Additionally, in one case (0.19%), an anomalous obtuse marginal branch was observed originating from the RCA, rather than its typical origin from the LCx.

Absence of the left coronary trunk

Among the 527 cases examined, four cases (0.76%) exhibited the absence of the left coronary trunk, and LAD and LCx were found to be separated from left sinus valsalva.

Anomalous course of coronary arteries

The interarterial course of the RCA, also known as a malignant course, was observed in five cases (0.94%), where

the artery had an aberrant origin from the left coronary sinus (Figure 5). In contrast, the interarterial course of the LMCA, where the LMCA originates from the right coronary sinus, was noted in two cases (0.38%).

Anomalous location of the coronary ostium

Among all participants, only one case (0.19%) exhibited high take-off of the RCA.

Intrinsic anomalies

Ectasia or aneurysm

Among all participants, only one case (0.19%) exhibited RCA ectasia.

Hypoplasia or agenesis

We observed a thin left main coronary artery (LM) in one case (0.19%), hypoplasia of the RCA was detected in 13 cases (2.46%), and hypoplasia of the circumflex artery was found in 17 cases (3.22%).

Anomalous origin of the posterior descending artery

In one case (0.19%), the posterior descending artery (PDA) was observed originating from the acute marginal branch.

Anomalies of termination

Fistula

Anomalies of coronary termination were identified in two cases (0.38%). One case (0.19%) involved a fistula between the RCA and the coronary sinus, while another case (0.19%) involved a fistula between the LAD and the pulmonary trunk.

All types of coronary artery anomalies detected in our study are summarized in Table 3.

Discussion

Coronary CT angiography allows for the non-invasive visualization of coronary vascular structures, reduces the cost and risks associated with conventional angiography, and has thus become the diagnostic test of choice for investigating coronary artery disease [14]. With the increasing use of coronary CT angiography in daily practice, the incidental detection of coronary artery anomalies and variations has become easier. While most of the cases are asymptomatic, some of the patients may experience chest pain, congestive heart failure, and in rare instances, sudden cardiac death [15].

Coronary dominance is determined by the coronary artery branch that gives rise to the PDA: if the PDA originates from the RCA, it is classified as right dominant; if it originates from the left LCx, it is classified as left dominant; and if it arises from both, it is classified as codominant [16]. The clinical importance of coronary dominance lies in its implications for the preparation of bypass surgery and the potential outcomes [17]. Approximately 70-80 % of the population exhibits right coronary dominance, 5-10 % exhibits left coronary dominance, and the remaining 10-20 % demonstrates codominance [17]. The results of

Table 3. The prevalence of coronary artery anomalies.

1. Anomalies of Origination and Course	Cases n (%)
1.1 Ectopic coronary origin from the contralateral coronary sinus	1 (0.19%)
Left coronary artery originating from the right coronary sinus	2 (0.38%)
Circumflex artery originating from the right coronary sinus	1 (0.19%)
Single Coronary artery	1 (0.19%)
Right coronary artery originating from the circumflex artery	1 (0.19%)
Circumflex artery originating from the right coronary artery	1 (0.19%)
Circumflex artery originating from diagonal branch	1 (0.19%)
Obtuse marginal branch from right coronary artery	1 (0.19%)
1.2.Absence of left coronary trunk	4 (0.76%)
1.3. Anomalous course of coronary arteries	
Interarterial course of right coronary artery	5 (0.94%)
Interarterial course of left main coronary artery	2 (0.38%)
2. Anomalous location of the coronary ostium	
High take-off of the right coronary artery	1 (0.19%)
3. Intrinsic anomalies	
3.1. Ectasia or Aneurysm	
Right coronary artery ectasia	1 (0.19%)
3.2. Hypoplasia or Agenesis	
Thin left main coronary artery	1 (0.19%)
Hypoplasia of right coronary artery	13 (2.46%)
Hypoplasia of circumflex artery	17 (3.22%)
3.3. Anomalous origin of the posterior descending artery	
Posterior descending artery from acute marginal branch	1 (0.19%)
4. Anomalies of termination	
4.1.Fistula	
Fistula between right coronary artery and coronary sinus	
Fistula between left anterior descending artery and pulmonary trunk	

n: number.

our study revealed a higher prevalence of right and left coronary dominance, while the incidence of codominance was lower than expected. The discrepancy in the dominance pattern can be attributed to the characteristics of the study cohort. Additionally, if the PDA originates from the LAD, it is referred to as “superdominant”, which is an extremely rare condition with a clinical significance, potentially leading to larger infarct, higher morbidity-mortality [18]. In our study group, there was only one case (0.19%) of superdominant LAD.

A myocardial bridge can be defined as a condition in which coronary arteries course through myocardium rather than over the epicardium [19]. The part of the coronary artery encased within the myocardium is referred to as a ‘tunneled artery,’ while the enveloping muscle is termed the ‘bridge’ [20]. Myocardial bridging is divided into two groups based on its depth: superficial and deep. If the depth is less than 2 mm, it is considered superficial, and if it is 2 mm or more, it is referred to as deep myocardial bridging [20]. There is ongoing debate regarding myocardial bridging, specifically whether it should be classified as a congenital anomaly or a normal anatomical variant

[5,14,21]. In the current study, we classified myocardial bridging as an anatomical variant because anatomical features of the coronary arteries should be considered variants rather than congenital anomalies when they occur in more than 1% of the general population [21-23]. We encountered 120 cases (22.8%) of myocardial bridging in this study, the majority of which were located superficially. Most patients with myocardial bridges are asymptomatic; however, some may experience symptoms such as angina, palpitations, myocardial ischemia, paroxysmal atrioventricular block, ventricular tachycardia, or sudden cardiac death [24]. On some occasions, the LMCA trifurcates and gives rise to the LAD, LCx, and ramus intermedius branch. There is controversial evidence regarding the increased risk of atherosclerosis or potential improvement in survival [25]. In our study, 9.3% of participants had a ramus intermedius branch, and their calcium scores were relatively higher compared to those without, though the difference was not statistically significant. Given the observed nonsignificant difference in means, further research is necessary to elucidate this trend towards higher values in patients with ramus intermedius branch.

Normal length of the LMCA varies from 2 to 40 mm according to an autopsy study [26,27]. A length of the LMCA less than 5 mm is considered a short LMCA, which is a normal variant [28]. In our study there are 11 cases (2.09%) with short LMCA, slightly less than previously reported [29,30]. There is a debate regarding the association between a short left main coronary artery (LMCA) and an increased risk of atherosclerosis, with studies reporting conflicting results [31,32]. Acute take-off of a coronary artery is another variant, which can be described as a sharp angle (less than 45 degree) between coronary artery and the aorta. This may lead to difficulty during coronary interventions [33].

There is no single standard approach regarding coronary artery anomalies classification, however most of the authors support anatomy based perspective to classify coronary artery anomalies [9,34–38]. The reported prevalence of coronary artery anomalies shows great variability [16]. In the current study, we used a classification system adapted from Angelini classification [12,13]. The most common anomalies were intrinsic anomalies (6.07 %) especially hypoplasia or agenesis followed by anomalies of origin and course (3.6 %). There is a need for a more consolidated approach to better describe and appreciate the clinical impact of coronary artery anomalies.

Limitations

The main limitations of this study are the relatively small number of the study group, absence of coronary catheter angiography images, and referral bias.

Conclusion

In conclusion, coronary artery anomalies and variations encompass a wide spectrum of abnormalities in coronary circulation. Most of these do not cause any symptoms, but some can lead to serious heart problems, including sudden cardiac death. Treatment is personalized based on the patient's condition and symptoms; therefore, an accurate diagnosis is crucial and can be achieved through coronary CT angiography.

Conflicts of interest

The authors declare that they have no conflict of interest. All authors have actively participated in this manuscript and approved the final article.

IRB statement

This study was performed in accordance with the Declaration of Helsinki and the institutional committee has approved the prospective design of the study.

Ethical approval

This study has been approved by the Clinical Research Ethics Committee of Bolu Abant İzzet Baysal University Decision No: 2023/332, dated November 7, 2023.

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Evaluation of malignancies and F18-FDG PET/CT imaging of patients living with HIV/AIDS in a university hospital

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Abstract

Aim: Human immunodeficiency virus (HIV) can cause tumoral changes in immune cells and lead to the development of AIDS-defining malignancies. As malignancies in HIV-infected individuals tend to be quite aggressive with a worse prognosis, making a definitive and early decision about HIV-related malignancies is very important. Therefore, the aim of this study was to examine HIV-related malignancies in HIV-positive patients and evaluate F18-FDG PET/CT results.

Materials and Methods: This single-centre, retrospective study was conducted on adult patients with HIV infection at a university hospital between January 2018 and December 2022. The demographic data, medication use, viral loads, and F18-FDG PET/CT results of the patients were accessed retrospectively from the automated hospital records system and patient files. F18-FDG PET/CT images of patients were evaluated for those with suspicion of cancer and staging of cancer.

Results: The study included 254 patients with median age (IQR) 33 (20) years, male sex: 81.9%. Malignancy was diagnosed in 3.1% (8/254) of HIV-positive patients. Non-Hodgkin lymphoma and Kaposi's sarcoma were the most common cancers. F18-FDG PET/CT was performed in 3.5% (9/254) of patients, of which 2.76% (7/254) had findings suggestive of malignancy. As a result of the biopsy, malignancy was detected in five of these patients, and HIV-related lymphadenopathy was detected in two. In two patients, there were no F18-FDG PET/CT imaging findings suggestive of malignancy.

Conclusion: F18-FDG PET/CT can be used in the evaluation of HIV-infected patients with suspected malignancy.



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Introduction

Human immunodeficiency virus (HIV) is a lentivirus that infects the CD4 cells primarily, thus greatly weakening the own defence of the body against disease [1,2]. In acquired immunodeficiency syndrome (AIDS), the number of CD4 T-lymphocytes of the host's immunity decreases. Consequently, the development of generalized lymphadenopathy (LAP) is the indicator of disease activity [3-5]. Long-term immune dysfunction malignant changes occur in immune cells, resulting in the development of AIDS-defining cancers. The term "HIV-related malignancy" is used to describe the group of malignancies (both non-AIDS-defining and AIDS-defining malignancies) that are increasing in frequency among patients with HIV infection. Aggressive B-cell lymphomas, Kaposi's sarcoma, and invasive cervical malignancy have been recognized as AIDS-defining

malignancies when they develop in HIV infected patients. In patients with HIV, all cancers are considered as non-AIDS-defining cancers except aggressive B-cell lymphoma, Kaposi's sarcoma, and invasive cervical cancer [6,7].

These type of cancers occurred in 30% or more of patients in AIDS patients before the improvement of effective anti-HIV treatment [8]. Cancer is the main cause of death in HIV infected patients in developed countries [9]. Fluorine 18-fluorodeoxyglucose (F18-FDG) PET/CT is used for diagnosis, staging, assessment of response to treatment, and restaging of tumors due to increase in glucose metabolism in most types of malignancies. F18-FDG PET/CT can detect metastases with a single whole-body imaging [10]. F18-FDG PET/CT is most often used in oncology, but glucose metabolism also increases in infectious or inflammatory conditions. Activated granulocytes, lymphocytes and macrophages also have increased SUVmax that indicates increased cell glycolysis [11].

As malignancies in individuals with HIV infection tend

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to be highly aggressive with poorer prognosis, it is crucial to make a definitive and early decision regarding HIV-associated malignancies [12,13]. Therefore, the aim of this study was to evaluate HIV-related malignancies in HIV-positive patients and examine the F18-FDG PET/CT results. To the best of our knowledge, there has been very limited research in Türkiye on the evaluation of malignancy in HIV-infected individuals.

Materials and Methods

Study population

This retrospective analysis, conducted in the Infectious Diseases Clinic of Hatay Mustafa Kemal University, was approved by the Non-Interventional Ethics Committee of Hatay Mustafa Kemal University Faculty of Medicine (date: 07.06.2023, decisions number: 15). All procedures were applied in accordance with the Declaration of Helsinki as revised in 2013.

The study included all of the patients aged >18 years who were followed up with a diagnosis of HIV in the Infectious Diseases and Clinical Microbiology Clinic of a single centre between January 2018 and December 2022. Patients were excluded from the study if they were aged <18 years, were co-infected with hepatitis B or C virus, or had a history of cancer before the diagnosis of HIV.

Variables and data collection

HIV-infected patients with suspicion or diagnosis of malignancy were scanned with the F18-FDG PET/CT automated system. Of the 254 patients followed up for HIV in this study, PET/CT was only used on nine patients with suspected LAP or staging of cancer.

For viral load determination of PLHIV, the HIV-RNA levels were studied using real-time polymerase chain reaction (Bosphore HIV-1 Quantification Kit, Anatolia genetworks, Turkey). The CD4⁺ T lymphocyte counts were examined with the flow cytometry method.

The primary outcome of the study was the frequency of cancer and the F18-FDG PET/CT findings of suspected LAP or a diagnosis of cancer in HIV-infected patients.

Statistical analysis

The descriptive statistics were presented as median, and interquartile range (IQR), frequency, and percentage. The Chi-squared test was employed to assess categorical variables. The normality of numeric data was assessed using the Shapiro-Wilk test. Whole parameters distributed non-normal. Mann-Whitney U test was employed for comparisons involving data that is not normally distributed. The statistical analyses were conducted using the SPSS (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL, USA) 21.0 software package. $p < 0.05$ was accepted as statistical significant.

Results

Basic characteristics of the sample and prevalence of malignancy

The study included 254 patients [median age (IQR) 33 (20) years, male sex: 81.9%]. Malignancy was diagnosed in 8

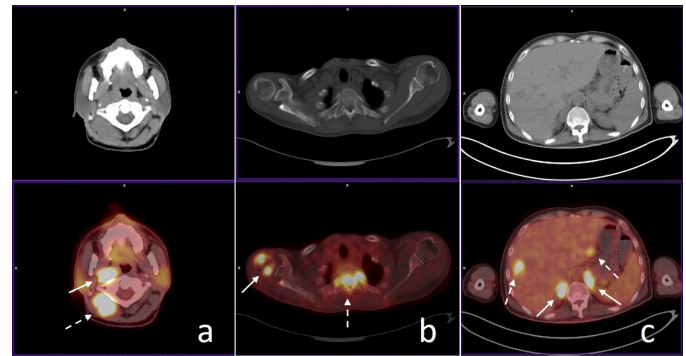


Figure 1. F18-FDG PET/CT was applied for the detection of the primary tumor after the observation of metastatic lesions in the liver and right adrenal gland on abdominal USG. PET/CT scan showing paravertebral (dashed arrow) and parapharyngeal hypermetabolic metastatic soft tissue masses (arrow) (a), right humerus (arrow) and T3 vertebrae lesions (dashed arrow) (b), liver (dashed arrow) and bilateral adrenal gland lesions (arrow) (c). The final diagnosis was Non-Hodgkin lymphoma after the biopsy of the cervical mass lesion.

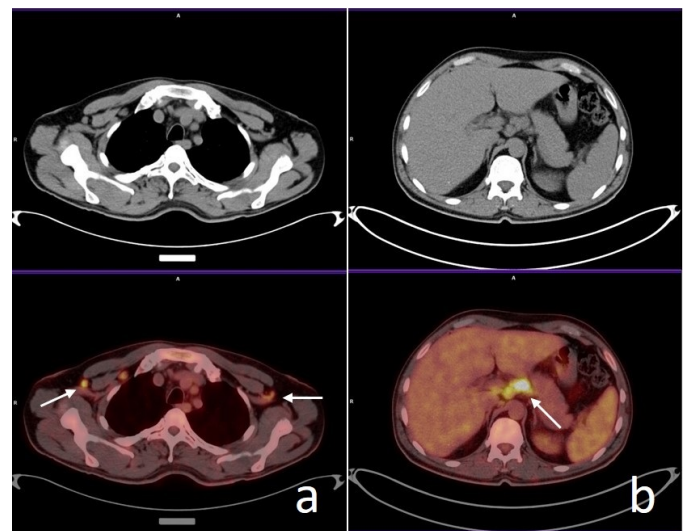


Figure 2. F18-FDG PET/CT was applied for the detection of the primary tumor after the observation of cervical and mediastinal lymphadenopathies on neck and thorax CT scans. PET/CT scans showing bilateral cervical (arrow) (a) and bilateral external iliac, obturator (dashed arrow) and inguinal lymphadenopathies (arrow) (b). The final diagnosis was Kaposi's sarcoma after the cervical lymph node biopsy.

(3.1%) HIV positive patients after biopsy and histopathological examination. Of the 254 patients followed up for HIV in this study, PET/CT was only used on 9 patients with suspected LAP or staging of cancer. The median (IQR) CD4 count of the cases at the time of initial admission was determined as 457 (415) cells/mm³. The median (IQR) HIV RNA at the time of first admission was 42,350 (132,590) copies/ml. Of the eight cases with malignancy, 6 (75%) were male, and 2 (25%) were female. Comparison of age, gender, CD4 and HIV RNA of patients with malignancy (n=8) and non-malignancy (n=246) is summarized in Table 1.

Malignancy classification

AIDS-defining malignancy was detected in four cases, and non-AIDS-defining malignancy was detected in four cases. Non-Hodgkin lymphoma was diagnosed in two patients,

Table 1. Comparison of age, gender, CD4 and HIV RNA load of patients with malignancy and non-malignancy.

	Malignancy			p
	No Median (IQR)	Yes Median (IQR)	Total Median (IQR)	
Age	32.5 (20)	48 (22.5)	33 (20)	0.076 ⁺
Male/female	201(81.7%)/45(18.3%)	7(87.5%)/1(12.5%)	208(81.9%)/46(18.1%)	0.675 [*]
CD4 count	462 (400)	200 (545)	457 (415)	0.058 ⁺
HIV RNA load	41360 (130928)	68300 (130470)	42350 (132590)	0.740 ⁺

⁺p was obtained from Mann Whitney U test, ^{*}p was obtained from Chi-Square test.

Table 2. Characteristics of patients with malignancy.

	Age	Gender	Type of malignancy	PET/CT	Time of diagnosis	Current status
1	29	M	NHL	+	Initial diagnosis	dead
2	52	F	Breast cancer	-	Follow-up	alive
3	31	M	Kaposi's sarcoma	+	Initial diagnosis	alive
4	54	M	Lung cancer	+	Follow-up	dead
5	28	M	Kaposi's sarcoma	+	Initial diagnosis	alive
6	54	F	NHL	-	Follow-up	alive
7	62	M	Colon cancer	+	Follow-up	alive
8	49	M	HL	+	Follow-up	alive

PET/CT: Positron Emission Tomography/Computed Tomography, NHL: Non-Hodgkin Lymphoma, HL: Hodgkin Lymphoma, M: Male, F: Female.

Table 3. Characteristics of patients who underwent PET/CT.

	Age	Gender	Sample Location	Pathology Result	PET/CT
1	29	M	Cervical LAP	NHL	Cervical mass, mediastinal, abdominal and pelvic LAP, liver and bone lesions (Figure 1).
2	24	M	-	-	Cervical, axillary and inguinal reactive lymph nodes.
3	28	F	-	-	Cervical, axillary and inguinal reactive lymph nodes.
4	31	M	Cervical LAP	Kaposi's sarcoma metastasis	Cervical, mediastinal, abdominal and pelvic LAP, pleural and subpleural metastatic lesions (Figure 2).
5	54	M	Lung biopsy	Lung cancer	Primary malignancy and wide spread metastases in the lung.
6	49	M	Periportal LAP	HL	LAP in the mediastinum and abdomen.
7	62	M	Colon biopsy	Colon cancer	Lymph node, liver and bone metastases.
8	57	M	Axillary LAP	HIV associated LAP	Cervical, mediastinal, axillary, abdominal, pelvic and inguinal LAP.
9	44	M	Right inguinal LAP	HIV associated LAP	Cervical, mediastinal, axillary, abdominal, pelvic and inguinal LAP (Figure 3).

LAP: Lymphadenopathy, M: Male, F: Female, PET/CT: Positron Emission Tomography/Computed Tomography, NHL: Non-Hodgkin Lymphoma, HL: Hodgkin Lymphoma.

Kaposi's sarcoma in two, and Hodgkin lymphoma, breast cancer, lung cancer and colon cancer each in one patient. The mean age of the patients with malignancy was 48 (28-62) years. The patients diagnosed with malignancy are summarized in Table 2.

PET/CT and antiretroviral therapy

PET/CT was performed in nine patients with suspicion of or a new diagnosis of malignancy (Five of eight patients with cancer). Three patients with cancer (One breast cancer, one Non-Hodgkin lymphoma and one Kaposi's sarcoma) did not undergo PET/CT. The demographic characteristics of the patients who underwent PET/CT are summarized in Table 3. Sample images of cases with PET/CT scans are shown in Figures 1-3. As the lymph

nodes were thought to be reactive on PET/CT in two of nine patients due to the absence of pathological FDG accumulation in the lymph nodes, it was decided not to perform a biopsy but to follow these patients conservatively. Malignancy did not develop in either of these two patients during the follow up. Five of the remaining seven patients who underwent biopsy were diagnosed with malignancy, and HIV-associated LAP was detected in the remaining two. The diagnoses of these five patients were Hodgkin lymphoma, Non-Hodgkin lymphoma, Kaposi's sarcoma, lung cancer and colon cancer. The SUVmax of the LAP in the cases with Hodgkin and Non-Hodgkin were 8.56 and 27.71, respectively. The SUVmax value of the LAP in the patient with Kaposi's sarcoma was 8.25. The SUVmax of the LAP in the two patients with HIV associated LAP were 6.40 and

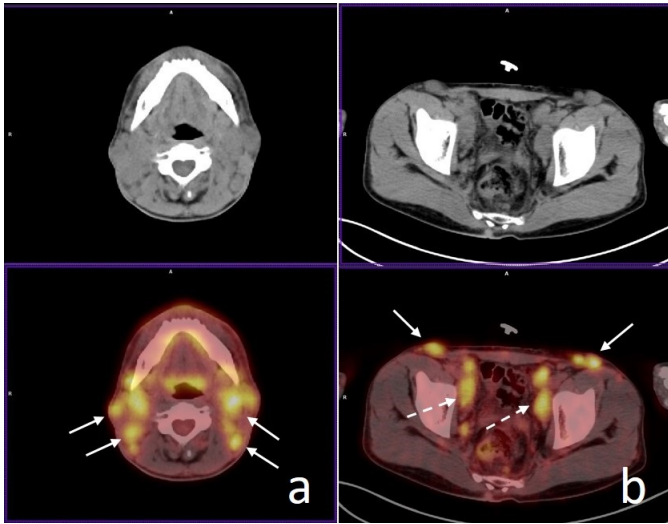


Figure 3. F18-FDG PET/CT was applied for the detection of the primary tumor after the observation of cervical and inguinal lymphadenopathies on USG. PET/CT scans showing bilateral axillary (arrow) (a) and paraaortic lymphadenopathies in the abdomen (arrow) (b). The biopsy from the inguinal lymph node was compatible with reactive lymph node.

5.05. The antiretroviral regimens (ART) used in the treatment of the cases diagnosed with malignancy were Tenofovir Alafanamide (TAF) + Emtricitabine (FTC) + Bictegravir sodium (BIC) in five patients, Tenofovir Disoproxil Fumarate (TDF) + Dolutegravir (DTG) + Emtricitabine (FTC) in two patients, Tenofovir Alafanamide (TAF) + Emtricitabine (FTC) + Elvitegravir /cobicistat (ELV/Co) in one patient.

Discussion

Although AIDS-defining malignancies have decreased with the use of antiretroviral therapies, one-third of the deaths in this population are due to malignancy [14,15]. A previous study found that the seroprevalence of malignancy in people living with HIV was 2.6% (48 patients) and 35 of these patients were diagnosed with AIDS-defining malignancy. While most AIDS-defining malignancies were diagnosed simultaneously with HIV, most non-AIDS-defining malignancies were diagnosed during patient follow-up [16]. In the current study, the seroprevalence of malignancy in people living with HIV was 3.1% (8 patients). Of these patients, four were diagnosed with AIDS-defining malignancy, three of which were diagnosed with malignancy concurrent with HIV. The high seroprevalence in this study may be due to the low number of patients.

LAP is frequent in HIV infection. Opportunistic infections, neoplasms, inflammatory conditions or reactive lymphoid hyperplasia may be the cause of LAP in HIV patients, whereas enlarged lymph nodes may be present at any stage of the disease [17,18]. Higher glucose metabolism is seen in the lymph nodes of HIV infected patients when compared with non-HIV infected individuals. High HIV RNA in the blood is associated with high viral replication in lymph nodes. LAPs have higher glucose utilization and dimension in lymphoma when compared with reactive LAP, but these conditions may overlap [19]. The etiology of LAP in HIV infected individuals should be defined. Fine needle aspiration biopsy of enlarged lymph nodes helps in

the classification of the underlying disease such as neoplastic lesions, non-neoplastic lesions and opportunistic infections. Lymphoma is mainly seen in the fourth decade of life and constitutes 9% of LAP cases in HIV infected individuals [18]. LAP is most common in the neck, followed by the axilla, reactive hyperplasia and atypical lymphocyte infiltration are the most common pathology, and tuberculosis and lymphoma are the main causes of LAP [20]. In the current study, F18-FDG PET/CT was performed on nine patients with suspicion of malignancy. The lymph nodes were thought to be reactive in F18-FDG PET/CT in two patients, a biopsy was not performed, and it was decided to follow up these patients. Five of the seven patients who underwent biopsy were diagnosed with malignancy, and HIV-associated LAP was detected in two patients.

Mhlanga et al. analyzed the F18-FDG PET/CT scans of 41 HIV-positive patients and suggested that together with the analysis of quantitative PET metabolic parameters, it is a valuable tool for the differentiation of benign adenopathy from lymphoma [19]. In another study, thirteen patients with HIV-infected Burkitt lymphoma were retrospectively examined and it was determined that 54% of the patients had F18-FDG uptake associated with HIV LAP [21]. Tatar et al. reported higher SUVmax values in lymphomas than in infectious or reactive nodes [22]. In the current study, there were no F18-FDG PET/CT imaging findings suggestive of malignancy in two patients. Considering malignancy in seven patients, biopsy was performed and malignancy was diagnosed in four of these patients and HIV-related LAP was diagnosed in two. According to these results, the high SUVmax of the lymph nodes of lymphoma makes it possible to distinguish between HIV-related infections and HIV-related generalized LAP.

The current ARTs used in the treatment of cases diagnosed with malignancy are less toxic and more reliable drugs than in the past. Somay et al., conducted a study to determine the ART-related follow-up results of patients receiving chemotherapy. During a 3-year follow-up period, the use of ART in combination with chemotherapy regimens led to better therapeutic outcomes and no mortality was observed [23]. However, one of the current study patients who received chemotherapy died during follow-up despite receiving ART. Follow-up and treatment are continuing for two of the patients in this study. As studies to date have included low numbers of patients, there is a clear need for further large-scale studies.

The mortality rate from AIDS-defining cancers decreases, but Non-Hodgkin lymphoma remains the most common cause of death due to cancer. The incidence of Non-Hodgkin Lymphoma has decreased in people with AIDS and survival has increased in the ART era, but patients still die from AIDS-related Non-Hodgkin Lymphoma [24]. In the current study, the most frequently detected HIV-related cancers were Kaposi's sarcoma and Non-Hodgkin lymphoma. Non-Hodgkin lymphoma was detected in one patient, who was newly diagnosed with HIV, but this patient died during follow-up.

Yin et al. mentioned that F18-FDG PET/CT showed involvement of multiple lymph node in -HIV-related Kaposi's sarcoma [25]. In another study by Tatar et al., Kaposi's sarcoma was characterized by multifocal widespread

lesions with different distribution, involving the nose, mouth, larynx and gastric mucosa, as well as cutaneous/subcutaneous nodules [22]. In the current study, F18-FDG PET/CT was performed to enable the detection of a primary tumor after cervical and mediastinal LAPs were seen on neck and thorax CT scans. Bilateral cervical and bilateral obturator, external iliac and inguinal LAPs were determined on PET/CT. The final diagnosis was Kaposi's sarcoma after the cervical lymph node biopsy. These studies demonstrated that F18-FDG PET/CT in HIV-positive patients was useful in identifying occult lesions in systemic Kaposi's sarcoma, which are difficult to identify with conventional imaging methods.

Since the introduction of ARTs, lung cancer has been the most common non-AIDS-defining cancer in AIDS patients who died. People infected with HIV who are also heavy smokers have an increased risk of lung cancer [26,27], and other factors such as frequent lung inflammation or infections contribute to the synergy with tobacco [28]. In the current study, one patient with lung malignancy died during follow-up.

Tatar et al. reported that the SUVmax values of HIV-related reactive LAP, HIV-related infections and HIV-related malignancy were not statistically significant with a SUVmax of 23.8 in lymphoma, 8.7 in Kaposi's sarcoma, 15.2 in tuberculosis, and 10.5 in benign LAP. However, the pattern of nodal or extranodal hypermetabolism may help in the differentiation of these conditions [22]. It has been previously reported that when the cut-off point of SUVmax of the lymph nodes was accepted as 8.0, specificity was 89.2% and sensitivity was 63.6% in the discrimination of malignant lymphoma and inflammatory LAP [29]. In the current study, the SUVmax of the LAP in two patients with HIV associated LAP were 6.40 and 5.05, whereas the SUVmax value of LAP was 8.56 in the patient with Hodgkin lymphoma and 8.25 in Kaposi's sarcoma. The SUVmax of the LAP in Non-Hodgkin lymphoma was very high with a value of 27.71. If the cut-off point of SUVmax is accepted 8.0, as in the study of Chen et al, patients can be discriminated as malignant or benign [29]. However, these results cannot be compared due to the low number of patients.

Limitations

Limitations of this study can be the retrospective design, with regional data, and a low number of cases.

Conclusion

F18-FDG PET/CT may significantly assist in the management of LAP in individuals with HIV. It has been predicted that as the survival rate in HIV-positive individuals increases, cancer cases will also increase. Non-HIV-related malignancies are becoming more common. Kaposi's sarcoma and Non-Hodgkin Lymphoma are AIDS-defined malignancies that are more common in HIV-positive patients and were also detected in this study. PET/CT imaging can demonstrate the distribution of HIV-associated LAPs and identify the area where the biopsy should be performed. The use of F18-FDG PET/CT can be considered preferable in the evaluation of HIV-related malignancies.

Ethical approval

Ethical approval was obtained for this study from the Hatay Mustafa Kemal University Non-Interventional Clinical Research Ethics Committee (date: 07.06.2023, decisions number: 15).

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Evaluation of sleep problems, quality of life and chronotype characteristics in children with primary headache

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Abstract

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Aim: Primary headaches are prevalent in children and negatively affects quality of life and sleep. This research aimed to compare the sleep, quality of life, and chronotype characteristics of children with primary headaches to healthy controls.

Materials and Methods: In this cross-sectional study, patients were selected based on a diagnosis according to the "International Headache Classification-3". The "Sleep Disorders Scale for Children (SDSC)" assessed sleep characteristics, the "Pediatric Quality of Life Inventory (PedsQL)" evaluated quality of life, and the "Childhood Chronotype Questionnaire (CCQ)" examined chronotype preferences of patients and controls. Statistical analysis considered a p-value less than 0.05 as significant.

Results: The study enrolled 50 patients with primary headaches and 50 healthy controls. Statistically significant differences were found between the groups in the physical, psychosocial, and total scores of the PedsQL, and in the sleep initiation, wakefulness transition, excessive sleepiness, and total scores of the SDSC ($p < 0.05$). However, no significant difference was found between the groups regarding chronotype preferences ($p = 0.401$). The SDSC sleep sweating score was a significant predictor of the CCQ score.

Conclusion: Our findings indicate that primary headaches in children and adolescents demonstrate an association with impaired quality of life and increased sleep problems, suggesting the need to address sleep and psychosocial issues in their evaluation. The lack of difference in chronotype preference may relate to the sample size and age range. Future studies with larger samples and specific age groups may provide more objective data.



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Introduction

Headaches, commonly observed in children, become more prevalent during adolescence. Headache is among the five most common diseases affecting individuals between the ages of 10-24 [1]. While headaches are classified into two groups, primary and secondary, there are also some particular types [2]. The diagnosis of primary headache requires the absence of an underlying pathology. In a recent meta-analysis, the combined prevalence of primary headaches in childhood was 62% [3]. The "International Classification of Headache Disorders-3rd Edition (ICHD-3)", published in 2018, is frequently used to diagnose headaches. In this classification system, headaches are divided into 14 subcategories and primary headaches are classified as migraine, tension-type headaches, trigeminal autonomic

headaches and other primary headache disorders [4]. Migraines and tension-type headaches are the most frequently encountered primary headache disorders. Among primary headaches, migraine is associated with the greatest functional impairment [5].

Sleep is a crucial necessity for children's physical and cognitive development. Sleep quality is associated with various medical and psychological disorders [6]. A reciprocal relationship exists between sleep problems and primary headaches in children, playing a crucial role in the clinical manifestations of primary headaches [7]. In a research involving 622 children with headaches, sleep problems were reported as the most common complaint, affecting 53.6% of participants [8]. Both headaches and sleep disorders are highly prevalent, suggesting that the relationship between the two disorders may arise from a common etiopathogenesis [9]. Extensive research highlights the significant impact of headaches on children's health-related quality of life. Primary headaches can impair children's overall qual-

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ity of life related to health, potentially causing a decline in their academic performance [10].

Chronotype refers to individual variations in circadian rhythm, determining preferences for morning, evening, or intermediate activity patterns. These evaluations classify individuals into three chronotypes: morningness, eveningness, and intermediate types [11]. Research indicates that chronotype influences pain sensitivity, with morning types experiencing lower pain sensitivity compared to evening types [12]. Individuals diagnosed with migraines are less likely to have a normal chronotype and tend to be more flexible regarding circadian rhythms. In the case of migraines, chronobiological mechanisms are directly tied to the underlying pathology and can influence the clinical manifestation, especially the timing of headaches. Migraine sufferers are less prone to exhibit typical chronotypes and have reduced adaptability to changes in circadian rhythm [13]. Research indicates that migraine attacks are more frequently observed in the morning among individuals with a morning chronotype, whereas those with an evening chronotype are more prone to experiencing migraine episodes during nighttime hours [14]. As a result, evaluating chronotype appears important when evaluating and managing patients with migraines.

Studies on primary headaches related to chronotype are limited in the literature, and expanding the existing data in this field could provide significant contributions to the management of headache treatment in affected individuals. This study aims to compare sleep problems, quality of life, and chronotype preferences in children with primary headaches and healthy controls, while also examining the association between chronotype scores and sleep disturbances.

Materials and Methods

This study is a single-centre, cross-sectional, prospective study involving a case group and healthy controls. The sample consists of children aged 6-18 who presented with headache complaints at the Department of Pediatric Neurology, Etlik City Hospital, between June 2023 and October 2023. Ethical approval for the study was obtained from the Etlik City Hospital Clinical Research Ethics Committee (Number: 2023-124, Date: 17.05.2023). The study was conducted under the Declaration of Helsinki and the Patient Rights Regulation. All patients and their families were provided with detailed information about the study, and written informed consent was obtained.

The inclusion criteria for the case group were as follows: a) age between 6 and 18 years, b) diagnosed with primary headache at the Pediatric Neurology Outpatient Clinics of Etlik City Hospital, c) regular neurological examination, d) written informed consent obtained from parents, and e) complete study data. Exclusion criteria for the case group were: a) presence of additional neurological disease, b) known sleep disorder diagnosis, c) being followed in psychiatry, d) having a psychiatric diagnosis, and e) use of medications that could cause headaches. The control group consisted of participants aged 6-18 without any known chronic illness, attending the paediatrics clinic of the same hospital. Inclusion criteria for the control group

were: a) being aged 6-18, b) regular neurological examination, c) providing verbal and written consent to participate in the study, and d) completing all study questionnaires. Exclusion criteria for the control group were: a) having an acute or chronic neurological disease, b) having visited child psychiatry, c) using medication in the past and/or currently, and d) experiencing any sleep problems.

During the study period, a total of 108 children aged 6-18 presented to the Pediatric Neurology Clinic at Etlik City Hospital with complaints of headache. Headache complaints were evaluated according to the ICHD-3 criteria, and 34 children were excluded from the study because they did not have primary headaches. The remaining 74 children with primary headaches were clinically evaluated by a child psychiatrist according to DSM-5 diagnostic criteria. Twenty-one children were excluded from the study due to psychiatric diagnoses (7 with major depressive disorder, 5 with anxiety disorder, 3 with enuresis nocturna, 3 with sleep disorders, 2 with obsessive-compulsive disorder, and 1 with developmental delay). Three cases were excluded for not consenting to participate, resulting in a total of 50 children included as the patient group in the study. For the healthy control group, 62 participants were reached initially; however, 7 were excluded due to previous visits to child psychiatry and the use of psychotropic medication, and 5 were excluded for not consenting to participate. Thus, a total of 50 healthy controls were enrolled in the study. The sample size was calculated post-hoc using the G*Power 3.1 program. Using a significance level of 0.05 and a moderate effect size of 0.6, the power of the study was determined to be 84% based on the total sample size of 100 participants included in our study.

Measurements

Sociodemographic data form

The form was created by the clinician to evaluate the sociodemographic characteristics of the children. It includes multiple-choice questions regarding age (in months and years), gender, educational status, parents' education levels and occupations, monthly income, place of residence, and family structure. The form is completed by the clinician.

Pediatric quality of life inventory (PedsQL)

In this study, the PedsQL was utilized to assess children's quality of life in physical, emotional, social, and school-related domains. The scale was developed by Varni and colleagues (1999) [15]. High scores reflect a better quality of life in that domain. Memik and colleagues (2007) [16] conducted the Turkish validity and reliability study of the scale. Patients aged 13 and above complete it themselves, while for younger age groups, it is completed by their parents.

The sleep disturbance scale for children (SDSC)

Bruni and colleagues (1996) designed the scale to evaluate sleep issues and specific sleep disorders in children/adolescents between the ages of 6 and 16 [17]. The Sleep Disturbance Scale for Children (SDSC) is a 26-item five-point Likert-type scale. The scale measures scores

from a minimum value of 26 up to a maximum value of 130. The cut-off score of the scale was found to be 39 points. The scale has a total of 6 subscales: disorders of initiating and maintaining sleep (DIMS), sleep-disordered breathing (SDB), disorders of arousal/nightmares (DA), sleep-wake transition disorders (SWTD), disorders of excessive somnolence (EDS), and hyperhydrosis during sleep. Bilmenoğlu and colleagues conducted the scale's Turkish validity and reliability test in 2019 [18]. The scale is completed by parents.

Childhood chronotype questionnaire (CCQ)

The questionnaire was developed by Werner et al. (2009) to determine chronotype in childhood [19]. The questionnaire consists of 27 items in total. Ten items assess morningness and eveningness characteristics in daily rhythms, and the last item gathers information about the chronotype. The validity and reliability study for the Turkish version was carried out by Dursun and his team [20]. Parents complete this questionnaire.

Statistical Analysis The data were analyzed using the SPSS.23 software program. The Kolmogorov-Smirnov test was applied first to assess whether the quantitative data followed a normal distribution. For datasets exhibiting normal distribution, the central tendency and variability were described using mean and standard deviation. Conversely, for those not exhibiting normality, median and interquartile range were utilized. Percentages were used for categorical data. We hypothesize that children with primary headaches will exhibit more pronounced sleep problems, lower quality of life, and distinct chronotype preferences compared to healthy controls. Additionally, we hypothesize that chronotype scores will be significantly correlated with the severity of sleep disturbances in children with primary headaches. To compare the patient and control groups, the Student's t-test was applied for data with a normal distribution, while the Mann-Whitney U test was employed for data without a normal distribution. The Chi-square test was applied for the analysis of categorical data; when necessary, Fisher's exact test and Yates' correction were performed. For effect size in the Chi-square test, Phi was used for 2x2 analyses and Cramer's V for others. The Pearson correlation test evaluated the relationship between total CCQ scores and PedsQL and SDSC sub-scores. Following the correlation analysis, linear regression analysis was used to determine the predictors of CCQ scores. The significance level was defined as $p < 0.05$.

Results

A total of 100 children, consisting of 50 patients and 50 healthy controls, were enrolled in the study during the research period. The mean age of the patients was 14.2 ± 2.4 years, while the mean age of the healthy controls was 12.9 ± 3.3 years ($p = 0.002$). A comparison of the two groups regarding gender revealed no statistically significant difference (Yates' correction, $X^2 = 0.686$, $p = 0.407$). Table 1 presents a summary of the sociodemographic features of both the patient and control groups.

The comparison of the PedsQL subscales and total score, as well as the SDSC subtests is shown in Table 2.

In our study, when chronotypes were evaluated according to the CCQ across the entire sample, it was found that 10 participants were of the morning type (< 23 points), 38 participants were of the intermediate type (24-32 points), and 32 participants were of the evening type (> 32 points). Table 3 displays the comparison of chronotype characteristics between the patient and control groups.

According to the data obtained through clinical interviews, when comparing the patient and control groups based on pain types, no statistically significant differences were observed between them regarding pain duration (over 5 hours or less), unilateral or bilateral location, pain severity (mild-moderate-severe), and changes with physical activity ($p > 0.05$).

When examining the correlation between CCQ total scores and PedsQL physical, psychosocial, and total scores, a mildly negative statistically significant correlation was found between the CCQ score and both the PedsQL psychosocial and total scores ($p = 0.001$ for both). Similarly, when examining the relationship between CCQ total scores and SDSC subscales, a mild to moderate negative statistically significant correlation was found between the CCQ total score and SDSC-DIMS, SDSC-DA, and SDSC-DOES ($p < 0.05$) (Table 4).

Finally, we conducted a linear regression analysis. In this analysis, our dependent variable was the Children's Chronotype Questionnaire (CCQ) score. Age, gender, SDSC subtests and total score, PedsQL physical and psychosocial subscales, and total score were taken as independent variables. The model was significant ($F(10,78) = 3.423$, $p = 0.001$). There was no autocorrelation among the data (Durbin-Watson coefficient = 1.578). According to this analysis, the SDSC-sleep sweating subtest emerged as a significant predictor of the CCQ total score. The regression results are shown in Table 5.

Discussion

Our study evaluated the comparison of patients with primary headaches and healthy controls in terms of sleep problems, quality of life, and chronotype preference. In the primary headache group, issues with sleep initiation and maintenance, arousal transitions, excessive daytime sleepiness, and physical and psychosocial quality of life were found to be greater than in healthy controls. No difference was observed in chronotype preference between the patient and control groups; however, sleep sweating was found to be a significant predictor of the chronotype total score.

In our study, the group with primary headaches was found to have more issues with sleep initiation and maintenance, arousal transitions, and excessive daytime sleepiness. Multiple studies have consistently demonstrated a strong association between primary headaches and sleep disturbances [21]. Initial studies on this relationship concentrated on the rise in parasomnias and sleep-disordered breathing in children with primary headaches. However, more recent research has demonstrated that this relationship is also linked to sleep disorders like restless legs syndrome, periodic limb movements during sleep, and narcolepsy [9]. A recent review evaluated the relationship

Table 1. Comparison of patient and healthy control groups in terms of sociodemographic characteristics.

	Patient Group (n=50)	Healthy Control Group (n=50)	Chi-square/ t-Z	P
Age (Mean±SD)	14.2±2.4	12.9±3.3	2.303	0.002
Gender (n-%)				
Female	34 (68%)	29 (58%)	0.686	0.407
Male	16 (32%)	21 (42%)		
Education level of the father (n-%)				
Primary School	7 (14%)	2 (4%)	11.267	0.010
Secondary School	5 (10%)	5 (10%)		
High school	26 (52%)	16 (16%)		
University and Beyond	12 (24%)	27 (54%)		
Education level of the mother (n-%)				
Primary School	13 (26%)	6 (12%)	8.278	0.100
Secondary School	11 (22%)	7 (14%)		
High school	14 (28%)	17 (34%)		
University and Beyond	12 (24%)	20 (40%)		
Father's Occupation (n-%)				
Civil Servant	9 (18%)	21 (42%)	9.613	0.022
Laborer	11 (22%)	3 (6%)		
Self-Employed	22 (44%)	20 (40%)		
Other	8 (16%)	6 (12%)		
Family Structure (n-%)				
Nuclear	38 (76%)	38 (76%)	1.955	0.582
Extended/Large	8 (16%)	6 (12%)		
Divorced-Single Parent	2 (4%)	5 (10%)		
Parental Loss-Single Parent	2(4%)	1(2%)		

Table 2. Comparison of sleep disturbance and quality of life scales between groups.

	Patient	Healthy Control	t-Z	P	Effect Size
PedsQL Physical (adolescent) (Mean±SD)	56.8±22.3	79.6±17.8	4.927	0.01*	1.13
PedsQL Social (adolescent) (Mean±SD)	62.4±17.5	79.9±14.8	4.692	0.01*	1.08
PedsQL Total (adolescent) (Mean±SD)	60.5±17.0	79.8±13.2	5.377	0.01*	1.21
PedsQL Physical (Child) (Mean±SD)	60.4±22.2	78.9±17.9	3.888	0.01*	0.92
PedsQL Social (Child) (Mean±SD)	65.0±17.5	80.1±14.8	3.884	0.01*	0.93
PedsQL Total (Child) (Mean±SD)	63.5±16.2	79.7±13.3	4.513	0.01*	1.09
SDSC – DIMS (Median-IQR)	13 (6)	11 (3)	3.357	0.001	0.36
SDSC – SBD (Median-IQR)	4 (2)	4 (4)	0.713	0.476	
SDSC – DA (Median-IQR)	3.5 (2)	3 (1)	1.420	0.156	
SDSC – SWTD (Median-IQR)	9.5 (5)	7 (4)	3.679	<0.001	0.37
SDSC – DOES (Median-IQR)	10 (7)	8 (6)	2.414	0.016	0.24
SDSC – Sleep hyperhydrosis (Median-IQR)	2 (2)	2 (2)	1.368	0.171	
SDSC – Total (Median-IQR)	45.5 (20.8)	39 (12.8)	3.242	0.001	0.32

PedsQL: The Pediatric Quality of Life Inventory, SDSC: The Sleep Disturbance Scale for Children , DIMS: Disorders of initiating and maintaining sleep, SBD: Sleep breathing disorders, DA: Disorders of arousal/nightmares, SWTD: Sleep wake transition disorders, DOES: Disorders of excessive somnolence, SD: Standard Deviation, IQR: Interquartile Range.

Table 3. Comparison of children with and without headache and total groups according to chronotype form.

	Patient n (%)	Healthy Control n (%)	Total n (%)	Chi Square	P
Morningness	3 (6)	7 (14)	10 (10)	1.828	0.401
Intermediate	20 (40)	18 (36)	38 (38)		
Eveningness	27 (54)	25 (50)	52 (52)		

Table 4. Correlations between chronotype total scores and the subscales of the PedsQL, SDSC.

	CCQ Total Score	PedsQL Physical	PedsQL Social	PedsQL Total
CCQ Total Score		r=-0.049 p=0.649	r=-0.342 p=0.001	r=-0.342 p=0.001
SDSC – DIMS	r=0.360 p<0.001	r=-0.109 p=0.310	r=-0.508 p<0.001	r=-0.374 p<0.001
SDSC – SBD	r=0.025 p=0.802	r=-0.159 p=0.138	r=-0.246 p=0.020	r=-0.231 p=0.029
SDSC – DA	r=0.129 p=0.201	r=-0.146 p=0.174	r=-0.318 p=0.002	r=-0.271 p=0.010
SDSC – SWTD	r=0.293 p=0.003	r=-0.256 p=0.015	r=-0.492 p<0.001	r=-0.432 p<0.001
SDSC – DOES	r=0.400 p<0.001	r=-0.409 p<0.001	r=-0.570 p<0.001	r=-0.400 p<0.001
SDSC – Sleep hyperhydrosis	r=-0.026 p=0.799	r=-0.259 p=0.014	r=-0.307 p=0.003	r=-0.315 p=0.003
SDSC – Total Score	r=-0.049 p=0.649	r=-0.332 p<0.001	r=-0.647 p<0.001	r=-0.567 p<0.001

CCQ: Childhood Chronotype Questionnaire, PedsQL: The Pediatric Quality of Life Inventory, SDSC: The Sleep Disturbance Scale for Children, DIMS: Disorders of initiating and maintaining sleep, SBD: Sleep breathing disorders, DA: Disorders of arousal/nightmares, SWTD: Sleep wake transition disorders, DOES: Disorders of excessive somnolence.

Table 5. Variables predicting CCQ score.

Variable	B	SEB	β
SDSC- Sleep hyperhydrosis	-0.880	0.439	-0.233*

$R^2 = 0.305$, $F(10,78)=3.423$, $p=0.001$ * $p<0.05$.

between the most common primary headache, migraine, and sleep symptoms. This review found that children with episodic migraines had shorter sleep durations, as well as higher rates of insomnia, sleep-related bruxism, and restless legs syndrome, according to both self-report scales and objective assessments such as polysomnography and actigraphy [22]. In a study on the relationship between daytime sleepiness and headaches, 69 patients aged 13-17 diagnosed with primary headaches were included, and the cases were assessed using the School Sleep Habits Questionnaire. Daytime sleepiness was found in 23.3% of the group with primary headaches [23]. In a study involving 21,177 adults, those with migraines had 1.42 times more significant daytime sleepiness than those without, and multivariate analyses showed that daytime sleepiness was 2.74 times greater in the headache group than those without headaches [24]. Comprehensive assessment of sleep disorders in children and adolescents with primary headaches is considered crucial for improving diagnostic accuracy and optimizing treatment outcomes [25]. The sleep problems observed in our study among those with primary headaches are consistent with the literature, emphasizing the importance of evaluating sleep issues in children and adolescents diagnosed with headaches.

Our study further revealed that children with primary headaches exhibit significant impairments in both physical and psychosocial dimensions of quality of life. A review

of 80 articles that evaluated the quality of life in primary headaches found that the physical and emotional/spiritual dimensions of quality of life were impaired [26]. In a recent study conducted in our country, 61 adolescents with primary headaches and 31 healthy controls were compared regarding quality of life. All quality of life scores were lower in the group with primary headaches [27]. In another study involving 466 primary and secondary school children diagnosed with primary headaches, it was found that students had school absenteeism related to headaches and that their families experienced work loss; approximately two-thirds of them also had difficulty coping with this pain [28]. Moreover, a retrospective study evaluating the sleep problems of children aged 7-17 with primary headaches showed that as sleep disorders increased, there was more significant functional impairment [29]. Our study's findings are consistent with the literature.

The results of our study indicated no significant discrepancy in chronotype preference between children with primary headaches and their healthy counterparts. A literature review shows that studies on chronotype and headaches are mainly focused on the migraine group. In a case-control study, researchers reported that individuals with migraines are more prone to early or late chronotypes than the control group. Moreover, individuals with migraines experienced more headache attacks in relation to their chronotype: those with early chronotypes tended to have more migraines in the morning, while those with late chronotypes experienced more attacks in the evening or at night [13]. Similarly, in a study comparing two groups with migraine and tension-type headaches, it was found that there was a significant relationship between chronotype and headache attack timing preference in participants with migraines but not in those with tension-type headaches.

The study proposed that circadian preference could serve as a modifiable factor for migraine management, highlighting the importance of chronotype assessment in tailoring treatment strategies [14].

Finally, our study found that as chronotype scores increased, sleep problems worsened, and quality of life decreased; additionally, sleep sweating could predict chronotype scores. There is evidence that the relationship between sleep disturbances and chronotype may mediate psychopathologies [10]. Individuals with an evening chronotype, in particular, are more likely to experience sleep-related issues such as hypersomnia and nightmares. Additionally, the risk of psychopathology is higher in those with an evening chronotype [30]. The predictive role of sleep sweating in chronotype scores observed in our study might be linked to the stronger association of higher chronotype scores with evening chronotypes and their increased susceptibility to parasomnias.

Our study has some limitations. First, the assessment tools used in our study are based on self or proxyreport scales. This may pose a risk of systematic bias. Second, the participants' sleep was evaluated based on information obtained from them. Third limitation is the lack of consideration for how cultural or regional differences might influence sleep patterns and chronotype characteristics. The age difference between the control and patient groups represents another limitation. Assessing participants' sleep parameters using more objective tools, such as polysomnography or actigraphy, could have minimized the risk of bias. Fourth, our study's single-center design limits the generalizability of the findings. Multi-center studies could yield more robust and externally valid results. Fifth, prospective studies involving a larger number of patients, categorizing primary headaches into tension-type and migraine, would provide much more robust data on this subject.

Conclusion

Our study found that patients followed up with a diagnosis of primary headache had sleep problems and both physical and psychosocial quality of life negatively affected. Although patients and healthy controls did not significantly differ in terms of chronotype preference, our finding that as chronotype scores increased (evening type), sleep problems increased, and quality of life was negatively impacted highlights the importance of evaluating sleep, quality of life, and chronotype in the approach to these patients. Additionally, our finding of 'sleep sweating' as a predictor of chronotype scores may provide insight into evaluating parasomnias.

Ethical approval

Ethical approval for the study was obtained from the Etlik City Hospital Clinical Research Ethics Committee (Number: 2023-124, Date: 17.05.2023).

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Evaluation of endoscopic and microscopic approaches in pediatric patients who undergo type 1 tympanoplasty

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Abstract

Aim: In the present study, we aimed to investigate endoscopic and microscopic type 1 tympanoplasty outcomes in pediatric patients.

Materials and Methods: In this retrospective analysis, we assessed a total of 134 patients. The study focused on the outcomes of 138 ears from these 134 pediatric patients who received type 1 tympanoplasty. Four patients had the procedure performed on both ears. The ages of the patients varied from 7 to 16 years. They were categorized into two groups based on the surgical technique employed. Group 1 consisted of patients who had type 1 tympanoplasty via an endoscopic method (n=58), while Group 2 included those who underwent the microscopic approach to type 1 tympanoplasty (n=80). The tympanic membrane was repaired using cartilage graft obtained from tragus cartilage in all patients. The outcomes (regaining hearing, graft success, duration of operation, etc.) of the patients in both groups were analyzed.

Results: The postoperative air-bone gap (ABG) in both groups was statistically lower than preoperative ABG. The pre-operative ABG of group 1 and group 2 were comparable with and no significant difference among the groups. Likewise, postoperative ABG of groups 1 and 2 showed no significant difference among the groups. The duration of operation of group 1 was statistically shorter than the duration of operation of group 2. The graft success rates of group 1 and group 2 were 94.55% and 94.74%, respectively; (p=0.309).

Conclusion: As in adults, type 1 tympanoplasty can be safely performed in children with an endoscopic approach. The most important advantage of this procedure is better visualization of the entire tympanic membrane in patients who have narrow external auditory canals. Although the regain rate of hearing of the groups were, endoscopic approach has shorter duration of operation and the duration of postoperative care were shorter than the microscopic approach.



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Introduction

Chronic otitis media (COM) is a common disease that can cause serious complications as a result of inadequate treatment. The preferred modality for treatment of COM is tympanoplasty surgery, which aims to eradicate ear infections, restore hearing, and repair the perforated tympanic membrane [1].

Tympanoplasty has been widely performed in pediatric patients for many years. Eradication of ear infections at an early age prevents long-term complications of COM. However, there may be some handicaps in performing this type of surgery on pediatric patients. First of all, the external auditory canal is narrow and curved, and postoperative

care is more difficult in pediatric patients [2,3]. Although the microscope is classically used in otologic surgery, the use of endoscopes has become increasingly popular due to advantages in the exposure of the operative site. Our aim was to evaluate the efficacy of the mode of access during tympanoplasty in pediatric patients.

In this study, we aimed to analyze the outcomes of endoscopic and microscopic tympanoplasty. Also, we investigated the advantages, disadvantages, and technical difficulties of endoscopic tympanoplasty in pediatric tympanoplasty.

Materials and Methods

The medical records of patients who were diagnosed with pediatric COM at our training and research hospital and underwent type 1 tympanoplasty from January 2010 to

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February 2019 were retrospectively reviewed. Ethical approval of the study was obtained from the institutional review board (decision no: 2020-08/6). The patients younger than 7 and older than 16 years of age, patients with previous ear surgery, and patients who have ossicular damage, cholesteatoma, and congenital ear malformation were excluded from evaluation in the preset study.

In total 138 ears of 134 pediatric patients were included for evaluation in our study. Four patients underwent tympanoplasty in bilateral ears. Five patients were excluded from the study due to insufficient records of the postoperative follow-up. The patients included in the study were divided into 2 groups: group 1 (n:58) consisted of patients who underwent type 1 tympanoplasty with the endoscopic approach, and group 2 (n:80) consisted of patients who underwent type 1 tympanoplasty with a microscopic approach. Postoperative outcomes were compared in both groups.

The demographic data, hearing gains, graft success, and operation duration were collected and examined for all the patients. Detailed findings of the ear, nose, and throat examination were evaluated and recorded. Tympanic membrane (TM) perforations were classified according to the size of the perforation. TM perforations affecting <25% of the surface of the TM were classified as small, perforations between 25% -50% of the TM surface was classified as moderate, perforations affecting 50% -75% of the TM membrane were classified as large, and > 75% TM perforations were classified as as near total.

The postoperative follow-up was performed on 1st, 6th, and 12th-month following the operation. The postoperative evaluation of the hearing, the physical examination findings, and the graft status were evaluated. The air-bone gaps (ABGs) in all patients were assessed before surgery and at 1, 6, and 12 months after the procedure. Hearing thresholds were recorded at frequencies of 0.5, 1.0, 2.0, and 4.0 kHz, and the average hearing levels were determined.

All patients underwent type 1 tympanoplasty under general anesthesia by an experienced otorhinolaryngologist. A microscope (Opmi Vario S88; Carl Zeiss) was used to operate the patients in Group 2, and the endaural approach was preferred. The graft obtained in the tragus cartilage of all patients was used as the graft material of the surgery. After the endaural incision, the tympanomeatal flap was elevated and accessed to the middle ear. The graft that was tailored according to the perforation size, and was placed. The cartilage graft was supported with Gelfoam above and below. Endoscopic system (Karl Storz, Tuttingen, Germany) and rigid endoscope (2.7 mm [6.0 cm]) were used in patients in group 1. The incision was performed about 6mm from the tympanic membrane lateral to the external ear canal, and the tympanomeatal flap was elevated. A cartilage graft obtained from the tragus was used for TM reconstruction, and the graft was supported by Gelfoam.

All patients received otomicroscopic and endoscopic evaluations at the 1st, 6th, and 12th months following surgery. The patients were assessed using audiometric measurements, examination of the TM, and ABGs. The postoperative audiologic results were assessed based on the audiograms obtained on the 12th month.

During the postoperative follow-ups, numeric rating scale of pain intensity (NRS-11, range 0 to10) were obtained from the patients on the postoperative 1st day.

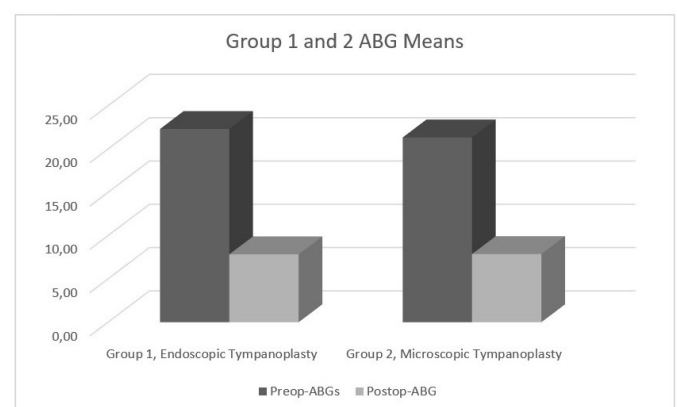
Statistical analysis

Statistical analyses were conducted utilizing the Statistical Package for Social Sciences, version 20 (SPSS v20) from IBM (USA). Continuous variables were described using the mean $\pm$ standard deviation (SD), along with the minimum and maximum values. Furthermore, 95% confidence intervals (CIs) were calculated for comparisons between groups. Categorical variables were represented as both the count of affected individuals and the corresponding percentage of the overall study population. To evaluate the normality of continuous variables, the Shapiro-Wilk test was applied. When comparing continuous variables between two groups (such as age and duration of surgery), the independent samples t-test was utilized for data that were normally distributed, while the Mann-Whitney U test was implemented for data that did not follow a normal distribution. For paired comparisons within the same groups (e.g., preoperative and postoperative air-bone gap), the paired samples t-test was used as needed. For categorical variables, such as gender distribution and condition of grafts, the Chi-square test was employed for comparison. If the expected frequencies were less than 5, Fisher's exact test was utilized for those comparisons. A p-value of less than 0.05 was determined to be statistically significant for all analyses.

Results

From total of 134 patients included in our study and 56 (41.79%) were female and 78 (58.21%) were male. The mean age in group 1 was 11.40 ± 2.34 years (range: 7–16 years), while the mean age in group 2 was 12.43 ± 2.03 years (range: 7–16 years). There was no statistically significant difference in age between the two groups ($p = 0.216$).

There were 56 patients in Group 1; 24 (42.9%) of these patients were female, and 32 (57.1%) were male. The bilateral ears of 2 male patients were operated and the data of a total of 58 ears were analyzed. In Group 2, 31 (39.74%) of



Alpha significance level was accepted as $p < 0.05$. Air-bone gap (ABG).

Figure 1. Preoperative and Postoperative ABGs following endoscopic and Microscopic Tympanoplasty.

Table 1. Distribution of General Features by Groups.

	Endoscopic Tympanoplasty (n:58) Group 1			Microscopic Tympanoplasty (n:80) Group 2			p*
	Mean±SD (CI:95%)	Min	Max	Mean±SD (CI:95%)	Min	Max	
Age (years)	11.40±2.34	7	16	12.43±2.03	7	16	0.216
Air-bone gap (dB)							
Preoperative	22.32±5.12	12	30	21.34±4.12	10	30	0.445
Postoperative	7.84±3.41	5	13	7.87±3.26	5	15	0.380
P*	0.000			0.000			
Operative duration (minute) p*	59.37±3.12	48	64	79.42±4.36	72	89	0.000**
Condition of the graft	n		%	n		%	p
Perforated	3		5.45	4		5.26	0.309
Nonperforated	55		94.55	76		94.74	

Abbreviations: n: number; SD: standard deviation; dB: decibel; CI: confidence interval; *: p-value by independent samples t-test; **: p-value by paired sample test.

Table 2. Distribution of Perforation Sizes and Locations in the study groups.

Perforation size	Locations	Endoscopic Tympanoplasty (n:58) Group 1	Microscopic Tympanoplasty (n:80) Group 2	Total
Small	Anterosuperior	3	2	5
	Anteroinferior	2	3	5
	Posterosuperior	5	7	12
	Posteroinferior	3	3	6
Moderate	Anterior	10	9	19
	Posterior	5	7	12
	Inferior	7	13	20
Large	Central	8	14	22
Near Total	Central	15	22	37

78 patients were female and 47 (60.26%) were male. The bilateral ears of 2 female patients were operated on, and the data of 80 ears were examined. found. There was no significant difference in terms of distribution of the gender among the groups (p=0.125). Demographic data and clinical findings are summarized in Table 1.

The external auditory canal’s width was sufficient for using an endoscope in all patients in group 1. In none of the patients, Conversion from the endoscopic to the microscopic access was not necessary in any of the patients. The distribution perforation size and localization of the tympanic membrane in the study are summarized in Table 2. After the operation, TM perforation was observed in 3 patients in group 1. Two patients had posterosuperior small perforation, and 1 patient had a large central perforation. In group 2, TM perforation was found in 4 patients in the operation. Anterior moderate perforation in 3 patients, posterosuperior small perforation in 1 patient. There wss no statistical difference in the frequency of pstoperarive TM perforation among the study groups (p=0.309) (Table 2).

In group 1, the preoperative and postoperative ABG were 22.32 ± 5.12 and 7.84 ± 3.41dB, respectively. In group 2, the preoperative and postoperative ABG 21.34 ± 4.12 and

7.87 ± 3.26 dB, respectively. The Preoperative ABG were significantly higher than the postoperative measurements in both groups (Table 1) (p<0.001). When the two groups were compared in terms of the preoperative and postoperative ABG, we found no statistically significant difference among the groups (respectively p=0.445, p=0.380) (Figure 1).

The mean duration of the operation in group 1 was 59.37 ± 3.12 min (range, 48-64 min), and it was 79.42 ± 4.36 min (range, 72-89 min)in group 2. The duration of operation in group 1 was statistically shorter than in the group 2 (p<0.001) (Table 1).

In tympanoplasty with a microscopic approach, external auditory canal curettage was performed to 7 patients, and anterior wall canaloplasty was performed to 5 patients to evaluate the ossicular chain.

NRS-11 scores obtained on the 1st postoperative day, were 1.02±21 in group 1 and 1.74±45 in group 2. There was a statisricaaly significant difference among the groups in terms of NRS-11 scores (p<0.001).

The postoperative follow-up period is between 19 to 27 months. The mean follow-up period in Groups 1 and 2 was 20.6 and 25.4 months, respectively.

Discussion

The main purpose of the tympanoplasty procedure is to eradicate the pathology and to restore the ventilation ability of middle ear. Tympanoplasty is a versatile procedure, with techniques adapted based on disease extent, middle ear condition, presence of cholesteatoma, and patient age. Surgical outcomes are therefore influenced by these factors, along with the chosen surgical technique, approach, and the surgeon's expertise. We included only the cases who underwent type 1 tympanoplasty to provide proper standardization of the groups. We excluded cases with cholesteatoma, ossicular chain repair, and the patients who required revisional surgery.

Various tympanoplasty methods have been described, but there is a trend towards minimally invasive approaches, especially in pediatric patients. Traditionally, tympanoplasty is performed under the microscope. Endoscopic approaches have become increasingly common recently. Although many studies suggest that the endoscopic approach is more advantageous in selected adult patients, data in the pediatric patients are not sufficient [4-6].

Despite the long-standing success of tympanoplasty performed with a microscope, challenges remain, notably in visualizing the anterior tympanic membrane and certain areas of the middle ear. For these reasons, surgeons have tried alternative methods such as tympanoplasty with an endoscopic approach. In endoscopic approach, the entire tympanic membrane can be observed [6,7]. Use of an angled endoscope is one of the additional advantages. All structures in the ear can be observed with angled endoscopes. It is a minimally invasive procedure that does not require additional procedures for exposure, thus the normal anatomy is not disturbed for exposure [8,9]. Karhuketo et al. [10] have evaluated 29 patients, and found that tympanoplasty with the endoscopic approach is advantageous because it preserves normal anatomical structures, does not require additional procedures, and the success of the operation is as high which is comparable with the microscopic approach.

One of the main advantages of the endoscopic approach is the shorter duration of operation. Since the restoration of hearing and success rates are comparable between the two methods. The postoperative pain is less intense and postoperative care are shorter in endoscopic approach, which is especially important in the pediatric patients [9,11]. Lade et al. [12] compared endoscopic and microscopic type 1 tympanoplasty in a randomized controlled study including 60 patients. Canaloplasty was performed in 5 patients who underwent microscopic tympanoplasty. In the endoscopic approach, the ossicular chain was easily examined, and no additional procedure was required. The success of the operation outcome was similar in the microscopic and endoscopic approaches, and they showed the endoscopic approach as an alternative method in their studies [12]. Similar results were obtained in our study. In our tympanoplasty with a microscopic approach, external auditory canal curettage was performed on 7 patients, and anterior wall canaloplasty was performed on 5 patients to evaluate the ossicular chain. In the endoscopic approach, extra intervention was not required.

In the study conducted by Osama et al. [13] the success

rate of tympanoplasty with the endoscopic approach was found to be 90%, and it was reported as 96% by Ayacha et al. [14]. In our study, the graft success rate in endoscopic tympanoplasty was 94.55%.

In the meta-analysis conducted by Manna et al. [15] it was stated that the results related to restoration of hearing in the endoscopic approach were not superior to microscopic, but the incidence of canaloplasty was low. Therefore, an endoscopic approach is recommended in tympanoplasty and stapes surgeries due to lower chorda tympani damage rate and pain levels.

Even if there is no difference in the success rates between the two approaches in the pediatric patient group, endoscopic approach is more superior due to lower rates of need for postoperative care. With the endoscopic approach, the need for canaloplasty has decreased, and in suitable procedures, the surgeries can be performed without elevating the external auditory canal flap [16]. Especially in pediatric patients, external auditory canal aspiration is a difficult procedure. Lower number of interventions in the external auditory canal results in faster recovery [17]. In our study, the duration of postoperative follow-up were lower in the endoscopic tympanoplasty group. Some of the patients were informed of their follow-up and did not come to the controls because they had no complaints. We observed less need for postoperative care and the need of external auditory canal aspiration in the endoscopic group.

A key challenge in pediatric otological surgery is the narrow external auditory canal. While children's canals typically achieve sufficient width for 2.7 mm endoscopes by age five [18, 19], the feasibility of endoscopic intervention through this narrow bony canal has been a subject of inquiry. Our study, focusing on type 1 tympanoplasty in children aged seven and older, demonstrated the adequacy of the endoscopic method in all cases. This aligns with findings by Ito et al. [18], who reported successful endoscopic middle ear surgery even in children with abnormally narrow canals. However, because our study focused exclusively on type 1 tympanoplasty, we cannot extrapolate these findings to more complex cases. The inherent limitations of single-handed endoscopic surgery, such as bleeding control, suction, and drilling, remain relevant considerations.

Several studies have compared endoscopic and microscopic type 1 tympanoplasty. Choi et al. [20] reported statistically lower postoperative pain in the endoscopic group on the first postoperative day, a finding corroborated by our own study. However, Kuo et al. [21, 22] found no significant difference in postoperative pain or complications. While audiologic outcomes may not differ significantly between approaches, factors influencing patient comfort, such as postoperative care and pain management, are important considerations. Furthermore, some publications suggest superior hearing outcomes with the endoscopic approach [23, 24]. A recent study by Kaur et al. [23] demonstrated higher graft acceptance rates and better hearing improvement with endoscopic transcanal tympanoplasty compared to the microscopic postauricular approach. They also suggested that platelet-rich plasma (PRP) may further enhance outcomes with both techniques. Ultimately, functional outcomes in middle ear

surgery are influenced by various factors, including disease severity and surgeon experience.

Conclusion

Postoperative surveillance is important after pediatric tympanoplasty. Endoscopic tympanoplasty offers a compelling treatment option, achieving similar hearing restoration rates to microscopic techniques but with reduced postoperative pain and care requirements.

Conflict of interest statement

None.

Ethical approval

Ethical approval was obtained from Acibadem University Clinical Research Ethics Committee for this study (decision no: 2020-08/6).

Author contributions

CH and DT contributed to the conception and design of the study, data collection, analysis, manuscript writing, and final approval. Specifically, CH was involved in the study design and gave final approval of the manuscript, while DT focused on data collection, analysis, and drafting the manuscript. All co-authors accept full responsibility for every aspect of the study and the completed manuscript.

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Borderline ovarian tumors: Importance of morphologic features, and contribution of MRI to diagnosis

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Abstract

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Aim: Differential diagnosis of borderline ovarian tumors (BOT) and malignant lesions with MRI is of great importance in terms of recognizing the chance of fertility preserving surgery. We aimed to describe and compare the MRI imaging findings and morphologic features of borderline and malignant ovarian tumors.

Materials and Methods: Patients who underwent pelvic MRI due to adnexal mass between 2019 and 2024 in the Radiology departments of two centers have been screened. Thirty-six lesions from 34 patients with BOT were identified and compared with the randomly selected 20 malignant adnexal tumors in 19 patients. Morphological features of lesions, contrast enhancement pattern, Apparent Diffusion Coefficients values, presence of ascites and peritoneal implants were evaluated for each lesion.

Results: Type 3 contrast enhancement pattern was reported in 8% of BOTs, and 26% of the malignant tumors. No significant difference was observed between BOT and malignant lesions in terms of contrast enhancement pattern ($p = 0.274$). In patients with BOTs, our rate of differentiating the ipsilateral ovary was higher than in malignant patients. Ipsilateral ovary was not discriminated in 25 (69.4%) of the BOTs, and 18 (90%) of the malignant lesions, with a borderline statistical significance ($p = 0.075$). Although the papillary lesions were commonly borderline and big-amorph lesions were commonly malignant, the difference did not reach statistical significance ($p = 0.078$).

Conclusion: Presence of solid tissue and the type of solid component are the most prominent features for the distinction of BOTs and malignant lesions. Time-intensity curves may provide additional information.



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Introduction

Borderline ovarian tumors (BOTs) are subgroup of epithelial ovarian tumors. They constitute approximately 15% of primary ovarian neoplasms [1, 2]. They have a better prognosis than malignant epithelial tumors and occur approximately 10 years earlier. They are usually seen between 40-50 years of age [3, 4]. Approximately 1/3 of the patients are under the age of 40 years [5, 6]. Since this patient group is in the reproductive period, it is important to have the chance of fertility preserving surgery.

Seventy-five percent of borderline tumors are at stage 1 at the time of diagnosis, and the 5-year survival rate is 95-97% [2]. Serous and mucinous borderline tumors constitute the majority of histologic subgroups. Less frequently seromucinous, endometrioid, clear cell, and Brenner tumors are encountered [7, 8].

Imaging modalities are of great importance in diagnosis. Ultrasound (US) is the first-line imaging modality. Computed Tomography (CT) contributes to staging and detection of regional lymph nodes and distant metastases in addition to the recognition of the lesion. Magnetic Resonance Imaging (MRI) provides more detailed information about the origin of the lesion, the presence of a solid component, and the type and signal characteristics of the solid component. The definitive diagnosis is made histopathologically but the diagnosis on the MRI report may be decisive for the selection of the appropriate treatment protocol, especially at young ages.

Differential diagnosis of BOT and malignant lesions with MRI is of great importance in terms of recognizing the chance of fertility preserving surgery, especially in young patients. In this study, we aimed to describe and compare the MRI imaging findings and morphologic features of borderline and malignant ovarian tumors.

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Materials and Methods

Population

Patients who underwent pelvic MRI due to adnexal mass between 2019 and 2024 in the Radiology departments of the Bilkent City Hospital, Ankara, and Başakşehir Çam and Sakura City Hospital, Istanbul, have been screened on the hospital databases. The images were evaluated by two radiologists with 15 and 8 years of experience in abdominal radiology.

This study was approved by the Ethics committee of the Bilkent City Hospital (12.06.2024; TABED 2-24-249). As the data were obtained from the images recorded in the hospital database informed consent was waived.

Thirty-six lesions from 34 patients with borderline tumors were identified and compared with the randomly selected control group of 20 malignant adnexal tumors in 19 patients. Size, number of septa, presence of solid component, type of solid component, contrast enhancement pattern, Apparent Diffusion Coefficients (ADC) values, presence of ascites and peritoneal implants were evaluated for each lesion. The number of the septa was categorized as less than 3, between 3-5 and more than 5 similar to previous studies. The ipsilateral ovary was assessed for differentiation from the lesion.

MRI examinations

MRI examinations were performed with 3T MRI scanners. The sequences and other acquisitions parameters used for imaging are shown in Table 1. MRI examinations that were performed without intravenous contrast material administration were excluded. Contrast enhancement was assessed visually and compared with the contrast enhancement of the outer myometrium at 30-40 s. and was evaluated as Type 1, 2 and 3.

Statistical analysis

Continuous variables were presented as median and interquartile range (IQR), and compared using Mann-Whitney-U test. Categorical variables were presented as frequency and percentages, and compared using chi-squared test or Fisher's exact test where appropriate. A p-value of <0.05 was considered statistically significant. Analyses were conducted using Stata v17 (StataCorp, TX, USA).

Results

The study group included 36 BOTs from 34 patients and 20 malignant lesions from 19 patients. There was no significant difference in age between patients with borderline lesions and malignant lesion (median [IQR] age 42 [35 – 55] vs. 46.5 [40.5 – 61.5] years; $p = 0.427$).

No significant difference was observed between BOT and malignant lesions in terms of contrast enhancement pattern ($p = 0.274$; Figure 1).

Ipsilateral ovary was not discriminated in 25 (69.4%) of the BOTs, and 18 (90%) of the malignant lesions, with a borderline statistical significance ($p = 0.075$).

Borderline tumors

The lesions frequently contained septa and solid components. Sixteen (44%) lesions had more than 5 septa, 16 (44%) lesions had less than 3 septa, and 1 (2.7%) lesion had between 3-5 septa. Solid components were present in 23 (64%) lesions.

Eleven of the lesions (30%) contained papillary projections, 2 (5.5%) contained papillary projections and branching papillae, 1 (2.7%) contained branching papillae, 2 (5.5%) contained mural nodules, and 7 (19%) contained large amorphous solid components. Of the tumors containing papillary projections, 1 was endometrioid and 1 was mucinous borderline tumor, and the remaining were serous or seromucinous tumors. The lesions containing large solid components were endometrioid and mucinous BOT.

All patients had variable amounts of pelvic fluid.

In terms of contrast enhancement pattern, 15 lesions (42%) had type 1, 6 (16%) lesions had type 2 and 3 lesions (8%) had type 3 contrast enhancement pattern. No significant difference was observed between BOT and malignant lesions in terms of contrast enhancement pattern ($p = 0.274$; Figure 1).

The ipsilateral ovary could be differentiated in 30% of the lesions.

Table 1. MR acquisition parameters.

Sequence	Plane	TR	TE	Slice thickness	FOV
T2	Coronal	5869	115	5.5	34x34
T2	Axial	3456	112	5.5	34x34
T2	Sagittal	4396	114	5.5	30x30
T1	Axial	812	10	5.5	34x34
T1+C	Coronal	691	8.9	5.5	34x34
T1+C	Sagittal	820	8.9	5.5	30x30
T1+C Lava	Axial	5.4	1.8	4	38x26.6

FOV, field of view; TE, time to echo; TR, time to repetition.

Figure 1. Contrast Enhancement Pattern of Borderline and Malignant Tumors.

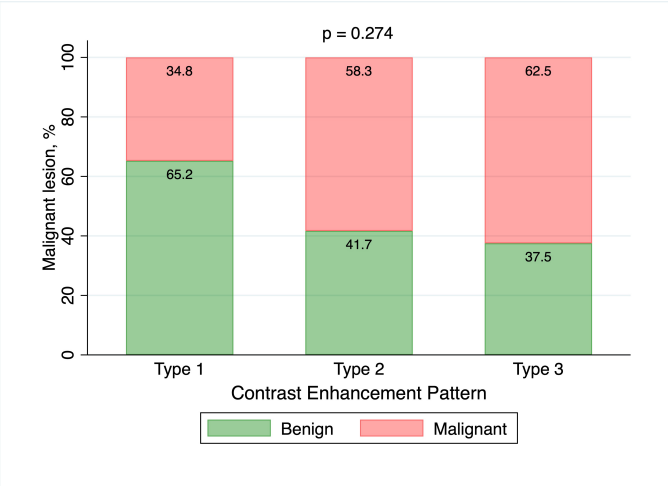




Figure 2. Coronal fat-suppressed contrast enhanced T1 image of mucinous malignant tumor with more than 5 septa.

Malignant tumors

Five of the malignant lesions had more than 5 septa (Figure 2), 3 had between 3-5 septa and 9 had less than 3 septa. Nineteen of the 20 lesions had a solid component. The only lesion without solid component was mucinous carcinoma with septa. Twelve (60%) of the lesions were large amorphous (Figure 3A, 3B, 3C), 1 had branching papilla (5%), 3 had papillary projection (15%) (Figure 4), 2 had solid mural nodules (10%) and 1 had papillary projection and branching papilla (5%).

Of the malignant lesions, 8 (40%) had type 1, 7 (35%) had type 2, and 5 (25%) had type 3 contrast enhancement patterns. Although the papillary lesions were commonly borderline, and big-amorph lesions were commonly malignant, the difference did not reach statistical significance ($p=0.078$; Figure 5).

The ipsilateral ovary could be differentiated in only one patient.

Ca-125 levels were lower in BOTs than in malignant lesions (median and IQR values 50 [16 – 106] vs. 133 [26 – 826]), but did not reach the statistical significance level ($p=0.108$). There was no significant difference between borderline and malignant tumors with regard to the O-RADS score ($p = 0.198$). Notably, 83.3% of the borderline tumors and 86.7% of the malignant tumors were either O-RADS 4 or 5.

Discussion

Ovarian epithelial tumors are categorized as benign, borderline and malignant. In the 2020 World Health Organization (WHO) classification, BOTs are defined as a sep-

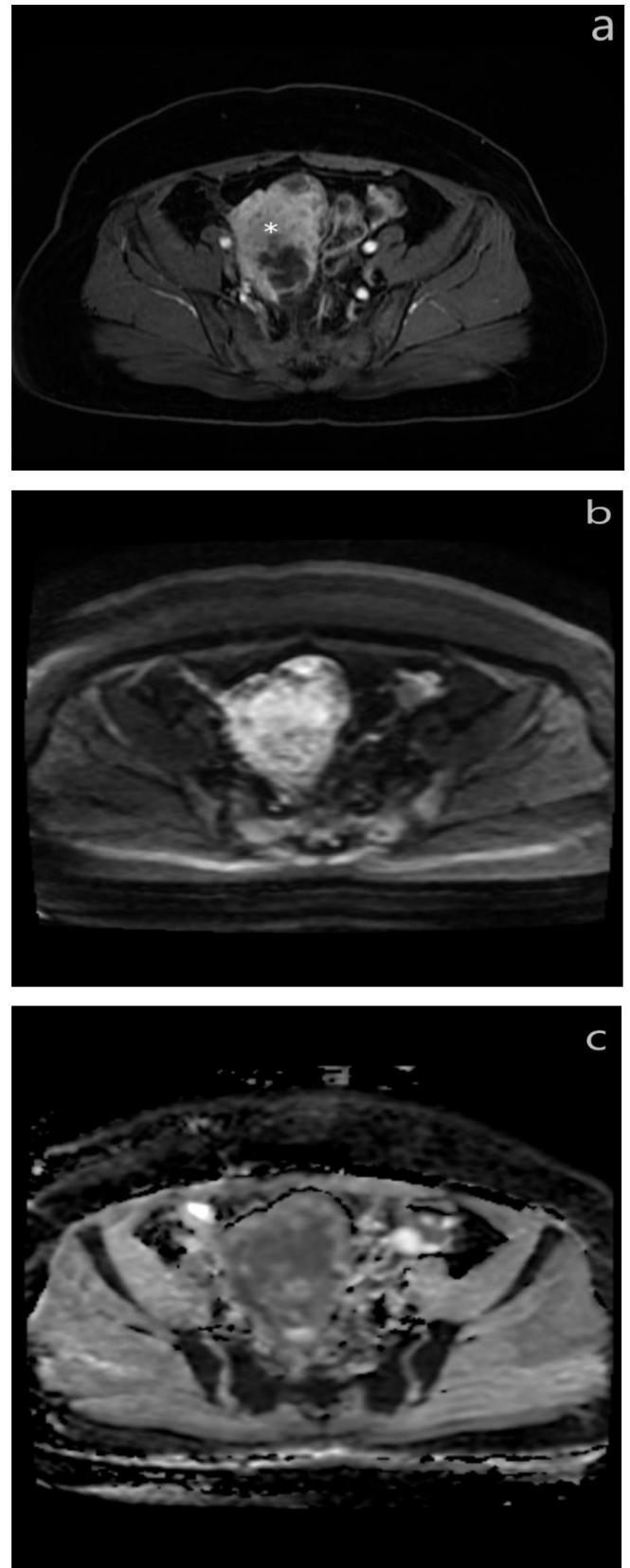


Figure 3. A: Axial fat-suppressed contrast enhanced T1 image of endometrioid malignant tumor with amorphous solid component, B: Axial diffusion weighted imaging of endometrioid malignant tumor with amorphous solid component, C: Apparent diffusion coefficient (ADC) imaging of endometrioid malignant tumor with amorphous solid component.

arate entity [9, 10, 11]. Borderline tumors constitute 15-20% of ovarian epithelial tumors [12].

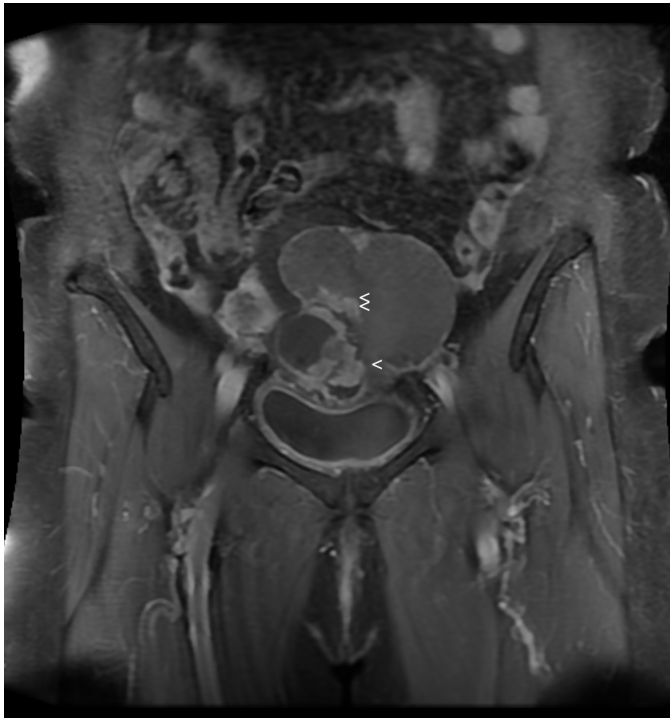


Figure 4. Coronal fat-suppressed contrast enhanced T1 image of serous malignant tumor with papillary projections (arrowheads).

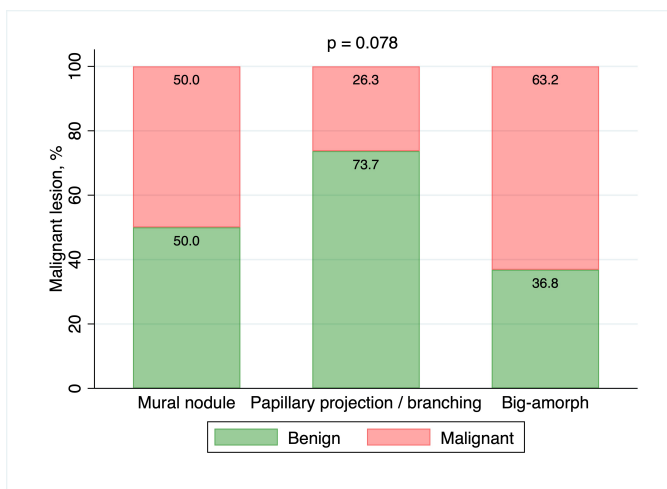


Figure 5. Type of solid components of Borderline and Malignant Tumors.

Borderline ovarian tumors may be detected incidentally or patients may present with nonspecific symptoms. Accurate recognition of these tumors is important to give the patient the chance for fertility-sparing surgery such as cystectomy or unilateral oophorectomy. MRI allows detailed evaluation of indeterminate adnexal masses due to its high soft tissue resolution and multiplanar imaging capacity. It allows differentiation of fat, hemorrhage, solid components and understanding of the fluid content of the lesion (fat, endometrioid, proteinaceous).

Borderline ovarian tumors may be purely cystic or may contain septa, mural solid nodules and large solid components. Only one BOT was purely cystic in our study. They usually have fewer septa and solid components than malignant tumors. Malignant tumors may contain a large number of contrast enhancing septa or large solid components,

their contours may be irregular and poorly circumscribed [13]. In our study, approximately 65% of BOTs had solid components whereas 89% of malignant lesions had solid components.

Papillary lesions were commonly borderline, and big-amorph lesions were commonly malignant. The borderline statistical significance might be due to low sample size.

Histological subtypes

Serous Borderline tumors are the most common type of borderline tumors. In our study, 37% of the lesions were serous BOT. Thomassin-Naggara et al. reported that papillary projections were more common in serous BOT and large solid tissue was more common in malignant tumors [14].

There are studies reporting that papillary projections and branching papillae support serous BOT [15]. Similarly, we detected papillary projections and branching papilla structures more frequently in serous and ceromusinous tumors. Of the 14 lesions with papillary projections and branching papillae, 12 were serous or ceromusinous tumors.

While it was previously considered that BOTs did not cause stromal invasion, later reports suggest that serous BOTs may cause microinvasions at a depth of less than 5 mm [16]. In our study, no peritoneal implant was seen on pelvic MRI in any of the borderline and malignant lesions. It is difficult to determine the presence of a peritoneal implant with MRI and upper abdominal slices need to be evaluated. Abdominal tomography is a more effective modality to search for implants.

It has been reported that ovarian stroma is preserved in 58% of cases in serous BOT [17]. Differentiation of the ovary on the side of the lesion may also support BOT. However, radiologic visualization of the ovary on the lesion side is also related to the size of the lesion. The relatively low rate of ipsilateral ovarian visualization in BOTs (1/3) in this study was considered to be related to lesion size. The mean size of a total of 25 lesions in which the ipsilateral ovary could not be differentiated was 136 mm.

Mucinous BOTs constitute approximately 1/3 of borderline ovarian tumors [18]. Consistent with the literature, the rate of mucinous BOTs was found to be 32% in this study. They are usually seen as multiloculated cystic masses. In mucinous borderline tumors, papillary projections are found less and irregular septations are found more [19, 20]. While hypointense microcysts on T2-weighted images and reticular contrast on MRI support mucinous BOT, solid component and mural nodules are more common in malignant tumors [21]. Nine of the 12 mucinous BOTs in our study did not have a solid component and all of them were multiseptated. In our study, 7 of the borderline BOTs were of the ceromucinous type. Only 1 lesion had a large solid component, and others contained papillary projections or branching papillae.

It has been reported in the literature that endometrioid BOTs constitute 2-3% of the borderline tumors [8, 9]. In our case, it constituted 8% of all BOTs.

Borderline Brenner tumors are also extremely rare [9]. It is difficult to differentiate malignant from borderline benign tumors according to imaging features [22]. In our series,

two patients with Brenner tumors were histopathologically benign and were excluded from the study as we did not include benign lesions.

Clear cells BOTs constitute less than 1% of BOTs [9]. There were no clear cells BOTs in our study.

MRI characteristics

Morphologic features are important in predicting histologic subtype of ovarian tumors. It is not possible to make a definitive diagnosis with MRI alone, intraoperative frozen section and histopathologic sampling are necessary, especially in making the decision for fertility-sparing surgery [23]. However, in mucinous BOTs, there is a possibility of misdiagnosis because of the large lesion size and heterogeneous structure [24, 25]. In such lesions, preoperative imaging has a high diagnostic contribution and may strengthen the histologic diagnosis.

Diffusion-weighted imaging and ADC values may give an idea about whether the lesion is benign, borderline or malignant. It has been shown that diffusion restriction is less and ADC values are higher in borderline lesions compared to malignant tumors. However, due to differences in technical parameters, it is difficult to determine a cut-off value that can be used in differential diagnosis in ADC [26, 27]. Since our study was conducted at two centers with MR images from three different MR devices, no comparison could be made in terms of ADC values.

The contrast enhancement pattern depends on the amount of fibrous or vascular component of the tumor. In dynamic contrast-enhanced MR, Type 2 time intensity curve (TIC) is more common in Borderline tumors and Type 3 TIC is more common in malignant tumors [14, 28]. In our study, Type 3 contrast enhancement pattern was reported in 8% of BOTs and 26% of the malignant tumors. Type 1 contrast enhancement in malignant lesions was higher in our study. This may be due to the fact that visual assessment was performed and dynamic contrast curves were not plotted and the total number of malignant lesions was low. Dynamic MRI examination may increase the accuracy in these lesions. In addition, if the size of the solid component is small, there is a possibility of misclassification in terms of the amount of contrast enhancement [29]. In our study, no significant difference was observed between BOT and malignant lesions in terms of contrast enhancement pattern ($p = 0.274$).

Ascites and peritoneal implants can also be seen in borderline tumors and do not confirm the diagnosis of malignancy. Especially in serous borderline tumors, ascites may be present in large amounts and is not a reliable finding in differentiation from malignant tumors [15, 30]. Peritoneal implants have been reported to be seen in 41% of malignant tumors and 10% of borderline tumors [13]. However, peritoneal implants are difficult to detect with MR. In our study, no peritoneal implant was detected in any of the patients on pelvic MRI.

While it is more difficult to see the same side ovary in malignant tumors, it is more possible to see the same side ovary in BOTs because of less stromal invasion and tissue destruction. In patients with BOTs, our rate of differentiating the ipsilateral ovary was higher than in malignant

patients. However, due to the large lesion size, ipsilateral ovary could not be differentiated in 25 /36 of the BOTs. In only one of the malignant lesions, ipsilateral ovary could be detected.

Increased tumor markers are seen in 25-60% of BOTs [31, 32] and have been reported especially in advanced tumors. In early stage tumors, the level of serum tumor markers may not be instructive. The normal level does not exclude the diagnosis of BOT [33]. There are variable values according to the stage of the disease and histologic subtype. This increases the importance of preoperative MRI examination. In the present study, while the Ca-125 levels were higher in malignant lesions compared with the BOTs, it does not reach the statistical significance level.

The low sample size is the major limitation of this study. With a conventional alpha level of 0.05, degrees of freedom of 2, and Cohen's effect size of 0.5, the power of the study is calculated to be 77%, which falls slightly below the conventional threshold of 80%. Therefore, the study's findings should be interpreted with this limitation in mind. This study provides preliminary data to inform the design of a future multicenter trial with a larger sample size, which is currently in the planning stage.

Conclusion

Since BOTs are seen at younger ages, fertility preserving surgery is important in this group. The presence of ascites and Ca-levels are not reliable in differentiating borderline from malignant. Peritoneal implants may be difficult to detect with MRI. In our study, the presence of solid tissue and the type of solid component were found to be the most reliable parameters evaluated for BOT-malignant differentiation in accordance with the literature. Drawing a TIC curve may be more reliable in the evaluation of contrast enhancement. Our study is important to emphasize what radiologists should pay attention to when evaluating lesions in MR reporting.

Ethical approval

This study was approved by the Ethics committee of the Bilkent City Hospital (12.06.2024; TABED 2-24-249).

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HOTAIR and HOXD gene expressions in patients diagnosed with leukemia

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Dear Editor,

Leukemias such as acute myeloid leukemia and chronic myeloid leukemia are hematologic cancers characterized by a various cytogenetic abnormalities resulting from disrupted myeloid differentiation and a varying course. Various researches have been carried out to examine the genetic characteristics of the disease to improve outcomes in patients diagnosed with leukemia. These studies aim to understand disease pathogenesis and identify potential therapeutic targets.

The homeodomain-containing (HD) transcription factors play a significant role in leukemogenesis in leukemia patients. HOX genes belong to this gene family and are known to be expressed in healthy hematopoietic cells, human CD34+ stem cells, and leukemic cells [1,2]. While only HOX A, B, and C from the HOX gene family are expressed in normal blood cells, all subtypes are expressed in leukemic cells. On the other hand, HOXD genes are not expressed during hematopoietic development. Expression of HOXD3 genes is observed particularly in erythroleukemic cells [3]. Additionally, HOXD13, which fuses with NUP98 in acute myeloid leukemia, was found in a very few leukemic cells [4,5]. In these cases, HOXD13 secretion is in both bone marrow cells and leukemic cells, and it is thought that the expression of HOXD13 genes may lead to clonal development in leukemic cells.

In addition, long non-coding RNAs (lncRNAs) play an important role in regulation of hematopoietic stem cells at different developmental stages. One of the most well-studied long non-coding RNAs (lncRNAs) is HOX transcript antisense RNA (HOTAIR) transcribed from the HOXC gene on 12q13.13.

The HOTAIR gene suppresses HOXD gene expression on the second chromosome [6]. HOTAIR gene expression a diagnostic and prognostic biomarker in various cancers [7]. Several studies suggest that it could be a biomarker in patients diagnosed with leukemia. In a study evaluating HOTAIR gene expression in blood specimens from individuals diagnosed with acute myeloid leukemia (AML), no statistically significant difference was found between newly diagnosed AML and healthy control groups using real-time reverse transcription-PCR (qRT-PCR) from blood specimens, indicating that HOTAIR gene expression is not a reliable biomarker in the diagnosis of AML [8]. Nonetheless, several studies have shown that HOTAIR gene expression is significantly elevated in patients with leukemia compared to the control group, and they postulated that it could be a poor prognostic marker [9]. HOTAIR and HOXD gene expression in patients diagnosed with leukemia may affect proliferation in leukemic cells. Therefore, it could be used as prognostic markers, but further studies on gene function and mechanism are needed.

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