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CONTENTS

Research Articles

Case Report

Research Article

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Expression profiles of LMAN2 in breast cancer cell lines in hypoxia and normoxia conditions

Hipoksi ve normoksi koşullarında meme kanseri hücre hatlarında LMAN2'nin ifade profilleri

Ceyda Okudu, Gökhan Ağtürk, Elif Kesim, Ayyub Ebrahimi

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Abstract

Purpose: Breast cancer is one of the most common and leading causes of death in women. The mechanism by which breast cancer develops is not fully understood. Understanding the mechanism of initiation and the genes and proteins involved in this process may help us to fight this type of cancer. The LMAN2 gene encodes VIP36, which transports properly folded proteins. The main aim of this study was to investigate the expression of the LMAN2 gene at the molecular level in breast cancer cells with different functional defects. Determining the level of LMAN2 gene expression under hypoxia, known as oxygen deprivation, has a significant impact on tumourigenesis and metastasis, and obtaining new data on the relationship between hypoxia and ER stress was identified as a secondary objective.

Material and methods: In this study, the expression level of the LMAN2 gene will be examined in breast cancer cell lines (SKBR3, MDA-MB-231, MDA-MB-468, MCF-7) and CRL4010 cell line as a control.

Results: LMAN2 gene expression level was evaluated at 48-hour periods by providing normoxic and hypoxic conditions. While the LMAN2 gene expression level in MCF-7, MDA-MB-231, and SK-BR-3 cells is significantly reduced, it was highly expressed in MDA-MB-468 in hypoxic and normoxic conditions. CHOP, HERP, and BiP gene expression levels were significantly higher in MDA-MB-468 under hypoxic conditions like LMAN2 expression.

Conclusion: LMAN2 and other ER stress response elements showed different expression profiles in SK-BR-3, MDA-MB-231, MDA-MB-468, and MCF7 cell lines under hypoxic conditions. Increased expression was found in MCF-7 and MDA-MB-468 cell lines, but decreased expression was detected in SK-BR-3 and MDA-MB-231 cell lines. The underlying reason for this difference is thought to be that the cell lines have different molecular properties, such as triple negative or HER2 (+/-) status.

Keywords: ER stress, hypoxia, LMAN2, breast cancer, apoptosis.

Okudu C, Agturk G, Kesim E, Ebrahimi A. Expression profiles of LMAN2 in breast cancer cell lines in hypoxia and normoxia conditions. Pam Med J 2025;18:474-485.

Öz

Amaç: Meme kanseri kadınlarda en sık görülen ve ölüme neden olan kanser türleri arasındadır. Meme kanserinin başlama mekanizması tam olarak açık değildir. Ortaya çıkış mekanizmasını ve bu süreçte yer alan genleri ve proteinleri anlamak, bu kanser türüyle mücadelemizde yardımcı olabileceğini düşünmekteyiz. LMAN2 geni, doğru katlanmış proteinleri taşıyan VIP36'yı kodlar. Bu çalışmanın temel amacı, çeşitli fonksiyonel bozukluklara sahip meme kanseri hücrelerinde LMAN2 geninin ekspresyonunu moleküler düzeyde incelemektir. Oksijen eksikliği koşulları olarak bilinen hipoksi altında LMAN2 gen ekspresyon düzeyinin saptanması, tümörigenez ve metastaz üzerinde önemli bir etkiye sahiptir ve hipoksi ile ER stresi arasındaki ilişki hakkında yeni veriler elde edilmesi de ikincil bir hedef olarak belirlenmiştir.

Gereç ve yöntem: Bu çalışmada, meme kanseri hücre hatlarında (SKBR3, MDA-MB-231, MDA-MB-468, MCF-7) ve kontrol olarak CRL4010 hücre hattında LMAN2 geninin ifade düzeyi incelendi.

Bulgular: LMAN2 gen ekspresyon düzeyi normoksik ve hipoksik koşullar sağlanarak 48 saatlik periyotta değerlendirilmiştir. MCF-7, MDA-MB-231 ve SK-BR-3 hücrelerinde LMAN2 gen ekspresyon düzeyi önemli ölçüde azalırken, MDA-MB-468'de hipoksik ve normoksik koşullarda yüksek düzeyde eksprese edildi. CHOP, HERP ve BiP gen ekspresyon seviyeleri, LMAN2 ekspresyonu gibi hipoksik koşullar altında MDA-MB-468'de önemli ölçüde daha yüksekti.

Sonuç: LMAN2 ve diğer ER stres yanıt elemanları hipoksik koşullar altında SK-BR-3, MDA-MB-231, MDA-MB-468 ve MCF7 hücre hatlarında farklı ekspresyon profilleri göstermiştir. MCF-7 ve MDA-MB-468 hücre

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hatlarında artmış ekspresyon bulunurken, SK-BR-3 ve MDA-MB-231 hücre hatlarında azalmış ekspresyon tespit edilmiştir. Bu farklılığın altında yatan nedenin, hücre hatlarının üçlü negatif veya HER2 (+/-) durumu gibi farklı moleküler özelliklere sahip olması olduğu düşünülmektedir.

Anahtar kelimeler: ER stres, hipoksi, LMAN2, meme kanseri, apoptoz.

Okudu C, Ağtürk G, Kesim E, Ebrahimi A. Hipoksi ve normoksi koşullarında meme kanseri hücre hatlarında LMAN2'nin ifade profilleri. Pam Tıp Derg 2025;18:474-485.

Introduction

Breast cancer (BC) has a significant socioeconomic impact due to its high incidence worldwide. Recent studies have shown that significant advancements in cancer prevention and treatment, as well as a better understanding of cancer biology, have been made thanks to the rapidly evolving technologies of today. In developed nations, cancer-related fatalities continue to be common and account for about 25% of all deaths [1]. BC is in the first place for cancer diagnoses in the United States, and despite lots of studies on this, it has the second place of mortality in this list after lung cancer [2]. BC is a heterogeneous disease with a wide range of histological and molecular characteristics caused by genetic, epigenetic, and transcriptomic changes, as well as a range of clinical manifestations, therapeutic responses, and formations. The diagnosis, prognosis, and treatment of BC are all impacted by this phenotypic variation [3]. It is not entirely clear how breast cancer begins [4]. Additionally, hormonal pathways affect breast cancer. Each evaluated molecular pathway enters an even more complex biological process with the effect of hormones. For this reason, elucidating the molecular infrastructure of breast cancer is becoming more and more important. With breast cancer cell lines with different characteristics (with different hormones and receptors active), it will be more possible to understand this complex mechanism and move on to clinical studies.

The endoplasmic reticulum (ER) is the most significant organelle in eukaryotic cells that is in charge of protein folding. Properly folded proteins in the ER are transported to other organelles or the cell membrane via cargo receptor proteins. LMAN2, also known as the VIP36 protein, is a leptin-type cargo receptor protein involved in this transport. Previous research has shown that gastric, ovarian, and prostate cancers have elevated levels of the LMAN2 gene expression [5]. Additionally, epigenetic changes in neurodegenerative diseases like multiple system atrophy cause a decrease in the control of protein folding. As a result, by facilitating the transport of glycoproteins folded in the ER, this cargo protein regulates the traffic between the ER, golgi, and cell membrane [6]. By ensuring that proteins that are misfolded and have escaped from control in the ER are returned to the ER, it also actively participates in the folding and transport of proteins in the cell [7]. The fact that the protein modifications between LMAN2 and cancer are among the types of diseases caused by the loss of control led us to examine the expression level of this gene in breast cancer. Another factor that causes ER stress is hypoxia, which is defined as a lack of oxygen in the body [8]. Due to insufficient oxygen levels, hypoxia causes protein misfolding, which then causes ER stress and activates the unfolded protein response (UPR) system [9]. ATF6, IRE1, and PERK are activated by BiP, an ER chaperone in the UPR pathway, which binds to misfolded proteins and cleaves them. The cell can enter the apoptosis pathway thanks to the activation of some nucleus-to-ER communication pathways by the released PERK, IRE1, and ATF6 [10].

The LMAN2 gene encodes VIP36, which acts as a cargo receptor protein in the endoplasmic reticulum. This protein, which transports folded proteins from the ER to other organs, is also responsible for bringing misfolded proteins back to the ER. Accumulation of misfolded proteins, which is one of the elements in cancer pathogenesis, may be caused by abnormalities in carrier proteins such as VIP36. This study aimed to reveal subcellular changes in breast cancer cells. The expression level of the LMAN2 gene, which encodes the cargo receptor protein in breast cancer cells, was investigated to reveal which changes at the ER level are associated with cancer formation. Advanced analyses were planned based on the results of this study.

It is aimed at determining the gene expression level of LMAN2 and ER stress elements in hypoxia conditions known as oxygen deficiency, which has an important effect on the formation of tumorigenesis and metastasis in breast cancer.

Materials and method

Culturing cell lines

Cancer cell lines SK-BR-3 (ATCC, HTB-30), MDA-MB-231 (ATCC, HTB-26), MDA-MB-468 (ATCC, HTB-132), and MCF7 (ATCC, HTB-22) and normal breast epithelial cell CRL-4010 (ATCC) as a control were used to determine the LMAN2 gene expression level.

The cells, which were frozen in cryotubes at -196°C in a liquid nitrogen tank, were transferred to 100 mm Petri dishes after thawing and in media containing 10% serum and 1% antibiotics; they were cultured in an incubator at 5% CO_2 and 37°C. DMEM-F12 (Gibco, 11320) medium for MDA-MB-231, MDA-MB-468, MCF-7, and CRL4010; RPMI1640 (Gibco, 11875) medium was used for SK-BR-3. Fetal bovine serum (Gibco, 26140079) was used as a serum, and penicillin/streptomycin (Gibco, 15140122) was used as an antibiotic. The medium was changed every 2-3 days until these cells were fully confluent (80-90%).

Hypoxia conditions

To determine the gene expression level of the LMAN2 in breast cancer cells under hypoxia and normoxia conditions, 21% of the oxygen level was used as normoxia, and 5% oxygen level was used as hypoxia in the incubator with 5% CO_2 and 37°C.

Determination of cell viability in hypoxia conditions

MTT [3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide] (Thermo Scientific, M6494) assay was performed to analyze the viability of cells under norm- and hypoxia conditions. After cells reached 80-90% confluency under appropriate conditions, they were counted and seeded in 1000 cells/well in 96-well cell culture plates. Then they cultured in norm- and hypoxia conditions for 24, 48, and 72 hours. At the end of the specified times, MTT solution was added to each well at 0.5 mg/mL final concentration and incubated for 3-4 hours. Then, DMSO (Sigma Aldrich, D2650) was added to the wells and incubated at room temperature for 10 minutes in a dark place to dissolve the formazan crystals formed by MTT. Each well was read with a microplate reader (Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer) at a wavelength of 570 nm, and the cytotoxicity level was determined according to the absorbance value read. The viability levels of the cells were calculated by dividing the tested wells by the control condition.

After calculation, all results were normalized with the control cell (CRL-4010 normal mammary epithelial cell) results. The time to stay in hypoxia, which has a 50% cytotoxic effect compared to the control, was accepted as cytotoxic hours.

Determination of gene expression of LMAN2 and ER stress markers in hypoxia conditions

Following the cell viability analysis, $3x10^5$ cells/well of cells were passed into a 6-well plate and incubated for 24 hours under normal conditions. Then, cells were incubated simultaneously for 48 hours under norm- and hypoxia conditions. Following incubation, cells were harvested for total RNA isolation, cDNA synthesis, and quantitative real-time polymerase chain reaction (qRT-PCR) analysis. After 48 hours of incubation, total RNAs were obtained with the total RNA isolation kit (Thermo Scientific, K0731) in line with the manufacturer's recommendations. The purity and concentration analysis of the obtained RNAs was performed using a spectrophotometer (Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer). To determine the expression level of the LMAN2 gene, cDNA synthesis using 1 µg of RNA was performed using a commercially available kit (Thermo Scientific, K1621). PCR cycling conditions for cDNA synthesis were 10 min. 25°C, 15 min. 42°C and 5 min. 85°C. The expression level of the obtained cDNAs and the genes whose primer sequences are given in Table 1 were checked. In the study, primers suitable for detecting the mRNA levels of the LMAN2 gene were determined using Primer3 and NCBI databases, and the synthesis of these primers was commercially performed by Sentromer DNA Technologies (Türkiye) as in Table 1.

Table 1. Primers and sequences used in RT-PCR

Target Gene	Primer Sequence
GAPDH	(F) 5' - CGA GAT CCC TCC AAA ATC AA - 3'
	(R) 5' - TTC ACA CCC ATG ACG AAC AT - 3'
LMAN2	(F) 5' - ACA ATG GCT CCC TGT CCT AC - 3'
	(R) 5'- CTC CCG TGA TGT CAA TGC AG - 3'
BIP	(F) 5' - GCT GAG GCT TAT TTG GGA AAG - 3'
	(R) 5' - TTA GGC CAG CAA TAG TTC CAG - 3'
CHOP	(F) 5' - AGA ACC AGG AAA CGG AAA CAG A - 3'
	(R) 5' - TCT CCT TCA TGC GCT GCT TT - 3'
HERP	(F) 5' - GGT TTA AGG CAA AGG GAA GTTC - 3'
	(R) 5' - AAA GCT GAA GCC ACC CAT AG - 3'

GAPDH: glyceraldehyde-3-phosphate dehydrogenase, LMAN2: lectin, mannose binding 2, BiP: Binding immunoglobulin protein CHOP: C/EBP homologous protein, HERP: Homocysteine-induced endoplasmic reticulum protein, F: Forward Primer Sequence R: Reverse Primer Sequence

The GAPDH gene was used as a housekeeping internal control. To evaluate the effect of hypoxia condition on ER stress, the expression levels of BiP, CHOP, and HERP genes belonging to the UPR system activated under ER stress conditions were also examined as positive controls.

RealQ Plus 2x Master Mix Green kit (Ampliqon, A323406) was prepared according to the manufacturer's recommendations and qRT-PCR was performed in 40 cycles in the thermal cycler (Bio-Rad, CFX Connect); 10 sec. 94°C, 30 sec. 55°C, 30 sec. 72°C using a temperature program. The cycle threshold values (threshold cycle/Ct) of the gene for at least two replicates of each sample examined in the study were normalized by GAPDH as the reference gene, and delta cycle threshold (ΔCt) values were calculated. ΔΔCt values were calculated by comparing them to the control group. Relative expression of each gene is calculated by using the formula $2^{-\Delta\Delta Ct}$. The represented data in the figures are the mean of technical and biological replicates ± Standard Deviation (SD).

Statistical analysis of data

All experiments were performed with at least two biological and two technical replicates. Statistical analysis of the data was done using the "SPSS 26 (IBM Inc., Chicago, IL, USA)" package program. Mann-Whitney U and Kruskal Wallis tests were used for data analysis. The statistical significance of the results obtained was evaluated over a *p*-value of 0.05, and values with a *p*-value of ≤0.05 were considered reliable.

This study has been designed as an in vitro and worked on human cell lines. This study does not require ethics committee approval.

Results

LMAN2 gene expression is differentially regulated in breast cancer cell lines

It was determined that LMAN2 gene expression decreased 0.06, 0.09, and 0.11-fold in MCF-7, MDA-MB-231, and SK-BR-3 cells, respectively, when compared to healthy breast cells. It was found that it increased 1.81 fold in MDA-MB-468 (Figure 1). According to the results obtained, LMAN2 is suppressed in MCF-7, MDA-MB-231, and SK-BR-3 breast cancer cell lines under normal conditions, while it is overexpressed in MDA-MD-468 cell lines. When the expression value of LMAN2 in different breast cancer cell lines under normoxia and hypoxia conditions was compared with the expression value in healthy breast cells, no significant result was obtained ($p>0.05$). The gene expression level for LMAN2 in different cell lines or different conditions did not show significant differences compared to the gene expression in CRL4010 under normoxia conditions.

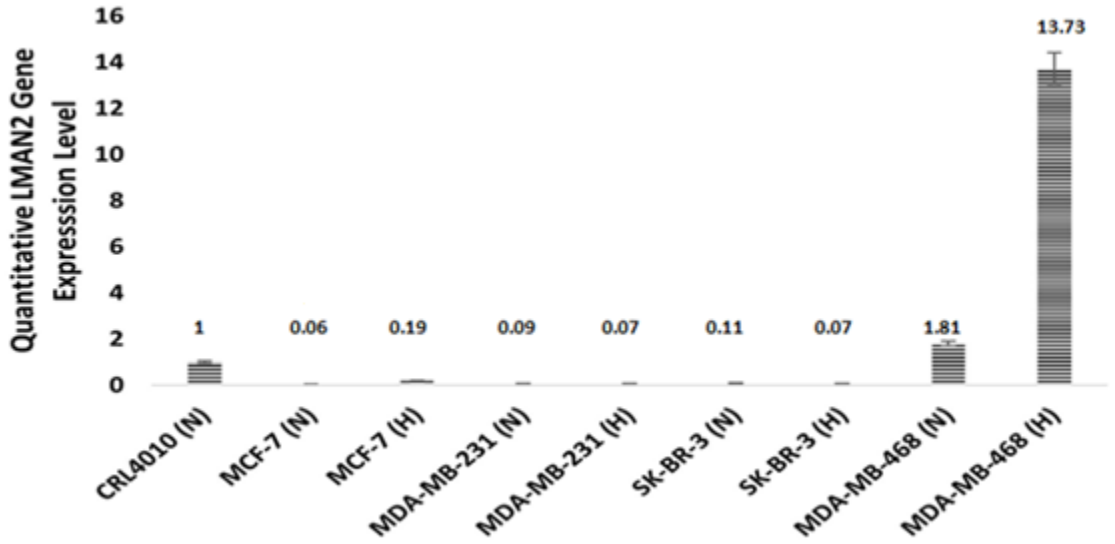

Figure 1. Regulation of LMAN2 gene expression under normoxia and hypoxia conditions

Cells were normalized with CRL4010, normal breast ephitelial cells, N: normoxia, H: hypoxia. LMAN2 gene expression in tumor and normal tissue under hypoxia and normoxia conditions was compared using the Kruskal-Wallis test. *p*=0.508, H=8.259

Hypoxia conditions affect cell viability in breast cancer cell lines

As a result of the cell viability test at the end of 24, 48, and 72 hours, breast cancer cells normalized with healthy breast epithelial cells as a negative control showed different survival patterns under norm- and hypoxia conditions (Figure 2). At the end of 24 hours, cell viability was decreased in MDA-MB-231, MDA-MB-468, and MCF-7 cells, while cell viability increased in SK-BR-3 cells under hypoxia conditions. After 48 hours, a decrease in cell viability was observed in all cancer cells, while no change was observed in the MCF-7 cell line. After 72 hours, a decrease in cell viability was detected in all breast cancer cell lines. Since the 48th-hour findings were statistically significant when the obtained data were analyzed, cells kept in hypoxic conditions for 48 hours were studied to determine gene expression levels.

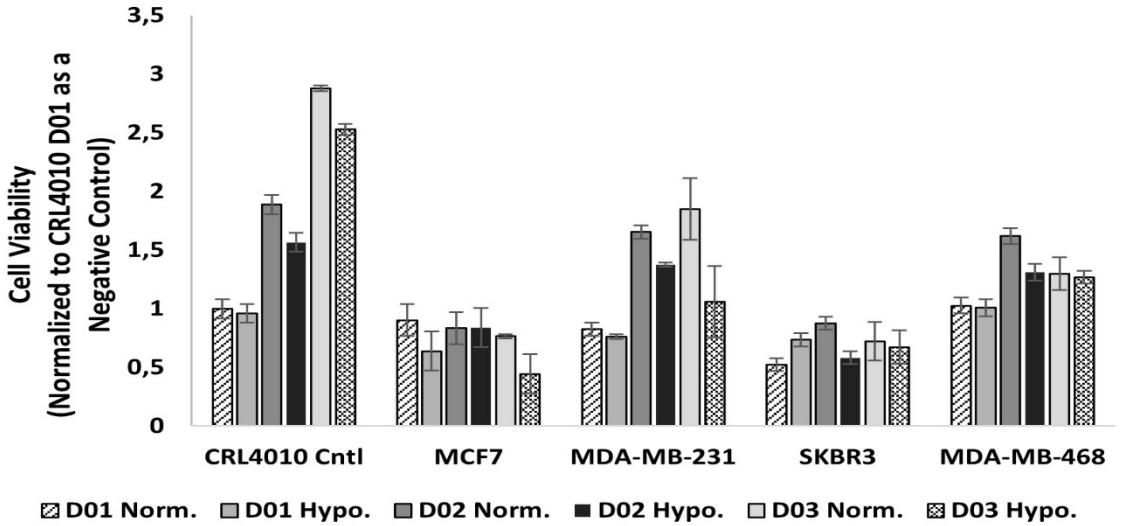

Figure 2. Comparison of cell viability under norm- and hypoxia conditions for 24, 48, and 72 hours

Cntl: control, Norm.: normoxia, Hypo.: hypoxia, D01: 24 hours, D02: 48 hours, D03: 72 hours

LMAN2 gene expression under hypoxia is differentially regulated in breast cancer cell lines

In determining the gene expression level under hypoxia conditions, the 48-hour hypoxia period was taken into account according to the cell viability analysis. It was observed that the LMAN2 gene expression level increased 1.28-fold in healthy breast epithelial cells under hypoxia conditions compared to normoxia conditions. CHOP gene expression, which is the other ER stress-regulating gene, was suppressed 0.91-fold, and on the other hand, HERP and BiP gene expression levels increased 1.41 and 1.89-fold, respectively (Figure 3). Mann-Whitney U test was performed to compare gene expression levels in hypoxia and normoxia conditions in the CRL4010 cell line. There were no statistically significant results (p=0.487).

Moreover, there were different LMAN2 gene expression levels in studied cancer cell lines. The LMAN2 expression level was increased 2.95 and 7.57-fold, respectively, in MCF-7 and MDA-MB-468 cell lines under hypoxia in comparison with control cell lines in normoxic conditions. However, it was found that this gene expression was downregulated in MDA-MB-231 and SK-BR-3 cell lines 0.75 and 0.65-fold, respectively (Figure 4). When these data were compared statistically with the Mann-Whitney U test, the increase in the LMAN2 gene only in the MCF-7 cell line among the 4 cell lines was found to be statistically significant (p=0.037).

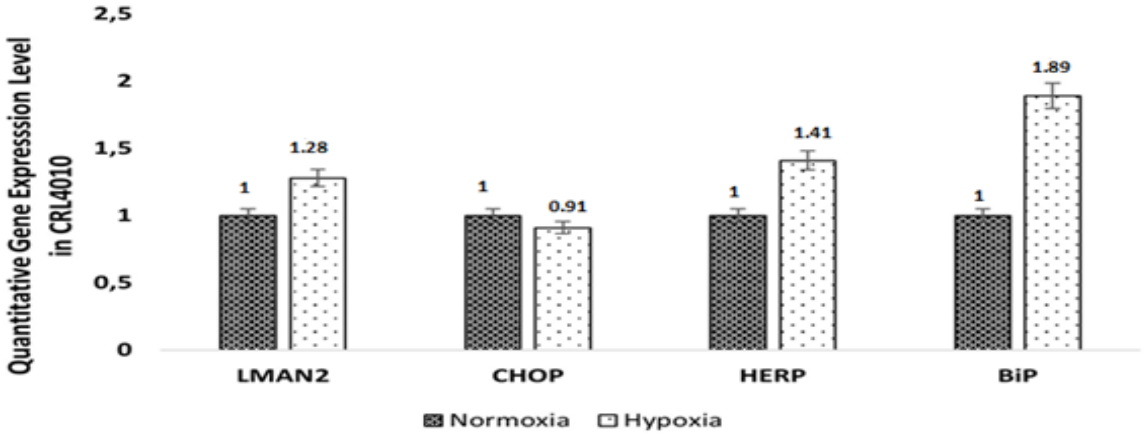

Figure 3. Gene expression levels of genes regulating LMAN2 and ER stress in healthy mammary epithelial cells under 48 hours hypoxia conditions

LMAN2: lectin, mannose binding 2, BiP: Binding immunoglobulin protein, CHOP: C/EBP homologous protein, HERP: Homocysteine-induced endoplasmic reticulum protein. Mann-Whitney U test was performed to compare gene expression levels in hypoxia and normoxia conditions in the CRL4010 cell line. p=0.487, U=3.000

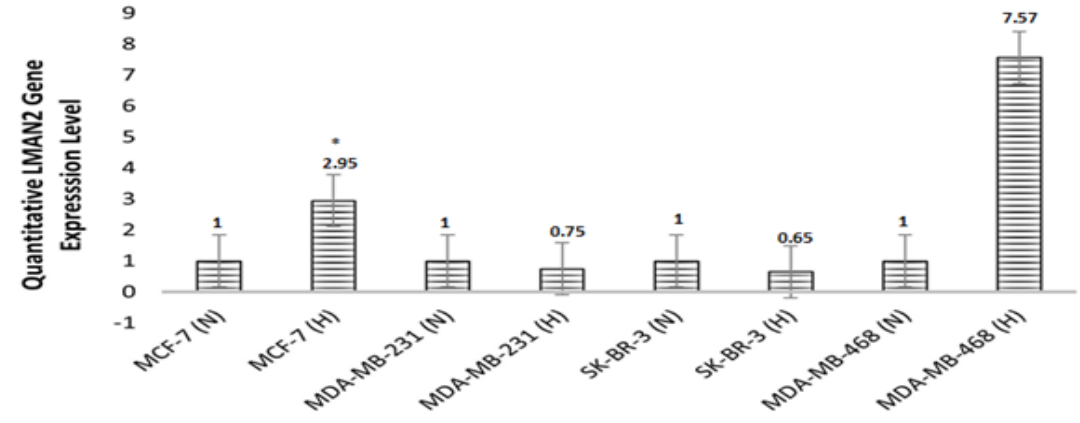

Figure 4. The gene expression level of LMAN2 gene expression at 48 hours hypoxia condition

Cancer cells normalized with normoxia levels. Comparison according to cell line in hypoxia and normoxia conditions was made using the Mann-Whitney U test. N: normoxia, H: hypoxia, *: statistically significant p=0.037 U=0.000

The fact that hypoxia is a factor triggering ER stress and the increased LMAN2 expression in healthy cells suggests that the LMAN2 gene is involved in the ER stress response. In addition, the difference in gene expression of the LMAN2 gene in cancer cells under hypoxia is thought to be due to the difference in the histopathological characteristics of these cell lines. When the CHOP gene expression level, which is one of the ER stress response elements, was normalized with CRL-4010 under hypoxia conditions, it was observed that it increased 9.45-fold in MDA-MB-468 cells and 1.18-fold in MDA-MB-231. On the other hand, it was determined that it decreased 0.12 and 0.10-fold in MCF-7 and SK-BR-3 cells, respectively (Figure 5). When the expression value of CHOP in different breast cancer cell lines under normoxia and hypoxia conditions was compared with the expression value in healthy breast cells, no significant result was obtained (p>0.05).

When CHOP gene expression was normalized with the expression level of each cancer cell line under normoxia conditions, it was suppressed 0.94, 0.59, 0.38-fold in MCF-7, MDA-MB-231, and SK-BR-3 cells, respectively. On the contrary, it was observed that it increased 3.36-fold in the MDA-MB-468 cell lines (Figure 6). This change in CHOP gene expression in different breast cancer cell lines was not found to be statistically significant (p>0.05).

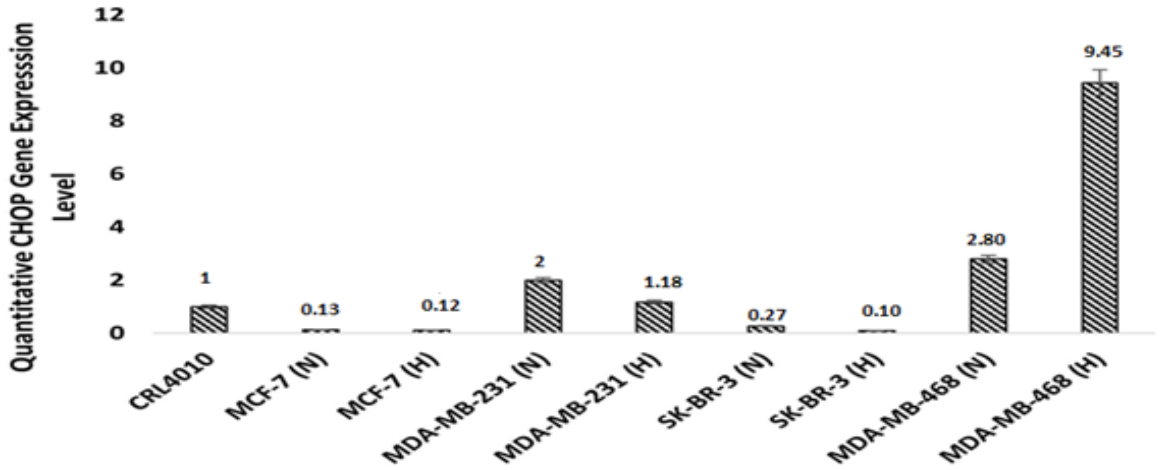

Figure 5. Regulation of CHOP gene expression under normoxia and hypoxia conditions

Cells were normalized with CRL4010, normal breast ephitelial cells, N: normoxia, H: hypoxia
CHOP gene expression in tumor and normal tissue under hypoxia and normoxia conditions was compared using the Kruskal-Wallis test. p=0.738, H=6.024

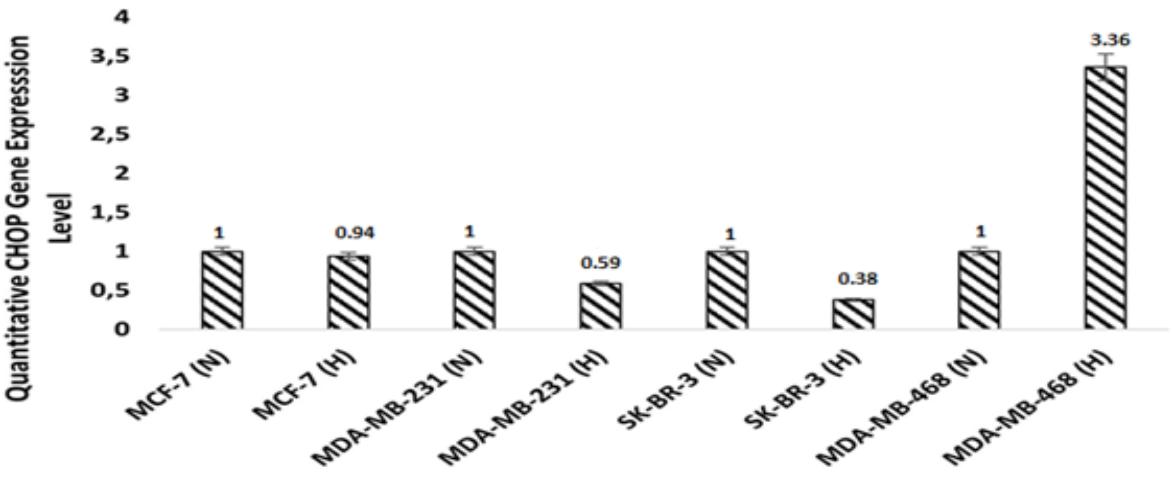

Figure 6. The gene expression level of CHOP gene expression at 48 hours hypoxia condition

Cancer cells normalized with normoxia levels, Comparison according to cell line in hypoxia and normoxia conditions was made using the Mann-Whitney U test. N: normoxia, H: hypoxia, p=0.487, U=3.000

It was also observed that the BiP gene expression level, which is one of the other ER stress response elements, increased 19.84 times in MDA-MB-468 cells and 3.41 times in MDA-MB-231 when normalized with CRL4010 under hypoxia conditions. It was determined that it decreased 0.35 and 0.93 in MCF-7 and SK-BR-3 cells, respectively (Figure 7). When the expression value of BiP in different breast cancer cell lines under normoxia and hypoxia conditions was compared with the expression value in healthy breast cells, no significant result was obtained (p>0.05). When BiP gene expression was normalized with the expression level of each cancer cell under normoxic conditions, it increased 2.04, 17.51, and 1.34-fold in MCF-7, MDA-MB-468, and SK-BR-3 cells, respectively, and in the other hand, it was found to be suppressed 0.35-fold in MDA-MB-231 cell lines (Figure 8). The increase in BiP gene only in the SK-BR-3 cell line was found to be statistically significant with the Mann-Whitney U test (p=0.037).

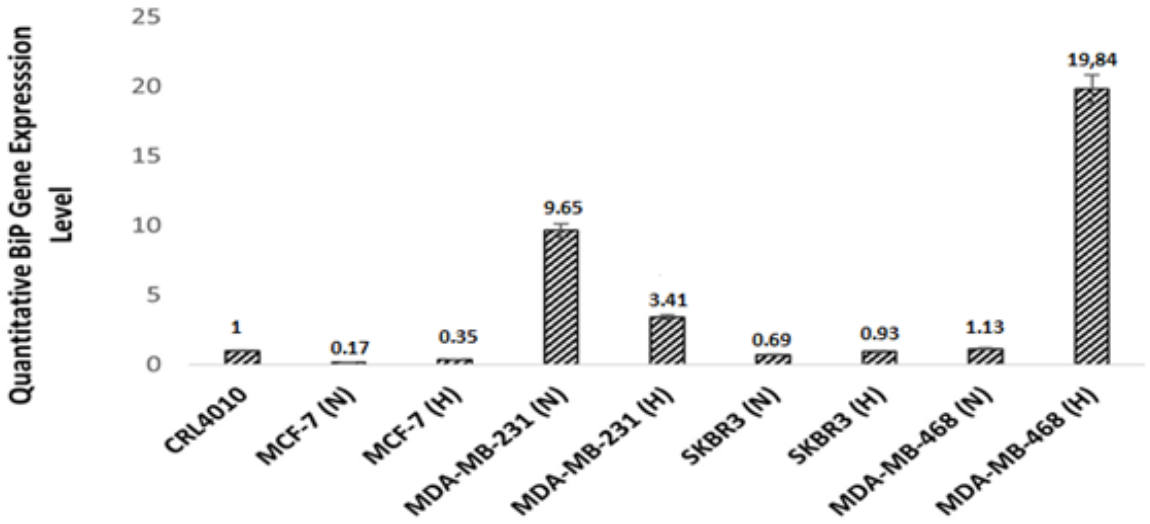

Figure 7. Regulation of BiP gene expression under normoxia and hypoxia conditions

Cells were normalized with CRL4010, normal breast ephitelial cells, N: normoxia, H: hypoxia
BiP gene expression in tumor and normal tissue under hypoxia and normoxia conditions was compared using the Kruskal-Wallis test. p=0.234, H=11.643

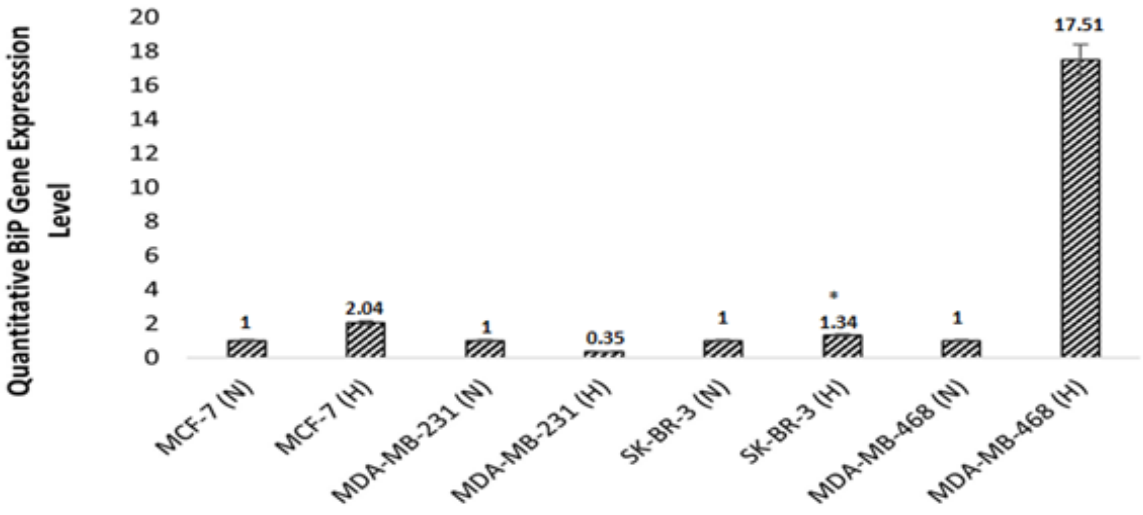

Figure 8. The gene expression level of BiP gene expression at 48 h hypoxia condition

Cancer cells normalized with normoxia levels, Comparison according to cell line in hypoxia and normoxia conditions was made using the Mann-Whitney U test. N: normoxia, H: hypoxia, p=0.487, U=3.000 for MCF-7, MDA-MB-231 and MDA-MB-468; p=0.037, U=0.000 for SK-BR-3

When HERP gene expression level, another ER stress response element, was normalized with CRL-4010 under hypoxia conditions, it was observed that it increased 13.45 times in MDA-MB-468 cells and 1.41 times in MDA-MB-231. It was determined that it decrease 0.24 and 0.23-fold in MCF-7 and SK-BR-3 cells, respectively (Figure 9). The gene expression level for HERP in different cell lines or different conditions did not show significant differences compared to the gene expression in CRL4010 under normoxia conditions (p>0.05).

On the other hand, when HERP gene expression was normalized with the expression level of each cancer cell under normoxic conditions, it was increased by 3.46 and 1.04 times in MDA-MB-468 and MDA-MB-231 cells, respectively, moreover, in MCF-7 and SK-BR-3 cells, it was found to be suppressed 0.97 and 0.77, respectively (Figure 10). The decrease in HERP gene only in the SK-BR-3 cell line was found to be statistically significant with the Mann-Whitney U test (p=0.037).

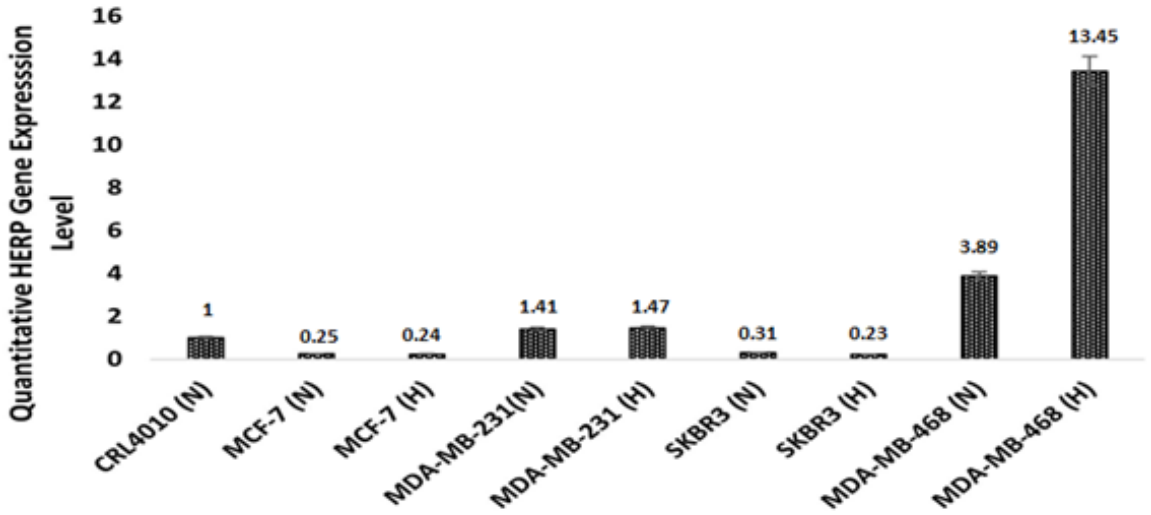

Figure 9. Regulation of HERP gene expression in normoxia and hypoxia conditions

Cells were normalized with CRL4010, normal breast ephitelial cells, N: normoxia, H: hypoxia
HERP gene expression in tumor and normal tissue under hypoxia and normoxia conditions was compared using the Kruskal-Wallis test. p=0.847, H=4.853

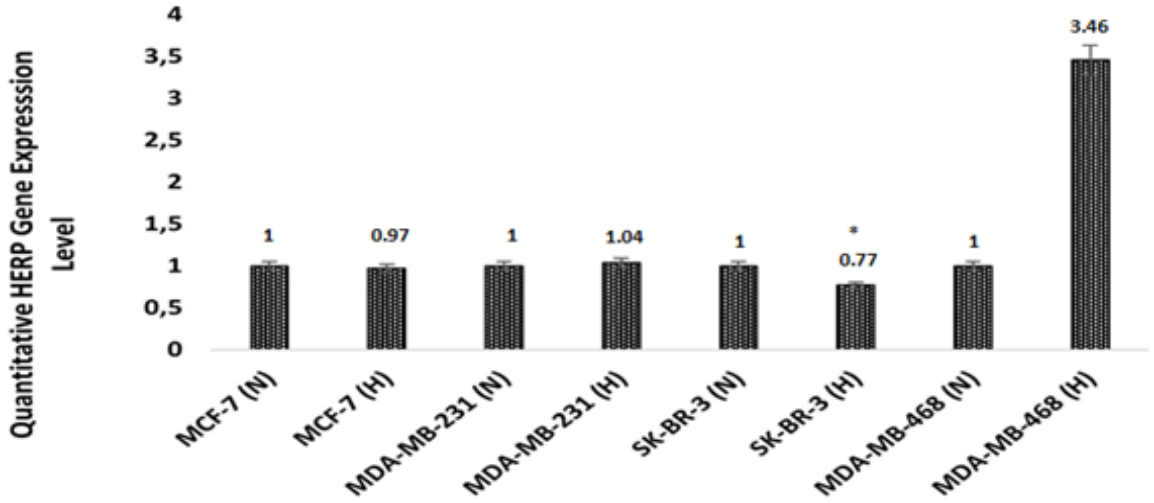

Figure 10. The gene expression level of HERP gene expression at 48 h hypoxia condition

Cancer cells normalized with normoxia levels, Comparison according to cell line in hypoxia and normoxia conditions was made using the Mann-Whitney U test. N: normoxia, H: hypoxia, p=0.487, U=3.000 for MCF-7, MDA-MB-231 and MDA-MB-468; p=0.037, U=0.000 for SK-BR-3

As a result, the gene expression level of LMAN2 differs according to the type of breast cancer under normal conditions, and it also differs according to the cell type in hypoxia conditions. While LMAN2 and BiP overexpressed in the MCF-7 cell line, HERP, and CHOP's gene expression levels were decreased. In the MDA-MB-231 cell line, LMAN2, CHOP, and BiP gene expression levels are decreased while HERP is increased. In SK-BR-3 cells, on the other hand, LMAN2, CHOP, and HERP gene expression levels were increased while BiP *was* downregulated. Interestingly, the expression levels of all genes are excessively increased in MDA-MB-468 cells.

Discussion

The endoplasmic reticulum is the organelle that is involved in the intracellular organization and the realization and control of folding, which is one of the post-translational modifications of proteins. This organelle, which has a unique system, arranges itself by regulating the release of misfolded proteins into or out of the cell. In this system, correctly folded proteins are transported by the cargo receptor protein VIP36. ER stress develops and the cell switches to apoptosis when there is an accumulation of folded proteins inside the cell. Cancer cells, which frequently resist apoptosis, are regulated differently from normal cells in terms of this physiological regulation. For instance, the cancer cell naturally escapes apoptosis and continues to exist in hypoxia, even though hypoxia is one of the conditions that lead to ER stress in the cell and triggers it to undergo apoptosis. In addition, hypoxia studies have shown that the microenvironment both promotes cancer cell metastasis and confers resistance to standard therapeutics [11]. This study aimed to determine how normoxia and hypoxia affect the gene expression level of LMAN2, which produces the VIP36 protein, one of the ER cargo receptors. One of the hypotheses put forward at the beginning of the study, "The pathogenesis of breast cancer under normal conditions is associated with the change in the expression level of the LMAN2 gene" was supported by the detection of different expression levels of LMAN2 in breast cancer cells showing different histopathological features under normoxia conditions. As a result of the experiments, LMAN2 gene expression was suppressed in MCF-7, SK-BR-3, and MDA-MB-231 cell lines. The MCF-7 cell line, which has a better prognosis than the others, has Receptor Tyrosine-protein Kinase erbB-2 (HER2-), Estrogen Receptor (ER +), and Progesterone Receptor (PR +/-) immune profiles and is in the luminal class A. LMAN2 was suppressed in SK-BR-3, which is in the HER2 (+) class, which has a moderate prognosis without ER and PR, and MDA-MB-231, which is known as triple negative with a poor prognosis and in the low claudin class [12]. However, it increased 1.18 times in the MDA-MB-468 cell line, which is also in the basal class with a triple-negative prognosis. However, this increase is very small compared to the decrease in other cell lines. In the studies conducted multiple databases, they found high levels of LMAN2 to be associated with poor prognosis in HER2+ breast carcinoma. They also found that LMAN2 gene expression was regulated at different levels according to the clinicopathological features of breast cancer [13, 14]. When studies on different cancer types were investigated, it was reported that LMAN2 gene expression level increased in the prostate, gastric, and ovarian cancers [6, 10, 15, 16].

As ER stress is induced in hypoxia conditions, increased gene expression of the transcription factor CHOP is observed because this transcription factor induces apoptosis by stimulating pro-apoptotic pathways [17]. However, it is expected that the expression of the CHOP factor, which stimulates the pro-apoptotic pathway, will decrease in cancer cells as it will help them escape from hypoxia apoptosis. In this study, CHOP was suppressed in MCF-7, MDA-MB-231, and SK-BR-3 cell lines under hypoxia conditions. A study conducted in MCF-7 cells showed that CHOP expression was increased by inducing ER stress with different external factors under hypoxia conditions [18]. Furthermore, CHOP was significantly increased in MDA-MB-468 cells. At this stage, the reason for the change in the CHOP gene expression level can be attributed to the molecular infrastructure and clinicopathological features. Under hypoxia conditions, the expression level of the BiP chaperone was increased in cells other than the MDA-MB-231 cell line. In the study, it was determined that BiP expression increased, apoptosis decreased, and migration triggered in hypoxic conditions in breast cancer cell lines [1]. While HERP was suppressed in MCF-7 and SK-BR-3 cells, it was increased in

MDA-MB-231 and MDA-MB-468 cells. HERP expression is one of the enzymes involved in protein degradation that induces ER stress, which is suppressed under hypoxia conditions in cancer cells, as in CHOP [19].

The most important limitation of the study is that it was only studied at the gene level, and studies are aimed to be conducted at the protein level. In addition, new studies are planned to investigate the relationship between LMAN2 and ER stress and apoptosis in more detail.

In conclusion, different expression profiles in cancer cell lines may be due to the molecular infrastructure of breast cancer. Under hypoxic conditions, LMAN2 gene expression was higher in MCF-7 and MDA-MB-468 cells than in MDA-MB-231 and SK-BR-3 cells. According to these findings, the LMAN2 gene is suppressed in cancer cells under normoxic conditions while its expression is increased under hypoxic conditions. There is no information in the literature about the level of LMAN2 under hypoxic conditions. Still, since LMAN2 is involved in this pathway, it can be assumed that its expression should decrease further and increase if it is engaged in the migration-promoting pathway.

In conclusion, hypoxia causes an increase in LMAN2 expression in healthy breast epithelial cells. It might be a protein involved in the ER stress pathway. To support these interpretations, more in-depth research is required.

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Research Article

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Radiological evaluation of calcific tendinitis with intraosseous migration: a single-center experience

İntraosseöz migrasyonlu kalsifik tendinitin radyolojik değerlendirmesi: tek merkez deneyimi

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Abstarct

Purpose: Migration of calcific deposits into adjacent tissues, such as bones, muscles, and the subacromial-subdeltoid bursa, is a rare complication that can lead to diagnostic challenges and unnecessary procedures. This study aims to describe the uncommon intraosseous migration of rotator cuff calcific tendinitis and evaluate these cases concerning demographic characteristics, associated pathologies, and radiological findings.

Materials and methods: This retrospective study, conducted between January 2021 and September 2024, reviewed 3.755 shoulder MRI scans. Exclusions included motion artifacts, pediatric cases, trauma or surgery history, and infections. Two radiologists independently evaluated cases of calcific tendinitis and intraosseous migration, analyzing findings against demographic data and related pathologies.

Results: Out of 3.000 scans, calcific tendinitis was found in 8.17% of cases. Intraosseous complications occurred in 0.5% of the total population and 6.12% of tendinitis cases. Most calcific tendinitis patients (73.77%) were female, with 86.67% of intraosseous cases being women (p=0.211 (Fisher's Exact Test)). The supraspinatus tendon was most frequently affected (63% of cases), with effusion being the most common pathology (55%). Patients with intraosseous complications showed higher rates of supraspinatus and infraspinatus involvement (both 73%) compared to those without complications (63% and 36%, respectively), and the difference was statistically significant (Fisher's Exact Test, p=0.005 for infraspinatus).

Conclusion: Calcific tendinitis can present rare intraosseous extensions, requiring careful imaging for accurate diagnosis. MRI and CT play crucial roles in identifying these cases. Untreated cases show persistent complications, while ultrasound-guided injections provide effective treatment. Proper differential diagnosis is necessary as calcific tendinitis may mimic tumors. Early diagnosis and appropriate treatment of intraosseous complications are essential. Future studies should explore larger populations and long-term follow-up for better evaluation of prognosis and outcomes.

Keywords: Calcific tendinitis, supraspinatus tendon, intraosseous migration, MRI.

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Öz

Amaç: Kalsiyum depositlerin kemik, kaslar ve subakromiyal-subdeltoid bursa gibi komşu dokulara göçü, tanısal zorluklara ve gereksiz prosedürlere yol açabilen nadir bir komplikasyondur. Bu çalışmanın amacı, rotator manşet kalsifik tendinitinin nadir görülen intraosseöz migrasyonunu tanımlamak ve bu vakaları demografik özellikler, ilişkili patolojiler ve radyolojik bulgular açısından değerlendirmektir.

Gereç ve yöntem: Bu retrospektif çalışma, Ocak 2021 ile Eylül 2024 tarihleri arasında gerçekleştirilmiş ve 3,755 omuz MRG incelemesi gözden geçirilmiştir. Hariç tutulanlar arasında hareket artefaktı olan hastalar, pediatrik vakalar, travma veya cerrahi öyküsü olanlar ve enfeksiyon vakaları yer almıştır. İki radyolog kalsifik tendinit ve intraosseöz migrasyon vakalarını bağımsız olarak değerlendirmiş, bulgular demografik veriler ve ilişkili patolojilerle analiz edilmiştir.

Bulgular: 3,000 tarama sonucunda vakaların %8,17'sinde kalsifik tendinit tespit edilmiştir. İntraosseöz komplikasyonlar toplam popülasyonun %0,5'inde ve kalsifik tendinit vakalarının %6,12'sinde gözlenmiştir. Kalsifik tendinit hastalarının çoğunluğu (%73,77) kadın olup, intraosseöz komplikasyonların %86,67'si kadın hastalarda görülmüştür (p=0,211 (Fisher's Exact Test)).

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İntraosseöz komplikasyonu olan hastalarda supraspinatus ve infraspinatus tutulum oranları (her ikisi de %73), komplikasyonu olmayan hastalara kıyasla daha yüksekti (sırasıyla %63 ve %36) ve bu fark infraspinatus için istatistiksel olarak anlamlıydı (Fisher's Exact Test, *p*=0,005).
Sonuç: Kalsifik tendinit, nadir görülen intraosseöz uzanımlar gösterebilir ve doğru tanı için dikkatli görüntüleme gerektirir. MRG ve BT, bu vakaların tanısında kritik bir rol oynar. Tedavi edilmeyen vakalarda komplikasyonlar devam ederken, ultrason rehberliğinde yapılan enjeksiyon tedavisi etkili sonuçlar vermektedir. Kalsifik tendinit, görüntülemede tümörleri taklit edebileceğinden doğru ayırıcı tanı gereklidir. İntraosseöz komplikasyonların erken teşhisi ve uygun tedavisi esastır. Gelecekteki çalışmalar, daha geniş popülasyonlar ve uzun dönem takiplerle prognoz ve tedavi sonuçlarını daha iyi değerlendirmelidir.

Anahtar kelimeler: Kalsifik tendinit, supraspinatus tendonu, intraosseöz migrasyon, MRG.

Tekinhatun M, Alver K, Akbudak İ, Turmak M, Deniz MA. İntraosseöz migrasyonlu kalsifik tendinitin radyolojik değerlendirmesi: tek merkez deneyimi. Pam Tıp Derg 2025;18:486-496.

Introduction

Calcific tendinitis is a relatively common condition associated with the deposition of calcium hydroxyapatite crystals in tendons [1-4]. It usually affects the pre-insertional portion of rotator cuff tendons, especially the supraspinatus tendon, in middle-aged women. Although benign and self-limiting, it can be an excruciatingly painful condition by triggering inflammatory changes. In rare cases, tendinous calcifications may show intraosseous, intramuscular and adjacent soft tissue migration [1-3, 5]. Ultrasound (US) and X-ray are the initial imaging modalities performed to patients having shoulder pain. Soft tissue calcifications and tendinopathy findings can be detected by US and X-ray. Despite percutaneous arthroscopic treatments have been reported less effective, US is also the preferential imaging modality for guiding these procedures. Computed tomography (CT) may be performed to patients not remembering trauma history or for any other reasons and findings like cortical erosion and intraosseous calcifications can be best detected with CT (Figure 1). Magnetic resonance imaging (MRI) is also frequently requested by clinicians for patients having persistent and nontraumatic shoulder pain immediately after X-ray or US imaging (Figure 2, 3). Additionally, while tendinopathy, cortical erosion and intraosseous calcifications can be detected by X-ray, US and CT, MRI is superior to other imaging modalities and highly sensitive in showing bone marrow edema and reactive inflamatory changes in perilesional soft tissues. However, intraosseous migration of intratendinous calcifications can mimic a neoplastic process like osteoid osteoma, osteoblastoma, chondroblastoma and periosteal osteosarcoma or infection, especially on MRI [6-8]. Furthermore, variable MR imaging characteristics due to temporal evolution of migrated intraosseous calcific deposits and associated bone changes may also contribute to diagnostic challenge [5]. So, radiologists should be aware of imaging findings of this rare clinical entity and they can reach confident diagnosis thanks to specific imaging characteristics in order to avoid misdiagnosis and biopsy which can cause unnecessary patient anxiety, further imaging and interventional procedures.

The aim of this study is to contribute to the identification of calcific tendinitis with intraosseous extension, which is defined as a rare complication in the literature.

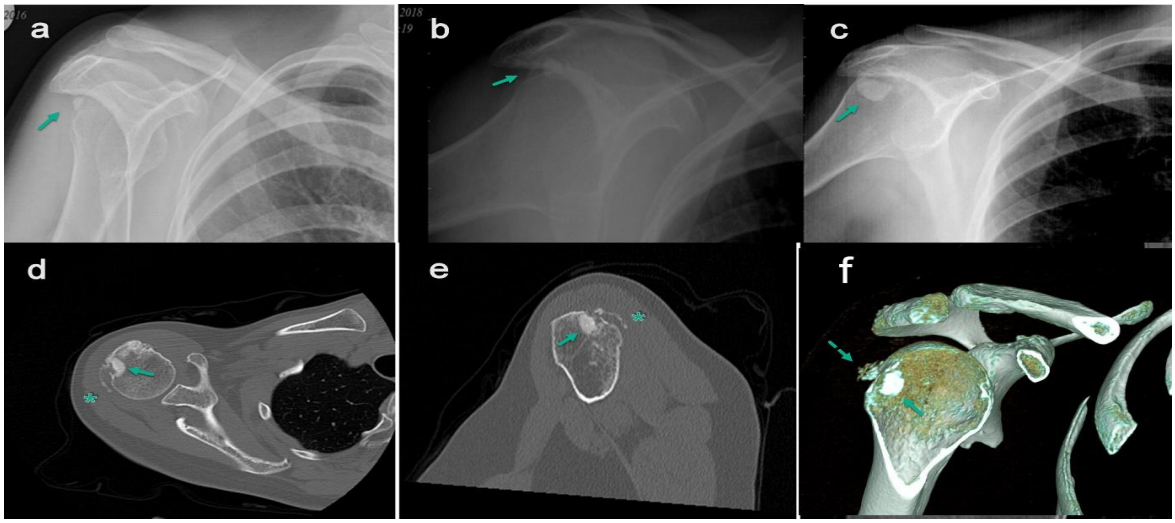

Figure 1. Radiographs of a 51-year-old female patient today (a), 2 years ago (b), and 4 years ago (c)

Migration of amorphous calcification (arrow) of musculoskeletal tendinitis. Computed tomography (CT) images—axial (d), sagittal (e), and volume-rendered (f)—reveal calcific tendinitis (* in d, e; dashed arrow in f) and its intraosseous migration (arrow in d, e, f)

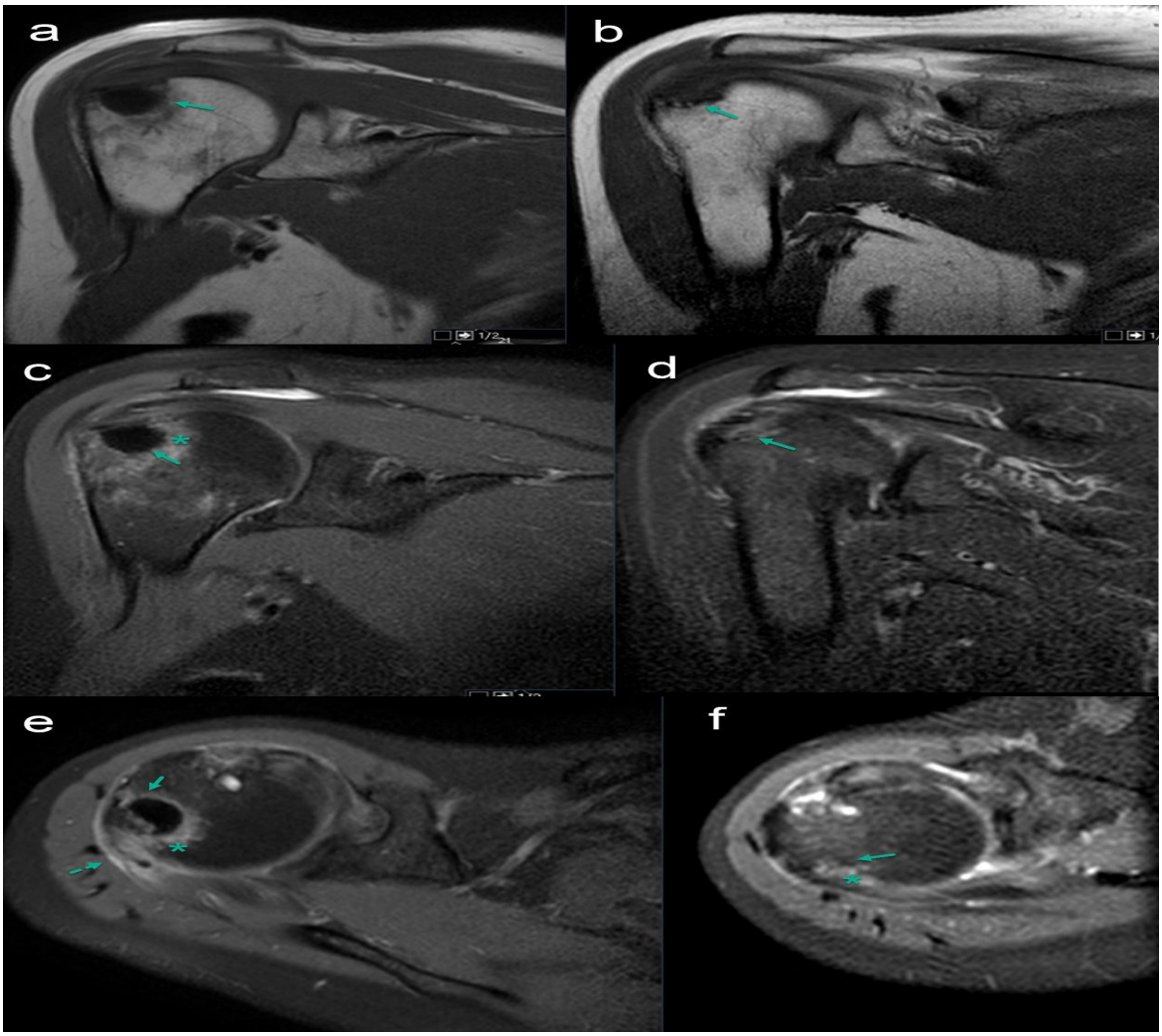

Figure 2. A 51-year-old female patient underwent MRI scans before and after treatment

Pre-treatment images—coronal T1-weighted (a), coronal proton density (PD) (c), and axial PD (e)—revealed intraosseous calcific tendinitis in the humeral head (arrow in a, c, e), accompanied by significant surrounding edema (* in c, e) and amorphous calcification foci of muscular tendinitis (dashed arrow in e). Post-treatment images—coronal T1-weighted (b), coronal PD (d), and axial PD (f)—demonstrated the resolution of calcifications (arrow in b, d, f) and a reduction in edema (* in f)

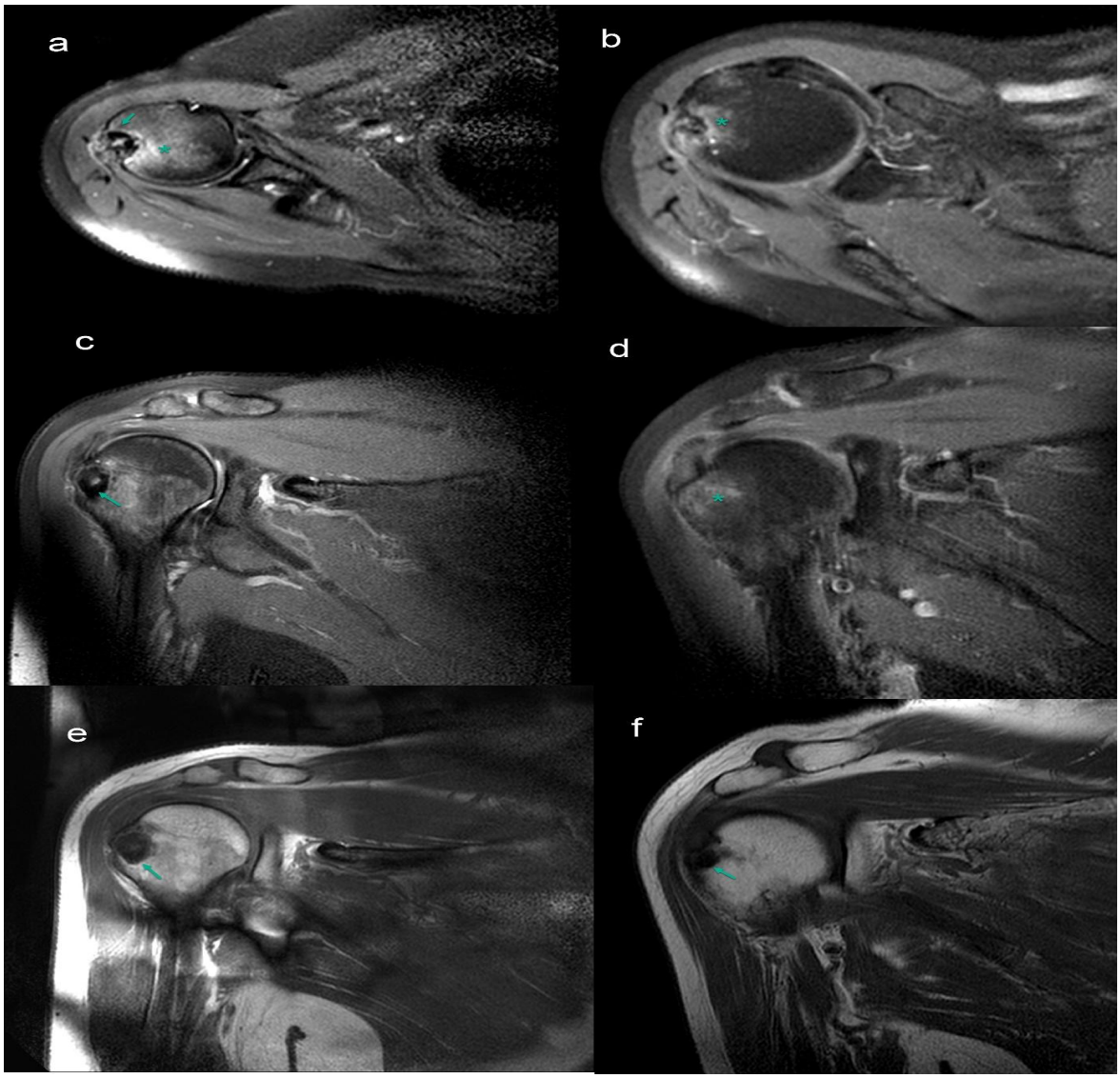

Figure 3. A 59-year-old female patient with known supraspinatus calcific tendinopathy presented with shoulder pain and limited range of motion

Initial and follow-up MRI scans, taken three months apart after the patient declined minimally invasive treatment, showed persistent intraosseous calcium in the greater tubercle (arrows in a, c, e, f) with marked surrounding bone marrow edema (* in a, b, d). The images included coronal (c, d) and axial (a, b) proton density sequences, as well as coronal T1-weighted images (e, f)

Material and methods

Ethics Committee approval from Dicle University Medical Ethics Committee (date:10.12.2020, and 60116787-020/73335) were obtained and was conducted in accordance with the principles of the Helsinki Declaration. A total of 3.755 shoulder MRI scans performed at our institution between January 2021 and September 2024 were retrospectively reviewed. Excluded from the study were scans with motion artifacts, pediatric patients, patients with a history of trauma or surgery, patients with infections or inflammatory pathologies, and those with masses detected in the shoulder region. As a result, 3.000 shoulder MRIs were independently evaluated by two experienced radiologists. The consensus method was used to ensure consistency between evaluations and validate results. In cases of discrepancies, final decisions were made through consensus.

In our study, calcific tendinitis was diagnosed based on MRI findings of hypointense signal changes on both T1- and T2-weighted images, consistent with calcium deposits within the rotator cuff tendons, and tendon thickening in muscles adjacent to the shoulder joint. Intraosseous calcific tendinitis was identified by the migration of these calcifications into the humeral head, accompanied by surrounding edema, which appeared hyperintense on T2-weighted sequences. A total of 245 cases of calcific tendinitis were identified. The MRI images were processed automatically through the picture archiving and communication system (PACS). Calcific tendinitis was classified according to the muscles involved, and the frequency of associated pathologies such as impingement, effusion, tendinopathy, rotator cuff tears, and the rare occurrence of intraosseous migration was investigated. All findings were correlated with patient demographic characteristics (age, gender, etc.).

Two patients with intraosseous complications had follow-up imaging available. One patient was treated by the interventional radiology unit, and detailed evaluations of both cases are described in the case examples section.

Magnetic resonance imaging (MRI) of the shoulder was performed using a 1.5T scanner (Magnetom Altea, Siemens Healthcare, Erlangen, Germany). The shoulder MRI protocol included two coronal oblique planes: a fluid-sensitive sequence with fat suppression (T2W FS) and a dark fluid sequence (T1W). Additionally, proton density (PD) sequences with a long TR and short TE were used for axial imaging, while bright fluid (T2W) sequences were applied for sagittal imaging (Table 1).

Table 1. Shoulder MRI sequence parameters

Sequence	Plane	TR (ms)	TE (ms)	Slice Thickness (mm)	FOV (mm)	Matrix	Time (min)
T2W FS	Coronal Oblique	4000	50	3.5	160	256x256	2:00
T1W	Coronal Oblique	600	12	3.5	160	256x256	1:40
PD (Long TR, Short TE)	Axial	3000	35	3.0	160	256x256	1:50
PD (Long TR, Short TE)	Sagittal Oblique	4000	50	3.5	160	256x256	2:10

TR= Repetition Time; TE= Echo Time; FOV= Field of View; T2W FS= T2-Weighted Fat-Saturated; T1W= T1-Weighted; PD= Proton Density

Statistical analysis

In this study, statistical analyses were performed to evaluate the differences in demographic data, muscle involvement, and associated pathologies among patients with calcific tendinitis and intraosseous complications.

Statistical analyses were conducted using the SPSS (Statistical Package for Social Sciences) for Windows version 22.0. Descriptive data were presented as n and % for categorical variables, and as mean ± standard deviation (mean ± SD) for continuous variables.

The normality of continuous variables (e.g., age) was assessed using the Kolmogorov-Smirnov test. Comparisons between two groups (e.g., age differences between patients with and without intraosseous complications) were performed using the Student's t-test when the data followed a normal distribution.

For the analysis of categorical data (e.g., gender distribution among patients with calcific tendinitis and those with intraosseous complications), Chi-square (χ^2) test or Fisher's Exact Test was used depending on the expected cell counts. Fisher's Exact Test was applied when the sample size was small.

The frequency of involvement of different muscles in calcific tendinitis cases was analyzed using Cochran's Q test, given that multiple muscles could be affected in the same patient.

Additionally, the occurrence of associated pathologies, such as effusion, tendinopathy, impingement, and tears, was evaluated using Cochran's Q test to compare their frequencies.

A *p*-value of <0.05 was considered statistically significant.

Results

A total of 3.000 shoulder MRIs were reviewed, and the prevalence of calcific tendinitis was found to be 8.17% (n:245). Intraosseous complications were observed in 0.5% (n:15) of the entire population. Among the calcific tendinitis cases, the incidence of intraosseous complications was calculated to be 6.12%. These findings suggest that intraosseous complications are rare.

In this study, the demographic data of patients with calcific tendinitis and intraosseous complications were analyzed (Table 2). Of the 245 patients, women made up 73.77% of the calcific tendinitis cases, while men accounted for 26.23% ($p=7.7\times10^{-14}$ (Chi-squared test)). Among the 15 patients with intraosseous complications, the percentage of women was even higher at 86.67% (*p*=0.211 (Fisher's Exact Test)). The average age in the general population was 51.78±12.10 years, while the average age of patients with intraosseous complications was 49.53±11.61 years (*p*=0.459) (student t test). These results indicate that calcific tendinitis and intraosseous complications are more common in women, and there is no significant age difference between the two groups (*p*=0.459) (student t test).

Table 2. Demographic data of calcific tendinitis and intraosseous complications

Feature	General Population	Intraosseous Cases
Total Cases	245	15
Female (%)	73.77%	86.67%
Male (%)	26.23%	13.33%
Age Mean ± Std Dev, Min-Max)	51.78±12.10, 20-92	49.53±11.61, 31-71

Gender distribution: Fisher's Exact Test, *p*=0.211 (not significant), age comparison: Kolmogorov-Smirnov test (D=0.047, *p*=0.20); levene's tetst (F=0.037, *p*=0.848); Student's t-test, t=-0.741, *p*=0.459 (not significant)

Muscle involvement

In calcific tendinitis cases, the most frequently affected muscle was the supraspinatus, found in 155 out of 245 patients (63%). The infraspinatus muscle was involved in 93 cases (38%), the subscapularis in 27 cases (11%), and the teres minor in 26 cases (11%). More rarely, the biceps was involved in 4 cases (2%) and the deltoid in just 1 case (<1%). The results of Cochran's Q Test (*p*=0.0001) demonstrate a statistically significant difference in the frequency of muscle involvement, with the supraspinatus and infraspinatus muscles being the most commonly affected (Table 3).

Table 3. Muscle involvement in calcific tendinitis

Muscle	Count	Percentage (%)
supraspinatus	155	63
infraspinatus	93	38
subscapulariss	27	11
teres minor	26	11
biceps	4	2
deltoid	1	0

Cochran's Q Test; Q=176.961, *p*=0.0001

In our patients with intraosseous complications, the involvement rates of the Supraspinatus and Infraspinatus muscles are respectively 73%, whereas these rates are observed as 63% and 36% in patients without complications (Figure 4). A comparison of muscle involvement rates between patients with and without intraosseous complications revealed statistically significant differences in the involvement of the infraspinatus (p=0.005) and teres minor muscles (p=0.013), based on Fisher's Exact Test.

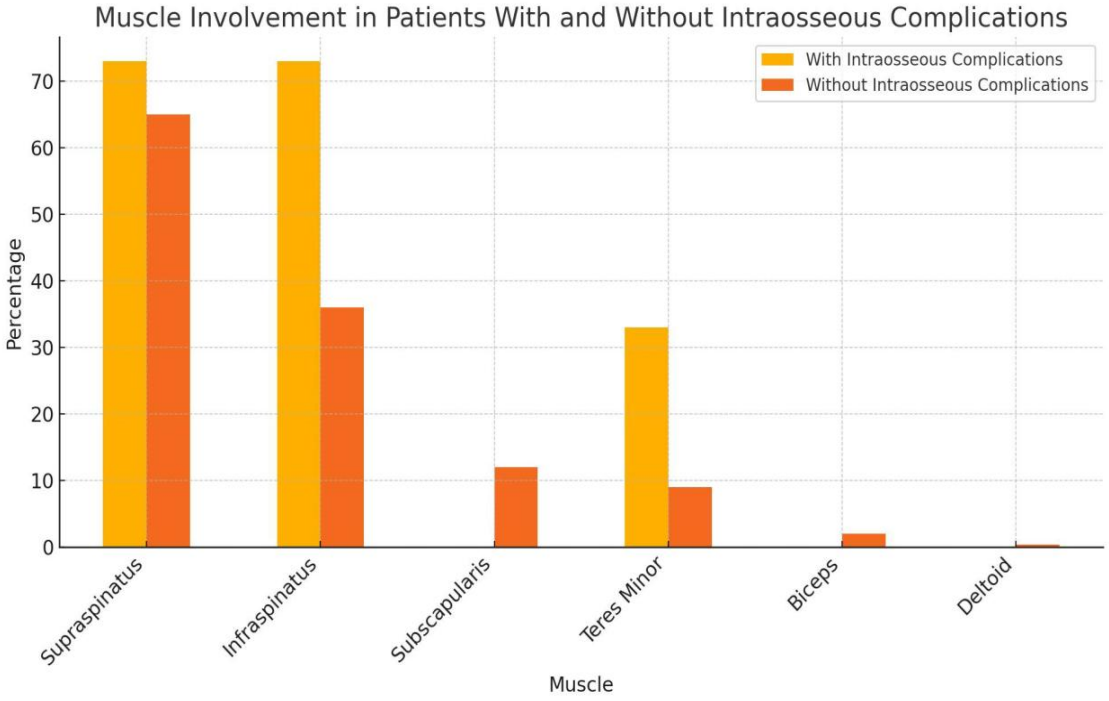

Figure 4. Comparison of muscle involvement rates in patients with and without intraosseous complications. Statistically significant differences were observed in the involvement of the infraspinatus (p=0.005) and teres minor muscles (p=0.013), based on Fisher's Exact Test

Accompanying diseases

When the associated pathologies in calcific tendinitis cases were examined, the most frequent finding was effusion, which was detected in 135 patients (55%). Tendinopathy was observed in 95 patients (39%), impingement in 60 patients (24%), and tears in 36 patients (15%). Intraosseous migration was observed in 15 patients (6%). Cochran's Q Test (p=0.0001) indicates a statistically significant difference in the distribution of associated pathologies, with effusion being the most frequently observed finding (Table 4).

In our patients with intraosseous complications, the prevalence rates of effusion, tendinosis, tears, and impingement are respectively 73%, 53%, 20%, and 33%. In patients without complications, these conditions are recorded at 54%, 38%, 14%, and 24% respectively (Figure 5). The differences were analyzed using the Chi-square test and found to be not statistically significant (χ^2=0.0059, p=0.995).

Table 4. Associated pathologies in calcific tendinitis

Pathology	Count	Percentage (%)
Impingement	60	24
Effusion	135	55
Tendinopathy	95	39
Tear	36	15
Intraosseous Migration	15	6

Cochran's Q Test; Q=225.614, *p*=0.0001

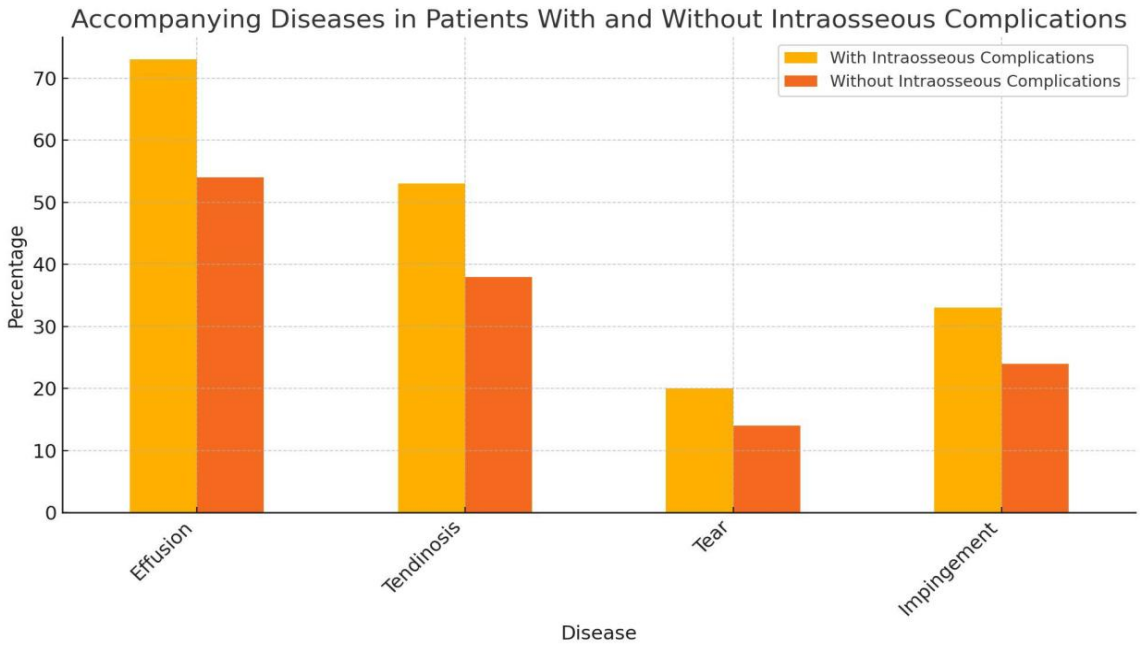

Figure 5. Comparison of accompanying diseases prevalence in patients with and without intraosseous complications. No statistically significant differences were observed (Chi-square test, χ^2=0.0059, *p*=0.995)

Discussion

The most significant finding of our study is the high involvement rates of the Supraspinatus and Infraspinatus muscles in patients with intraosseous complications of calcific tendinitis, observed at 73% each. Previous studies have shown that calcific tendinitis most commonly affects the Supraspinatus tendon. Furthermore, intraosseous migration is frequently reported in patients with calcific tendinitis involving the Supraspinatus and Infraspinatus tendons. However, there is limited data in the literature on this aspect. Case reports often describe involvement of both the Supraspinatus and Infraspinatus tendons in patients with intraosseous complications, which aligns with the findings of our study [9-14].

Calcific tendinopathy, also known as hydroxyapatite deposition disease or calcific tendinitis, accounts for approximately 3% to 7% of all cases of painful shoulder [4, 9]. Its clinical presentation ranges from asymptomatic to severe pain and symptoms do not always correlate with imaging findings [2, 3, 9, 10]. Adhesive capsulitis, rotator cuff tears, migration of calcific deposits to bones and soft tissues, humeral head osteolysis and ossifying tendinitis are the well-defined complications of calsific

tendinitis [11]. In our study, intraosseous complications were observed in 15 out of 240 cases, highlighting a notable prevalence of this specific complication.

In the literature, treatment methods involving intraosseous extension and those without it for patients with rotator cuff calcific tendinopathy (RCCT) have shown that treatments performed under ultrasound guidance yield less favorable outcomes in cases with intraosseous spread compared to those with only tendinous or peritendinous disease [12]. In our study, follow-up imaging of one treated patient with intraosseous calcific tendinitis among the 15 cases showed significant regression of findings after treatment. However, in another patient with intraosseous calcific tendinitis who did not undergo treatment but had follow-up imaging, the findings persisted.

The radiologic diagnosis of calcific tendinitis is made by the characteristic and classical imaging appearance of calcific deposits in the involved tendon, particularly pre-insertional portion of supra and infraspinatus tendons. US and X-ray imaging findings can be considered sufficient for the diagnosis in majority of cases. However, demonstration of continuity between the migration of calcific deposits to the humeral head and initial calcific tendinitis plays the crucial role in the diagnosis. Our study observed that follow-up imaging with plain X-rays was sufficient to confirm the diagnosis in some cases. However, in cases where intraosseous migration was suspected, CT imaging provided a detailed evaluation of cortical discontinuity and the precise localization of calcific deposits. These findings emphasize the importance of advanced imaging modalities in complex cases.

Furthermore, well-defined hypointens MR focus on all pulse sequences corresponding to hyperdense foci in CT images accompanying to marked bone marrow oedemia in humeral head and periarticular calcifications seem very specific to this condition. In our cohort, MRI was instrumental in diagnosing patients where bone marrow oedema and periarticular changes were prominent. This allowed for a more comprehensive assessment of disease progression and provided valuable information for clinical management.

For these reasons, additional imaging with CT and MRI should be performed in patients having calcific tendinitis diagnosis with suspected intraosseous migration for depicting classical imaging findings and continuity of disease [15]. We believe that both CT and MRI imaging should be performed due to CT is superior to detect cortical discontinuity and sites and morphology of intraosseous calcifications while bone marrow and soft tissue oedema can be best demonstrated with MRI. In our study, the use of MRI follow-up in select patients proved essential for monitoring the evolution of the disease, assessing changes in bone marrow oedema—which is closely related to pain symptoms—and excluding other pathologies such as tumors.

As Zampa et al. [8] stated in the recent study, CT and MRI images performed 3 months after first presentation and initial X-ray examination contributed much to reach correct diagnosis in our cases too. Similarly, in our cases, follow-up imaging, including both CT and MRI, clarified the temporal evolution of the disease and associated bone changes. In addition, Paruthikunnan et al. [1] struggled to define temporal evolution of disease and associated bone changes and stated that imaging findings may vary because of nature of the disease and this can lead to diagnostic difficulties. Consistent with these observations, follow-up with MRI in our study also enabled us to track the migration of calcific deposits and identify diverse imaging characteristics confidently.

In some cases which patients have nocturnal pain symptoms and CT features of an osteolytic lesion without osseous extrusion of the tendon calcification, it can be challenging to exclude tumors like osteoid osteoma, osteoblastoma or any other malign lesions [1]. Physcians may request further imaging such as nuclear bone scan in such cases however its contribution to diagnosis is limited due to inflammation around migrated calcific deposits in humeral head also shows increased bone tracer activity. In our cases, presence of cortical discontinuity in greater tuberositas, demonstration of calcific deposits migration from tendons to humeral head, absence of symptoms like nocturnal pain responding to aspirin therapy and lack of a soft tissue mass did not necessitate any further imaging.

Bone involvement in calcific tendinitis is a very rare complication and humeral head is the most affected site, but other areas of involvement such as the wrist, elbow, hip, and knee have also been reported in the literature [3]. In 76% of cases, migration of calcific deposits to humeral head originate from supraspinatus tendon while other rotator cuff muscles are less commonly affected [16]. In our cases, both the supraspinatus and infraspinatus tendons were affected, with involvement rates of 73% each. These findings indicate a broader pattern of tendon involvement in calcific tendinitis with intraosseous migration. A comparison of muscle involvement rates in patients with and without intraosseous complications revealed statistically significant differences in the involvement of the infraspinatus (p=0.005) and teres minor muscles (p=0.013), based on Fisher's Exact Test. These results emphasize the need to consider multiple tendon involvements in similar cases and highlight the diagnostic importance of advanced imaging.

This study has several limitations. First, the retrospective design of the study may lead to missing or inaccurate data, as it does not allow for direct intervention in the data collection process. Additionally, retrospective studies carry the risk of selection bias, as patients included in the study are chosen based on specific criteria, limiting the generalizability of the results. Since this study was conducted in a single center, the findings may not be applicable to different populations or centers. Moreover, the lack of long-term follow-up data restricts the ability to evaluate the long-term outcomes of calcific tendinitis.

Future studies should aim to overcome these limitations by being designed as prospective and multicenter studies involving larger patient populations. Additionally, the inclusion of long-term follow-up data would allow for a more detailed assessment of the prognosis and response to treatment of calcific tendinitis.

In conclusion, despite its rarity, it is crucial for radiologists to be familiar with the imaging findings of intraosseous extension of calcific tendinitis mentioned above in detail for avoiding misdiagnosis which may lead to further unnecessary imaging, biopsy and any other surgical interventions. Additionally, the injection treatment guided by ultrasound is practical and therapeutic.

Our findings emphasize the importance of advanced imaging modalities such as CT and MRI in confirming intraosseous migration and assessing its extent. Furthermore, routine follow-up with MRI can aid in monitoring disease progression, evaluating response to treatment, and detecting any residual or recurrent changes. A comprehensive understanding of the characteristic imaging findings will not only improve diagnostic accuracy but also help in guiding appropriate management and preventing overtreatment.

Abbreviations and acronyms: US, ultrasound; CT, computed tomography; MRI, magnetic resonance imaging; PACS, picture archiving and communication system; PD, proton density.

Consent for publication: Informed consent is routinely obtained from all patients undergoing MRI.

Availability of data and materials: The data supporting this study's findings are available on request from the corresponding author.

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

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Research Article

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Types and clinical features of 140 newly diagnosed cases of diabetes in childhood: a single-center experience

Çocukluk çağında yeni tanı almış 140 diyabet olgusunun tanı tipleri ve klinik özellikleri: tek merkez deneyimi

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Abstract

Purpose: Type 1 diabetes (T1DM) accounts for the majority of childhood diabetes mellitus (DM). However, in recent years, there has been an increase in the prevalence of type 2 diabetes mellitus (T2DM) and the diagnosis of monogenic diabetes (MD). The aim of this study was to evaluate the clinical and laboratory findings, as well as the types of DM, in patients diagnosed between the ages of 0 and 18.

Materials and methods: In the study, 140 patients diagnosed with DM in our clinic were evaluated retrospectively.

Results: During the 3-year period, 140 patients (n=76, 54.3% male) were diagnosed with diabetes. The mean age at diagnosis of the patients was 10±4.19 years. 93.6% of patients were diagnosed with T1DM, 2.8% of patients with T2DM and 3.6% of patients were diagnosed with MD. It was observed that the cases of T1DM peaked in the 5-9 (36.6%) and 10-14 (37.4%) age groups. the prevalence of diabetic ketoacidosis (DKA) was 61.8%. The majority of patients 64.9% with T1DM were diagnosed in the autumn/winter months. 75% of the patients with T2DM were female, and the mean age at diagnosis was 15.05±1.11years. Two of the cases of MD were neonatal DM, two were GCK-MODY and one was CEL-MODY.

Conclusion: Although the majority of childhood diabetes cases are T1DM, the frequency of T2DM tends to increase, especially in obese adolescents. It should be kept in mind that obesity may also occur in autoantibody-positive T1DM patients. It was determined that T1DM cases were more common in the winter season, in the 10-14 age group, and that DKA was higher. Genetic examination should be performed in cases with suspected MD.

Keywords: Diabetes mellitus, type 1 diabetes, type 2 diabetes, monogenic diabetes, childhood.

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Öz

Amaç: Çocukluk çağındaki diyabetes mellitusun (DM) çoğunluğunu tip 1 diyabet (T1DM) oluşturur. Ancak son yıllarda tip 2 diyabetes mellitus (T2DM) sıklığında ve monogenik diyabet (MD) tanısı koymada artış gözlenmektedir. Bu çalışmada 0-18 yaş arasında tanı konulan diyabetli hastalarda klinik ve laboratuvar bulgularının, DM tiplerinin değerlendirilmesi amaçlandı.

Gereç ve yöntem: Çalışmada kliniğimizde DM tanısı alan 140 hasta retrospektif olarak değerlendirildi.

Bulgular: Üç yıllık süreçte 140 hastaya (76 erkek, %54,3) diyabet tanısı konuldu. Hastaların ortalama tanı yaşı 10±4,19 yıldı. Hastaların %93,6'sına T1DM, %2,8'ine T2DM, %3,6'sına MD tanısı konuldu. Tip 1 diyabetes mellitus vakalarının 5-9 (%36,6) ve 10-14 (%37,4) yaş gruplarında zirve yaptığı görüldü. Diyabetik ketoasidoz (DKA) prevalansı %61,8 idi. Yine bu olguların %64,9'u sonbahar/kış aylarında tanı aldı. Tip2 diyabetli hastaların %75'i kadındı ve ortalama tanı yaşı 15,05±1,11 idi. Monogenik diyabetli olgularının ikisi neonatal DM, ikisi GCK-MODY ve biri CEL-MODY idi.

Sonuç: Çocukluk çağı diyabet vakalarının çoğunluğu T1DM olmasına rağmen, özellikle obez ergenlerde T2DM sıklığı artma eğilimindedir. Otoantikor pozitif T1DM'li hastalarda da obezitenin olabileceği akılda tutulmalıdır. Tip1 diyabet olgularının daha çok kış mevsiminde, 10-14 yaş grubunda başvurduğu ve DKA sıklığının yüksek olduğu saptandı. Monogenik diyabet şüphesi olan olgulara genetik inceleme yapılmalıdır.

Anahtar kelimeler: Diyabetes mellitus, tip 1 diyabet, tip 2 diyabet, monogenik diyabet, çocukluk çağı.

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Introduction

Diabetes mellitus is a metabolic disease characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Deficient insulin action in tissues leads to abnormalities in carbohydrate, fat, and protein metabolism. The main diagnostic criterion in all types of diabetes in children is the biochemical increase in plasma glucose level in the presence of hyperglycemia symptoms. In the presence of overt symptoms, a significant increase in fasting plasma glucose (≥126 mg/dl) or random plasma glucose (≥200 mg/dl), or an OGTT 2-hour plasma glucose level of ≥200 mg/dl and an HbA1c value of ≥6.5% confirm the diagnosis of diabetes [1].

Type 1 diabetes (T1DM) is usually characterized by the loss of endogenous insulin production as a result of damage to β cells by an autoimmune mechanism. There is the presence of one or more autoantibodies. These cases are defined by the presence of at least one of the following: islet cell autoantibodies (ICA), insulin autoantibodies (IAA), anti-glutamic acid decarboxylase 65 autoantibodies (anti-GAD), and β-cell-specific zinc transporter 8 autoantibodies (ZnT8). Some patients have autoantibody negativity and are referred to as idiopathic [1-3]. T1DM accounts for 90% of diabetes in children [1]. It occurs at a rate of 52.2 per 100.000 in Finland, where the highest incidence has been observed [1, 4]. In our country, the incidence of T1DM has been reported as 11.3 per 100.000 in the 0-14 age group and 10.8 per 100.000 in the 0-18 age group [5].

Type 2 diabetes (T2DM) results from inadequate insulin response in the presence of increased insulin resistance [2]. It is a metabolic disorder that predominantly affects adults. However, its prevalence has been on the rise in recent years, particularly among obese children and adolescents. T2DM has a multifactorial etiology consisting of genetics, physiology, a high-calorie diet, low physical activity, and a sedentary lifestyle. The possibility of microvascular complications is higher in cases of T2DM than in cases of T1DM [6]. In Canada, which is one of the countries with a high incidence of T2DM, the incidence in the 0-18 age group is 821/100.000; and in Australia, the incidence of T2DM is 670/100.000 in the under 24 age group. Low incidence rates have been reported in Europe and the United Kingdom (0.6-1.4/100.000) [7].

Monogenic diabetes (MD) is caused by a defect in a single gene involved in β-cell development or function. It constitutes 1-6% of the cases. It can occur as neonatal diabetes (NDM), maturity-onset diabetes of the young (MODY), and various syndromes. MODY is the most common type. Since the clinical features in cases of MODY are similar to T1DM and T2DM, patients may sometimes get misdiagnosed [1]. Neonatal diabetes is an MD that occurs in the first 6 months of life. Neonatal diabetes should also be considered in patients between 6-12 months of age who have no findings of autoimmunity [8]. Neonatal diabetes can be transient/permanent. Today, dysfunctions in more than 40 genes have been identified to cause MD [9]. Monogenic diabetes is identified more in childhood with the increase in genetic testing. Monogenic diabetes should be considered in cases of diabetes occurring especially in the first 12 months of life, in the presence of autosomal dominant familial hyperglycemia/diabetes, and in patients with extrapancreatic findings (elevated liver enzymes, congenital heart disease, diarrhea, brain malformations, optic atrophy, deafness, etc.) [1, 8].

In this study, it was aimed to determine the clinical and laboratory features on admission and types of diabetes in patients who were diagnosed with diabetes in Mardin Training and Research Hospital between the ages of 0-18.

Materials and methods

Study population and laboratory

In the study, 140 patients between the ages of 0-18 who were diagnosed with diabetes between 2020 and 2023 in the Pediatric Endocrinology Clinic of Mardin Training and Research Hospital were included. Records of the patients were examined retrospectively. Diabetes was diagnosed according to the criteria of the American Diabetes Association (ADA) and International Society for Pediatric and Adolescent Diabetes (ISPAD) guidelines [1, 10].

The age, gender, body weight (BW), BW standard deviation score (SDS), height, height SDS, body mass index (BMI), and BMI SDS of the patients at the time of admission were obtained. Obesity was defined as a BMI ≥95th percentile for age and gender. Calculations of oxological data were made using the growth curves of Turkish children [11]. In addition, the season of admission, consanguinity between parents, number of siblings, whether the patients were from rural or urban areas, and family history of diabetes were recorded. The plasma glucose levels, C-peptide levels, blood ketones, blood pH, glycated hemoglobin (HbA1c) values, Anti-GAD, IAA and ICA at the time of admission were obtained from the medical records of the patients and evaluated. ZnT8 autoantibodies could not be measured. The presence of one or more autoantibodies against β-cells was considered positive autoimmunity [1]. The cases were defined as ketosis or diabetic ketoacidosis (DKA) according to blood gas values and presence of ketones. Patients with a beta-hydroxybutyrate (BOHB) level of ≥3 mmol/L in the blood or a ketone level of ≥+2 in urine were considered positive for ketones, a venous blood pH of <7.30 and bicarbonate (HCO3) of <18meq/L were considered DKA. Diabetic ketoacidosis was classified according to the pH and HCO3 values of the patients (patients with a pH of <7.3 and a HCO3 of <18 mEq/L were considered to have mild DKA, patients with a pH of <7.2 and a HCO3 of <10 meq/L were considered to have moderate DKA, and patients with a pH of <7.1 and a HCO3 of <5 meq/L were considered to have severe DKA) [12].

The patients were evaluated during the diagnosis/follow-up in terms of thyroid stimulating hormone (TSH), free thyroxine (fT4), anti-thyroglobulin (anti-Tg), anti-thyroid peroxidase (Anti-TPO), celiac autoantibodies (tissue transglutaminase IgA and IgG antibodies) and accompanying autoimmune diseases. The cases of T2DM were evaluated in terms of hyperlipidemia, hypertension, and fatty liver. Genetic analysis for MD was performed in patients with negative diabetes autoantibodies, family history of autosomal dominant diabetes, patients younger than 12 months, and patients with low insulin requirement (such as 0.5U/kg/day) 1 year after diagnosis. [1].

Approval for the study was obtained from Mardin Artuklu University Faculty of Medicine Ethics Committee (approval date: 19/04/2023-94149, approval number: 2023/4-7).

Statistical analysis

SPSS 11.0 for Windows® package software was used in the analyses. Data were expressed as mean±SD (range) or median (interquartile range, IQR). In the comparison of the data of two independent groups: if the group distribution was normal, the independent samples t test, and if the distribution was not normal, the Mann-Whitney U test was used. A *p* value less than 0.05 was considered statistically significant.

Results

Of the 140 patients included in the study, 93.6% were diagnosed as T1DM, 2.8% as T2DM and 3.6% as MD. Of all patients, 64 (45.7%) were female, 76 (54.3%) were male and the mean age at diagnosis was 10±4.19 years (Table 1). A minimum of one autoantibody-positive result was observed in 69.2% of patients, while 44.2% of patients exhibited two or more autoantibody-positive results. Of the 83 patients (59.2%) who had DKA at presentation, 42.1% (n=35) had severe DKA. Six percent (n=5) of all DKA patients were younger than three years old. Cerebral edema developed clinically in 3 patients who presented with severe DKA. There was no mortality in any of the patients.

Type 1 diabetes cases

Type 1 Diabetes was diagnosed in 93.6% (n=131) of our patients. Of the T1DM cases, 58 (44.3%) were female and 73 (55.7%) were male. The prevalence of DKA was found to be 61.8% in all patients with T1DM. From the medical records of the patients, it was determined that 20.6% had a family history of T1DM. There was a female predominance in patients under the age of 10 and a male predominance in patients over the age of 10. The majority of patients (64.9%) were diagnosed in the autumn/winter months (Table 2). A total of 25.2% (n=33) of patients exhibited least one antibody positivity, while 48.9% (n=64) were positive for more than one antibody. These cases were classified as autoimmune T1DM. A total of 25.9% (n=34) of the patients were found to be autoantibody-negative, and these cases were classified as non-autoimmune T1DM (idiopathic).

Table 1. Sociodemographic and clinical characteristics of patients at the time of diagnosis.

	Total
Age at diagnosis[a] (years)	10±4.19
Gender (Female/Male)	64 (45.7%) / 76 (54.3%)
Family history of diabetes	20%
Parental consanguinity	37.8%
Living in the city center	37.9%
Mean number of children of the families	4.2
Prepubertal	49.2%
Plasma glucose[a] (mg/dL)	436.9±154.9
C-peptide[b] (ng/ml)	0.35 (0.4)
HbA1c[b] (%)	11.8% (3.38)

SDS: standard deviation score, [a]: mean± standard deviation, [b]: median (IQR), HbA1c: Glycated hemoglobin

Table 2. Characteristics of type 1 diabetes cases

Variable	**Category**	**n (%)**
Gender	Female	58 (44.3%)
	Male	73 (55.7%)
Age at diagnosis	0-4 years old	14 (10.7%)
	5-9 years old	48 (36.6%)
	10-14 years old	49 (37.4%)
	15-18 years old	20 (15.3%)
Season of diagnosis	Winter	49 (37.4%)
	Autumn	36 (27.5%)
	Spring	31 (23.7%)
	Summer	15 (11.4%)
Diabetic ketoacidosis	Present	81 (61.8%)
	Absent	50 (38.2%)

At the time of diagnosis, 8 (6.1%) patients were found to have autoantibody positivity for Hashimoto's thyroiditis, and the patients were euthyroid. In 11 (8.3%) patients, celiac serology positivity was found. In 3 (27.2%) of these patients, spontaneous normalization was detected, while celiac disease was identified by biopsy in 8 (6.1%) patients.

Of the autoantibody-negative patients, 53% presented with DKA, 23.5% with ketosis, and 23.5% with hyperglycemia. The number of obese patients diagnosed with T1DM was 4. Only one of these patients had autoantibody negativity, and the remaining three patients presented with DKA. There was no difference between autoantibody-positive and negative cases in terms of age, weight SDS, height SDS, and BMI SDS. The mean plasma glucose levels, median C-peptide and HbA1c levels of both groups at the time of admission were similar (Table 3).

Table 3. Clinical and laboratory features of T1DM cases

	Total (n=131)	Antibody+ n=97)	Antibody- (n=34)	t, z value	*p* value
Age[a]	10.2±3.94	9.88±3.89	10.44±4.12	t=-0.71	0.477
Weight SDS[a,b]	-0.53 (1.64)	-0.45±1.16	-0.40±1.33	t=-0.23	0.814
Height SDS[a]	-0.08±1.07	-0.11±1	-0.0003±1.26	t=-0.53	0.596
BMISDS[a]	-0.59±1.27	-0.54±1.25	-0.73±1.34	t=0.73	0.464
Glucose[a]	446.2±146.9	444.7±144.3	450.5±156.2	t=-0.19	0.844
C-peptide[b]	0.34 (0.34)	0.32 (0.31)	0.35 (0.59)	z=-1.31	0.190
HbA1c[a,b]	12.1 (3.2)	12.4 (3.65)	12.21±2.12	z=-0.38	0.700

SDS: standard deviation score, [a]: mean± standard deviation, [b]: median (IQR), HbA1c: Glycated hemoglobin, BMI: Body mass index
t: Independent samples t test, z: Mann-Whitney U test

Type 2 diabetes cases

Four (2.8%) patients were diagnosed with T2DM. The mean age at diagnosis of the patients was 15.05±1.11 years, the mean BMI SDS was 3.36±1.16, the mean C-peptide level was 2.63±0.39 ng/ml, and the mean HbA1c value was 6.32±1.15 %. The patients were pubertal. They were asymptomatic at the time of diagnosis. Three of the patients, who presented with obesity, were diagnosed by high fasting plasma glucose levels. In the other patient, diabetes was detected by the OGTT. Acidosis or ketosis was not observed in the patients. All patients were autoantibody-negative and had acanthosis nigricans, hyperlipidemia, and fatty liver. Hypertension or other microvascular complications were not detected. All of the patients had a family history of T2DM. Metformin was administered to three patients, and metformin+ insulin treatment was administered to one patient.

Monogenic diabetes cases

This group consisted of 5 (3.6%) patients. Two patients were diagnosed with neonatal diabetes. One of them presented with hyperglycemia in the neonatal period. A heterozygous mutation of c.692G>T (p.Trp231Leu) was detected in the ABCC8 gene in this patient. Following a brief course of treatment, the patient achieved remission at six months of age. When the same mutation was detected in the 21-year-old uncle of this patient, who was receiving insulin treatment, his treatment was changed to sulfonylurea (SU). The other patient presented with severe DKA at 5 months of age. In the genetic analysis, a homozygous mutation was detected in the lipopolysaccharide-responsive beige-like anchor (LRBA) gene. Immunodeficiency was not observed in the patient who was receiving insulin treatment and had autoantibody negativity.

A c.214G>A (p.Gly72Arg) heterozygous mutation was detected in the GCK gene in a 23-month-old patient presented with hyperglycemia, who had a family history of diabetes in his mother, grandmother, and aunts. Low-dose insulin treatment was initiated in the patient whose hyperglycemia continued despite the diet. Plasma glucose regulation was achieved with insulin treatment. In the genetic analysis of a 13-year-old girl who presented with fasting hyperglycemia, c.661G>A (p.E221K) heterozygous mutation was detected in the GCK gene. In this patient, blood sugar was regulated simply by adjusting the diet. In the genetic analysis performed on a patient who presented with hyperglycemia and obesity, and had a family history of diabetes and autoantibody negativity, c.1776dup (p.Val593Argfs*6) heterozygous mutation was detected in the CEL (carboxyl ester lipase) gene. Treatment was initiated in the patient who required insulin.

Discussion

Type 1 diabetes is the most common type of diabetes in childhood. According to 2021 data from the International Diabetes Federation (IDF), it is estimated that 108.200 children and adolescents under the age of 15 are diagnosed with T1DM every year [13]. It is stated that T1DM has two peaks, at ages 5-7 and during puberty. It is thought that the first peak occurs due to infections during the school starting period, and the second peak occurs due to the increase in insulin-counteracting hormones during puberty [14]. In our study, it was observed that T1DM peaked in the 5-9 and 10-14 age groups, and this result was consistent with previous studies conducted in Türkiye [3, 5, 15].

Some patients cannot be clearly classified at the time of diagnosis. Accurate identification of the type of diabetes can help determine treatment approaches [1]. The presence of autoantibodies against pancreatic islet cells is used as the best diagnostic tool for T1DM [1, 16]. In our T1DM series consisting of 131 cases, the rate of autoantibody positivity at the time of diagnosis was found as 74.1%. In a study conducted in China, the rate of autoantibody positivity at the time of diagnosis was reported as 61.01% in patients with T1DM [17]. With the increase in obesity in the general population, patients with autoimmune T1DM may present with overweight/obesity as well. Detection of autoimmune markers is useful in the diagnosis of T1DM, especially in patients, in whom the distinction is not clear, such as obese adolescents. However, studies show that autoantibody positivity may be present in 10-20% of the patients who have been clinically diagnosed with T2DM [7].

It has been stated that approximately 10% of children and adolescents with T1DM do not have autoimmunity [16, 18]. 25.9% of our patients with T1DM were autoantibody-negative. In a study, genes responsible for MD were identified in 10.5% of patients requiring insulin therapy and exhibiting no autoantibodies [19]. Monogenic diabetes may be one of the reasons for the high number of autoantibody-negative patients in our society, where the rate of parental consanguinity is high. However, genetic analysis could not be performed in all of these patients.

The risk of developing T1DM is higher among relatives of patients with T1DM than in the general population. The incidence rate of diabetes in the sibling of a child with T1DM is between 6-7% [1]. A total of 20.6% of patients with T1DM had a family history of T1DM, which is consistent with the findings of previous studies [20]. The aetiology of T1DM is influenced by a number of factors, including environmental factors and viral infections. In our study, similar to previous studies [21, 22], it was observed that the patients presented predominantly in the autumn/winter seasons, when viral infections were more common (64.9%). In some publications, male predominance has been reported in T1DM cases diagnosed during adolescence and later. In our T1DM cases, there was a male predominance over the age of 10 [23].

Diabetic ketoacidosis is a life-threatening complication of diabetes. Morbidity and death may occur during the follow-up of patients. It has been observed that the prevalence of DKA is lower in regions where socioeconomic status is higher and better health services are provided. In a study conducted in 13 countries, the prevalence of DKA at diagnosis was reported as 29.9% [24]. The frequency of DKA was reported at varying rates in North America and Europe (15-70%) [12]. In the SEARCH study conducted in 2021, it was found that the prevalence of DKA had increased over the years [25]. In our study, the frequency of DKA in patients with T1DM was found to be 61.8%. In a recent study conducted in the neighboring province of Diyarbakır, the frequency of DKA was found high (64.9%) [26]. The previously reported rates in our country were found to be lower [3, 27, 28]. The high rate of DKA in our region was attributed to the late presentation/diagnosis of the patients. In addition, since the starting year of the study overlapped with the COVID-19 pandemic, cases may have presented with DKA more frequently. Early diagnosis of diabetes and reducing the frequency of DKA through effective education will reduce the incidence of fatal complications such as cerebral edema.

Type 1 diabetes is frequently accompanied by other autoimmune diseases in children. Studies have reported thyroid autoantibody positivity in approximately one-quarter of patients with

TIDM [29]. In our study, autoantibody positivity was detected in 6.1% of the patients, and all of these patients were euthyroid. The incidence of celiac disease in patients with T1DM has been reported to vary between 1-10% [30]. In a study conducted in our country, positive celiac serology was found in 15.4% of the patients and spontaneous recovery occurred in 23.3% of these patients [30]. In our study, positive celiac serology was detected in 8.3% of the patients, the frequency of spontaneous normalization (27.2%) and biopsy-confirmed celiac disease was found in 6.1% of all patients with T1DM; these results were consistent with the literature [30].

The incidence of T2DM in children and adolescents has increased significantly in the last 20 years in parallel with obesity. It can be observed in all racial/ethnic groups. However, higher incidence rates have been reported in the USA, Canada, Australia, East/South Asia, and the Middle East [7]. T2DM is more common in the 10-19 age group and girls due to hormonal changes that occur during adolescence [31]. Studies have shown that β-cell function decreases by an average of 20-35% per year in cases of T2DM that develop at a young age. These data show substantially higher rates than the 7–11% annual decline rates in β-cell function that have been reported in adults with T2DM [7, 32]. In children with T2DM, β-cell function deteriorates faster than in adults, therefore complications occur earlier [33]. Similar to the literature, the majority of our patients with T2DM were female and were over the age of 10 years at the time of diagnosis [31]. Our patients had obesity, insulin resistance, acanthosis nigricans, and a family history of T2DM. No pathology was found in the screening for microvascular complications.

Two of our patients with monogenic diabetes had neonatal diabetes and the other three had MODY. The patient, who was diagnosed with diabetes in the neonatal period and had a heterozygous mutation in the ABCC8 gene, had TNDM. Oral treatments such as SU can be initiated in patients with KATP mutations. Since the genetic analysis result of our patient was delayed and the patient was in remission by the time the result was received, SU treatment could not be initiated. The follow-up of the patient in remission is continuing. SU treatment was beneficial in the uncle of the patient, who had the same mutation. Homozygous LRBA mutation was detected in the patient who presented with severe DKA at 5 months of age. LRBA deficiency can be accompanied by endocrine, hematological, autoimmune diseases and recurrent infections. No immunological abnormality has been observed in our patient, who is still receiving insulin treatment and being followed up in our clinic. However, the patient had a sibling who was diagnosed with diabetes in the neonatal period and died due to sepsis at the age of 5. The death was possibly associated with immunodeficiency, but there was no genetic diagnosis in this patient.

Heterozygous inactivating mutations in the GCK gene are a common cause of MODY. They cause slightly elevated HbA1c levels and a stable fasting hyperglycemia that does not require treatment. Patients requiring pharmacological treatment have been reported in the literature [34, 35]. Two of our patients with MODY had GCK-MODY. Plasma glucose regulation was achieved in one of the patients with diet only and with low-dose insulin treatment in the other patient. The mother of the patient who was receiving insulin treatment also had diabetes and was receiving insulin treatment, however, genetic analysis could not be performed in the mother. In patients with CEL-MODY, there is exocrine pancreatic dysfunction, which usually occurs in childhood. This is followed by diabetes in adulthood [36]. Our patient with CEL-MODY presented with hyperglycemia. Insulin treatment was initiated in the patient whose exocrine pancreas functions were normal. His mother also had diabetes, but genetic analysis could not be performed. The patient, in whom plasma glucose has been regulated with insulin treatment, is still being followed up in our clinic.

The small number of cases was a limitation of our study. Another limitation was that genetic analysis could not be performed in all cases considered to have monogenic diabetes.

In conclusion, although the majority of childhood diabetes cases are T1DM, the frequency of T2DM tends to increase, especially in obese adolescents. However, it should be kept in mind that autoantibody-positive patients with

T1DM may also have obesity. It was determined that T1DM cases were more common in the winter season, in the 10-14 age group, and the prevalence of DKA was high. Early diagnosis and treatment can reduce the risk of childhood complications such as DKA and cerebral edema. As observed in our cohort, which consisted of a small number of children and adolescents with diabetes, the presence of monogenic diabetes, the rate of which reaches up to 4%, should not be overlooked. It should always be kept in mind that the types of diabetes diagnosed in childhood and adolescence may be different. It should be taken into consideration that different types of diabetes cases may require different treatment options and therefore, different parameters may need to be scanned during follow-up.

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Authors contributions: A.A.K. and M.N.O. constructed the main idea and hypothesis of the study. A.A.K. and R.Y. developed the theory and arranged/edited the material and method section. A.A.K. and G.T. have evaluated the data in the results section. The discussion section of the article was written by A.A.K., A.A.K., and M.N.O. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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

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Research Article

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Evaluation of infarct types and related cerebral vessels in the presence of different risk factors by three-dimensional (3D) imaging methods in patients with ischemia

İskemi hastalarında farklı risk faktörlerinin varlığında enfarktüs tipleri ve ilişkili beyin damarlarının üç boyutlu (3D) görüntüleme yöntemleri ile değerlendirilmesi

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Abstract

Purpose: The factors that predispose to stroke are defined as risk factors. The subtypes of stroke can be classified by considering the changeable risk factor and its relationship with stroke. The aim of this study is to examine the infarction types and related brain vessels in the presence of different risk factors in ischemia patients by rendering three-dimensional (3D) cross-sectional ischemic damaged brain regions on magnetic resonance (MR) and computed tomography (CT) images obtained from patients.

Material and methods: 105 patients (53 male, 52 female) with ischemia and 50 normal (23 male, 27 female) members of control group were participated in the study. A number of cross-sectional images (transverse, sagittal and coronal sectiones) were reconstructed on computer. Infarct types were classified as atherosclerotic, cardioembolic, lacunar, cryptogenic and transient ischemic attacks. The infarct size was determined in infarct types in patients with hypertension, diabetes, smoker and coronary artery disease. The arteries that irrigate the infarct area were classified. The most infarcted arteries and the largest infarcted arteries were evaluated statistically.

Results: Infarct types were anatomically correlated with their infarct size and the arteries causing infarction. Thus, which arteries cause which types of infarcts in which risk factors are described firstly in the literature.

Conclusion: The infarct size in the brain is of great clinical importance. It has been observed that clinical findings become more pronounced as infarct size increases. This situation varies according to the localization of ischemia. The risk factors and types of infarcts vary in men and women. These results are thought to be the basis for explaining the mechanisms of clinical findings.

Keywords: Brain, image, 3D, infarct type, risk factor.

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Öz

Amaç: İnmeye zemin hazırlayan faktörler risk faktörleri olarak tanımlanmaktadır. İnmenin alt tipleri, değişken risk faktörü ve bunun inme ile ilişkisi dikkate alınarak sınıflandırılabilir. Bu çalışmanın amacı iskemi hastalarında farklı risk faktörlerinin varlığında enfarktüs tipleri ve ilgili beyin damarlarının, hastalardan elde edilen manyetik rezonans (MR) ve bilgisayarlı tomografi (BT) görüntüleri üzerinde kesitsel iskemik hasarlı beyin bölgelerinin üç boyutlu (3D) hale getirilerek incelenmesidir.

Gereç ve yöntem: Çalışmaya 105 iskemi hastası (53 erkek, 52 kadın) ve 50 normal (23 erkek, 27 kadın) kontrol grubu üyesi katıldı. Bir dizi kesitsel görüntü (enine, sagittal ve koronal kesitler) bilgisayarda yeniden oluşturuldu. Enfarktüs tipleri aterosklerotik, kardiyoembolik, laküner, kriptojenik ve geçici iskemik ataklar olarak sınıflandırıldı. Hipertansiyon, diyabet, sigara içen ve koroner arter hastalığı olan hastalarda enfarktüs tipine göre enfarktüs boyutu belirlendi. Enfarktüs alanını sulayan arterler sınıflandırıldı. En çok enfarktüslü arterler ve en büyük enfarktüslü arterler istatistiksel olarak değerlendirildi.

Bulgular: Enfarktüs tipleri anatomik olarak enfarkt boyutu ve enfarktüse neden olan arterlerle ilişkiliydi. Böylece literatürde ilk olarak hangi arterlerin hangi tip enfarktüslere, hangi risk faktörlerine neden olduğu anlatılmaktadır.

Sonuç: Beyindeki enfarktüs büyüklüğü klinik açıdan büyük önem taşımaktadır. Enfarktüs boyutu arttıkça klinik bulguların daha belirgin hale geldiği gözlenmiştir. Bu durum iskemi lokalizasyonuna göre değişmektedir. Risk faktörleri ve enfarktüs türleri kadın ve erkeklerde farklılık gösterir. Bu sonuçların klinik bulguların mekanizmalarını açıklamada temel oluşturacağı düşünülmektedir.

Anahtar kelimeler: Beyin, görüntüleme, 3B, enfarktüs tipi, risk faktörü.

Sağtaş E, Özdemir MB. İskemi hastalarında farklı risk faktörlerinin varlığında enfarktüs tipleri ve ilişkili beyin damarlarının üç boyutlu (3D) görüntüleme yöntemleri ile değerlendirilmesi. Pam Tıp Derg 2025;18:508-522.

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Introduction

Stroke includes all diseases in which the vessels supplying the brain are directly affected by a pathological process and subsequently caused by a temporary or permanent involvement of a part of the brain due to ischemia or bleeding [1, 2]. The infarct in any part of the brain causes insufficiency in the functions of the human body depending on the regional anatomical and physiological effects of the brain. Stroke with sudden onset of nonconvulsive focal neurological deficits is the most common form of stroke [2]. Thrombotic or embolic vascular occlusion resulting from the passage of a clot, plaque, or agglutinated platelets into the circulation, or systemic reduction in blood flow such as cardiac arrest or shock, leads to ischemia, particularly in the delicate border regions between the main cerebral blood vessels, such as the middle cerebral and posterior cerebral arteries.

Ischemic strokes are manifested by different neurological symptoms depending on the function of the vessel disturbed by blood flow and the brain area irrigated by this vessel. It is possible to identify infarct subtypes reflecting the location and width of the infarct by assessing basic neurological findings and thus predict prognosis [3].

A detailed examination of the neurological findings in these diseases with advanced methods will allow the discovery of new information not only related to the mechanism of the diseases but also to the anatomical and physiological functions of the brain. 3D anatomical imaging is widely used in medicine today. Firstly, the computer-aided 3D program “Surf-Driver”, developed by anatomist Scott Lazonof, has found wide usage. The body of a male prisoner in death was divided into 1 mm sections as cadavers and photographs were transferred to the computer with the Visible Human Project, developed in 1986, and the 3D reconstructions were made in the Surf-Driver program for the first time [4]. These kinds of works were later made in Korea and China. Nowadays, computer-aided 3D programs have developed with computer technology. Osirix is one of these programs [5]. Nowadays, real-time simulations are performed with 3D methods, and physicians develop difficult operations by practicing in 3D environments. At the same time, radiologists can easily identify pathologies that they have difficulty understanding in two-dimensional (2D) images in a 3D environment. 3D methods are also used in medical education. Imaginary dissection is possible with the 3D method. Thus, it was possible to be easier to understand by making them 3D. The patients with ischemia were generally evaluated by 2D radiological imaging. The areas of ischemia with 3D have not been previously studied with clinical findings. Our aim is to examine the damage of patients with ischemia in 3D and provide the basis for clinical studies.

Material and method

A total of 105 patients with ischemia (53 male and 52 female) and 50 normal control patients (23 male and 27 female) were included in the study (Table 1). The ages of males and females were compared. 2D MR images of the patient with ischemia were obtained (Figure 1). Cross-sectional images were reconstructed on a computer (Figure 2-4). Computer-assisted (Apple-Mac) 3D program Osirix was used for this three-dimensional (3D) examination. The infarct fields were identified. The volumes of infarct dimensions were calculated automatically. The cross-sectional calculations were made with the Cavalieri method at the same time. The total number of points was calculated in these two-dimensional and three-dimensional calculations and compared with each other.

Table 1. Number of patients

	No	Percent
Patient (M)	53	34.2
Patient (F)	52	33.5
Control (M)	23	14.8
Control (F)	27	17.4
Total	155	100.0

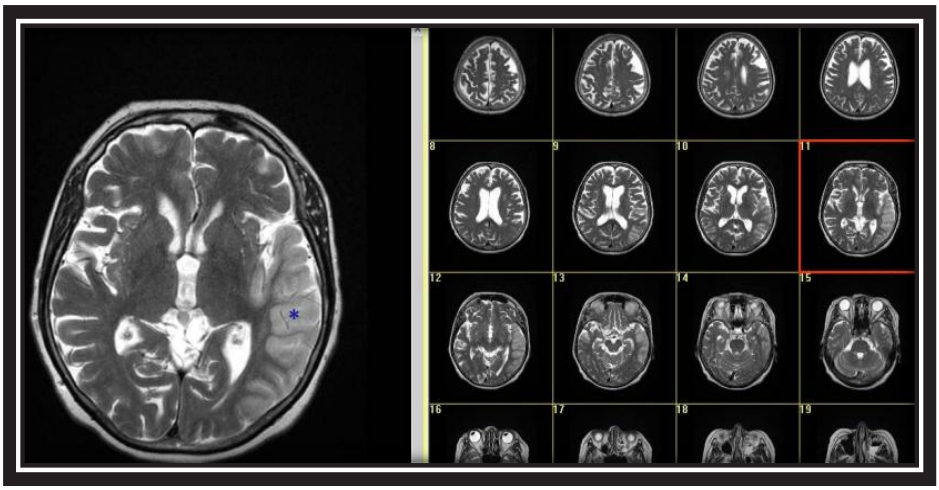


Figure 1. MR images of a patient with ischemia

A large infarct area (*) is shown in the right hemisphere. Transverse serial section of the patient is located on the right side These sections were transferred to computer and used for 3D images

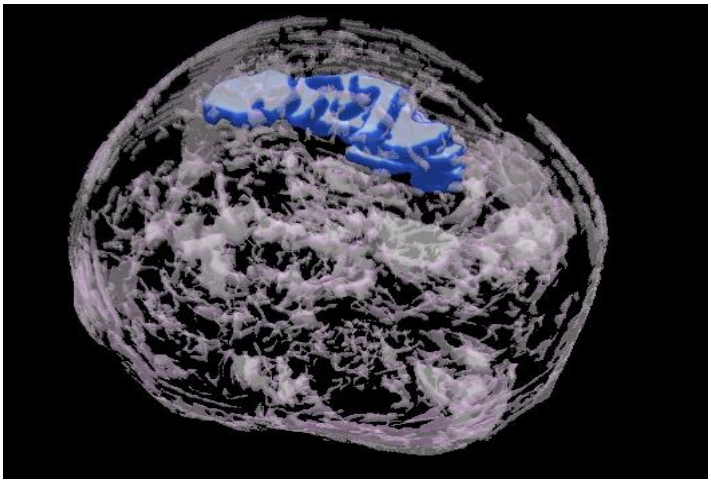


Figure 2. The infarct area is shown in blue in 3D

The relationship between the infarct area and brain gray matter (neuron bodies) is shown (top view)

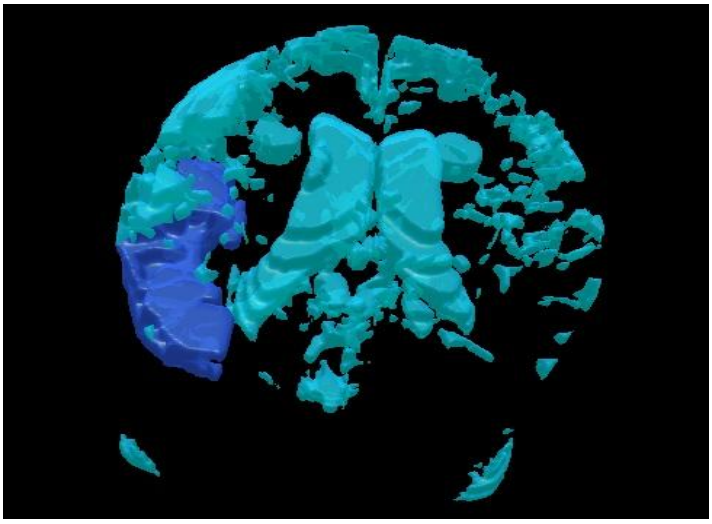


Figure 3. The infarct area is shown in blue in 3D

The relationship between the infarct area and CSF circulation is shown (anterior view)

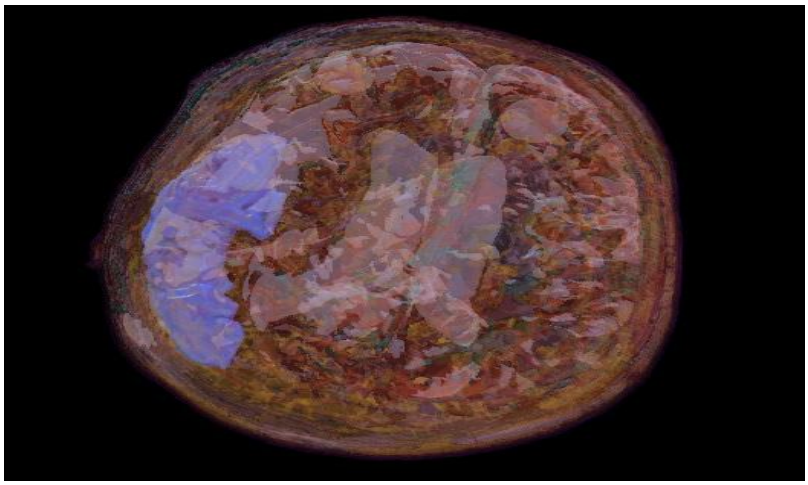


Figure 4. The infarct area is shown in blue in 3D

The relationship between the infarct area and all brain structures is shown (top view)

The patients were classified according to the presence of risk factors such as hypertension, diabetes mellitus, smoking, and coronary artery disease. The infarct types were classified as atherosclerotic, cardioembolic, lacunar, cryptogenic, and transient ischemic attacks. The effects of these personal characteristics on infarct type and size were evaluated. The infarct size was determined in infarct types in patients with hypertension, diabetes, smoking, and coronary artery disease.

The arteries irrigating the infarct area were identified. The deep arteries that descended into the deeper part of the cerebrum and irrigated the capsular interna and basal nuclei were called “central arteries”. Multiple arteries were responsible for multiple infarct areas and large infarct areas. The responsible artery was evaluated as “normal” in patients with neurological findings, but no infarction was detected on imaging tools.

The vessels with the highest infarct and the largest infarct were evaluated statistically. The infarct-forming multiple arteries were not examined. Only one responsible artery was looked at.

Two-dimensional (2D) follow-up and three-dimensional (3D) follow-up were correlated with infarct volumes. SPSS 17.0 program was used for statistical calculations. Independent Sample t-test were applied to compare the means. Pearson test was used for correlations. Pearson correlation coefficient was given as “r” with stars. $p<0.05$ was evaluated as statistically significant.

The data of the patients consists of Pamukkale University Faculty of Medicine archives between 2016 and 2019.

Results

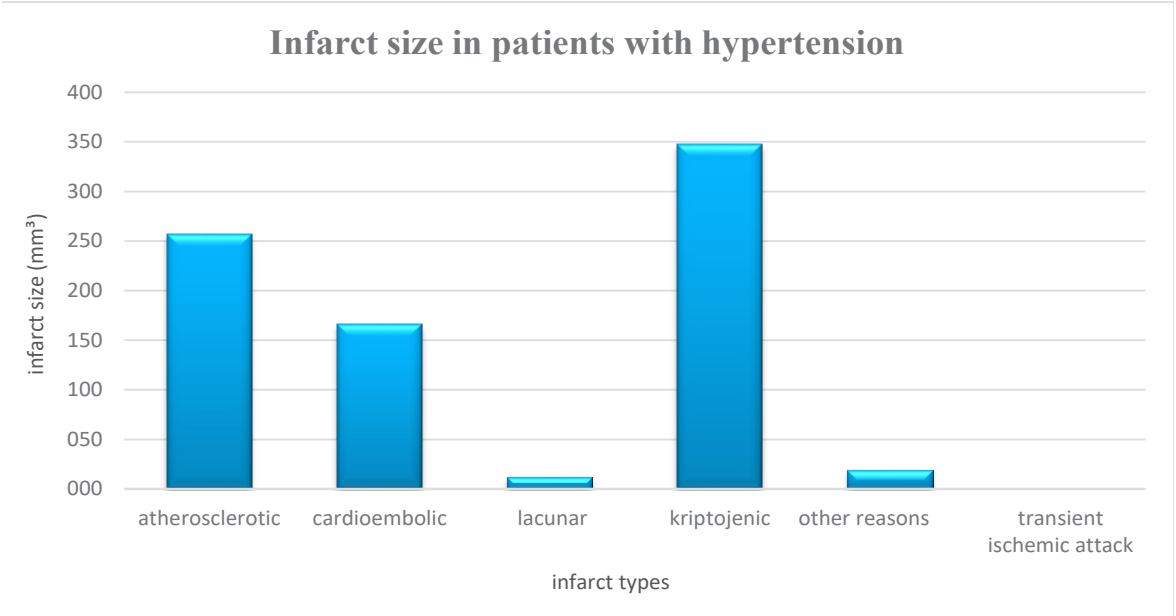
53 male and 52 female patients with ischemia were studied. 23 male and 27 female normal individuals were used for the control group. There were sufficient patient and control groups, and the distribution of male and female was very close to each other (Table 1). There was no statistically significant difference between the control and patient groups ($p=0.89$). The mean age of the patients was 66.26±15.12. The minimum age was 19, and the maximum age was 89. There was no statistically significant difference between men and women in terms of age ($p=0.67$).

The infarct types of patients were determined. We investigated whether hypertension, diabetes, smoking, and coronary artery diseases were present in the patients. Infarct types and hypertension, diabetes, smoking, and coronary artery diseases were compared in terms of infarct size. In terms of hypertension, the largest infarct area was detected in the cryptogenic infarct type. The mean infarct size was 257.03±263.98 mm^3. The second size of infarction area was in atherosclerotic type. The cardioembolic type was following them. Lacunar infarcts had a very small size (Table 2, Graph 1). Table 2 shows the infarct dimensions of infarct types in patients with and without hypertension.

Table 2. Comparison of infarct size and infarct types in patients with hypertension

Hypertension	Infarct Type	Mean (mm³)	N	SD*
	Atherosclerotic	257.03	18	263.98
	Cardioembolic	166.15	35	304.77
	Lacunar	12.00	11	9.19
Exist	**Kriptojenic**	347.22	1	.
	Other reasons	18.94	1	.
	Transient isch.attack	0.00	8	0.00
	Total	147.84	74	261.82
	Atherosclerotic	463.02	12	726.22
	Cardioembolic	308.61	11	360.43
	Lacunar	6.49	3	2.77
Absence	**Kriptojenic**	52.08	2	24.55
	Other reasons	192.55	1	.
	Transient isch.attack	0.00	2	0,00
	Total	298.94	31	516.19
	Atherosclerotic	339.43	30	501.43
	Cardioembolic	200.21	46	320.66
	Lacunar	10.82	14	8.46
Total	**Kriptojenic**	150.46	3	171.28
	Other reasons	105.75	2	122.76
	Transient isch.attack	0.00	10	0.00
	Total	192.45	105	360.24

*SD: Standart Deviation



Graph 1. Infarct size in patients with hypertension

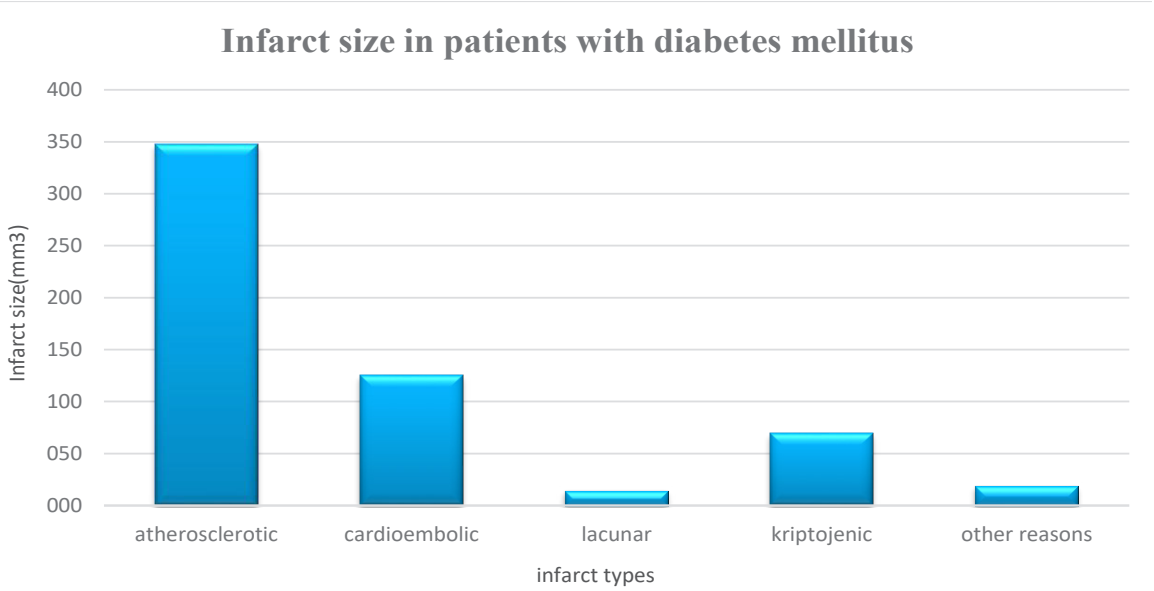
The largest infarct area in atherosclerotic infarct type was determined in terms of diabetes. The mean infarct size was found to be 347.70±316.24 mm³. The second most common cause of infarction in these patients was the cardioembolic type, followed by the lacunar type. Cryptogenic infarcts were very small in size (Table 3, Graph 2). Table 3 shows the infarct size of infarct types in patients with and without diabetes.

In terms of smoking, the largest infarct area was detected in atherosclerotic infarct type. The mean infarct size was 535.26±706.97 mm³. The second common cause of large infarction was cardioembolic type. The lacunar type was following them. The cryptogenic type of infarcts were very small in size (Table 4, Graph 3). Table 4 shows the infarct dimensions of infarct types in smokers and nonsmokers.

Table 3. Comparison of infarct size and infarct types in patients with diabetes mellitus

Diabetes Mellitus	Infarct Type	Mean (mm³)	N	SD*
Exist	**Atherosclerotic**	347.70	10	316.24
	Cardioembolic	125.38	16	197.04
	Lacunar	14.21	2	6.70
	Kriptojenic	69.44	1	.
	Other reasons	18.94	1	.
	Total	186.66	30	256.18
Absence	**Atherosclerotic**	335.29	20	579.95
	Cardioembolic	240.13	30	367.06
	Lacunar	10.25	12	8.84
	Kriptojenic	190.97	2	220.97
	Other reasons	192.55	1	.
	Transient isch.attack	0.00	10	0.00
	Total	194.76	75	395.79
Total	**Atherosclerotic**	339.43	30	501.43
	Cardioembolic	200.21	46	320.66
	Lacunar	10.82	14	8.46
	Kriptojenic	150.46	3	171.28
	Other reasons	105.75	2	122.76
	Transient isch.attack	0.00	10	0.00
	Total	192.45	105	360.24

*SD: Standart Deviation

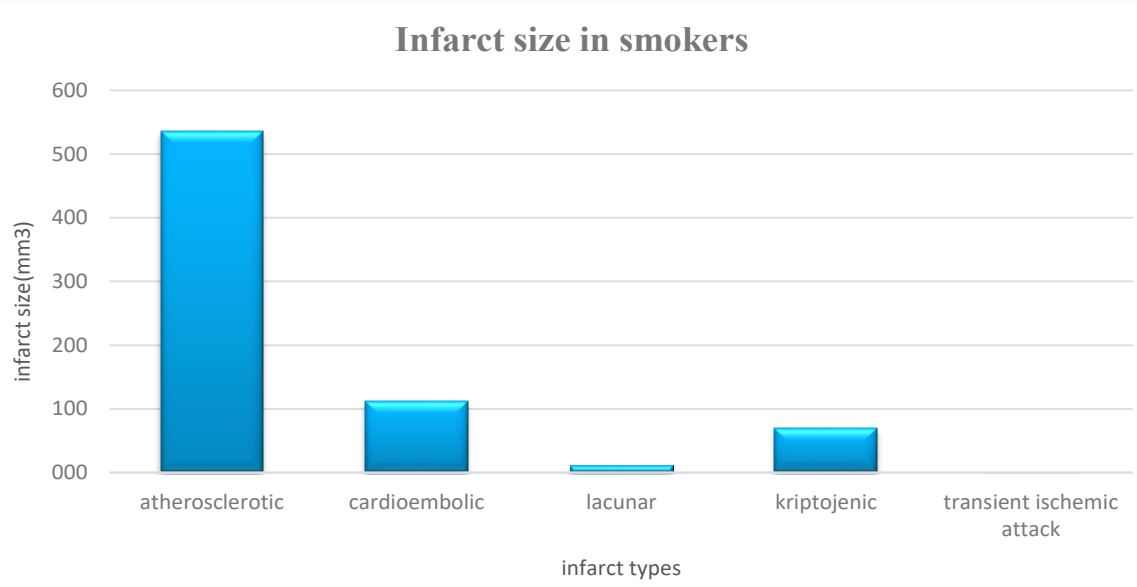


Graph 2. Infarct size in patients with diabetes mellitus

Table 4. Comparison of infarct size and infarct types in patients with smokers

Smoke	Infarct Type	Mean (mm^3)	N	SD*
Exist	**Atherosclerotic**	535.26	13	706.97
	Cardioembolic	111.36	12	183.18
	Lacunar	11.14	6	12.20
	Kriptojenic	69.44	1	.
	Transient isch. attack	0.00	2	0.00
	Total	247.97	34	497.14
Absence	**Atherosclerotic**	189.67	17	160.85
	Cardioembolic	231.58	34	353.76
	Lacunar	10.58	8	5.15
	Kriptojenic	190.97	2	220.97
	Other reasons	105.75	2	122.76
	Transient isch.attack	0.00	8	0.00
	Total	165.86	71	272.18
Total	**Atherosclerotic**	339.43	30	501.43
	Cardioembolic	200.21	46	320.66
	Lacunar	10.82	14	8.46
	Kriptojenic	150.46	3	171.28
	Other reasons	105.75	2	122.76
	Transient isch.attack	0.00	10	0.00
	Total	192.45	105	360.24

*SD: Standart Deviation



Graph 3. Infarct size in smokers

In terms of coronary artery disease, the largest infarct area was determined to be atherosclerotic infarct type. The mean infarct size was 306.81±293.13 mm³. The type that caused the second-degree infarction was cardioembolic. Lacunar infarcts had very small size (Table 5, Graph 4). Table 5 shows the infarct dimensions of infarct types in patients with and without coronary artery disease.

In terms of gender, the atherosclerotic infarct type was the largest infarct in men, while cardioembolic infarct was the largest infarct type in women. The mean size of an atherosclerotic infarction was 437.63±587.28 mm³ in men and 257.11±386.81 mm³ in women. The second common cause of infarction in men was cardioembolic type followed by lacunar infarction. Cryptogenic type infarcts had a very small size. The second most common cause of infarction in women was cryptogenic, followed by the atherosclerotic type. Lacunar infarcts had very small size (Table 6, Graph 5). The infarct size of the male and female infarct types was statistically different (*p*=0.01). However, there was no statistically significant difference found between male and female (*p*=0.93). Sick male and sick female infarct types and sizes are shown in Table 6.

The most common artery causing the cerebral infarction is the left middle cerebral artery (20%). This was followed by the right middle cerebral artery (13%). The central branches of the left middle cerebral artery (11%) and basilar artery (8%) were listed as other causative arteries (Table 6, Graph 6). In addition, 18 cases with arteries causing multiple infarcts were detected in our study (18%) (Table 7). The distribution of all vessels causing infarction is shown in Table 7.

The artery causing the most infarction is the left middle cerebral artery, while the infarct size of the artery causing the largest volume is the right middle cerebral artery. The mean size was 445.77±350.31 mm³ (Table 7, Graph 7). The distribution of infarct sizes by vessels is shown in Table 7.

In the evaluation of the data, the following findings were obtained regarding statistical analysis:

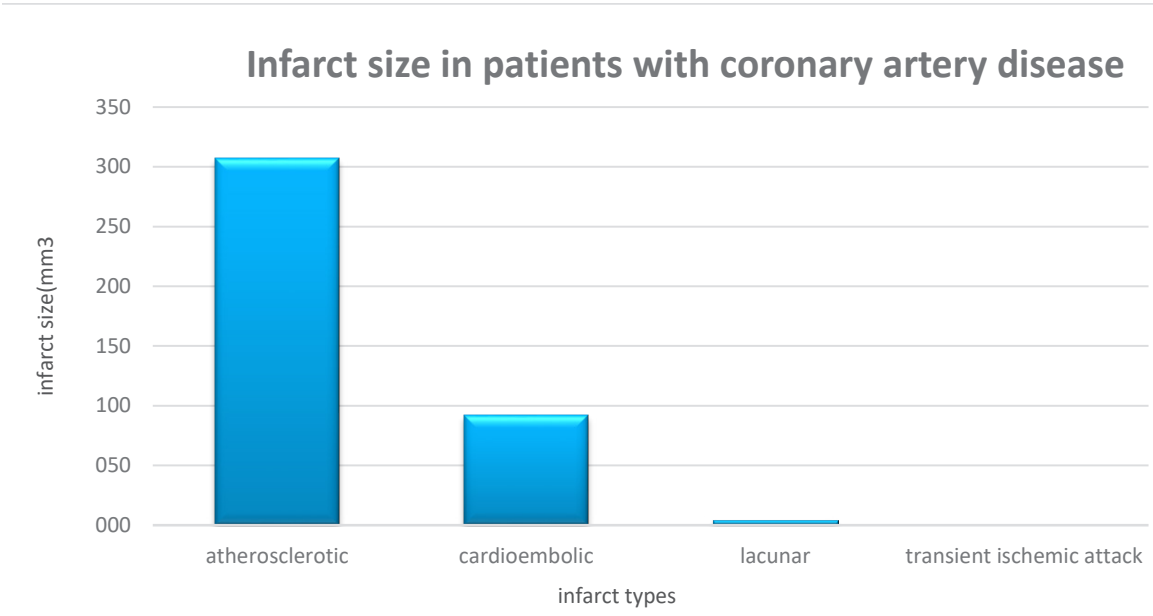
The correlation between two-dimensional (2D) follow-up and infarct area was statistically significant (*p*=0.01, r=0.992*).

The correlation between three-dimensional (3D) follow-up and infarct volume was statistically significant (*p*=0.01, r=0.950*).

Table 5. Comparison of infarct size and infarct types in patients with coronary artery diseases

Coronary Artery D.	Infarct Type	Mean (mm³)	N	SD*
Exist	Atherosclerotic	306.81	6	293.13
	Cardioembolic	92.11	11	126.06
	Lacunar	4.00	1	.
	Transient isch.attack	0.00	2	0.00
	Total	142.90	20	210.04
Absence	Atherosclerotic	347.58	24	545.89
	Cardioembolic	234.19	35	355.59
	Lacunar	11.34	13	8.57
	Kriptojenic	150.46	3	171.28
	Other reasons	105.75	2	122.76
	Transient isch.attack	0.00	8	0.00
	Total	204.10	85	387.26
Total	Atherosclerotic	339.43	30	501.43
	Cardioembolic	200.21	46	320.66
	Lacunar	10.82	14	8.46
	Kriptojenic	150.46	3	171.28
	Other reasons	105.75	2	122.76
	Transient isch.attack	0.00	10	0.00
	Total	192.45	105	360.24

*SD: Standart Deviation

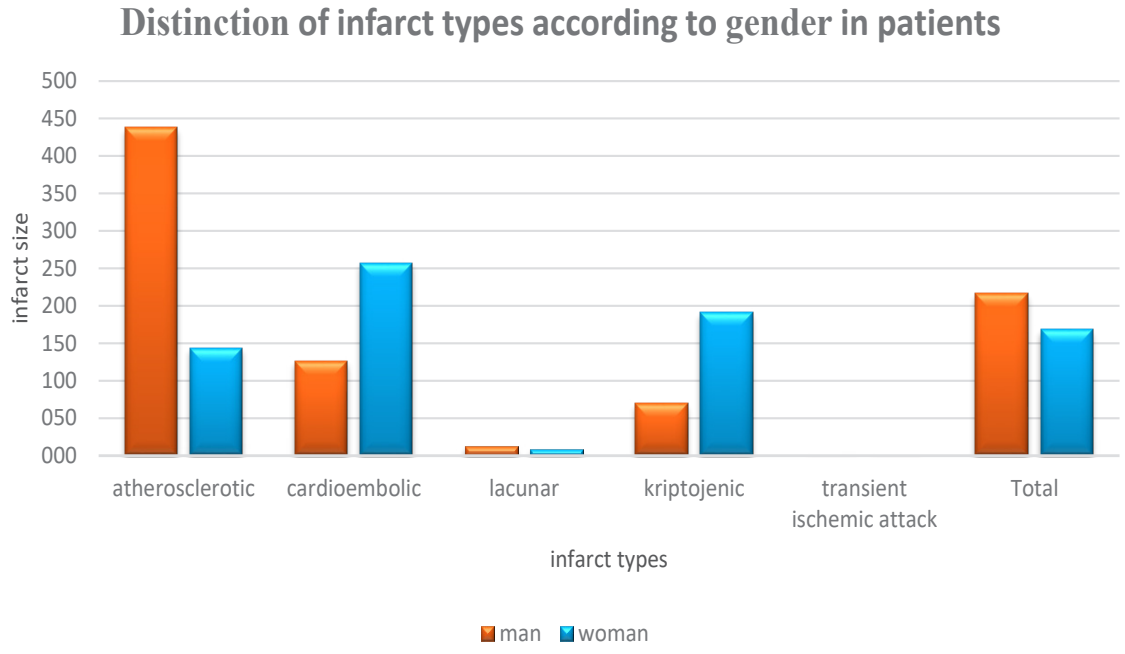


Graph 4. Infarct size in patients with coronary artery disease

Table 6. Comparison of infarct size and infarct types in patients with gender

Sex	Infarct Type	Mean (mm³)	N	SD*
Patient Male	Atherosclerotic	437.63	20	587.28
	Cardioembolic	126.25	20	190.97
	Lacunar	12.08	9	10.42
	Kriptojenic	69.44	1	.
	Transient isch.attack	0.00	3	0.00
	Total	216.14	53	414.31
Patient female	Atherosclerotic	143.02	10	133.24
	Cardioembolic	257.11	26	386.81
	Lacunar	8.55	5	2.33
	Kriptojenic	190.97	2	220.97
	Other reasons	105.75	2	122.76
	Transient isch.attack	0.00	7	0.00
	Total	168.29	52	297.39

*SD: Standart Deviation

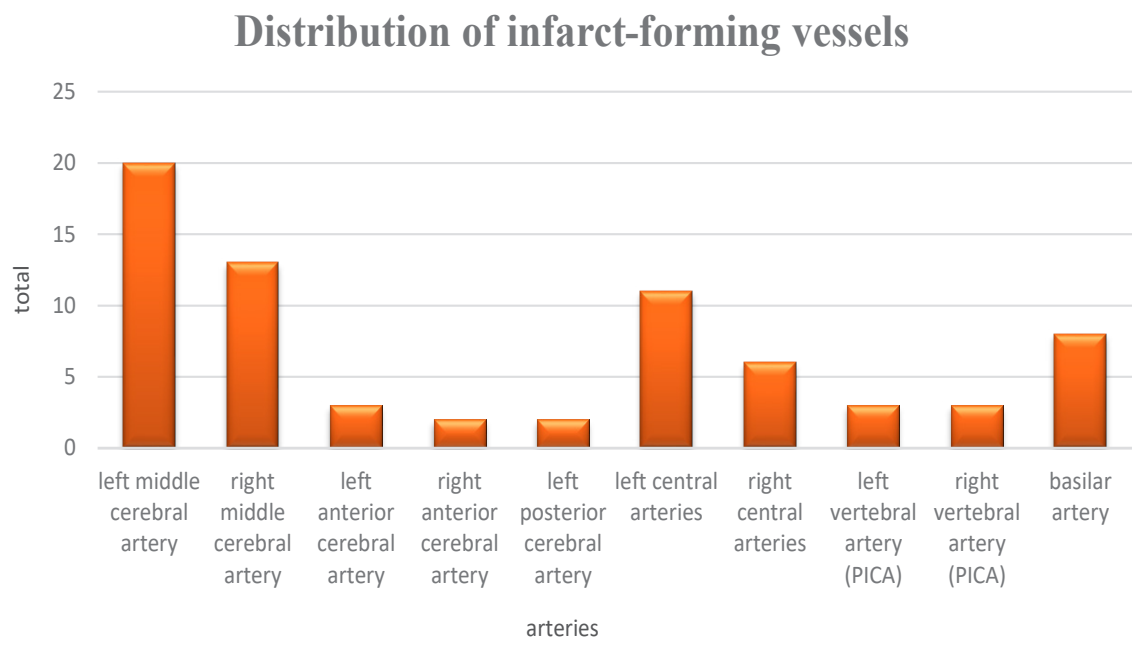


Graph 5. Distinction of infarct types according to gender in patients

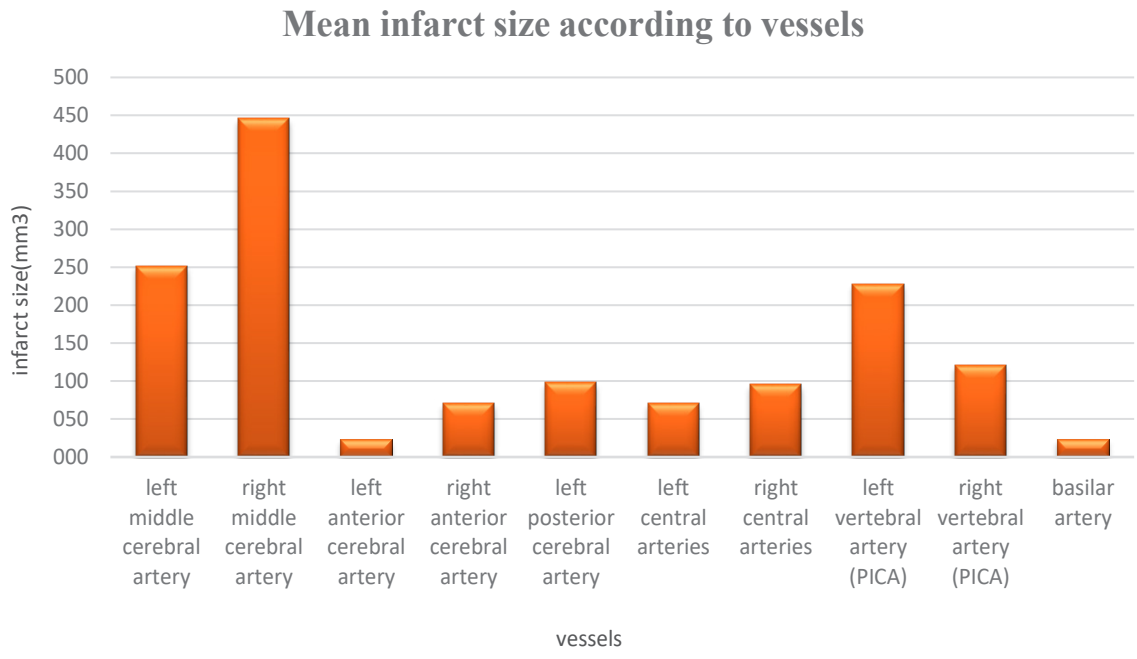
Table 7. Frequency of vessel retention and relation with infarct size

VESSELS	Mean (mm^3)	N	SD*
Normal	1.00	10	1.63
Left middle cerebral artery	252.03	20	409.10
Right middle cerebral artery	445.77	13	350.31
Left anterior cerebral artery	23.15	3	4.82
Right anterior cerebral artery	71.03	2	87.05
Left posterior cerebral artery	98.24	2	98.75
Left central arteries	70.70	11	126.46
Right central arteries	95.75	6	88.24
Left vertebral artery (PICA)	228.33	3	204.14
Right vertebral artery (PICA)	120.49	3	91.33
Basilar artery	22.92	9	18.24
Left middle cerebral artery and left posterior cerebral artery	64.69	3	64.76
Right middle cerebral artery and right posterior cerebral artery	299.87	1	.
Left middle cerebral artery and basilar artery	114.36	4	41.19
Left middle cerebral artery and left basilar artery	655.85	6	1123.93
Left posterior cerebral artery and left basilar artery	344.07	1	.
Right vertebral artery (PICA) and basilar artery	154.68	1	.
Middle cerebral artery and right posterior cerebral artery	205.47	4	79.93
Right posterior cerebral artery and right central artery and right vertebral artery (pıca)	34.72	1	.
Left middle cerebral artery and left central artery and right central artery	239.91	1	.
Right middle cerebral artery and right central artery	202.02	1	.
Total	195.50	105	367.37

*SD: Standart Deviation



Graph 6. Distribution of infarct-forming vessels



Graph 7. Mean infarct size according to vessels

Discussion

Factors that predispose to stroke are defined as risk factors. The subtypes of stroke can be classified by considering the change in risk factor and its relationship with stroke. The aim of our study was to relate these factors to the anatomical features of the brain and vessels. In our study, we performed the stroke types according to the most common ones with the suggestion of the Neurology Department of Pamukkale University Faculty of Medicine. Stroke was divided into subtypes. These were divided into atherosclerotic, cardioembolic, lacunar, cryptogenic, and transient ischemic attacks in our study. We correlated infarct types anatomically with infarct size and vessels causing infarct. Thus, we first identified which vessels cause which types of infarcts in which risk factors. Approximately 40% of ischemic strokes do not have a definite etiological cause, and these cases are called cryptogenic strokes [6]. Some of the cryptogenic strokes are undetectable paradoxical emboli (PDE) [7, 8]. The second is an atherosclerotic type. The cardioembolic type is the most common infarct type in patients with hypertension (33%). Atherosclerotic type is the second most common type of infarction in patients with hypertension (20%). Accordingly, we can say that embolic brain damages cause large brain ischemia. Atherosclerotic infarction is the most common cause of stroke in all risk groups. Stroke was less common in females in literature, and this suggests that estrogen has a protective effect against stroke, according to the current literature [9]. But this was not statistically significant in our study. On the other hand, atherosclerotic infarction is statistically significantly less, while cardioembolic and cryptogenic infarct is significantly higher in women. This information proves that the protective effect of direct estrogen is in atherosclerotic infarcts. There are studies in the literature that estrogen prevents atherosclerosis. However, the findings showing the protection of atherosclerotic infarct in this way were first presented in our study.

It has been shown that infarct types vary according to risk groups. In patients with hypertension, cardioembolic infarct appear more frequently, followed by atherosclerotic infarcts. Although the infarct patterns in patients with diabetes mellitus (DM) and coronary artery disease were similar to those with hypertension, cardioembolic infarct were not as common. In contrast, atherosclerotic infarction is the highest in smokers. However, in our study, smoking was found to have a greater impact on atherosclerotic infarctions than the other three major risk factors. The size of the infarcted area caused by infarct types may also vary according to four major risk factors. While the cryptogenic infarctions cause the greatest infarct size in patients with hypertension, the infarct size caused by the atherosclerotic infarct is statistically significant in the other three major types. This suggests that cryptogenic infarcts are more dangerous in patients with hypertension. In such patients, this may be attributed to individual anatomical features of the patient. These may be related to the presence and frequency of anostomoses between these vessels, or structure of the vessel walls. In the light of this information, anatomical characteristics of the person may be determinative in diagnosis and treatment in future studies. In order to do this, computerized neuroscience has started to work. With the development of technology in the last 30 years, human anatomy has been digitalized with computer-aided 3D programs parallel to imaging techniques.

Although the incidence of cardioembolic infarct was higher in DM, atherosclerotic infarct caused the largest infarcted area in DM. Among the risk factors for cerebrovascular diseases, DM is among the most common factors after hypertension [10]. Determination of the size and type of infarct in DM is new information in the literature.

Smoking is another risk factor for infarction. Many large-scale studies investigating stroke risk factors (Framingham, Cardiovascular Health Study, The Honolulu Heart Study) have shown that smoking is a risk factor for ischemic stroke and increases the risk by about two-fold compared to other risk factors [11, 12]. In our study, the most common infarct type caused by smoking was again cardioembolic infarct (12%). Among all infarction types, the atherosclerotic type was associated with the largest infarct volume.

Symptomatic and asymptomatic cardiac diseases have been reported to be strongly associated with cerebrovascular diseases [13, 14]. Myocardial infarction predisposes a risk for

the development of atrial fibrillation and may be a source of cardiogenic embolism. Acute coronary syndrome is rarely associated with stroke [15]. In our study, the most common infarct type in coronary artery disease was cardioembolic. However, atherosclerotic type was the infarct type that caused the largest infarct in volume.

When the risk factors and infarct types were evaluated in terms of infarct size, the most common infarct type was found to be atherosclerotic type in all risk factors except hypertension. In hypertension, this is the cryptogenic type.

One of the important results in our study is that women and men differ in terms of risk factors and infarct types. In terms of atherosclerotic type, infarct was seen in a much higher volume in males and less in females. The life time risk of stroke is considered to be higher in men, regardless of any age group [9, 16]. However, recent studies show that the risk of stroke is increasing in women. According to the studies, the rate of stroke has increased threefold in middle-aged women, while it has remained constant in men. While the lifetime risk of stroke is about 20% in women aged 55-75 years, it is between 14-17% in men [9]. In our study, in terms of infarct size, it was observed that males developed larger infarcts than females. But, this finding is not statistically significant. The lower incidence of stroke in women and the smaller infarct volume in our study compared to men may be attributed to estrogen.

In our study, the arteries that irrigate infarct areas were also studied in detail. The frequency of infarct formation of each artery was determined in this study. Left middle cerebral artery was the most common artery causing infarction (20%). This was followed by right middle cerebral artery.

After middle cerebral artery, the most common bleeding arteries were left central arteries (10%). These were followed by right central arteries (7%).

In our study, the third most common artery in infarcts was the basilar artery. Right and left vertebral artery involvement is 6%. Right and left involvement is equal. In our study, the anterior cerebral arteries were the least responsible arteries in the lesions.

In the literature, infarct sizes caused by vessels were compared in each other in this study. The right cerebral medial artery is the vessel that causes the largest infarct in volume. This was followed by the left cerebral medial artery. The left vertebral artery followed by the right vertebral artery, together with the central arteries, are the most common arteries to cause infarcts.

These results will be the basis for explaining the mechanisms of these clinical findings if they are evaluated in comparison clinical conditions of the patients with the whose images used in this study.

Infarct areas and clinical findings should be evaluated together to determine whether the symptoms and clinical findings are related to anatomic localization or infarct size.

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Research Article

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Anatomical and demographic findings in symptomatic osteochondral lesions of the talus

Semptomatik talus osteokondral lezyonlarında anatomik ve demografik bulgular

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Abstract

Purpose: Talar osteochondral defects involve damage to both the chondral surface and the underlying subchondral bone tissue. The primary etiological factors are thought to be major trauma or repetitive microtrauma. Clinically, patients often report ankle pain, swelling, and restricted joint mobility, particularly after prolonged standing or physical activity. This study aims to examine the demographic characteristics of patients who were diagnosed and treated for talar osteochondral lesions presenting with symptoms at our medical center.

Materials and methods: A retrospective evaluation was conducted on patients diagnosed with osteochondral lesions of the talus, who had been examined and managed by a specialized foot and ankle surgeon within the orthopedic and traumatology department of an university hospital over the past five years. Key parameters recorded included the patients' age, sex, the laterality of the affected ankle (right or left), and the precise anatomical location of the osteochondral lesion, distinguishing between medial and lateral involvement of the talar dome.

Results: This study included a total of 42 patients, with 27 being female and 15 male. The age distribution of the study cohort spanned from 18 to 70 years, with an average age calculated at 46 years. In terms of lesion localization, 36 cases were located on the medial talus, while 6 were found on the lateral aspect. Statistical analysis revealed a significant tendency for osteochondral lesions to occur on the medial side of the talus (p=1.87x10^{-11}). When assessing the affected ankle, 24 cases involved the left ankle, while 18 were in the right ankle. However, there was no statistically significant difference in laterality (p=0.175).

Conclusion: Talar osteochondral defect is a condition that affecting both chondral and subchondral tissue, appear to be more frequently located medially in symptomatic patients and tend to be more common in females.

Keywords: Talus, demographic, osteochondral.

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Öz

Amaç: Talusun osteokondral defektleri hem kıkırdak yüzeyin hem de alttaki subkondral kemik dokusunun hasarını içerir. Başlıca etiyolojik faktörlerin büyük travmalar veya tekrarlayan mikrotravmalar olduğu düşünülmektedir. Klinik olarak hastalar sıklıkla ayak bileği ağrısı, şişlik ve özellikle uzun süre ayakta durma veya fiziksel aktivite sonrasında eklem hareket kısıtlılığı bildirmektedir. Bu çalışma, tıp merkezimizde semptomlarla başvuran ve tanı konularak tedavi edilen talus osteokondral lezyonları bulunan hastaların demografik özelliklerini incelemeyi amaçlamaktadır.

Gereç ve yöntem: Son beş yıl içinde bir üniversite hastanesinin ortopedi ve travmatoloji bölümünde ayak ve ayak bileği cerrahisi konusunda uzmanlaşmış bir cerrah tarafından incelenen ve yönetilen, talusun osteokondral lezyonu tanısı almış hastalar retrospektif olarak değerlendirildi. Kayıt altına alınan temel parametreler arasında hastaların yaşı, cinsiyeti, lezyonun etkilenen ayak bileğinde (sağ veya sol) yerleşimi ve osteokondral lezyonun anatomik konumu (medial veya lateral talus kubbesi) yer aldı.

Bulgular: Çalışmaya toplamda 42 hasta dahil edildi; bunların 27'si kadın, 15'i erkekti. Çalışma grubunun yaş dağılımı 18 ile 70 yıl arasında değişmekte olup, ortalama yaş 46 olarak hesaplandı. Lezyon lokalizasyonu açısından 36 olgu medial talusta, 6 olgu ise lateral talusta bulundu. İstatistiksel analiz, osteokondral lezyonların medial talusta belirgin şekilde daha sık görüldüğünü ortaya koydu (p=1,87x10^{-11}). Etkilenen ayak bileği açısından 24 olgu sol ayak bileğinde, 18 olgu ise sağ ayak bileğinde görüldü. Ancak laterallik açısından istatistiksel olarak anlamlı bir fark bulunmadı (p=0,175).

Sonuç: Talusun osteokondral defekti hem kıkırdak hem de subkondral dokuyu etkileyen bir durum olup, semptomatik hastalarda daha sık olarak medialde lokalize olduğu ve kadınlarda daha yaygın görüldüğü tespit edilmiştir.

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Anahtar kelimeler: Talus, demografik, osteokondral.

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Introduction

Talar osteochondral defects involve damage to both the chondral surface and the underlying subchondral bone tissue [1]. The primary etiological factors are thought to be major trauma or repetitive microtrauma [2]. Due to the limited regenerative capacity of chondral tissue, osteochondral lesions can develop progressively after trauma [3]. In addition to mechanical factors, some cases may have underlying conditions such as rheumatic diseases, prolonged systemic medication use, or infectious pathologies contributing to the lesion formation [4].

Clinically, patients often report ankle pain, swelling, and restricted joint mobility, particularly after prolonged standing or physical activity. A thorough examination is essential to assess potential ligamentous injury or joint instability, with specific tests such as the medial and lateral compression test and tenderness evaluation [5]. Various imaging techniques, such as X-rays, computed tomography (CT), and magnetic resonance imaging (MRI), are frequently employed to evaluate the anatomical structure of the ankle and detect the presence of osteochondral lesions [6].

This study aims to examine the demographic characteristics of patients who were diagnosed and treated for talar osteochondral lesions presenting with symptoms at our medical center.

Materials and methods

This study received ethical approval from the Pamukkale University Non-Interventional Clinical Research Ethics Committee (approval no: 01, date: 07.01.2025). A retrospective evaluation was conducted on patients diagnosed with osteochondral lesions of the talus, who had been examined and managed by a specialized foot and ankle surgeon within the orthopedic and traumatology department of our university hospital over the past five years. The primary objective of this review was to collect and analyze demographic and clinical data related to these patients. Key parameters recorded included the patients' age, sex, the laterality of the affected ankle (right or left), and the precise anatomical location of the osteochondral lesion, distinguishing between medial and lateral involvement of the talar dome. By systematically reviewing these variables, we aimed to identify potential demographic trends and anatomical patterns that could contribute to a deeper understanding of the distribution and characteristics of talar osteochondral lesions. The study included individuals aged 18 and above who had a confirmed diagnosis of osteochondral lesions in the talus. Those with insufficient imaging records, a history of oncologic conditions, or systemic rheumatic diseases were excluded. Statistical significance was determined using the One-Sample Proportion Z-Test and Chi-Square Test.

Results

This study included a total of 42 patients, with 27 being female and 15 male. The collected demographic and clinical data are comprehensively presented in Table 1. Although there was a noticeable predominance of female participants compared to males, statistical evaluation revealed that this difference did not reach a level of significance (p=0.053). The age distribution of the study cohort spanned from 18 to 70 years, with an average age calculated at 46 years. This broad range highlights the diverse age profile of the affected individuals, suggesting that osteochondral lesions of the talus may develop across various stages of adulthood regardless of gender. Further evaluation of age distribution in relation to the presence of osteochondral lesions did not show any statistically meaningful correlation (p=0.270). These findings suggest that while there may be a slight tendency for osteochondral lesions to occur more frequently in females, this trend does not reach statistical significance.

Table 1. Patients' age, gender, right-left ankle, and medial-lateral locations of the talus

Patient No	Age	Side	Location	Gender
1	36	left	medial	female
2	62	left	medial	female
3	42	right	lateral	female
4	51	right	medial	female
5	51	left	medial	female
6	44	left	medial	female
7	26	left	medial	male
8	56	left	medial	male
9	48	left	lateral	female
10	38	right	medial	male
11	55	left	medial	female
12	60	right	lateral	female
13	20	right	lateral	male
14	57	right	medial	female
15	47	left	medial	female
16	42	right	medial	female
17	47	left	medial	female
18	48	left	medial	male
19	45	left	medial	female
20	41	right	medial	female
21	32	left	medial	male
22	59	right	medial	female
23	41	left	lateral	female
24	33	right	medial	male
25	38	left	medial	male
26	55	left	medial	female
27	58	right	medial	female
28	36	right	medial	female
29	67	right	medial	female
30	34	left	medial	male
31	42	right	medial	female
32	18	left	medial	female
33	41	right	medial	male
34	58	right	medial	female
35	40	left	medial	male
36	51	left	medial	male
37	50	left	medial	male
38	59	right	medial	female
39	48	right	medial	female
40	30	left	medial	male
41	68	left	lateral	male
42	70	left	medial	female

In terms of lesion localization, 36 cases were located on the medial talus, while 6 were found on the lateral aspect (Figures 1 and 2). Statistical analysis revealed a significant tendency for osteochondral lesions to occur on the medial side of the talus (p=1.87x10^{-11}). When assessing the affected ankle, 24 cases involved the left ankle, while 18 were in the right ankle. However, there was no statistically significant difference in laterality (p=0.175).

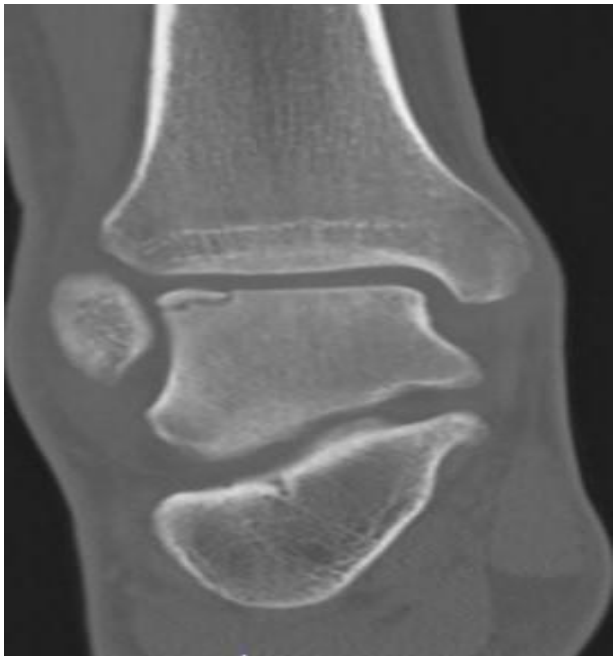

Figure 1. An osteochondral lesion located laterally on the talus is seen in the coronal section of an ankle CT scan

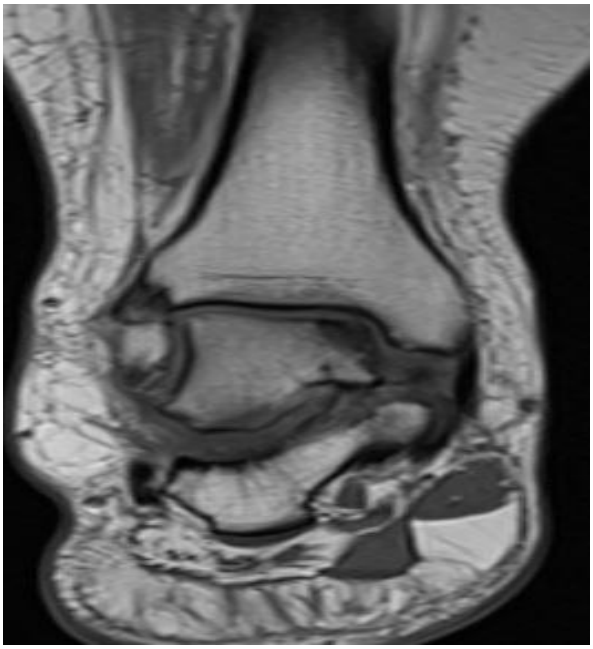

Figure 2. An osteochondral lesion located medially on the talus is seen in the coronal section of an ankle MRI in the T1 sequence

Discussion

This study evaluated the demographic characteristics of 42 patients with osteochondral lesions of the talus, assessed by a foot and ankle surgeon. No notable statistical association was detected between patient age and osteochondral lesion occurrence, indicating that the condition might be influenced by other demographic or biomechanical factors rather than age alone. A similar conclusion was reported in a retrospective study by Kim et al. [7], which analyzed 364 patients over a 10-year period and also failed to identify a significant correlation.

Although our findings suggest that osteochondral lesions were more common in female patients, this difference did not reach statistical significance. Kim et al. [7] also observed a higher prevalence of osteochondral lesions in females, but their study did not find a statistically meaningful relationship. Likewise, research conducted by Boz et al. [8] using MRI imaging found no significant association between gender and osteochondral lesion occurrence.

To classify lesion locations, we applied the Orr classification system [9]. In the Orr classification, the talus is divided into three regions: medial, lateral, and central. Our analysis demonstrated that osteochondral lesions were significantly more frequent on the medial talus. A retrospective study involving 90 patients similarly reported a predominance of medial lesions, though statistical significance was not observed [10]. Furthermore, Kim et al. [7] found that 87.9% of lesions in their study were located medially on the talus.

When evaluating the distribution of osteochondral lesions between the right and left ankles, our study did not reveal a statistically significant difference in laterality. The data suggest that the occurrence of lesions was relatively balanced between both sides, indicating that neither the right nor the left ankle is inherently more susceptible to developing these lesions. Consistent with our findings, Boz et al. [8] also reported no statistically meaningful variation in lesion prevalence between the right and left ankle in their study. These results imply that factors other than laterality, such as biomechanics, individual activity levels, or underlying anatomical differences, may play a more critical role in the development of osteochondral lesions in the talus.

A large-scale retrospective study using data from the U.S. military reviewed records spanning ten years, covering approximately 14 million personnel. The incidence of osteochondral lesions of the talus was reported as 27 per 100,000 individuals [11]. The study also found a statistically significant increase in incidence among females and individuals of Caucasian descent. In our research, the proportion of female patients was also higher. However, our study did not aim to determine incidence rates, as it focused solely on patients diagnosed with talar osteochondral defects.

Certain constraints should be acknowledged in this research. The retrospective design inherently restricts the control over potential influencing factors on osteochondral lesion development. However, as all evaluations were performed by a single researcher specialized in foot and ankle surgery, this limitation was minimized. Additionally, one of the notable strengths of this research is that all symptomatic patients were included, regardless of the treatment approach. The inclusion of a diverse patient cohort, rather than focusing on a single treatment modality such as conservative management, ligament repair, mosaicplasty, or microfracture, adds robustness to our findings.

Talar osteochondral defect is a condition that affects both chondral and subchondral tissue, appears to be more frequently located medially in symptomatic patients and tends to be more common in females.

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Authors' contributions: The core concept and hypothesis of the study were formulated by A.N.A. The theory was formulated, and the materials and methods section was structured by M.Y. The data analysis in the results section was conducted by M.Y. The discussion section was written by A.N.A. and later reviewed, revised, and approved by M.Y. Additionally, all authors actively contributed to discussions throughout the study and approved the final version of the manuscript.

Conflict of interest: The authors have stated that they have no conflicts of interest to disclose.

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Research Article

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Association of PTEN expression with hormone receptor status, tumor subtype, histological grade, and clinicopathological parameters in endometrial carcinomas

Endometriyal karsinomlarda PTEN ekspresyonunun hormon reseptörü durumu, tümör alt tipi, histolojik derece ve klinikopatolojik parametrelerle ilişkisi

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Abstract

Purpose: This study investigates the relationship between PTEN expression and tumor characteristics and clinical outcomes in endometrial carcinoma (EC). EC is the most common malignancy of the female genital tract, typically classified into Type I (endometrioid, hormone-sensitive, favorable prognosis) and Type II (serous, aggressive, poor prognosis). PTEN is a tumor suppressor gene that regulates cell growth. Loss of PTEN expression is frequently observed in Type I EC and is associated with early tumorigenesis.

Materials and methods: A retrospective analysis was conducted on 186 EC cases. PTEN expression was evaluated immunohistochemically, and its association with tumor size, histological subtype, stage, hormone receptor status, and survival outcomes was analyzed.

Results: Loss of PTEN expression was detected in 81.2% of cases. While PTEN loss was more prevalent in tumors >3 cm in size, it did not show a significant correlation with stage, grading, myometrial invasion, or metastasis. p53 mutation and high-grade tumors were associated with poorer survival rates. Estrogen receptor (ER) and progesterone receptor (PR) expression were predominantly observed in endometrioid carcinoma.

Conclusions: Although PTEN loss is frequently observed in endometrioid EC, it does not directly impact survival outcomes. Hormone receptor status and genetic alterations play a crucial role in EC pathogenesis. Further studies on PTEN and other molecular markers may contribute to the development of personalized treatment strategies. These findings suggest that while PTEN loss plays a role in early tumor development, it is not a definitive prognostic factor in EC.

Keywords: Endometrial cancer, PTEN, tumor cells**.**

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Öz

Amaç: Bu çalışma, PTEN ekspresyonunun tümör özellikleri ve hastalık progresyonu ile ilişkisini incelemektedir. Endometriyal karsinom (EK), kadın genital sisteminin en yaygın malignitesidir ve genellikle Tip I (endometrioid, hormon duyarlı, iyi prognoz) ve Tip II (seröz, agresif, kötü prognoz) olarak sınıflandırılır. PTEN, hücre büyümesini düzenleyen bir tümör supresör genidir. PTEN ekspresyon kaybı özellikle Tip I EK'de sık görülmekte ve erken tümör gelişimi ile ilişkilendirilmektedir.

Gereç ve yöntem: Bu çalışmada 186 EK vakası retrospektif olarak analiz edilmiştir. PTEN ekspresyonu immünohistokimyasal olarak değerlendirilmiş ve tümör boyutu, histolojik alt tip, evre, hormon reseptör durumu ve sağkalım sonuçları ile ilişkisi araştırılmıştır.

Bulgular: PTEN ekspresyon kaybı olguların %81,2'sinde tespit edilmiştir. PTEN kaybı 3 cm'den büyük tümörlerde daha sık görülmesine rağmen, evre, derecelendirme, myometrial invazyon veya metastaz ile anlamlı bir ilişki göstermemiştir. p53 mutasyonu ve yüksek dereceli tümörler daha kötü sağkalım oranları ile ilişkilendirilmiştir. Östrojen (ER) ve progesteron (PR) reseptör ekspresyonu ise ağırlıklı olarak endometrioid karsinomda gözlenmiştir.

Sonuç: PTEN kaybı endometrioid EK'de sık görülmekle birlikte sağkalım üzerinde doğrudan bir etkisi bulunmamaktadır. Hormon reseptör durumu ve genetik değişiklikler EK patogenezinde önemli bir rol oynamaktadır. PTEN ve diğer moleküler belirteçler üzerine yapılacak ileri çalışmalar, kişiselleştirilmiş tedavi stratejilerinin geliştirilmesine katkı sağlayabilir. Bu bulgular, PTEN kaybının erken tümör gelişiminde rol oynadığını ancak EK için kesin bir prognostik faktör olmadığını göstermektedir.

Anahtar kelimeler: Endometrial kanser, PTEN, tümör hücreleri.

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Introduction

Endometrial carcinoma (EC) is the most common cancer of the female genital tract and the fourth most commonly diagnosed cancer overall, after breast, lung, and colorectal cancers. Understanding the molecular genetic mechanisms that drive tumor progression is crucial for improving early diagnosis, assessing prognosis, and developing effective treatment strategies for endometrial carcinoma [1]. According to global data, approximately 420.368 women are diagnosed with endometrial carcinoma annually, with 97.723 deaths attributed to the disease [2].

Endometrial carcinoma is traditionally categorized into two subtypes based on their biological behavior and prognosis. Type I EC is commonly associated with risk factors such as obesity, hyperlipidemia, and hyperestrogenism, and predominantly consists of well to moderately differentiated endometrioid tumors. In contrast, Type II EC encompasses poorly differentiated tumors, including serous carcinoma, clear cell carcinoma, and carcinosarcoma. Type II tumors typically exhibit a more aggressive clinical course, with a greater propensity for deep myometrial invasion and are often diagnosed at more advanced stages. These tumors are characterized by a higher recurrence rate and are associated with a poorer prognosis [3].

Phosphatase and Tensin Homolog (PTEN) is a gene located at the 10q23.31 locus, composed of nine exons encoding a 403-amino acid protein product with phosphatidylinositol, tyrosine, and serine/threonine protein phosphatase activity [4]. PTEN protein produces a tumor-suppressor phosphatase that regulates cell division and inhibits oncogenesis. As a dual-specificity phosphatase, PTEN suppresses cell proliferation and induces apoptosis via an AKT-dependent pathway [5]. PTEN is the most frequently mutated tumor suppressor gene in endometrial carcinoma, with particularly high mutation rates in the endometrioid subtype. In contrast, PTEN mutations are rarely observed in serous carcinoma. PTEN loss of function serves as an indicator of early carcinogenesis in the endometrium and is associated with favorable prognosis [6]. Even a slight reduction in PTEN protein levels and activity can lead to increased cancer susceptibility and tumor malignancy [6]. PTEN exerts its effects through the Akt signaling pathway by inhibiting cell proliferation and inducing apoptosis. PTEN mutations or deletions have been identified in approximately 80% of endometrioid carcinoma cases. Functional inactivation of PTEN is linked to cancer progression and the development of a malignant phenotype [7]. The physiological fluctuation of PTEN levels throughout the menstrual cycle suggests that it is regulated by hormonal factors [8].

This study aims to explore the association between PTEN expression and tumor characteristics, as well as its impact on disease prognosis in endometrioid endometrial carcinoma. The findings are expected to provide valuable insights that can aid in clinical decision-making, particularly in determining the necessity of adjuvant therapy.

Materials and methods

Cases

This study presents a retrospective analysis of 186 cases of endometrial cancer, all diagnosed between 2018 and 2024. Histopathological data, including patient age, tumor type, histological grade, nuclear grade, tumor diameter, depth of invasion, lymphovascular invasion, perineural invasion, cervical stromal invasion, endocervical gland involvement, and uterine serosal involvement, were obtained from pathology reports. Additionally, immunohistochemical findings for markers such as p53, Ki-67, estrogen receptor (ER), progesterone receptor (PR), HER2, and PTEN, as well as treatment details, disease-free survival, and overall survival, were collected from the hospital automation system and patient follow-up records of the Department of Obstetrics and Gynecology.

The ethical approval for this study was granted by the Non-Interventional Clinical Research Ethics Committee of Pamukkale University in its meeting dated February 22, 2022 (meeting no: 04).

Immunohistochemistry

A representative tumor sample that best reflected the tumor tissue was selected for each case. From the selected paraffin blocks, 5-micron-thick sections were obtained and mounted on positively charged slides for PTEN antibody staining. The tissue samples were

incubated at 60°C overnight for deparaffinization and subsequently stained using an automated staining protocol with the VENTANA Benchmark XT system. Automated staining was performed using pre-diluted PTEN antibody (SP-218, Ventana, Rabbit Monoclonal Antibody) to visualize the targeted proteins.

The immunohistochemical expression of PTEN in tumor-dominant blocks was analyzed using a light microscope. When assessing PTEN expression in the endometrial carcinoma regions, the adjacent endometrial stroma was used as an internal positive control. PTEN reactivity was evaluated based on its extent and distribution through a semi-quantitative scoring system for nuclear staining. Cytoplasmic staining intensity was classified as either moderate to strong (positive) or faint (negative, indicating loss of expression) [9].

Statistical analysis

Statistical analyses were conducted using SPSS software (version 23.0, SPSS Inc., Chicago, IL, USA). Demographic and clinical data are presented as mean ± standard deviation (SD) or frequency (percentage). A *p*-value of <0.05 was considered statistically significant. Kaplan-Meier survival analysis, Cox regression analysis, Mann-Whitney U test, and Chi-square test were used for statistical assessment.

Results

Demographic and clinical data

A total of 186 endometrial cancer cases were included in the study. Histologically, 163 cases (87.6%) were diagnosed as endometrioid carcinoma, while 23 cases (12.4%) were classified into other subtypes, including serous carcinoma (n=11), mixed carcinoma (serous carcinoma + endometrioid carcinoma) (n=5), clear cell carcinoma (n=2), carcinosarcoma (n=3), and dedifferentiated carcinoma (n=2). Among these cases, 127 (68.3%) underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy (TAH+BSO), while 59 cases (31.7%) were diagnosed through curettage specimens. The patients' ages ranged from 31 to 89 years, with a mean age of 60.27±10.38 years. Tumor diameter ranged from 1 to 10 cm, with a mean size of 3.61±2.01 cm. 61 cases (32.8%) had tumors <3 cm, while 106 cases (57%) had tumors ≥3 cm. 123 cases (66.1%) were <65 years old, while 61 cases (32.8%) were ≥65 years old.

In the immunohistochemical analysis, the following marker expressions were observed: ER: 176/186 cases (94.6%), PR: 166/186 cases (89.2%), HER2, score 3: 1/181 cases (0.5%), p53 (mutant expression): 16/186 cases (16%), PTEN expression loss: 151/186 cases (81.2%), Ki-67 proliferation index >20%: 122/186 cases (65.6%).

In terms of tumor invasion, 53 cases (28.5%) exhibited invasion beyond the inner half of the myometrium. Serosal invasion was detected in 2 cases (1.1%), lymphovascular invasion in 19 cases (10.2%), and perineural invasion in 2 cases (1.1%). Malignant peritoneal fluid was identified in 3 cases (1.6%).

Staging analysis revealed that 74 cases (39.8%) were in early-stage (Stage I-II), while 9 cases (4.8%) were in advanced-stage (Stage III-IV). No recurrences were observed in the cohort. Six patients (3.2%) died during follow-up, with an overall survival duration ranging from 31 to 89 months and a mean survival of 26.5±14.88 months.

Association between tumor type and clinicopathological features

Endometrioid carcinomas were more frequently observed in patients younger than 65 years, whereas other tumor subtypes were predominantly found in patients older than 65 years (p=0.003). Perineural invasion was most commonly detected in the non-endometrioid group, with a statistically significant difference (p=0.000). Lymphovascular invasion was observed more frequently in endometrioid carcinoma compared to other subtypes (p=0.044) (Figure 1).

Immunohistochemical analysis showed that the Ki-67 proliferation index was higher in serous carcinoma, clear cell carcinoma, and mixed-type carcinomas than in endometrioid carcinoma, with a trend indicating it may exceed 20% (p=0.094) (Figure 2a, 2b). P53 mutation was most frequently detected in serous carcinoma, and this difference was statistically significant (p=0.000) (Figure 2c, 2d).

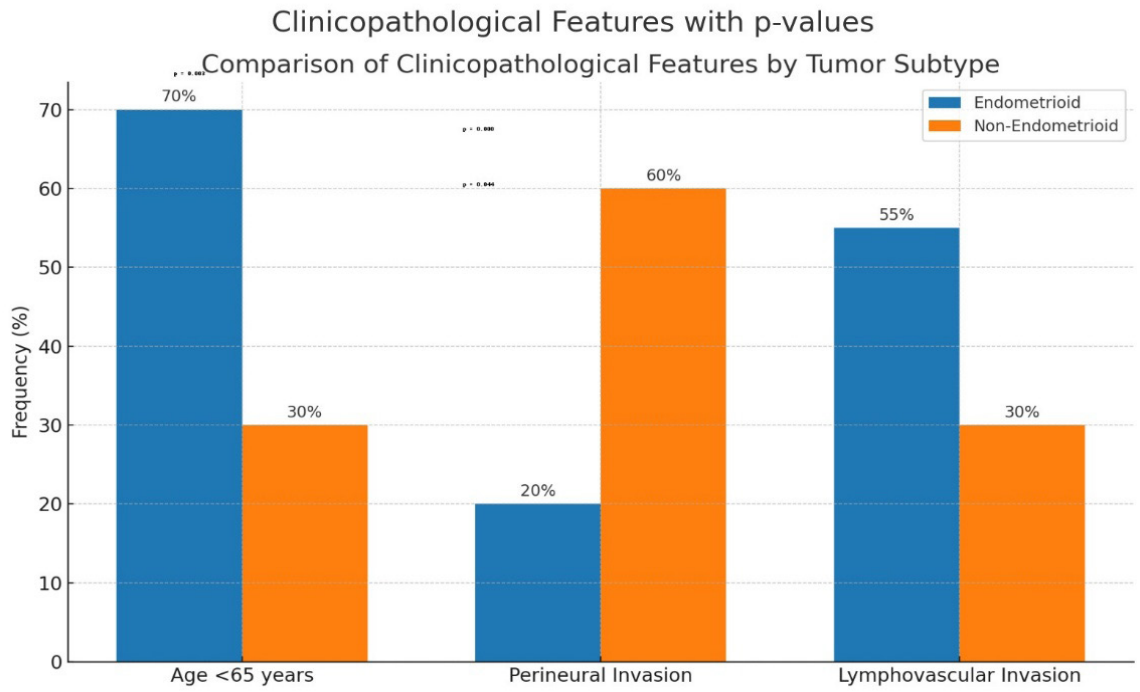

Figure 1. Comparison of clinicopathological features by tumor subtypes

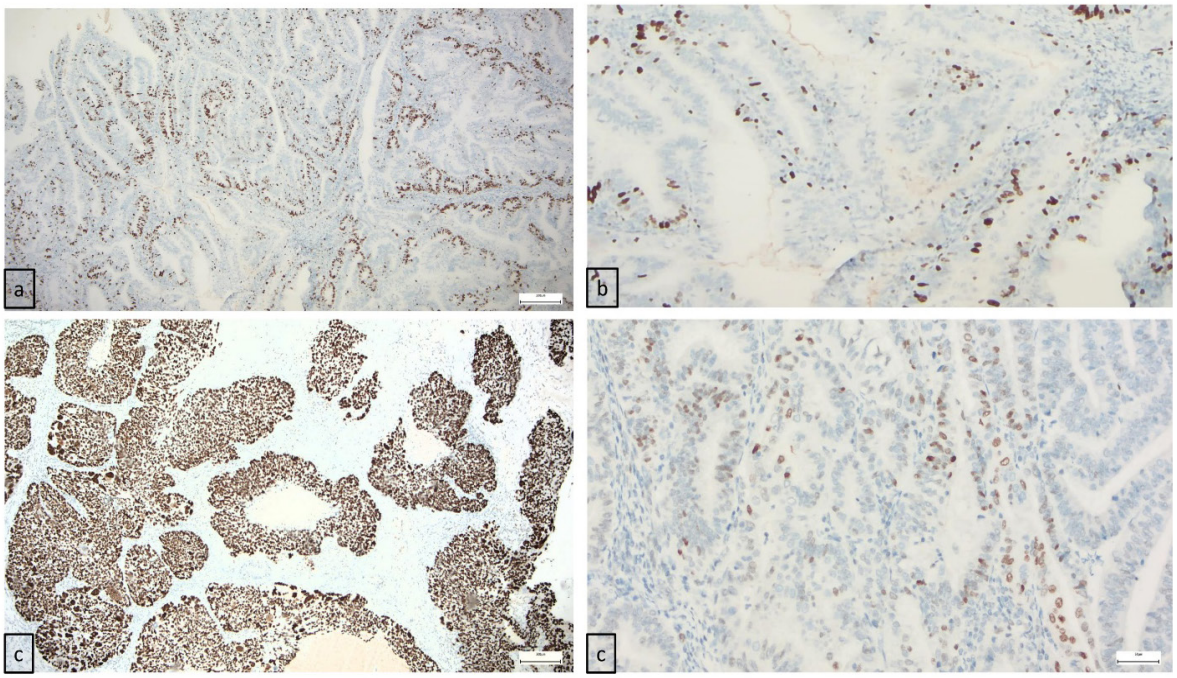

Figure 2. a. Immunohistochemically, Ki-67 proliferation index > 20% in a high-grade tumor, IHC, x100 b. Ki-67 proliferation index < 20%, IHC, x200 c. Strong nuclear p53 positivity, mutant, IHC, x100 d. Rare nuclear p53 positivity, wild-type, IHC, x200

HER2 positivity was identified in serous carcinoma (p=0.000). In contrast, estrogen receptor (ER) and progesterone receptor (PR) expression was most commonly observed in endometrioid carcinoma (p=0.000). The mortality rate was significantly higher in patients diagnosed with serous carcinoma and other aggressive subtypes, including carcinosarcoma and dedifferentiated carcinoma (p=0.004). The clinicopathological distribution of the cases is presented in Table 1.

Table 1. Distribution of cases according to their clinicopathological features

Clinicopathological Features	Number of Patients Endometrioid Carcinoma n=163 (87.6%)	Number of Patients Others n=23 (12.4%)	Total n=186 (10%)	*p*	**X^2 value
Age					
<65	115 (62.5%)	8 (4.3%)	123 (66.8%)	0.000*	12.195
≥65	46 (25%)	15 (8.2%)	61 (33.2%)		
Tumor diameter					
<3 cm	55 (32.9%)	6 (3.6%)	61 (36.5%)	0.634	0.194
≥3 cm	93 (55.7%)	13 (7.8%)	106 (63.5%)		
Histological grade					
Low grade	122 (70.1%)	0 (0%)	122 (70.1%)	0.000*	59.086
High grade	30 (17.2%)	22 (12.6%)	52 (29.9%)		
Myometrial invasion					
Inner half invasion	105 (62.1%)	11 (6.5%)	116 (68.6%)	0.161	1.960
Outer half invasion	44 (26%)	9 (5.3%)	54 (31.4%)		
Lymphovascular invasion					
No	142 (76.3%)	18 (9.7%)	160 (86.0%)	0.044*	6.249
Yes	17 (9.1%)	2 (1.1%)	19 (10.2%)		
Perineural invasion					
No	158 (88.3%)	19 (10.6%)	177 (98.9%)	0.000	3.702
Yes	1 (0.6%)	1 (0.6%)	2 (1.1%)		
Endocervical gland involvement					
No	142 (76.3%)	16 (8.6%)	158 (84.9%)	0.030*	7.014
Yes	7 (3.8%)	4 (2.2%)	11 (5.9%)		
Stage					
I-II	68 (81.9%)	6 (7.2%)	74 (89.2%)	0.375	0.787
III-IV	9 (10.8%)	0 (0%)	9 (10.8%)		
Patient survival					
Alive	160 (86%)	20 (10.8%)	182 (52.3%)	0.004*	8.103
Ex	3 (1.6%)	3 (1.6%)	166 (47.7%)		

*p<0.05 statistically significant difference; chi-square analysis

Correlation between PTEN expression and tumor characteristics

Loss of PTEN expression was most commonly observed in the endometrioid carcinoma subtype (Figure 3a-3d). PTEN expression loss was also observed in the other tumors (Figure 4a-4c). Additionally, Chi-square analysis revealed that loss of PTEN expression was more prominently observed in tumors with a diameter >3 cm (p=0.16). Loss of PTEN expression was not significantly associated with clinical-pathological parameters, such as age, perineural invasion, lymphovascular invasion, endocervical gland involvement, cervical stromal invasion, metastasis, recurrence, or immunohistochemical markers like Ki-67, p53, ER, PR, and HER2 (p>0.05) (Table 2) (Figure 5).

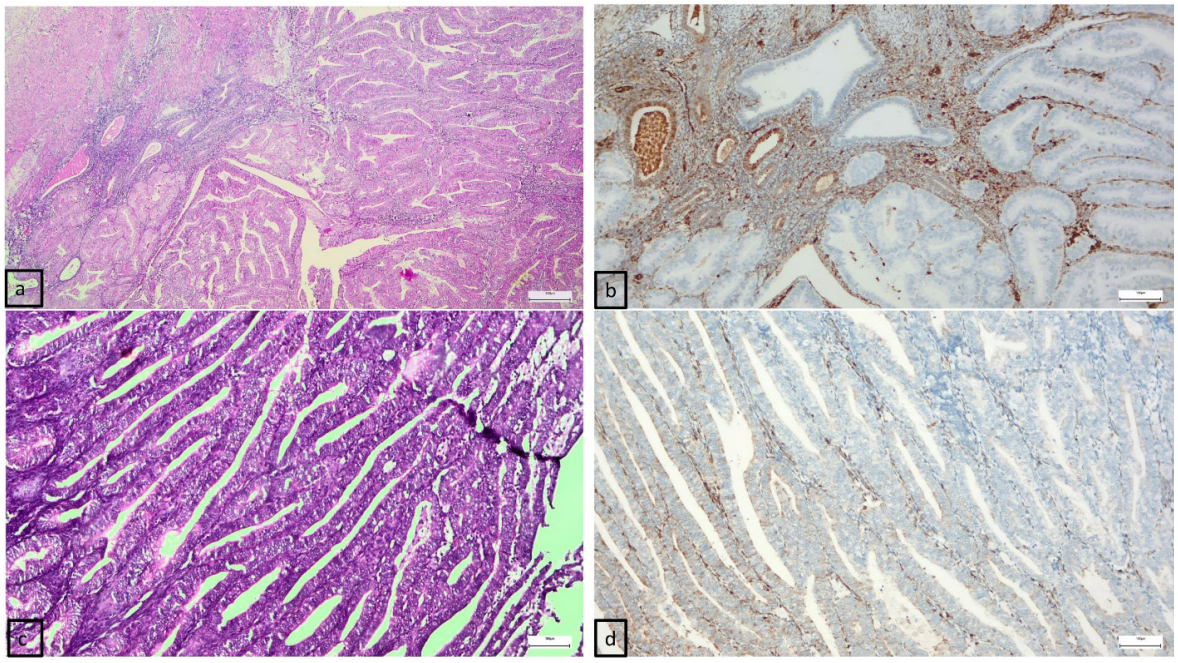

Figure 3. a. Endometrioid carcinoma, H&E, x100 b. Loss of PTEN immunoexpression observed in tumor areas, with no loss in normal glands, IHC, x200 c. Endometrioid carcinoma, H&E, x200 d. Weak staining areas with PTEN observed, but no loss, IHC, x200

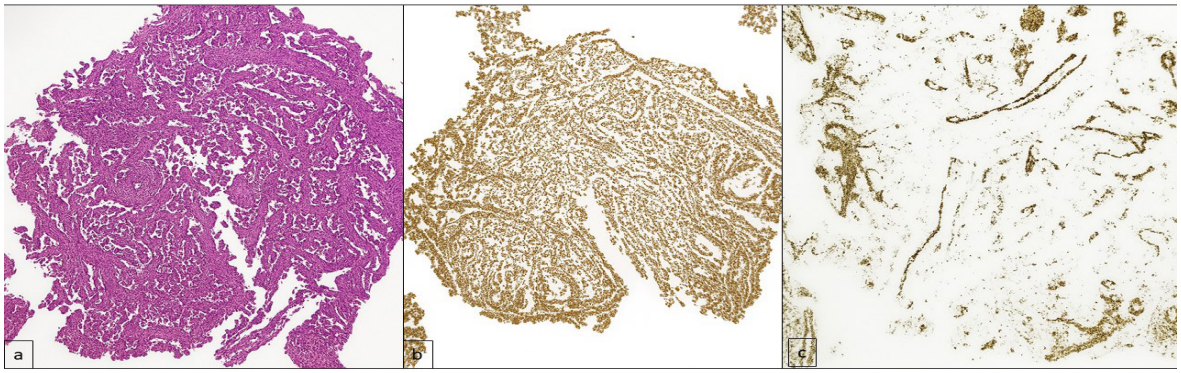

Figure 4. a. High-power microscopic image showing a papillary architecture with hierarchical branching, fibrovascular cores, and nuclear atypia, consistent with serous papillary carcinoma. H&E, x100. b. Immunohistochemical staining for p53 demonstrating strong and diffuse nuclear positivity in tumor cells, consistent with a mutant (aberrant) expression pattern, supporting the diagnosis of serous papillary carcinoma, IHC, x100. c. PTEN showing complete loss of cytoplasmic and nuclear expression in tumor cells, while surrounding stromal and inflammatory cells retain expression, IHC, x200

Table 2. Relationship of PTEN immunoexpression with clinicopathological features

Clinicopathological Features	Number of Patients Loss of PTEN Immunoexpression n=151 (81.2%)	Number of Patients No loss of PTEN Immunoexpression n=35 (18.8%)	*p*	**X^2 value
Age				
<65	100 (54.3%)	23 (12.5%)	0.874	0.025*
≥65	49 (50.5%)	12 (6.5%)		
Tumor type				
Endometrioid	135 (72.6%)	28 (15.1%)	0.128	2.319
Others	16 (8.6%)	7 (3.8%)		
Tumor size				
<3 cm	44 (26.3%)	17 (10.2%)	0.019*	5.506
≥3 cm	92 (55.1%)	14 (8.4%)		
Biopsy type				
Endometrial curettage	52 (28%)	7 (3.8%)	0.098	2.734
TAH+BSO	99 (53.2%)	28 (15.1%)		
Tumor grade				
Low grade	99 (56.9%)	23 (13.2%)	0.810	0.058
High grade	43 (24.7%)	9 (5.2%)		
Histological grade				
1	12 (14%)	5 (5.8%)		
2	41 (47.7%)	13 (15.1%)	0.822	0.392
3	12 (14%)	3 (3.5%)		
Lymphovascular invasion (LVI)				
No	128 (68.8%)	32 (17.2%)	0.578	1.096
Yes	17 (9.1%)	2 (1.1%)		
Myometrial invasion				
Inner half invasion	94 (55.6%)	22 (13%)	0.757	0.096
Outer half invasion	44 (26%)	9 (5.3%)		
Evre				
I-II	66 (79.5%)	8 (9.6%)	0.299	1.077
III-IV	9 (10.8%)	0 (0%)		
Patient survival				
Alive	144 (77.4%)	35 (18.8%)	0.194	1.437
Ex	7 (3.8%)	0 (0%)		

**p*<0.05 statistically significant difference; chi-square analysis

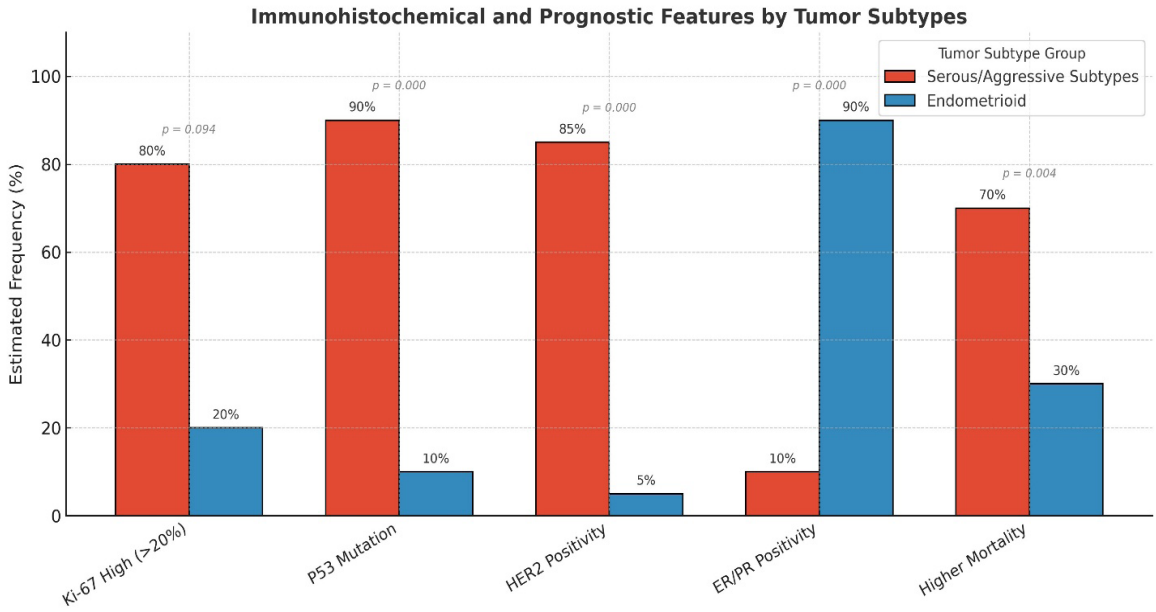

Figure 5. Immunohistochemical and prognostic features by tumor subtypes

In this study, various clinicopathological parameters were compared according to the group_pten variable using the Mann-Whitney U test. The analysis revealed a statistically significant difference between the groups only in terms of tumor size (U=1611.5, Z=-2.395, *p*=0.017). No significant differences were observed for the other variables (*p*>0.05). The effect size (Cohen's r) was also calculated for the tumor size variable and found to be r=0.176.

PTEN positivity and disease outcome

Kaplan–Meier survival analysis was conducted to evaluate the impact of various clinical and pathological parameters on overall survival (OS) and disease-free survival (DFS). The loss of PTEN expression did not exhibit a statistically significant association with either OS or DFS (*p*=0.219). However, patients with high-grade tumors demonstrated significantly lower survival rates compared to those with low-grade tumors (*p*=0.048; low-grade: 65.893±0.776 months vs. high-grade: 59.153±2.322 months). Furthermore, patients with perineural invasion exhibited markedly poorer survival outcomes (*p*=0.000; perineural invasion present: 29±4.950 months vs. absent: 65.509±0.737 months). Additionally, the presence of a p53 mutation was associated with a significant reduction in survival (*p*=0.033; wild-type p53: 65.373±0.803 months vs. mutant p53: 39.917±3.331 months).

Conversely, variables such as age, lymphovascular invasion, endocervical gland involvement, cervical stromal invasion, metastasis, recurrence, and immunohistochemical markers (including Ki-67, ER, PR, and HER2) were not found to have a significant impact on survival. Kaplan-Meier survival curves were generated for each of these parameters; however, statistical analysis using the log-rank test and Cox regression model revealed no significant association with survival time (*p*>0.05).

Discussion

This study aimed to evaluate the relationship between PTEN expression and various tumor characteristics in endometrial cancer, such as stage, grade, and patterns of invasion and recurrence. These findings will contribute to understanding disease prognosis and will aid in future targeted individualized therapies.

Loss of PTEN function is a significant event in endometrial carcinogenesis, which may develop in response to known endocrine risk factors. Additionally, PTEN is a useful immunohistochemical biomarker in the evaluation of premalignant diseases. In cases where PTEN expression is impaired, the underlying genetic modification is typically associated with mutations [2, 10]. Moreover,

PTEN IHC results showed positive staining in 9 of 33 endometrioid adenocarcinoma cases (27.27%) and negative staining in 24 cases (72.73%) [2, 9]. PTEN inactivation has been reported in 83% of endometrioid endometrial cancer cases and in 55% of precancerous lesions, characterized by loss of expression [11, 12]. PTEN inactivation driven by mutations is linked to early-stage disease and a more favorable prognosis. Patients with PTEN mutations have a 5-year survival rate of approximately 80%, while the survival rate is around 50% in patients without such mutations [11]. A study referencing The Cancer Genome Atlas (TCGA) database found that PTEN was mutated in 57% of 530 EC patients [13]. A similar finding was also observed with multigene next-generation sequencing (NGS) [14, 15]. In our study, PTEN inactivation was observed in 82.8% of endometrioid endometrial cancer cases, but it was not associated with survival.

In 2013, The Cancer Genome Atlas Research Network classified EC into four distinct molecular categories. In the 'ultramutated', 'hypermutated', and 'low copy number' categories, which are typically endometrioid, PTEN mutations were found in 94%, 88%, and 77% of cases, respectively. In the last category, mainly consisting of serous EC ('high copy number'), PTEN mutations were found in only 15% of cases [16]. In our study, PTEN inactivation was seen in 63.6% of other carcinoma cases, which is higher than reported in the literature, possibly due to the smaller sample size. A higher rate of PTEN loss was observed in stage III cancers [11], although no such relationship was found in our study.

The normal endometrium goes through cycles of growth and changes in response to hormonal fluctuations. Interestingly, studies from two different research groups have shown that during the proliferative phase of the menstrual cycle, when estradiol is more dominant, PTEN protein levels are highest in the uterine epithelial cells [17, 18]. Previous studies suggest that long-term exposure to high estrogen levels, along with the accumulation of genetic mutations, plays a key role in the progression of hyperplastic endometrium to type I endometrial carcinoma [19, 20]. However, PTEN mutations may contribute to the early development of atypical endometrial hyperplasia, and additional mutations in KRAS and PIK3CA are linked to the transformation of this hyperplasia into cancer [21]. It is important to note that PTEN mutations alone are not enough to cause cancer. In six out of eight cases, the carcinomas had mutations not found in surrounding healthy tissue, indicating that other mutations are also necessary for the cancer to develop [11]. However, this study did not assess the relationship between PTEN inactivation and hormone receptor status.

Tumor molecular biology research has supported the development of anti-tumor compounds. In the new approach to drug discovery, the selection of molecular targets has become a key issue. The mechanism of the PTEN gene and its associated signaling pathways has become a hot topic in targeted research. Further studies on the PTEN gene signaling pathway will provide new ideas and foundations for the diagnosis and treatment of tumors at the genetic level [6].

A limitation of our study is the smaller number of cases in the non-endometrioid group. Further studies with larger patient cohorts could yield different results. Moreover, PTEN was analyzed using immunohistochemistry, and genomic methods could provide more effective results.

In conclusion, our study found that the loss of PTEN expression did not have a significant direct effect on survival. However, other clinical and genetic factors, such as high-grade tumors, perineural invasion, and p53 mutations, significantly impacted survival. These findings suggest that to better understand the prognosis of endometrial cancer, other genetic and clinical factors should be considered. This highlights the need for personalized clinical management, where each patient is treated according to their genetic and clinical characteristics. Additionally, the prognostic significance of PTEN expression in EEC should be further investigated using genomic and proteomic approaches. A detailed study of PTEN status and metabolic conditions may improve the management of EEC patients in gynecological clinical settings.

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Authors contributions: Y.A.K. formulated the study's main concept and hypothesis. Y.A.K. and Ö.K.C. developed the theoretical framework and organized the materials and methods. Y.A.K. conducted the data analysis. Both authors wrote and approved the discussion section. All authors participated in discussions and approved the final manuscript.

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

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Prevalence of lumbosacral transitional vertebra on lumbar CT and associated degenerative imaging findings in symptomatic patients

Semptomatik hastalarda lomber BT'de lumbosakral transisyonel vertebra prevalansı ve eşlik eden dejeneratif görüntüleme bulguları

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Abstract

Purpose: This study aimed to assess the prevalence and distribution of lumbosacral transitional vertebra (LSTV) subtypes in patients with low back pain and investigate associated degenerative changes using lumbar CT imaging.

Material methods: A retrospective review of 1.035 patients who underwent lumbar CT for low back pain between January 2023 and April 2024 was conducted. 133 with LSTV were identified and classified according to the Castellvi classification. The degenerative changes at the LSTV level, including pseudoarticular degeneration, disc narrowing, facet joint arthropathy, foraminal stenosis, and scoliosis, were evaluated.

Results: LSTV was present in 12.85% of the patient population. Degeneration at the pseudoarticulation was observed in 67.7% of cases, with Types II and IV showing significantly higher rates (98.2% and 100%) compared to Types I (60%) and III (0%). Disc narrowing at the cephalad level was most common in Type III (90%). Facet joint arthropathy was observed in 85.7% of cases, while foraminal stenosis was only found in Type II (9.8%). Scoliosis was more prevalent in Types II (50.9%) and IV (50%) than in Types I (24%) and III (4.5%). Statistically significant differences were observed in pseudoarticular degeneration, disc narrowing, foraminal stenosis, and scoliosis.

Conclusion: LSTV is common in low back pain patients, with Types II and IV showing early degeneration at the pseudoarticulation level that may contribute to symptoms, particularly in young and middle-aged individuals. Nerve root compression due to degenerative hypertrophy is common in Type II and requires careful examination of the LSTV region with imaging methods in symptomatic cases.

Keywords: Lumbosacral transitional vertebra, computed tomography, pseudoarticulation, low back pain.

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Öz

Amaç: Bu çalışmanın amacı, bel ağrısı nedeniyle lomber BT çekilen hastalarda lumbosakral transisyonel vertebra (LSTV) yaygınlığını ve alt tiplerinin dağılımını değerlendirmek ve ilişkili dejeneratif değişiklikleri araştırmaktır.

Gereç ve yöntem: Ocak 2023 ile Nisan 2024 arasında bel ağrısı nedeniyle lomber BT çekilen 1.035 hastanın retrospektif incelemesi yapıldı. LSTV'li 133 hasta belirlendi ve Castellvi sınıflandırmasına göre sınıflandırıldı. Psödoartiküler dejenerasyon, disk daralması, faset eklem artropatisi, foraminal stenoz ve skolyoz dahil olmak üzere LSTV seviyesindeki dejeneratif değişiklikler değerlendirildi.

Bulgular: LSTV hasta popülasyonunun %12,85'inde saptandı. Psödoartiküler dejenerasyon vakaların %67,7'sinde gözlendi ve Tip II ve IV'te Tip I (%60) ve III (%0) ile karşılaştırıldığında önemli ölçüde daha yüksek oranlar (%98,2 ve %100) görüldü. Sefalad disk daralması Tip III'te (%90) en yaygındı. Faset eklem artropatisi vakaların %85,7'sinde gözlenirken, foraminal stenoz yalnızca Tip II'de (%9,8) bulundu. Skolyoz Tip II'de (%50,9) ve IV'te (%50) Tip I (%24) ve III'e (%4,5) göre daha yaygındı. Psödoartiküler dejenerasyon, disk daralması, foraminal stenoz ve skolyozda istatistiksel olarak anlamlı farklılıklar gözlendi.

Sonuç: LSTV bel ağrısı hastalarında yaygındır. Tip II ve IV'te psödoartikülasyon seviyesinde erken dejenerasyon görülür ve bu özellikle genç ve orta yaşlı bireylerde semptomlara katkıda bulunabilir. Dejeneratif hipertrofiye bağlı sinir kökü sıkışması Tip II'de yaygındır ve semptomatik vakalarda görüntüleme yöntemleriyle LSTV bölgesinin dikkatli bir şekilde incelenmesini gerektirir.

Anahtar kelimeler: Lumbosakral transisyonel vertebra, bilgisayarlı tomografi, psödoartikülasyon, bel ağrısı.

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Introduction

Low back pain is a significant health problem on a global scale, affecting individuals of all age groups, with a lifetime prevalence rate of up to 84% reported [1].

Lumbosacral transition is a relatively common pathology that is frequently overlooked in the diagnostic process of low back pain [2]. Lumbosacral transitional vertebra (LSTV) is defined as the presence of a unilateral or bilateral lumbar transverse process that articulates with the upper surface of the sacrum [3]. The diagnosis of this condition is made when there are varying degrees of fusion between the transverse process of the terminal lumbar vertebra and the sacrum, and this is recognized as a mechanical cause of low back pain [4].

The prevalence of LSTVs in the general population has been documented as ranging from 4% to 35.5% [5]. Patients who present with chronic lower back pain or functional impairment due to congenital deformity caused by LSTV are diagnosed with Bertolotti's Syndrome. The condition was first described by Mario Bertolotti in 1917 [6, 7].

The most widely employed classification system for imaging is the Castellvi classification, which divides the condition into four types based on the relationship of the transverse process with the sacrum [8]. According to Castellvi, Type I includes hyperplastic transverse processes measuring at least 19 mm. Type II exhibits incomplete lumbarization/sacralization with enlarged transverse processes that form a joint with the sacrum. Type III describes lumbarization/sacralization with loss of the joint space formed by the transverse process and complete bony fusion to the sacrum. Type IV is characterized by the presence of Type II LSTV on one side and Type III LSTV on the other [9, 10].

The recommended course of treatment comprises a range of approaches, including the use of oral medication, physical therapy, steroid injections at the site of pain, and surgical resection or fusion [6].

Computed tomography (CT) and magnetic resonance imaging (MRI) can provide more accurate diagnosis and classification than plain radiography. CT provides the highest accuracy in evaluating bony anatomy and is the most useful modality for evaluating LSTVs [11].

The present study aims to determine the prevalence and distribution of LSTV types according to the Castellvi classification in patients with lower back pain and to calculate the rates of vertebral degenerative changes among the subtypes. This study is the first to investigate pseudoarticular degeneration at the level of the LSTV and degenerative changes at other levels with lumbar CT. Previous studies have predominantly been performed with reconstructions from abdominal CT [12, 13].

Materials and methods

Study design and patient population

In this study, the images of 1.132 patients who were admitted to our center with the complaint of "low back pain" between January 2023 and April 2024 and underwent lumbar CT imaging were retrospectively scanned using our hospital's picture archiving and communication system (PACS). We excluded 25 patients who did not provide optimal imaging conditions due to motion and beam-hardening artifacts. 57 patients had a history of surgery. Additionally, 15 patients with spinal involvement classified as either malignant or benign were excluded from the evaluation. Of the remaining 1.035 patients, 133 patients with LSTV were divided into four groups according to the Castellvi classification and evaluated in terms of degenerative and anatomical spinal, vertebral, and disc changes (degenerative findings between the transverse process and the sacral surface, narrowing in the cephalad intervertebral disc, degenerative changes in the facet joints, foraminal stenosis at the transition level, degenerative spondylolisthesis, and scoliosis).

Permission was obtained from the Pamukkale University Non-Interventional Clinical Research Ethics Committee for the study (approval number: E-60116787-020-353871 and approval date: 30.04.2024).

Imaging

CT image acquisition of the lumbosacral region was performed using a multidetector CT scanner (Philips Ingenuity 128, Philips Healthcare, Cleveland INC, United States). The following parameters were utilized for the axial lumbosacral CT acquisition: a collimation width of 64 × 0.625 mm, a matrix size of 512 × 512, a slice thickness of 1.5 mm, a tube voltage of 120 kV, and a tube current of 140 mA. The CT images were reconstructed in both the sagittal and coronal planes.

Imaging analysis

Lumbalization and sacralization terms were not used because of the frequent lack of full spinal imaging and the difficulties in precisely identifying the fifth vertebra. The evaluation was conducted by a radiologist with 28 years of experience in the field of radiology, using a structured report form on a PACS (Picture Archiving and Communications System) workstation.

“Degeneration at the LSTV level’’ or pseudoarticulation refers to narrowing the distance between the transverse process and the sacrum, sclerosis of the bony surfaces, and/or osteophyte formation (Figure 1).

“Disc narrowing” was evaluated according to the four-degree scale defined by Videman et al. [14]. Except for the L5–S1 disc, cases were considered significant for disc narrowing from grade 1, where the disc height is greater than the cephalad disc.

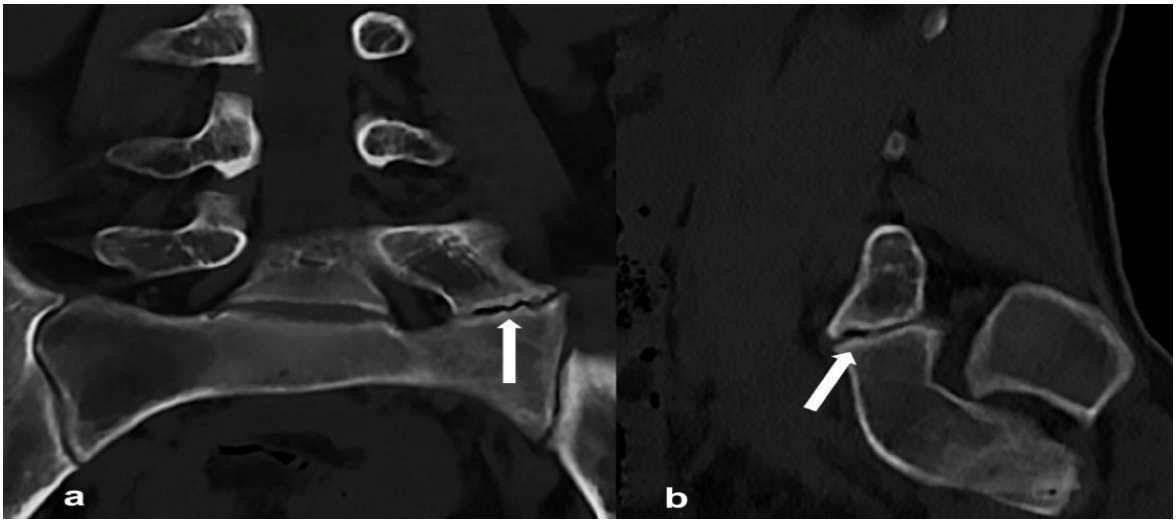

Figure 1. A 20-year-old female patient with Castellvi Type II shows irregularities in the bony surfaces, subchondral sclerosis, and degenerative vacuum phenomena in the joint space at the left pseudoarticulation level

Coronal (a) and sagittal CT image (b)

“Facet joint arthropathy” has been evaluated according to the classification published by Kalichman et al. [15]. Changes such as joint space narrowing, osteophyte formation, hypertrophy of the joint protrusion, subarticular sclerosis, subchondral cysts, and the vacuum phenomenon have been considered. Accordingly, images have been considered positive from grade one (mild degenerative disease, characterized by narrowing of the joint space, small osteophyte formation, or mild hypertrophy of the joint) [16].

Nerve root canal stenosis” describes the obliteration of the fat tissue in the intervertebral foramina. It has been considered narrowed when the dimension measured at the level of the nerve canal between the pseudoarticulation and the lateral side of the vertebral body is less than 3 mm. [17] (Figure 2).

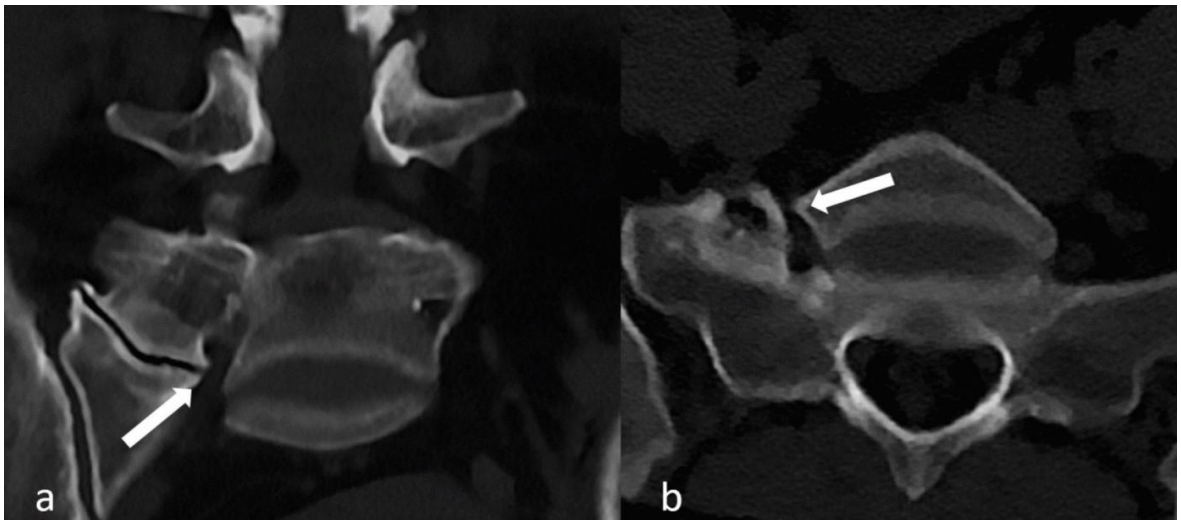

Figure 2. A 58-year-old woman with Castellvi Type II shows narrowing of the neural foramen and compression of the nerve root due to hypertrophy and degenerative changes at the level of the right pseudoarticulation

Coronal (a) and axial CT image (b)

Scoliosis is defined as a structural sideways curvature of the spine. A small deviation (<10 degrees) is sometimes called spinal asymmetry, while "true" scoliosis is characterized by a deviation greater than 10 degrees [18].

The term "anterolisthesis" refers to the forward displacement of one vertebral body relative to the vertebral body below it. In the study, anterolisthesis was evaluated according to the Meyerding classification. Vertical lines are drawn along the upper and lower vertebral posterior cortex, and a measurement is taken between them [19]. Sagittal reconstruction images were used to detect anterolisthesis, and its presence or absence was recorded as a binary value.

Statistical analysis

The analysis was conducted using the SPSS 25.0 (IBM SPSS Statistics 25 software (Armonk, NY: IBM Corp.)) package program. Continuous variables are presented as mean ± standard deviation and median (IQR: 25^{th}-75^{th} percentiles), and categorical variables are presented as counts and percentages. Kruskal-Wallis variance analysis was used to examine differences between groups for numerical data, while the Chi-square test was used for categorical data. The relationships between continuous variables were examined using the Spearman correlation coefficient. A *p*-value <0.05 was considered statistically significant.

Results

The frequency distributions of CT findings for 133 patients are presented in Table 1. Of the 1035 patients included in the study, 133 (12.85%) (66.2% female, 33.8% male; age range 15-85 years; mean 58; median 59) were found to have LSTV. The gender disparity was not statistically significant (*p*=0.257).

The prevalence of degeneration at the LSTV level was 67.7% (90 out of 133) among the patient population. The presence of degeneration was observed in 98.2% of patients in Type II (56 out of 57), in all patients in Type IV (4 out of 4), in 60% of patients in Type I (30 out of 50), and no degeneration was observed in any patient in Type III. Accordingly, these changes were significantly higher in Types II and IV, whereas they were not observed in Type III.

Table 1. Distribution of lumbar CT findings

Types	Patients (n:133)	Degeneration of LSTV level	Narrowing of Cephalad Disc	Facet Arthropathy	Foraminal Stenosis	Anterior Spondylolisthesis	Scoliosis
Type 1	50	30	32	41	0	9	12
Type 2	57	56	45	48	13	13	29
Type 3	22	0	20	21	0	4	1
Type 4	4	4	2	4	0	1	2
Total	133	90 (67.6%)	99 (74.4%)	114 (85.7%)	13 (9.7%)	27 (20.3%)	44 (33.0%)
***p* value**		0.0012	0.041	0.252	0.0033	0.96	0.0013

A *p*-value <0.05 was considered statistically significant

The prevalence of intervertebral disc narrowing at the upper level of the transitional vertebra was 74.4% (99 out of 133) in the study population. This rate exceeded 90% (20 out of 22) in Type III LSTV, although a high prevalence was noted across all LSTV types.

Facet arthropathy was observed in 85.7% (114 out of 133) of patients, with no significant intergroup difference.

The prevalence of foraminal stenosis was 9.8% (13 out of 133), with all cases occurring in the Type II group.

The prevalence of anterior spondylolisthesis was 19.5% (26 out of 133 patients), and there was no notable difference between groups in the presence of anterior spondylolisthesis.

Scoliosis was identified in 33.1% of patients (44 out of 133), with a higher prevalence observed in Types II and IV. Scoliosis was observed in 50.9% (29 of 57) of patients in Type II, 50% (2 of 4) of patients in Type IV, 24% (12 of 50) of patients in Type I, and 4.5% (1 of 22) of patients in Type III.

Statistically significant differences were observed among LSTV patient intergroups in terms of degeneration of LSTV level (p=0.0012), foraminal stenosis (p=0.0033), narrowing of cephalad disc (p=0.041), and scoliosis (p=0.0013). In contrast, no statistically significant difference was observed between the LSTV patient groups with respect to gender (p=0.276), age (p=0.346), facet arthropathy (p=0.252), and anterior spondylolisthesis (p=0.960).

Discussion

LSTV variation was detected in 12.85% of the total sample of 1035 patients in the present study. Several notable differences were identified between the LSTV patient groups regarding LSTV pseudoarticular degeneration, foraminal stenosis, cephalad disc narrowing, and scoliosis. However, no differences were observed in terms of gender, age, facet arthropathy, and anterior spondylolisthesis. While degenerative changes at the pseudoarticulation level were detected at a higher rate in Type II and Type IV compared to other types, cephalad disc narrowing was more common in Type III, foraminal stenosis was high only in Type II, and scoliosis was detected at a much lower rate in Type III. These differences between types are important in the differential diagnosis of low back pain.

Previous studies have associated Type II and Type IV LSTV with an increased prevalence of low back and hip pain, as well as lower levels of physical activity [20]. The mechanism behind spinal biomechanical abnormalities caused by LSTV remains unclear [10]. In this study, the detection of pseudoarticular degeneration in almost all Type II and all Type IV patients suggests that LSTV pseudoarticular degeneration begins at an early age (Figure 1). This could explain why LSTV is a common cause of low back pain in younger and middle-aged individuals [11]. The development of degenerative changes at other levels due to the shift of the center of gravity to the upper levels, which is often discussed in the literature, is thought to play a secondary role.

In the present study, more than half of Type I LSTVs (60%) had pseudoarticular degeneration. This suggests that with age, the loss of height in the disc space also reduces the distance between the transverse process and the sacrum, and degeneration develops at this level over time due to friction. L4-5 height loss was found at almost the same rate in Type I LSTVs (64%). This finding shows that Type I LSTV can progress to Type II LSTV over time.

Foraminal and extraforaminal stenosis due to degenerative hypertrophy at the pseudoarticulation level was detected in 13 (9.7%) of the patients, all of whom had Type II LSTVs. Similar rates of 13% have been reported in previous studies [21]. Nerve root compression may result from stenosis at this level [22], so patients with LSTV should undergo a detailed examination for this condition, particularly when clinical signs of L5 nerve root compression are present. Coronal MRI sequences or additional CT imaging can help evaluate the neural foramen.

As demonstrated by numerous studies, the discs situated above the transitional vertebra are more susceptible to early degeneration, while those located between the transitional vertebra and the sacrum exhibit a reduced risk of degeneration [12, 23]. In the present study, it was observed that the loss of height of the intervertebral disc above the transitional vertebra was more prevalent in Type II and Type III. This finding suggests that patients with these types may experience a greater incidence of back pain due to degenerative changes.

Scoliosis was found to be more common in the Type II and Type IV patient groups than in the other types. It was found in about half of the patients in both groups. Scoliosis was found in 24% of the type I group and only 4.5% of the type III group. The presence of fixed vertebrae at the lumbosacral level due to fusion in Type III may be responsible for a reduced incidence of scoliosis, as observed in Type I. In contrast, the presence of asymmetry, degenerative changes, and instability at the pseudoarthrosis level, as seen in Types II and IV, may contribute to the development of scoliosis. A literature review did not reveal any relevant data on this issue.

There are some limitations to our study. Firstly, it was a retrospective study conducted in a single center, which is prone to selection bias. Another limitation was that the patients included were not asymptomatic, so a control group could not be included in the study. Despite these limitations, the study included a large and homogeneous number of patients. Inter-reader reliability of different degeneration parameters was not performed because it was not the subject of this study.

In conclusion, LSTV has been identified as a prevalent incidental finding, with a prevalence of 12.85% in lumbar CT scans obtained due to low back pain. The present findings indicate that degeneration at the pseudoarticulation level occurs at an early age in Type II and Type IV patient groups, thus providing a rationale for the low back pain observed in young and middle-aged patients with LSTV. Furthermore, the study noted that nerve root compression due to degenerative hypertrophy at the pseudoarticulation level is prevalent, particularly in the Type II patient group. Therefore, care should be taken not to overlook transition areas on MRI or CT scans, especially in symptomatic cases.

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Authors contributions: E.S. constructed the main idea and hypothesis of the study. E.S. and H.P. developed the theory and arranged/edited the material and method section. H.P. and E.S. have evaluated the data in the results section. The discussion section of the article was written by E.S. and H.P. reviewed, corrected and approved. In addition, both authors discussed the entire study and approved the final version.

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

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Research Article

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Evaluation of inflammation-related prognostic scores, CRP/albumin, LDH/albumin and lactate/albumin ratios in patients with sepsis

Sepsis hastalarında inflamasyon ilişkili prognostik skorlar CRP/albumin, LDH/albumin ve laktat/albumin oranlarının değerlendirilmesi

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Abstract

Purpose: Results regarding the clinical usefulness and predictive accuracy of inflammation-based parameters, including C-reactive protein (CRP), lactate dehydrogenase (LDH), and lactate in septic patients are conflicting. In our study, we aimed to evaluate the relationships of the combination of these inflammatory parameters with albumin, disease severity and prognosis.

Materials and methods: 98 patients diagnosed with sepsis were categorised as survivors (n=68) and nonsurvivors (n=30) according to their intensive care unit (ICU) mortality. Prognostic factors were evaluated with the receiver operating characteristic curve and Cox proportional hazard regression model. Survival was analyzed with the Kaplan-Meier method.

Results: Compared to survivors, nonsurvivors had increased CRP and lactate (13.5 vs 10.0, *p*=0.044; 2.2 vs 1.7, *p*=0.006, respectively), while LDH and albumin levels were not significantly different (246.0 vs 245.0, *p*=0.624; 2.9 vs 3.0, *p*=0.061, respectively). When combined factors were evaluated, CRP/albumin and lactate/albumin were significantly higher in nonsurvivors (4.9 vs 3.0, *p*=0.018; 0.8 vs 0.5, *p*=0.001, respectively), while LDH/albumin was similar in both groups (93.1 vs 78.9, *p*=0.148). Lactate/albumin had the highest AUC of 0.709 (*p*=0.001), while CRP had the lowest AUC of 0.628 (*p*=0.044). In a multivariate analysis of possible predictors of ICU mortality, only lactate could be an independent predictor of poor outcome (HR:1.998; *p*=0.020), while other variables were not independently associated.

Conclusion: Increased CRP, lactate, CRP/albumin and lactate/albumin levels in septic patients were associated with poor outcomes. Lactate level was an independent predictor of mortality.

Keywords: Albumin, C-reactive protein, lactate, prognosis, sepsis.

Ilban A, Ilban O. Evaluation of inflammation-related prognostic scores, CRP/albumin, LDH/albumin and lactate/albumin ratios in patients with sepsis. Pam Med J 2025;18:550-560.

Öz

Amaç: Septik hastalarda C-reaktif protein (CRP), laktat dehidrogenaz (LDH) ve laktat gibi inflamasyona dayalı parametrelerin klinik yararlılığı ve tahmin doğruluğuna ilişkin sonuçlar çelişkilidir. Çalışmamızda bu inflamatuvar parametrelerin albumin ile kombinasyonunun, hastalık şiddeti ve prognoz ile ilişkilerini değerlendirmeyi amaçladık.

Gereç ve yöntem: Sepsis tanısı konulan 98 hasta, yoğun bakım ünitesi (YBÜ) mortalitelerine göre yaşayanlar (n=68) ve ölenler (n=30) olarak sınıflandırıldı. Prognostik faktörler receiver operating characteristic curve ve Cox proportional hazard regression model ile değerlendirildi. Sağ kalım Kaplan-Meier methodu ile analiz edildi.

Bulgular: Yaşayan hastalarla karşılaştırıldığında, ölen hastalarda CRP ve laktat düzeyleri artmıştı (sırasıyla 13,5'e karşı 10,0, *p*=0,044; 2,2'ye karşı 1,7, *p*=0,006), ancak LDH ve albumin düzeyleri anlamlı derecede farklı değildi (sırasıyla 246,0'ya karşı 245,0, *p*=0,624; 2,9'a karşı 3,0, *p*=0,061). Kombine faktörler değerlendirildiğinde, CRP/albumin ve laktat/albumin ölen hastalarda anlamlı derecede yüksek iken (sırasıyla 4,9'a karşı 3,0, *p*=0,018; 0,8'e karşı 0,5, *p*=0,001), LDH/albumin ise her iki grupta benzerdi (93,1'e karşı 78,9, *p*=0,148). Laktat/albumin 0,709 ile en yüksek AUC'ye sahipken (*p*=0,001), CRP 0,628 ile en düşük AUC'ye sahipti (*p*=0,044). YBÜ mortalitesinin olası belirleyicilerinin çok değişkenli analizinde, yalnızca laktat kötü sonucun bağımsız bir belirleyicisi olabilirken (HR:1,998; *p*=0,020), diğer değişkenler bağımsız olarak ilişkili değildi.

Sonuç: Septik hastalarda CRP, laktat, CRP/albumin ve laktat/albumin düzeylerindeki artış kötü sonuçlarla ilişkilidir. Laktat düzeyi mortalitenin bağımsız bir belirleyicisidir.

Anahtar kelimeler: Albumin, C-reaktif protein, laktat, prognoz, sepsis.

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Introduction

Sepsis, defined as an uncontrolled host response to infection, leads to death as a result of cellular and organ dysfunction caused by dysregulated inflammation [1]. The intensity of the infection and the inflammatory response are the main factors determining the outcome in critically ill patients with sepsis [2]. Various biomarkers have been used for diagnosis, treatment and prognostic evaluation of infections in sepsis patients. The ability to detect the disease with high accuracy using sepsis biomarkers may help to initiate appropriate antibiotic therapy early and evaluate treatment efficacy [3].

Sepsis patients are a heterogeneous population and have different risk factors including age, underlying diseases, infection pattern and organ dysfunction. Due to the wide and complicated structures of immune mediators and the influence of different conditions, it is difficult to find biomarkers with high predictive value based on immune response in critically ill patients. Simultaneous assessment of several sepsis-associated biomarkers may reduce the limitations of any one biomarker. Although some inflammation-based parameters, including C-reactive protein (CRP), lactate dehydrogenase (LDH), lactate and albumin and their combinations have been investigated as potential indicators, mixed results have been obtained regarding their clinical usefulness and predictive accuracy [3-6].

Considering the potential effects of the inflammatory response on the clinical outcome of septic patients, we hypothesised that the combinations of these inflammatory parameters with albumin, a negative acute phase reactant, would be superior in predicting intensive care unit (ICU) mortality. To test this hypothesis, we evaluated the associations of CRP/albumin, LDH/albumin and lactate/albumin ratios with disease severity and their prognostic performance.

Materials and methods

Study design

We conducted a retrospective cohort study involving an analysis of the medical records of patients diagnosed with sepsis within the first 48 hours of admission to the Anesthesiology and Reanimation ICU of Ordu University for a period of 1 year. This study was conducted in accordance with the Declaration of Helsinki between June 2023 and June 2024, with the approval of the Ordu University Non-Interventional Clinical Research Ethics Committee (approval number: 108/2024 and approval date: July 26, 2024). Informed consent was not obtained due to its retrospective nature.

Inclusion and exclusion criteria

Medical patients aged 18 years and over, diagnosed with clinical sepsis according to the SEPSIS-3 definition and confirmed microbiologically were included in the study [7]. Patients with intoxication, malignancy, preexisting immunodeficiency, steroid use (prednisolone equivalent above 0.3 mg/kg/day), chronic hepatic failure, renal replacement therapy, blood product transfusion and malnutrition were excluded from the study.

Study protocol

Demographic, clinical data, and laboratory values were documented. Only the first admission records were used to analyse patients with multiple ICU admissions. In patients diagnosed with sepsis, disease severity determined by the sequential organ failure assessment (SOFA) score, mechanical ventilation requirement and biochemical parameters were measured within the first 48 hours after admission. Microbiological results in samples obtained 48 hours before or after ICU admission were evaluated. Standard microbiological methods were used in the isolation of the samples. Identification and antimicrobial susceptibilities of the growing colonies were determined by Becton Dickinson Phoenix (USA) automated system. Antimicrobial resistance status was defined according to the study of Magiorakos et al. [8]. Minimal inhibitory

concentration (MIC) breakpoints, as defined by the European Committee on Antimicrobial Susceptibility Testing, were used to assess MIC results (EUCAST 2023) [9]. Pathogens with intermediate antimicrobial susceptibility were defined as resistant. Empiric antimicrobial therapy with adequate doses of antimicrobial drugs covering likely pathogens was considered appropriate. CRP, LDH, and albumin levels were measured with Cobas 8000 (Roche-Hitachi, Tokyo, Japan), and lactate levels were measured with ABL800 Flex (Radiometer, Copenhagen, Denmark). All the patients received routine sepsis treatment as defined by current sepsis guidelines [7]. The study period was 1 year, and the primary endpoint was 30-day ICU mortality.

Sample size

In our preliminary analysis of 26 patients (18 were survivors and 8 were non-survivors), the areas under the curve (AUC) of lactate/albumin's receiver operating characteristic (ROC) analysis to predict death were 0.68. To determine if a statistically significant difference in lactate/albumin values existed between the groups, with an alpha of 5%, relating to a null value of 0.5 and power of 80%, at least 67 survivors (negative cases) and 29 non-survivors (positive cases) were required.

Statistical analysis

Data analysis was conducted using SPSS software (version 26.0). Continuous variables were summarised using mean values ± SD or medians (interquartile ranges), and categorical variables were expressed using percentages. To compare potential predictors between survivors and non-survivors, the Mann-Whitney U test was employed. Spearman's rank correlation test was utilised to examine the association between inflammatory markers and SOFA scores. The predictive performance in mortality discrimination of the variables found significant in the univariate analysis was evaluated by ROC analysis, and the AUC values were calculated. The optimal cut-off values were established using Youden's index with maximisation of sensitivity and specificity. Differences between ROC curves were analysed using the DeLong et al. [10] method. The independent predictors of mortality in critically ill patients were defined by the Cox proportional hazard regression model. Covariates, including age, sex, and SOFA score, were used for adjustment. Lactate-related variables that reached the highest AUC values in the ROC analysis were further examined. Kaplan-Meier survival curves were created using the prognostic cut-off values of these parameters and analysed with the log-rank test. Statistical significance was defined as two-sided *p*-values lower than 0.05.

Results

Out of 118 patients admitted to the ICU, 98 medical patients met the study criteria and were included in the statistical analysis. The median age of these participants was 76.5 years, 45.9% male and 54.1% female. Clinical data and some laboratory parameters are shown in Table 1. In this cohort, a median stay of 15 days was spent in the ICU; hypertension was the most common comorbidity with 44.9%; the median SOFA score was 8 and 41.8% of patients received mechanical ventilation.

When the sources of infection were evaluated, the most common infection site responsible for the development of sepsis was pneumonia, with 31.6%. Of the 113 microorganisms identified in septic patients, 67 were gram-negative, 37 were gram-positive, and 9 were fungal agents. When the responsible bacteria were evaluated according to their antimicrobial resistance status, the incidence of multi-drug resistant (MDR) agents was highest in gram-negative pathogens with 54, while it was found to be 27 in gram-positive pathogens (Table 1).

CRP, LDH, lactate and albumin determined in the blood withdrawn within the first 24 hours of admission to the ICU are shown in Table 2. Compared to survivors, non-survivors presented increased CRP and lactate (13.5 vs 10.0, p=0.044; 2.2 vs 1.7, p=0.006, respectively), while LDH and albumin levels were not significantly different (246.0 vs 245.0, p=0.624; 2.9 vs 3.0, p=0.061, respectively). When combined factors were evaluated, CRP/albumin and lactate/albumin were significantly higher in non-survivors (4.9 vs 3.0, p=0.018; 0.8 vs 0.5, p=0.001, respectively), while LDH/albumin was similar in both groups (93.1 vs 78.9, p=0.148).

Table 1. Characteristics of study cohort

Characteristic	Value
Age	76.5 (67-87)
Gender, male, n (%)	45 (45.9)
Comorbidity, n (%)	
Diabetes mellitus	25 (25.5)
Hypertension	44 (44.9)
Renal failure	12 (12.2)
Heart disease	21 (21.4)
Pulmonary disease	19 (19.4)
Neurological disease	15 (15.3)
Vasopressor use	42 (42.9)
SOFA	8 (5-10)
Mechanical ventilation, n (%)	41 (41.8)
Hemoglobin (g/dL)	11.4 (9.5-12.7)
White Blood Cells (10^9/L)	13.3±5.1
Procalcitonin (ng/mL)	7.9 (5-12)
Serum creatinine (mg/dL)	0.9±0.3
Alanine aminotransferase, (IU/L)	38 (19-51)
Primary infection site, n (%)	
Pneumonia	31 (31.6)
Urinary tract	20 (20.4)
Skin/soft tissues	13 (13.3)
Abdominopelvic	10 (10.2)
Other	24 (24.5)
Identified microorganisms, n (%)	
Gram negative bacilli / MDRO	67 (68.4) / 54 (80.6)
Gram positive cocci / MDRO	37 (37.8) / 27 (73.0)
Fungi	9 (9.2)
Appropriate antimicrobial treatment, n (%)	64 (65.3)
Length of ICU stay (days)	15 (8-24)

n=98 patients. Data shown as mean ± standard deviation, median (interquartile ranges) or n (%)
SOFA: sequential organ failure assessment, MDRO: multi-drug resistant organism, ICU: Intensive care unit

Table 2. Comparison of selected possible predictors of mortality

Predictor	Total (n=98)	Survivors (n=68)	Nonsurvivors (n=30)	*p* value	z
CRP (mg/dL)	11.4 (4-18)	10 (3-16)	13.5 (7-81)	0.044*	-2.012
LDH (U/L)	245.5 (200-343)	245 (193-321)	246 (207-403)	0.624	-0.489
Lactate (mmol/L)	1.8 (1-3)	1.7 (1-2)	2.2 (2-4)	0.006*	-2.763
Albumin (g/dL)	3 (2-3)	3 (3-4)	2.9 (2-3)	0.061	-1.873
CRP/albumin	3.7 (2-7)	3 (1-6)	4.9 (2-29)	0.018*	-2.366
LDH/albumin	82.6 (67-122)	78.9 (64-113)	93.1 (67-151)	0.148	-1.445
Lactate/albumin	0.6 (0.4-0.9)	0.5 (0.3-0.7)	0.8 (0.4-1.4)	0.001*	-3.303

Data shown as median (interquartile ranges), * Survivors vs Nonsurvivors group ($p<0.05$)
CRP: C-reactive protein, LDH: lactate dehydrogenase z was the test value of the Mann-Whitney U test

Correlation coefficients of SOFA score and inflammatory parameters were evaluated by bivariate analysis. SOFA score determined in the initial stage of sepsis was positively correlated with CRP, lactate, CRP/albumin and lactate/albumin ratio (r=0.314, p=0.002; r=0.278, p=0.006; r=0.300, p=0.003; r=0.320, p=0.001, respectively). Compared to other parameters, the relationship between the lactate/albumin ratio and SOFA score was more pronounced. Nonetheless, no statistically significant association between SOFA score and LDH, albumin, and LDH/albumin ratio was observed (r=0.180, p=0.076; r=-0.117, p=0.252; r=0.191, p=0.060, respectively) (Table 3). Additionally, a significant positive association was observed between the lactate/albumin ratio and other important predictors of mortality, namely CRP, lactate, and CRP/albumin ratio (r=0.259, p=0.004; r=0.225, p=0.010; r=0.279, p=0.002) (data not shown).

Table 3. Correlation between SOFA scores and inflammatory biomarkers

Variable	SOFA	
	rs#	*p* value
CRP	0.314	0.002*
LDH	0.180	0.076
Lactate	0.278	0.006*
Albumin	-0.117	0.252
CRP/albumin	0.300	0.003*
LDH/albumin	0.191	0.060
Lactate/albumin	0.320	0.001*

SOFA: sequential organ failure assessment, CRP: C-reactive protein, LDH: lactate dehydrogenase, #Spearman correlation, *$p<0.05$

The usefulness of each indicator in predicting critically ill patient mortality was evaluated by ROC analysis (Table 4, Figure 1). Lactate/albumin had the highest AUC of 0.709 (p=0.001), while CRP had the lowest AUC of 0.628. CRP/albumin had the best sensitivity (63.4%) in predicting death with a cut-off value of 4.5, and lactate had the best specificity (95.6%) with a cut-off value of 3.4. When ROC curves were compared pairwise with lactate/albumin, which had the highest performance for mortality prediction, no significant difference between AUC values was observed. Therefore, the predictive ability of all variables in the mortality of critically ill patients was similar.

Table 4. Performance of significant variables in predicting ICU mortality

Variables	Cut-off	AUC (95% CI)	Sensitivity (%)	Specificity (%)	*p* value
CRP	12.3	0.628 (0.524-0.723)	63.3	61.8	0.308
Lactate	3.4	0.675 (0.573-0.767)	33.3	95.6	0.168
CRP/albumin	4.5	0.650 (0.548-0.744)	63.4	69.1	0.453
Lactate/albumin	0.75	0.709 (0.608-0.796)	56.7	76.5	

DeLong et al. Receiver operating characteristic plot analysis of significant variables with respect to prediction of ICU mortality
The *p* values correspond to the difference between the AUC of the parameters and the AUC of Lactate/albumin ratio
AUC: Area under the receiver operating characteristic curve, CI: Confidence interval, CRP: C-reactive protein

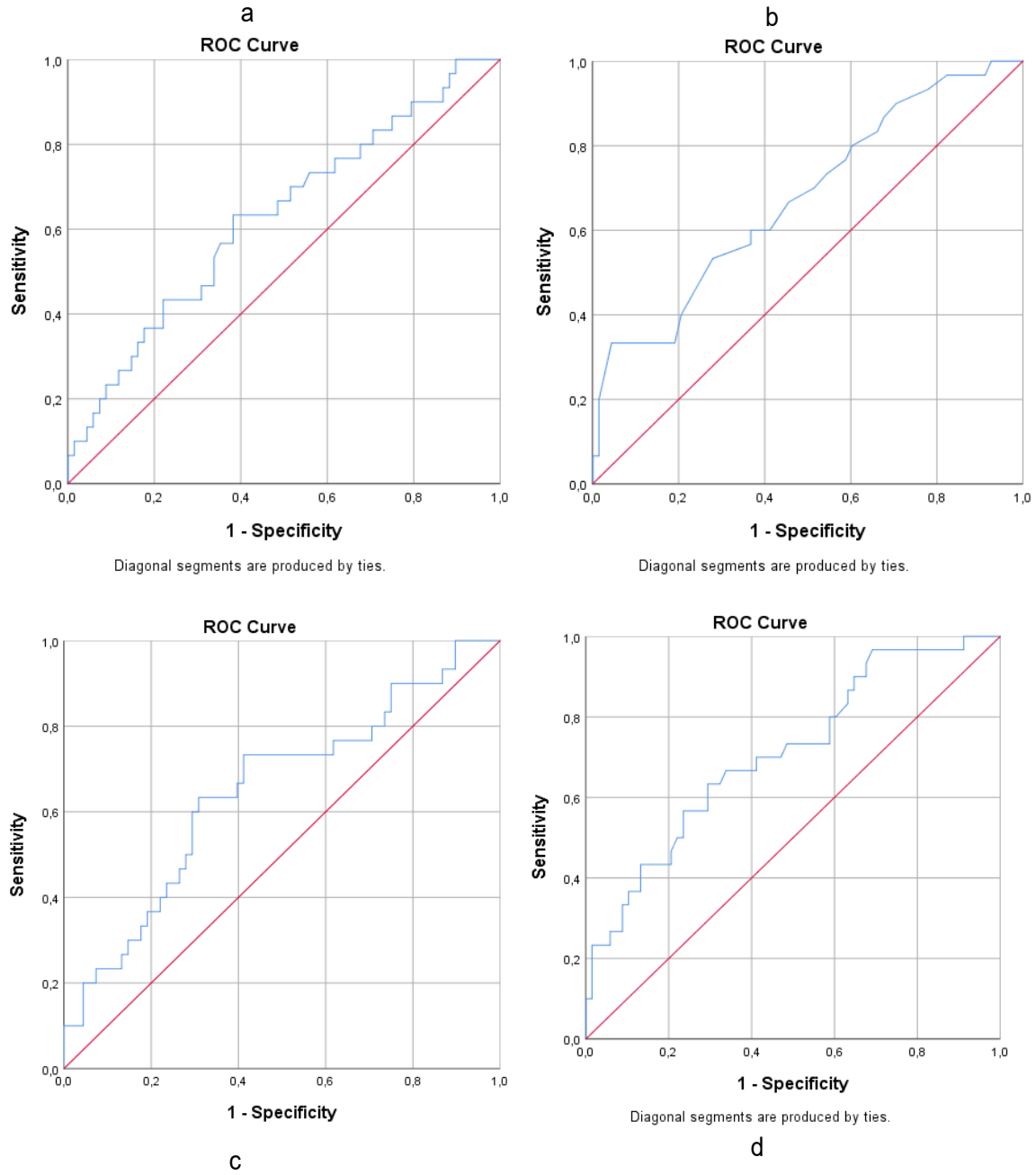

Figure 1. Receiver operating characteristics curves for a) C-reactive protein, b) Lactate, c) CRP/albumin and d) Lactate/albumin in predicting ICU mortality

In a multivariate analysis of possible predictors of critically ill ICU mortality, in addition to demographic factors, including age and sex, we checked the SOFA score, which included disease severity. When adjusted for these parameters, only lactate could be an independent predictor of poor outcome (HR:1.998; p=0.020), while the other variables were not independently associated (Table 5).

Table 5. Independent variables for predicting ICU mortality by multivariate Cox regression analysis

Predictor	B-value	HR	95% CI	*p* value
CRP	0.007	1.008	0.985-1.031	0.523
Lactate	0.692	1.998	1.114-3.581	0.020*
CRP/albumin	-0.004	0.996	0.943-1.051	0.878
Lactate/albumin	-0.972	0.378	0.107-1.341	0.132

Hazard ratio (HR) was calculated using a Cox proportional-hazards model adjusted by age, sex and SOFA score
The HR indicates the risk of ICU mortality, *p<0.05, CI: Confidence interval, CRP: C-reactive protein

Mortality at 30 days from ICU admission was analysed using the Kaplan-Meier method, using the cut-off values for lactate and lactate/albumin variables obtained from our data. Using a lactate cut-off value of 3.4, the survival probability of critically ill patients with <3.4 was significantly higher than those with ≥3.4 (p=0.040, log-rank test). However, when the cut-off value of 0.75 for lactate/albumin was used, the mortality rates of critically ill patients did not differ significantly (p=0.107, log-rank test) (Figure 2).

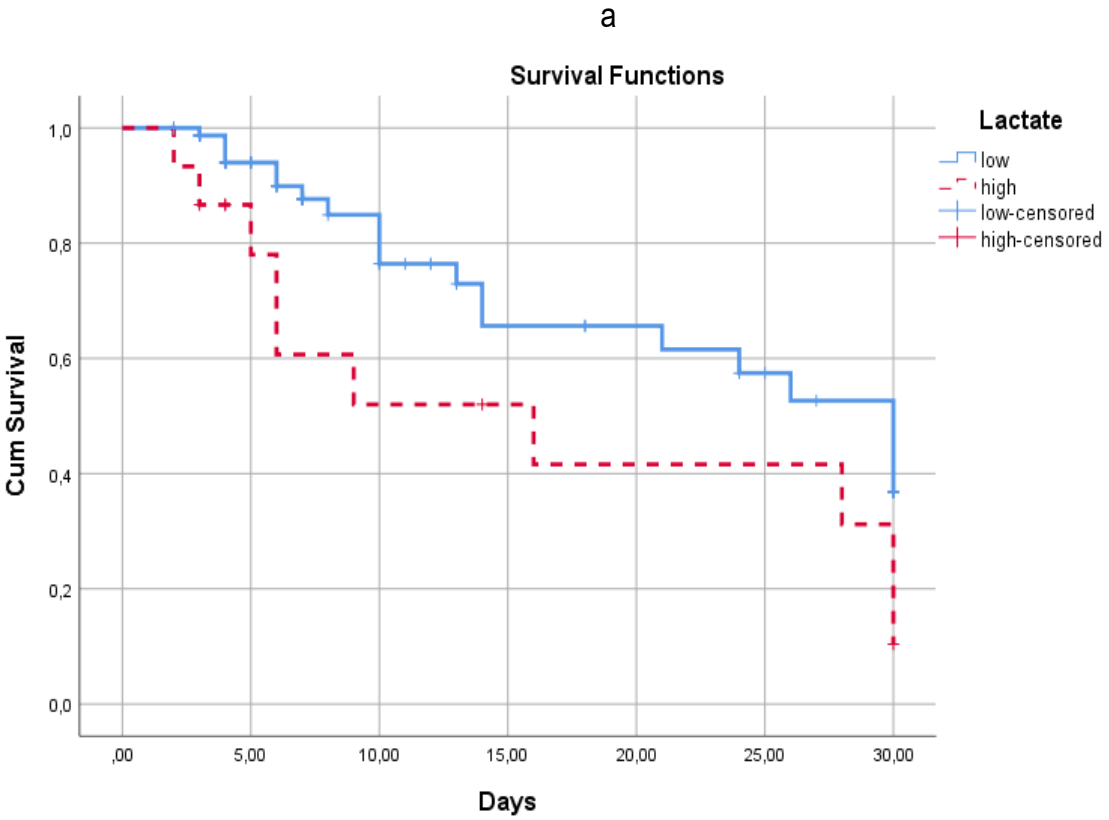

Figure 2. Kaplan-Meier survival curves of the critically ill patients stratified by

a) Lactate

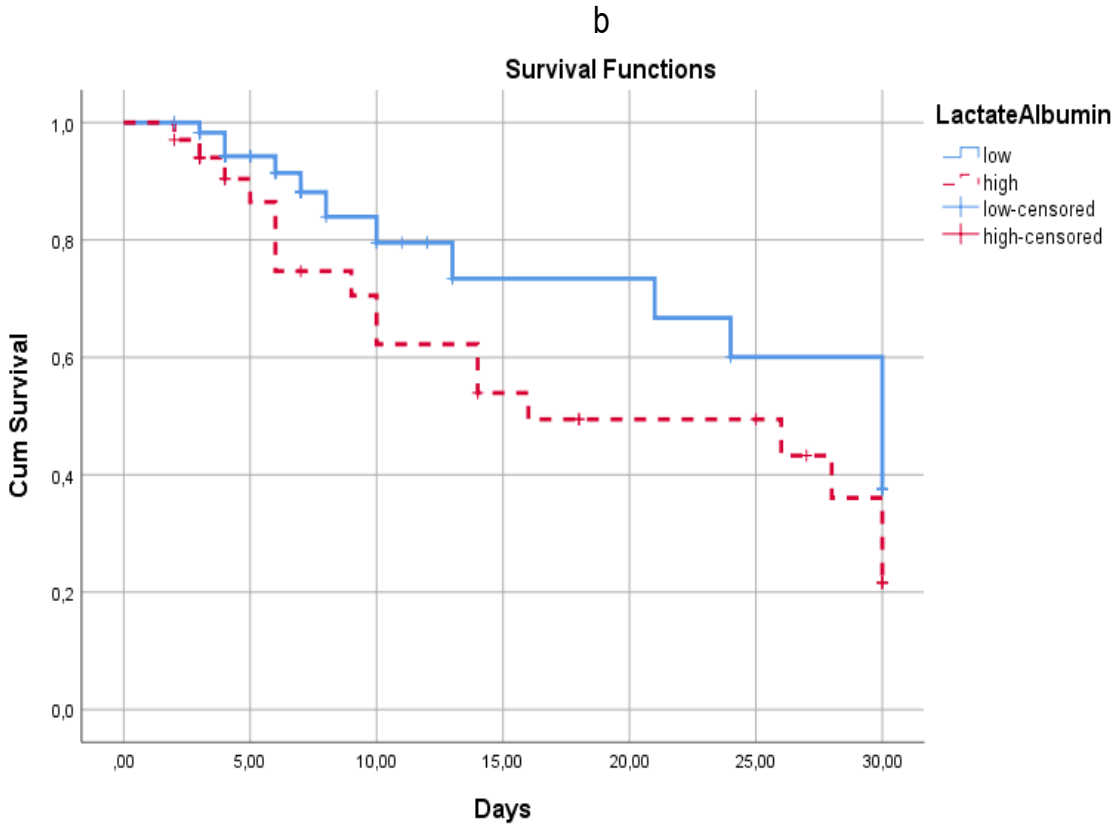

Figure 2. Kaplan-Meier survival curves of the critically ill patients stratified by b) Lactate/albumin ratio

Discussion

In this study, lactate levels were independently associated with time to death censored right at day 30 in critically ill patients experiencing sepsis, and the risk of mortality increased approximately twofold after adjusting for confounding factors. At the same time, lactate/albumin presented similar predictive values to other prognostic parameters, namely CRP, lactate and CRP/albumin, in distinguishing mortality in septic patients.

CRP is an acute-phase reactant synthesised in the liver by the significant stimulation of interleukin-6 in response to infection and inflammation [11]. CRP levels, which start to increase in the first 2 hours in acute conditions, can reach peak levels within 48 hours. The relatively short half-life of CRP, 19 hours, is important in the follow-up of infectious diseases and inflammatory conditions [12]. In the study by Ye et al. [13] examining the relationships of different markers with infection and sepsis, CRP performed superiorly to procalcitonin. In the study by Zhang et al. [14], CRP elevation was independently linked with a poorer prognosis in patients with sepsis. In our study, CRP levels, although reaching higher values in the non-survivors group, were not independent predictors of mortality.

Serum albumin levels, a nutritional parameter, are an indirect indicator of the inflammatory response and vary according to the severity of inflammation [15]. In a study conducted by Artero et al. [16] in community-acquired sepsis patients, hypoalbuminemia was identified as the most important risk factor associated with mortality. In another prospective study, Yin et al. [15] examined the prognostic significance of serum albumin levels in sepsis patients who did not receive exogenous albumin supplementation. They revealed that the risk of mortality was increased in patients with serum albumin below 29.2 g/L. Similarly, in our study, where we excluded patients receiving replacement therapy and prevented the potential confounding effect, although albumin levels were lower among non-survivors, the

difference between the groups was at the limit of statistical significance. This difference may also be related to the fact that albumin levels are affected by other variables, such as chronic inflammation and nutritional status [17].

The study sought to enhance predictive capacity by combining single parameters that have been shown to be independent predictors of mortality. The CRP/albumin ratio is being examined as a prognostic score in patients with systemic inflammation, sepsis and cancer. In the study conducted by Basile Filho et al. [18] in critically ill surgery patients, the CRP/albumin ratio, although it reached higher AUC values than CRP and albumin in predicting mortality, was not a prognostic indicator for the mortality of septic patients. Ranzani et al. [19], in their study conducted on medical ICU patients, investigated the association between the CRP/albumin ratio and the likelihood of death within 90 days of patients with sepsis. They showed that residual inflammation assessed by the CRP/albumin ratio at the discharge from the ICU was an independent predictor of long-term mortality after a sepsis episode and that the results had higher prognostic accuracy than single CRP measurements. In our study, CRP/albumin ratio had prognostic significance for 30-day ICU mortality in septic patients; its poor predictive value was similar to CRP but was not independently associated with mortality. These findings align with the results of earlier research, which suggest that residual effects may persist for up to 90 days post-ICU in septic patients and that inflammatory parameters could offer superior insight into long-term prognosis [20, 21].

Results regarding the prognostic significance of serum LDH levels in sepsis patients considered an indicator of anaerobic glycolysis, are contradictory. In the study conducted by Erez et al. [22] in medical patients, isolated LDH elevation, in addition to being associated with the severity of the underlying disease and infection, was an independent predictor of mortality. On the contrary, Miglietta et al. [23] showed that despite a higher incidence of mortality, LDH levels were lower in patients with systemic candidiasis than in patients with bacterial sepsis. The fact that LDH levels were not correlated with mortality in septic patients in our study was consistent with the second-mentioned study.

Low peripheral oxygenation, which leads to increased anaerobic glycolysis due to inadequate oxygen delivery in sepsis, increases lactate production [24]. However, lactate levels, generally considered an indicator of tissue hypoxia, also increase in conditions other than tissue oxygenation [25]. Hyperlactatemia observed in septic patients with normal oxygen delivery and tissue perfusion is associated with increased aerobic glycolysis due to overstimulation of the Na^+-K^+-ATPase pump in the skeletal muscle [26]. In addition, hepatic or renal dysfunction may increase lactate levels by decreasing lactate elimination [27]. Previous studies have shown that in patients with sepsis, along with the diagnostic value, increased lactate levels are linked to disease severity and mortality [28].

However, the fact that lactate levels are affected by different diseases may limit their diagnostic and prognostic use. The fact that lactate and albumin are associated with different mechanisms in the septic process suggests that a ratio in which inflammatory and nutritional parameters with prognostic importance are evaluated together may reach a higher predictive value. Wang et al. [29] assessed in a prospective study the clinical use of the lactate/albumin ratio in predicting the development of organ failure and mortality in patients with sepsis. Although lactate levels were higher in patients with multiple organ dysfunction syndrome (MODS), the risk of mortality was not increased. An increase in the lactate/albumin ratio was shown to be associated with MODS and mortality. In another study, Lichtenauer et al. [30] examined the importance of the lactate/albumin ratio in risk stratification of septic patients. Lactate levels were higher in non-survivors, and its predictive value in the short-term analysis, including in-hospital mortality, was comparable to that of the lactate/albumin ratio. However, in the long-term study, including post-discharge mortality, the lactate/albumin ratio had a higher prognostic value. Therefore, it was suggested that the combination of lactate, which indicates the severity of acute disease, and albumin, a long-term indicator such as nutritional status, may increase the prognostic capacity. In our study, admission lactate levels assessed by time-to-event analysis were linked to an elevated risk of 30-day ICU mortality in septic patients. Although the lactate/albumin

ratio reached the highest predictive value, its prognostic capacity was similar to serum lactate.

The increase in inflammation parameters observed in septic patients due to the acute phase response generated by the innate immune system is related to the severity of the clinical condition [1]. Since disease severity is an indicator of organ dysfunction caused by increased inflammation in sepsis patients, the significant correlation found in our study between the SOFA score and the highest predictive marker, the lactate/albumin ratio, suggests that our results are consistent with the relationship between increased inflammatory responses and the lactate/albumin ratio [31]. Hypothetically, disease severity assessed by the SOFA score, similar to other inflammatory conditions, is an important indicator of changes in inflammatory mediators observed in the acute phase of sepsis patients. Therefore, in accordance with the positive relationships between the well-known inflammatory markers CRP, lactate and CRP/albumin ratio and the lactate/albumin ratio, a significant relationship can be expected between the lactate/albumin ratio and the SOFA score.

There were some limitations in this study. The fact that our study was a single centre restricts the generalisation of our results to other institutions. Although we tried to measure the severity of disease in critically ill patients with sepsis using the SOFA score, unmeasured confounding variables and other causes of inflammation may prevent more reliable measurements of prognostic markers. Due to the lack of nutritional data such as body mass index or total protein, it was not possible to evaluate to what extent the parameters used for mortality risk estimation are related to nutritional status or sepsis-induced inflammation. In our results, only the relationship of inflammatory indicators with short-term mortality was evaluated. Therefore, multicenter prospective studies are needed to better assess the prognostic scores examined in our study as mortality predictors in septic patients.

In conclusion, increased CRP, lactate, CRP/albumin and lactate/albumin levels in sepsis patients were associated with a poor outcome and had similar prognostic values. However, only lactate was an independent predictor of mortality. Using these parameters in the early stages of sepsis may help identify high-risk patients and determine specific treatments.

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Authors' contributions: A.I. constructed the main idea and hypothesis of the study. O.I. collected the data. A.I. and O.I. developed the theory and arranged the material and method section. O.I. has done the evaluation of the data in the results section. Discussion section of the article was written, reviewed, corrected and approved by A.I. and O.I. In addition, all authors discussed the entire study and approved the final version.

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

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Research Article

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Surgical timing for proximal femur fractures does not affect early mortality: single center experience

Proksimal femur kırıklarında cerrahi zamanlama erken mortaliteyi etkilemez: tek merkez deneyimi

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Abstract

Purpose: This study investigates the relationship between surgical timing and 30-day and 90-day mortality in geriatric patients with proximal femur fractures. It also evaluates other parameters potentially affecting mortality, addressing the ongoing debate in the literature about the ideal surgical timing for such fractures.

Patients and methods: A retrospective analysis of 181 patients aged 65 and older with femoral neck or intertrochanteric femur fractures was conducted. Data on demographics, comorbidities, fracture type, surgical timing, and mortality were collected. Patients were categorized into four groups based on surgery timing: within 24 hours, 24-48 hours, 48-72 hours, and after 72 hours. Statistical analyses included t-tests, Mann-Whitney U tests, Chi-square tests, and Bonferroni-corrected post hoc analyses. A significance level of $p<0.05$ was used.

Results: The overall 30-day and 90-day mortality rates were 3.86% and 11.04%, respectively. Surgical timing did not significantly affect 30-day and 90-day mortality. The highest 30-day mortality rate (6.7%) was observed in patients operated on within the first 24 hours, potentially due to rushed preoperative preparations. The group with the highest 90-day mortality rate (22%) consisted of patients undergoing surgery after 72 hours, likely influenced by comorbidities or anticoagulant use.

Conclusion: Surgical timing does not significantly affect mortality in proximal femur fractures, though the safest interval appears to be 24-72 hours. While early surgery can reduce complications related to immobilization, sufficient time for preoperative optimization is crucial. A balanced approach focusing on patient readiness rather than rigid timing guidelines ensures better outcomes.

Keywords: Hip fracture, surgical timing, mortality.

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Öz

Amaç: Bu çalışmada, proksimal femur kırığı olan geriatri hastalarında cerrahi zamanlama ile 30 günlük ve 90 günlük mortalite arasındaki ilişkiyi araştırmak amaçlanmıştır. Ayrıca, literatürde bu tür kırıklar için ideal cerrahi zamanı konusundaki devam eden tartışmayı ele alarak, mortaliteyi potansiyel olarak etkileyebilecek diğer parametreler de değerlendirildi.

Hastalar ve yöntem: 65 yaş ve üzeri femoral boyun kırığı veya intertrokanterik femur kırığı olan 181 hastanın retrospektif analizi yapıldı. Demografik veriler, komorbiditeler, kırık tipi, cerrahi zamanı ve mortalite hakkında veriler toplandı. Hastalar, ameliyat zamanlamasına göre dört gruba ayrıldı: 24 saat içinde, 24-48 saat içinde, 48-72 saat içinde ve 72 saatten sonra. İstatistiksel analizler t-testleri, Mann-Whitney U testleri, Ki-kare testleri ve Bonferroni düzeltmeli post hoc analizlerini içeriyordu. $p<0,05$ anlamlılık düzeyi kullanıldı.

Bulgular: Toplam 30 günlük ve 90 günlük ölüm oranları sırasıyla %3,86 ve %11,04'tü. Cerrahi zamanı, 30 günlük ve 90 günlük mortaliteyi önemli ölçüde etkilemedi. En yüksek 30 günlük ölüm oranı (%6,7), ilk 24 saat içinde ameliyat edilen hastalarda gözlemlendi; bu durum, muhtemelen aceleci preoperatif hazırlıklardan kaynaklanıyor. En yüksek 90 günlük ölüm oranına sahip grup (%22), muhtemelen komorbiditeler veya antikoagülan kullanımı tarafından etkilenmiş olan 72 saatten sonra ameliyat olan hastalardan oluşuyordu.

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Sonuç: Cerrahi zamanlama, proksimal femur kırıklarında mortaliteyi önemli ölçüde etkilemez, ancak en güvenli aralık 24-72 saat gibi görünmektedir. Erken cerrahi, immobilizasyonla ilgili komplikasyonları azaltabilirken, preoperatif optimizasyon için yeterli zaman kritik öneme sahiptir. Hasta hazırlığına odaklanan dengeli bir yaklaşım, katı zamanlama yönergeleri yerine daha iyi sonuçlar sağlar.

Anahtar kelimeler: Kalça kırığı, cerrahi zamanlama, mortalite.

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Introduction

One of the main causes of death and morbidity in the elderly population is hip fractures, which are widespread [1]. According to published research, 30-day death rates following hip fractures range from 8% to 13% [2]. In addition to mortality, hip fractures lead to pain, limited mobility, deformity, and a decrease in quality of life in both the early and late stages [3].

In studies related to surgical timing for proximal femur fractures, definitions of early and late surgery vary, but surgery performed within the first 24-48 hours after admission is often considered early surgery [4]. It is argued that early surgery reduces mortality and avoids complications such as pressure sores, thromboembolism, pneumonia, and urinary tract infections, which often occur secondarily to immobilization [5-7]. In addition, there are studies indicating that surgical timing does not affect mortality, but early surgery may be preferred to avoid potential perioperative complications [8-11].

In some countries, national guidelines have been published for the timing of surgery in hip fractures, and they are evaluating this as a quality indicator in healthcare service delivery [12, 13]. However, there are also authors who argue that early surgery cannot be used as an indicator of quality and that providing sufficient time for preoperative medical optimization for patients is safer [14].

Regarding the impact of surgical scheduling on mortality following hip fractures, there is disagreement in the research. In this study, the relationship between surgical timing and mortality rates at 30 days and 90 days was investigated. Additionally, other parameters that could affect mortality were also evaluated.

Materials and methods

Following the approval from the Scientific Research Evaluation and Ethics Committee of Ankara Etlik City Hospital (approval number: AEŞH-BADEK-2024-038, approval date: January 10, 2024), patients diagnosed with proximal femur fractures who applied to our hospital between October 2022 and December 2023 were retrospectively examined. Patients aged 65 and over, diagnosed with femoral neck fracture (FNF) and intertrochanteric femoral fracture (ITF) after falling from their own height, were included in the study. Patients younger than 65 years, polytrauma patients with accompanying fractures or other systemic injuries, and patients with pathological fractures were excluded from the study. When patients were evaluated according to these criteria, the study comprised a total of 181 patients. Data on patients' age, gender, comorbidities, use of oral anticoagulants, types of fractures, consultations requested during the preoperative preparation process, times of hospital admission and surgery, and types of surgeries performed (proximal femur nail, hemiarthroplasty, and total hip arthroplasty) were collected from our hospital's archive records. Patients were divided into 4 groups based on the timing of the surgical procedure: the first 24 hours, 24-48 hours, 48-72 hours, and after 72 hours. Again, the patients' death statuses were queried through the phone numbers registered in the archive, and the dates of death of the deceased were recorded.

The results of tests conducted on variables in the study were evaluated with a 95% confidence interval, and a *p*-value of <0.05 was considered significant. The Kolmogorov-Smirnov test was applied for normality analysis of the data. To detect differences between numerical variables and groups, Independent Sample t-tests were conducted for variables where the normality condition was met, and Mann-

Whitney U Test procedures were conducted for variables where the condition was not met. For the analysis of differences between groups for categorical variables, a non-parametric test, Chi-square Test, was applied. Post Hoc Analysis was conducted to determine which groups had significant differences in analyses involving more than two categorical groups. In this context, Adjusted Residual determination was made, and new *p*-values were determined with Bonferroni correction. Detection and interpretation of differences between groups were made based on these *p*-values.

Results

Among the 181 patients in the study, 71 (39.2%) were male and 110 (60.8%) were female. The mean age of the patients was determined to be 80.2. Femoral neck fractures occurred in 63 (34.8%) and intertrochanteric femur fractures in 118 (65.2%) of the patients. A total of 24 patients with femoral neck fractures performed hemiarthroplasty, 39 patients underwent total hip arthroplasty, and all patients with intertrochanteric femur fractures were treated with proximal femoral nailing.

Upon assessing the comorbidities of the patients, hypertension (116 patients), diabetes mellitus (68 patients), and coronary artery disease (42 patients) were identified as the most prevalent concomitant conditions. The most commonly requested preoperative consultations during surgical preparation were cardiology (173 patients), pulmonology (169 patients), and internal medicine (68 patients). Each patient requested an average of 2.74 preoperative consultations, and the average completion time for these consultations by the relevant departments, following the initial assessment by the anesthesiology department, was 84.6 minutes. Table 1 presents the comorbidities of the patients and the departments for which preoperative consultations were solicited.

Table 1. Patients' comorbidities and preoperative consultations

Comorbidities	n (%)	Preoperative Consultations	n (%)
Hypertension	116 (64.1)	Cardiology	173 (95.5)
Diabetes Mellitus	68 (37.5)	Pulmonology	169 (93.3)
Coronary Artery Disease	42 (23.2)	Internal Medicine	68 (37.5)
Stroke	18 (9.9)	Neurology	54 (29.8)
Alzheimer	18 (9.9)	Psychiatry	9 (4.9)
Chronic Obstructive Pulmonary Disease	15 (8.2)	Neurosurgery	8 (4.4)
Parkinson's Disease	11 (6)	Thoracic Surgery	5 (2.7)
Heart failure	8 (4.4)	Endocrinology	4 (2.2)
Thyroid Dysfunction	5 (2.7)	Nephrology	3 (1.6)
Chronic Renal Failure	4 (2.2)	General Surgery	3 (1.6)

The mean time from hospital admission to surgery for the patients was found to be 51.4 hours. The 30-day mortality rate for all patients was found to be 3.86% (7/181). Further, the death rate after 90 days was discovered to be 11.04% (20/181). The relationship between all the investigated variables and mortality rates at 30 days and 90 days was examined (Table 2, Table 3).

The mean age of patients who survived the first 30 days was 79.9, whereas the mean age of patients who died was 86.4, indicating a significant correlation between age and 30-day mortality (p=0.05). No correlation was identified between age and mortality within the first 90 days. No significant correlation was identified between gender, the length of preoperative consultations, and fracture type and 30-day and 90-day death rates.

Table 2. Relationship between variables and 30-day mortality

		30-day mortality			
		Survived	**Death**	**Total**	***p***
Age (Mean±SD)		79.9±8.54	86.4±8.58	80.24±8.61	0.05* (t=-1.953)
Preoperative Consultation Time (Mean±SD)		85.7±67.5	56.7±32.5	84.6±66.72	0.085 (u=375.00)
		Survived	**Death**	**Total**	***p***
Gender n (%)	**Female**	105 (95.4)	5 (4.6)	110 (100)	0.706¥
	Male	69 (97.1)	2 (2.9)	71 (100)	
Fracture Type n (%)	**FNF**	61 (96.8)	2 (3.2)	63 (100)	0.636 (v=0.000)
	ITF	113 (95.7)	5 (4.3)	118 (100)	
Comorbidities n (%)	**HT**	113 (97.4)	5 (2.6)	116 (100)	0.134 (v=0.000)
	DM	61 (89.7)	6 (10.3)	68 (100)	0.046¥*
	CAD	38 (90.4)	4 (9.6)	42 (100)	0.05¥*
	Stroke	18 (100)	0 (0)	18 (100)	0.398¥
	Alzheimer	17 (94.4)	1 (5.6)	18 (100)	0.534¥
	COPD	13 (86.6)	2 (13.4)	15 (100)	0.047* (v=3.942)
	Parkinson's Disease	11 (100)	0 (0)	11 (100)	0.416¥
	HF	6 (75)	2 (25)	8 (100)	0.032¥*
	TD	5 (100)	0 (0)	5 (100)	0.142¥
	CRF	4 (100)	0 (0)	4 (100)	0.096¥
Surgical Timing n (%)	**<24 h**	84 (93.3)	6 (6.7)	90 (100)	0.211 (v=4.512)
	24-48 h	24 (100)	0 (0)	24 (100)	
	48-72 h	25 (96.2)	1 (3.8)	26 (100)	
	>72 h	41 (100)	0 (0)	41 (100)	
Total Mortality n (%)		174 (96.14)	7 (3.86)	181 (100)	

FNF: Femoral neck fracture, ITF: Intertrochanteric femur fracture, HT: Hypertension, DM: Diabetes mellitus, CAD: Coronary artery disease COPD: Chronic Obstructive Pulmonary Disease, HF: Heart failure, TD: Thyroid Dysfunction CRF: Chronic renal failure
t: Independent Samples Test, u: Mann Whitney U Test, v: Pearson Chi-Square test, ¥Chi-Square Test – Fisher Exact *p* value,
*: $p<0.05$ statistically significant

Table 3. Relationship between variables and 90-day mortality

		90-day mortality				
		Survived	**Death**	**Total**	***p***	
Age (Mean±SD)		80.09±8.76	81.4±7.38	80.24±8.61	0.508 (t=-0.663)	
Preoperative Consultation Time (Mean±SD)		83.24±63.25	95.5±91.3	84.6±66.72	0.121 (u=1267.00)	
		Survived	**Death**	**Total**	***p***	
Gender n (%)	**Female**	100 (90.9)	10 (9.1)	110 (100)	0.295 (v=1.095)	
	Male	61 (85.9)	10 (14.1)	71 (100)		
Fracture Type n (%)	**FNF**	56 (88.8)	7 (11,2)	63 (100)	0.491 (v=0.000)	
	ITF	105 (88.9)	13 (11.1)	118 (100)		
Comorbidities n (%)	**HT**	103 (88.7)	13 (11.3)	116 (100)	0.315 (v=0.001)	
	DM	61 (89.7)	6 (10.3)	68 (100)	0.061 (v=0.549)	
	CAD	35 (83.3)	7 (16.7)	42 (100)	0.243¥	
	Stroke	17 (94.4)	1 (5,6)	18 (100)	0.437¥	
	Alzheimer	16 (88.8)	2 (11.2)	18 (100)	0.704¥	
	COPD	10 (66.6)	5 (33.4)	15 (100)	0.014¥**	
	Parkinson's Disease	10 (90.9)	1 (9.1)	11 (100)	0.368¥	
	HF	6 (75)	2 (25)	8 (100)	0.547¥	
	TD	5 (100)	0 (0)	5 (100)	0.287¥	
	CRF	4 (100)	0 (0)	4 (100)	0.155¥	
Surgical Timing n (%)	**<24 h**	81 (90)	9 (10)	90 (100)	0.05* (v=7.588)	0.68§
	24-48 h	23 (95.8)	1 (4.2)	24 (100)		0.23§
	48-72 h	25 (96.2)	1 (3.8)	26 (100)		0.19§
	>72 h	32 (78)	9 (22)	41 (100)		0.012§
Total Mortality n (%)		161 (88.96)	20 (11.04)	181 (100)		

FNF: Femoral neck fracture, ITF: Intertrochanteric femur fracture, HT: Hypertension, DM: Diabetes mellitus, CAD: Coronary artery disease COPD: Chronic Obstructive Pulmonary Disease, HF: Heart failure, TD: Thyroid Dysfunction CRF: Chronic renal failure
t: Independent Samples Test, u: Mann Whitney U Test, v: Pearson Chi-Square test, ¥Chi-Square Test – Fisher Exact p value
§Post Hoc Analysis was conducted to determine which surgical timing groups had significant differences and new p-value were determined with Bonferroni correction as 0.008. Posthoc analysis revealed no difference between surgical timing groups, *:$p<0.05$ statistically significant

Upon evaluating the correlation between comorbidities and early death, it was noted that the individuals who suffered from diabetes mellitus, coronary artery disease, chronic obstructive pulmonary disease, or chronic renal failure had a 30-day death rate that was significantly higher as compared to the others; however, the sole comorbidity associated with a considerably increased 90-day death rate was chronic obstructive pulmonary disease.

Patients were categorized based on the timing of surgical intervention: 90 patients underwent surgery within the first 24 hours post-admission, 24 patients within the 24-48 hour range, 26 patients within the 48-72 hour range, and 41 patients after the 72nd hour. The timing of the surgical procedure was shown to have no impact on the first 30-day mortality. The evaluation of 90-day mortality revealed a borderline significance value (p=0.05).

Post-hoc analysis and Bonferroni correction revealed that the time of the surgery did not influence the 90-day mortality rate.

Discussion

This study examined the impact of surgical scheduling on 30-day and 90-day mortality rates in proximal femur fractures among patients aged over 65 years. Furthermore, additional criteria that may influence early death were assessed. A Canadian study utilizing administrative management data revealed 30-day and 90-day mortality rates of 4.9% and 8.5%, respectively [5]. In the study conducted using data from the Danish fracture database, the 30-day and 90-day mortality rates were reported as 7.8% and 15.2%, respectively [7]. Our investigation revealed a total 30-day death rate of 3.9% and a total 90-day mortality rate of 11.04%, consistent with existing literature.

The literature lacks consensus on the impact of surgical timing on mortality. In high-patient-volume review and meta-analysis studies, some research indicates that early surgery decreases mortality, whereas other studies suggest no correlation between surgical time and death [5-11]. Various studies establish distinct time frames within which surgery must be conducted to be considered early surgery. Studies established this limit at 24, 48, or 72 hours, assessing its influence on mortality and surgical complications [15-17]. Consequently, rather of establishing one specific time limit in our study, we categorized our patients based on the 24-hour intervals of their surgical procedures and completed our analysis, revealing that the timing of the surgery did not influence the 30-day and 90-day death rates. Our analysis revealed that the group with the highest 30-day mortality rate comprised individuals who underwent surgical intervention during the first 24 hours (6.7%). Despite the approval from the appropriate departments indicating that all patients were prepared for surgery, there may have been undue rush during the preoperative preparation and the decision-making over the time of surgery. Nonetheless, the heterogeneous distribution of patients among the categories may render the acquired results false and misleading. Nevertheless, this rate cannot be deemed high when compared with the 30-day mortality rates reported in other studies within the literature [5, 7]. The group with the highest 90-day mortality rate was identified as those operated on 72 hours or later (22%). The elevated mortality rate in this group may be attributed to the presence of patients with significant comorbidities.

In some countries, national guidelines exist for the timing of surgery in hip fractures, and performing the surgery within the time frame specified in the guidelines is considered a quality indicator in healthcare delivery. In the latest 2021 update of the American Academy of Orthopaedic Surgeons (AAOS) guidelines for the management of hip fractures in older adults, it is recommended that surgery be performed within the first 48 hours [13]. In the latest guidelines updated in 2023 in the United Kingdom, it is recommended that patients diagnosed with a hip fracture undergo surgery on the day of admission or, at the latest, the following day [12]. The Association of Anaesthetists of Great Britain and Ireland has defined <8 hemoglobin, electrolyte imbalance, uncontrolled diabetes, arrhythmia, heart failure, pneumonia, and coagulopathy as acceptable reasons for delaying surgery after a hip fracture [18]. Although there is no consensus report and guidelines in our country, the approach we apply in our clinic is to operate on patients as early as possible to avoid potential perioperative complications, provided they are physiologically stable, ready for surgery, and their preoperative preparations are completed. In parallel, we found that approximately half of the patients who participated in our research (90/181) underwent surgery within the first 24 hours. The most significant reason for the delay in surgery for patients operated on 72 hours and later was determined to be the use of oral anticoagulants. All 32 patients in our research who used oral anticoagulants underwent surgery 72 hours or later. In a recent study involving 1803 patients, it was shown that the most common medical factor leading to surgical delays was the use of oral anticoagulants [19]. Another important reason for the delay in surgery could be that the patients in this group are uncontrolled in terms of comorbid diseases, and more time is spent to achieve their physiological stabilization. In another study investigating the relationship between surgical timing and mortality, it was reported that the proportion of patients with a high number of comorbidities was higher in the group that underwent delayed surgery compared to the group that underwent early surgery [20].

Due to potential ethical issues, it is inherently difficult to plan this study prospectively, but its main limitations are its design as a retrospective study and the lack of a large patient population. Additionally, the failure to examine whether mortality developed due to surgical complications or comorbidities can also be identified as a limitation.

In summary, there is no agreement regarding the optimal surgical timing for proximal femur fractures, which have seen a steadily increasing incidence over the years and have become a significant cause of mortality, especially in the aging population. Based on the results of our research, there is no effect of surgical timing on mortality rates; however, although not statistically significant, the safest interval for surgery appears to be between 24-72 hours. Performing surgery hastily and early just to adhere to a rule is as dangerous as planning it too late, which increases the risk of complications arising from the fracture itself or secondary to immobilization. The safest way to determine the timing of surgery is to ensure that the patient has enough time for preoperative medical optimization and to perform the surgery at the earliest moment the patient is ready for surgery.

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Research Article

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Global research trends on the links between primary health care and diabetes from 1980 to 2024: a machine learning-based science mapping

1980-2024 yılları arasında birincil sağlık hizmetleri ile diyabet arasındaki ilişkiler üzerine küresel araştırma eğilimleri: makine öğrenmesi tabanlı bilimsel haritalama çalışması

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Abstract

Purpose: A systematic and detailed examination of studies on diabetes can guide the evaluation of healthcare services, the identification of issues, and the implementation of preventive interventions. Therefore, this study aims to assess diabetes research in the primary health care (PHC) field using machine learning-based bibliometric methods.

Materials and methods: In this study, articles related to diabetes in the PHC field were obtained from the Web of Science Core Collection on March 15, 2024. These articles were analyzed using bibliometric methods and the Latent Dirichlet Allocation (LDA) topic modeling technique.

Results: The analysis of the studies revealed that 3.355 articles on diabetes in the PHC field were produced by researchers from 114 different countries, 4.226 institutions, and 12.426 individual researchers. Recent years have shown a growing interest in topics such as obesity, hypertension, chronic diseases, exercise, and physical activity within the PHC field. Topic modeling identified eight distinct latent topic clusters: (1) Diabetes management in PHC, (2) Risk factors and management of diabetes in PHC, (3) Acute and chronic complications of diabetes in PHC, (4) Gestational diabetes, (5) Prediabetes and Type 1 diabetes, (6) COVID-19 and diabetes, (7) Quality of life, awareness, and health education, (8) Current treatment methods and guideline resources.

Conclusion: Primary Care Diabetes and Journal of Family Medicine and Primary Care are the leading journals in PHC-related diabetes research. The studies show a significant overlap between diabetes research and topics such as hypertension and obesity. Future studies in the PHC field are recommended to focus on diabetic retinopathy and diabetic wound research.

Keywords: Primary health care, diabetes, data analysis, science mapping, topic modeling.

Yasli G, Alici S, Damar M. Global research trends on the links between primary health care and diabetes from 1980 to 2024: a machine learning-based science mapping. Pam Med J 2025;18:572-589.

Öz

Amaç: Diyabetle ilgili yapılan çalışmaların sistematik ve ayrıntılı olarak incelenmesi, sağlık hizmetlerinin değerlendirilmesine, sorunların belirlenmesine ve önleyici müdahalelere rehberlik edebilir. Bu nedenle çalışmamız kullanılan makine öğrenmesi destekli bibliyometrik yöntem ile birincil sağlık hizmetleri (BSH) araştırma alanındaki diyabet araştırmalarını değerlendirmeyi amaçlamıştır.

Gereç ve yöntem: Çalışmamızda 15 Mart 2024 tarihinde Web of Science Core Collection veri kaynağından elde edilen diabet konulu birinci basamak sağlık hizmetleri araştırma alanındaki makaleler bibliyometrik yöntemler ve Latent Dirichlet Allocation (LDA) konu modelleme yöntemi ile analiz etmektedir.

Bulgular: Çalışmalar incelendiğinde BSH alanındaki diyabet konusunda 3,355 makalenin 114 farklı ülkeden, 4,226 farklı kurum ve 12,426 farklı araştırmacı tarafından üretildiği görülmüştür. Son yıllarda birinci basamak sağlık hizmetleri alanında obezite, hipertansiyon, kronik hastalık, egzersiz, fiziksel aktivite konu başlıklarının yoğun ilgi gördüğü görülmüştür. Yapılan konu modellemesi ile sekiz farklı gizli konu kümesi bulunmuştur. Bunlar sırasıyla; (1) BSH'de diyabet yönetimi, (2) BSH'de diyabetin risk faktörleri ve yönetimi (3) BSH'de diyabetin akut ve kronik komplikasyonları (4) gestasyonel diyabet (5) prediyabet, tip 1 diyabet (6) COVID-19 ve diyabet (7) yaşam kalitesi, farkındalık ve sağlık eğitimi, (8) güncel tedavi yöntemleri ve rehber kaynaklar şeklindedir.

Sonuç: Primary Care Diabetes ve Journal of Family Medicine and Primary Care dergileri BSH alanında diyabet araştırmalarında öne çıkan dergilerdir. Diyabet araştırmaları ile hipertansiyon ve obezite konularının yoğun şekilde birlikte işlendiği görülmüştür. Gelecek çalışmalar için BSH alanında diyabetik retinopati ve diyabetik yara çalışmalarının yapılması önerilmektedir.

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Anahtar kelimeler: Birinci basamak sağlık hizmetleri, diyabet, veri analizi, bilim haritalama, konu modelleme.

Yaslı G, Alıcı S, Damar M. 1980-2024 yılları arasında birincil sağlık hizmetleri ile diyabet arasındaki ilişkiler üzerine küresel araştırma eğilimleri: makine öğrenmesi tabanlı bilimsel haritalama çalışması. Pam Tıp Derg 2025;18:572-589.

Introduction

Diabetes mellitus (DM), commonly referred to as diabetes, is a chronic and debilitating condition characterized by persistently elevated blood glucose levels due to insufficient insulin production or the body's inability to utilize the insulin it produces effectively [1]. Diabetes affects individuals across all ages, genders, and geographic locations, making it one of the leading global causes of mortality and morbidity. Both genetic and environmental factors contribute to the etiopathogenesis of Type 2 diabetes, which accounts for more than 90% of all cases [2, 3]. The global prevalence of diabetes has reached pandemic proportions, with the International Diabetes Federation (IDF) reporting a prevalence rate of 9% (463 million adults) in its 9th edition in 2019. According to IDF estimates, the number of people living with diabetes increased to 537 million worldwide in 2021, a trend largely attributed to the aging population [4].

In addition to its high prevalence, morbidity, and mortality, diabetes also poses a significant economic burden, making it a critical public health issue. It is observed that diabetes increases healthcare expenditures, imposing financial strains on national healthcare systems. Global healthcare expenditures related to diabetes reached $966 billion in 2021 for the 20-79 age group and are expected to rise to $1.05 trillion by 2045 [5, 6]. Without adequate glycemic control, diabetes may lead to complications that complicate treatment, increase healthcare costs, and result in higher mortality rates. Therefore, establishing a healthy lifestyle and achieving glycemic control through medical treatment are crucial.

Systematic and detailed examination of studies related to diabetes can guide the evaluation of healthcare services, identification of issues, and preventive interventions. For this purpose, bibliometric analyses can be utilized. Bibliometric analysis can assess the productivity trends of a research topic over time, as well as serve as a valuable tool for comparing research outputs of academics, leading institutions, countries, and journals in a specific field of interest [7-9]. Currently, the body of literature on diabetes research within the context of primary health care (PHC) remains limited in providing comprehensive and relevant information for traditional literature analysis. This article aims to reveal the overall structure of the scientific literature on diabetes—a significant topic in PHC research and one of the most common modifiable contributors to global morbidity and mortality—by analyzing publications indexed in the Web of Science (WoS).

Background

Primary healthcare services play a critical role in the provision of healthcare, encompassing preventive, promotive, and curative interventions. The primary goal of primary healthcare is not only to address diseases but also to intervene in and prevent the underlying risk factors that contribute to their development. Within a tiered healthcare system, the fundamental function of primary care institutions is to deliver preventive health services for all. Primary prevention focuses on preventing individuals from developing diseases [10]. However, many issues that could be resolved at the primary care level are often referred to secondary care, and problems manageable in secondary care are frequently

transferred to tertiary care institutions. This practice leads to unnecessary overcrowding in institutions, reduces employee productivity, and diminishes service quality. Efforts to improve primary healthcare services significantly contribute to advancing public health. Stronger PHC will contribute significantly to health system performance and public health.

The early diagnosis and treatment of DM are of critical importance due to several factors: Type 2 DM represents a significant public health issue, has an asymptomatic phase in its early stages, and is associated with progressive microvascular damage in undiagnosed patients. Additionally, it has been demonstrated that strict glycemic control can delay or prevent the development and progression of diabetic complications [11]. To reduce diabetes-related morbidity and mortality, primary care physicians must have a comprehensive understanding of how to manage diabetic patients effectively [12]. Consequently, primary care physicians should assess all patients for diabetes risk factors and implement preventive measures to reduce the likelihood of diabetes development. They should perform diabetes screening in at-risk individuals and, considering that prevention and treatment require a multidisciplinary approach, refer patients to nephrology, ophthalmology, cardiology, or other relevant specialties when necessary [13].

A review of the literature reveals that numerous bibliometric studies have been conducted on various aspects and topics related to diabetes. For instance, bibliometric studies have been performed on postoperative diabetes mellitus in kidney transplant recipients [7], the bibliometric analysis of experimental research on diabetic nephropathy [8], the bibliometric analysis of research on diabetic foot ulcer therapy [14], and gestational diabetes and long-term cardiovascular health [15]. Similarly, bibliometric analyses have been conducted on many diabetes-related topics. A Web of Science (WoS) search revealed that 55 bibliometric articles on diabetes were published in 2024, compared to 28 in 2023. This highlights that diabetes is a highly prominent topic in the literature, and research analyses on this subject are conducted under a wide range of subtopics, drawing considerable interest. Additionally, diabetes is a significant public health issue, particularly for primary healthcare services [16, 17]. However, our detailed review of the literature did not identify any bibliometric studies that analyze discussions on diabetes within the context of primary healthcare research.

Materials and method

Data sources and search string

In our study, the Web of Science (WoS) Core Collection was selected as the data source, focusing on research articles and review articles that include the term “diabetes” in either the title or the author’s keywords within the “primary health care” research area. All articles on diabetes published between 1980 and 2024 were included in our study. Document types such as book chapters, letters, and proceedings were excluded from the analysis. The primary reason for selecting Web of Science (WoS) as the bibliometric data source is the presence of a specific category named “primary health care” within WoS [9]. The existence of such a category defined by WoS allows us to conduct searches directly through journals explicitly associated with the primary health care category. A similar category is not available in the Scopus database. This ensures that publications unrelated to primary health care are included at a minimal level in the study.

Data for our analyses were collected on April 15, 2024. During data acquisition, we applied a sequential filtering process as shown in Figure 1 below, with the final dataset being retrieved using query number four. No ethics committee permission is required for the use of data obtained from such data sources. The relevant WoS data are publicly available and extracted from the journals indexed by such data sources. Therefore, ethics permission was not obtained in our study.

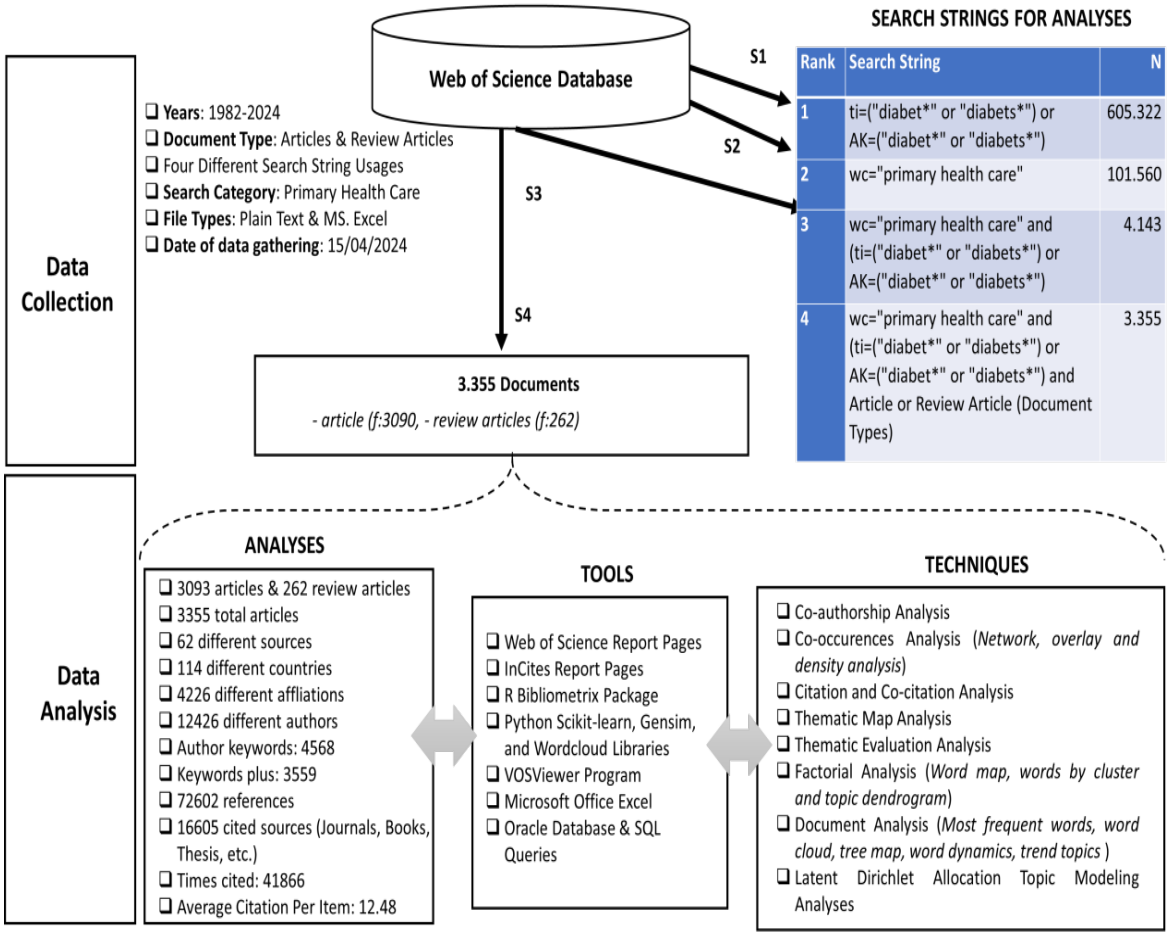

Figure 1. Research methodology and study design

Statistical analysis and latent dirichlet allocation (LDA) topic modeling

The tools used for statistical analysis were Microsoft Office Excel, SQL, Hypertext Preprocessor (PHP) programming language, the Python programming language, Oracle Database, R Bibliometrix package Biblioshiny program, and VOSviewer software. Microsoft Excel was used to quickly make modifications and revisions to the data. Additionally, SQL query language and the PHP programming language were employed to transfer the bibliometric data obtained from Web of Science (WoS) in Plain Text format into an Oracle database. Through the studies conducted using this setup, we were able to quickly access article content and classify the articles, which proved particularly beneficial in the discussion section. VOSviewer and Biblioshiny programs were used to perform analyses such as co-authorship by country, co-authorship by institutions, co-authorship by authors, co-citation references, co-citation sources, trend topics, and co-occurrence of author keywords on the data obtained from WoS. Analyses were conducted using data obtained from the WoS bibliometric database in Plain Text and Excel formats. In some instances, the WoS reporting tool was also utilized during the analyses. The rationale for selecting two software packages was the observation that the different programs occasionally produced varying results in data analyses.

Also, we use the Python programming language for machine learning-based analyses. Additionally, for topic modeling, both Topics Analyses and LDA topic modeling were employed. LDA is a fundamental technique in text mining and natural language processing, representing words, documents, and corpora as mixtures of topics. Using LDA, topics within documents can be identified, providing a clear representation of their content [9]. In our study, LDA was applied to the abstracts and titles of research articles to perform topic modeling. This analysis was conducted using the Python, with libraries such as Scikit-learn, Natural

Language Toolkit (NLTK), Gensim, Matplotlib, and Wordcloud. NLTK (Natural Language Toolkit) is a Python library used for natural language processing. Scikit-learn is a Python library that integrates various machine learning algorithms, including classification, regression, clustering, and decision trees. Gensim is an open-source Python library focused on topic modeling for natural language processing. LDA analysis utilized all three libraries. Additionally, Matplotlib and Wordcloud libraries were used for the visualization of the results. Sometimes, the bibliometric data obtained may include copyright information or details about the journal in which the article was indexed. Therefore, researchers need to clean such expressions from the abstract section.

In the LDA analysis, the Perplexity and Coherence metrics were used to evaluate the model's performance. In this study, the Perplexity metric was calculated as 2875.808, while the Coherence score was determined to be 0.490. These scores indicate that the overall performance of the model is satisfactory; however, experimenting with different hyperparameter settings may further enhance coherence and topic interpretability. The obtained results demonstrate that clustering was successfully achieved in the eight-topic dataset. The Perplexity value indicates that the model effectively represents the text and aligns well with the test data, while the Coherence score suggests that the topics exhibit an adequate level of semantic meaning. However, the literature suggests that Coherence scores above 0.5 generally indicate stronger model consistency [18]. Therefore, based on the obtained scores, it can be inferred that the model is generally successful but could be further optimized. Additionally, elements such as copyright information and journal indexing details present in the bibliometric data were removed from the abstracts to achieve a more meaningful content analysis. In this context, experts refined the relevant word blocks to enhance the interpretability of the results.

Results

Since 1980, a total of 3.355 research and review articles on diabetes have been produced in the field of PHC research. These documents are indexed as follows: Science Citation Index Expanded (SCI-Expanded) with 2.533 articles, Emerging Sources Citation Index (ESCI) with 727 articles, and Social Sciences Citation Index (SSCI) with 571 articles. The total citation count for these documents is 41.866 (without self-citations: 39.633), with an average citation count of 12.48 per document and an h-index of 78 (Figure 2). Overall, interest in the topic of diabetes has increased over the years, with 2022 being the most productive year, contributing 336 articles (10.01%). The first article on this topic was published in 1980. Although it is early in 2024, six articles on diabetes have already been published.

It was found that 48.97% (n=1.643) of studies on diabetes were published as open access. The sustainability goals of these studies are as follows: good health and well-being (n=3.143, 93.68%), gender equality (n=168, 5.00%), zero hunger (n=21, 0.62%), quality education (n=18, 0.53%), no poverty (n=12, 0.35%), decent work and economic growth (n=4, 0.11%), sustainable cities and communities (n=4, 0.11%), life on land (n=2, 0.06%), reduced inequality (n=1, 0.03%), and peace, justice, and strong institutions (n=1, 0.03%).

Analysis of countries, institutions, and researchers

Upon reviewing the studies, it was found that the 3.355 articles were produced by 114 different countries, 4.226 different institutions, and 12.426 different researchers. The top five countries with the highest volume of research in this field were as follows: USA (n=951, 28.34%, Average Citation Per Article (ACPA): 17.56), England (n=325, 9.68%, ACPA: 19.69), Australia (n=229, 6.82%, ACPA: 12.36), India (n=225, 6.70%, ACPA: 4.34), and Spain (n=184, 5.48%, ACPA: 5.54) (Appendix 1). The five countries with the highest citation counts were USA, England, Australia, Canada, and the Netherlands. In the field of PHC research on diabetes, the top five institutions with the most contributions were: The University of California System (USA, n=64), the University of Leicester (England, n=58), the University of London (England, n=57), Utrecht University (Netherlands, n=53), and the US Department of Veterans Affairs (USA, n=46) (Appendix 2).

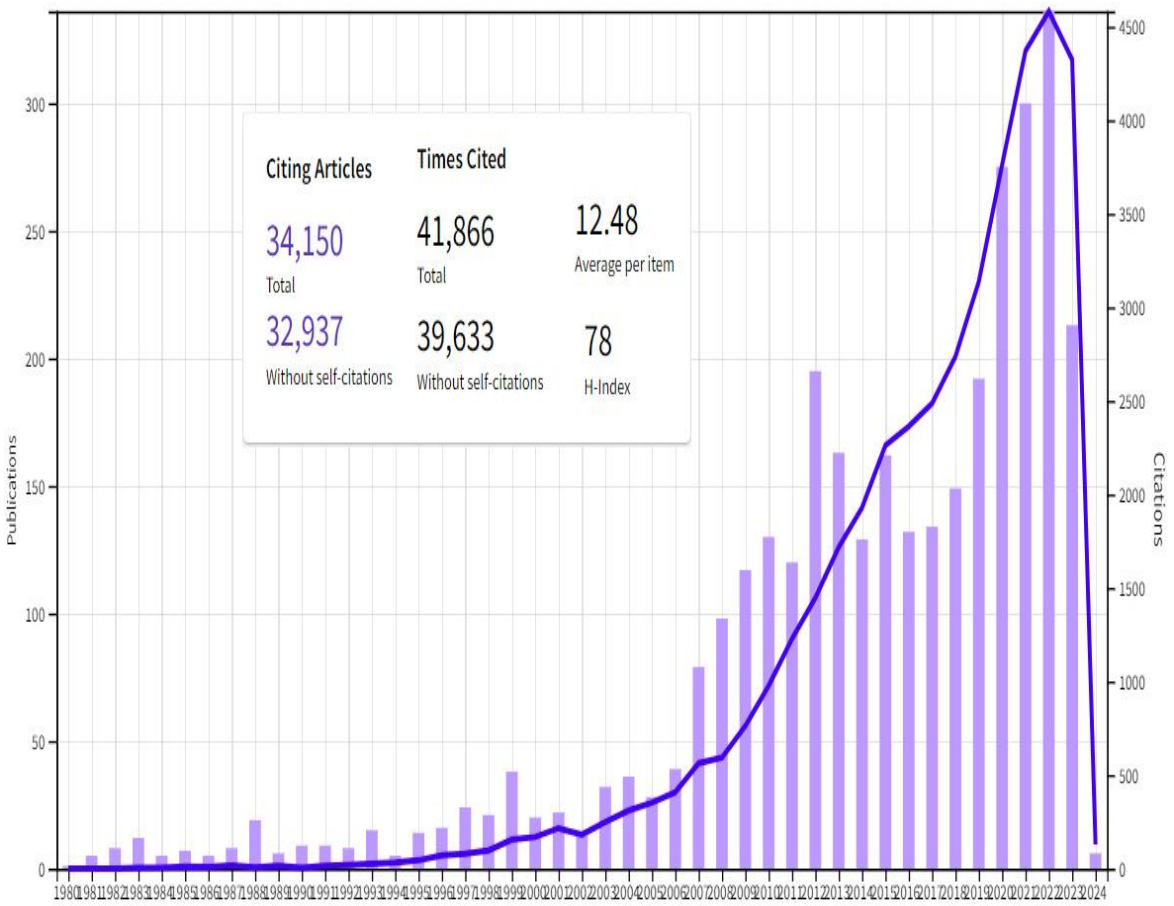

Figure 2. Total cited and publications over time

Appendix 1. Top 30 countries with the most publications on diabetes in the PHC research field

Rank	Countries	HI	ACPA	N	%	Rank	Countries	HI	ACPA	N	%
1	USA	67	17.56	951	28.34	16	Germany	15	13.46	70	2.08
2	England	42	19.69	325	9.68	17	New Zealand	13	7.74	58	1.72
3	Australia	28	12.36	229	6.82	18	Finland	14	12.35	57	1.69
4	India	16	4.34	225	6.70	19	Türkiye	13	11.14	50	1.49
5	Spain	16	5.54	184	5.48	20	Belgium	13	10.74	42	1.25
6	Canada	31	16.69	172	5.12	21	Italy	10	9.08	38	1.13
7	Netherlands	29	16.76	151	4.50	22	Wales	16	25.76	37	1.10
8	Saudi Arabia	12	6.03	110	3.27	23	France	11	8.83	36	1.07
9	Poland	4	1.72	108	3.21	24	Norway	11	10.37	35	1.04
10	China	19	11.72	101	3.01	25	Scotland	15	24.00	34	1.01
11	Sweden	21	14.89	101	3.01	26	Malaysia	9	12.39	33	0.98
12	Denmark	18	12.05	84	2.50	27	Mexico	8	8.76	33	0.98
13	Iran	16	12.86	74	2.20	28	Israel	10	14.93	30	0.89
14	South Africa	14	8.68	74	2.20	29	Japan	7	5.82	28	0.83
15	South Korea	9	5.63	73	2.17	30	Brazil	7	8.00	25	0.74

ACPA: Average Citation per Articles, N= Document Count, HI: H-index

Appendix 2. Top 15 institutions with the most publications on diabetes in PHC research area

Rank	Afliations	Country	TC	HI	ACPA	N	%
1	University of California System	USA	1.628	23	25.44	64	1.90
2	University of Leicester	England	1.340	20	23.10	58	1.72
3	University of London	England	1.208	20	21.19	57	1.69
4	Utrecht University	Netherlands	753	17	14.21	53	1.58
5	US Department of Veterans Affairs	USA	1.668	20	36.26	46	1.37
6	Veterans Health Administration	USA	1.668	20	36.26	46	1.37
7	Utrecht University Medical Center	Netherlands	649	16	14.42	45	1.34
8	University Hospitals of Leicester NHS Trust	England	1.019	18	23.70	43	1.28
9	University System of Ohio	USA	432	11	10.80	40	1.19
10	State University System of Florida	USA	454	11	11.64	39	1.16
11	Aarhus University	Denmark	540	13	14.21	38	1.13
12	University of Toronto	Canada	547	13	14.78	37	1.10
13	University of Cambridge	England	915	16	25.42	36	1.07
14	Leicester General Hospital	England	784	15	22.40	35	1.04
15	Maastricht University	Netherlands	555	14	16.32	34	1.01

ACPA: Average Citation per Articles, N= Document Count, HI: H-index, TC: Times Cited

The most prominent researchers in the field, along with their average citation per article (ACPA) and number of articles, were as follows: Seidu S (University of Leicester, England, ACPA=14.45, n=20), Davies MJ (University of Leicester, England, ACPA=13.44, n=18), Kostev K (University Hospital of Giessen and Marburg, Germany, ACPA=13.50, n=16), Gorter KJ (Utrecht University, Netherlands, ACPA=16.07, n=15), Griffin SJ (University of Cambridge, England, ACPA=19.07, n=15), Lauritzen T (Aarhus University, Denmark, ACPA=18.87, n=15), and Kinmonth AL (University of Cambridge, England, ACPA=22.50, n=14). The countries of these researchers, as well as the keywords they frequently used in their articles, are shown in Figure 3.

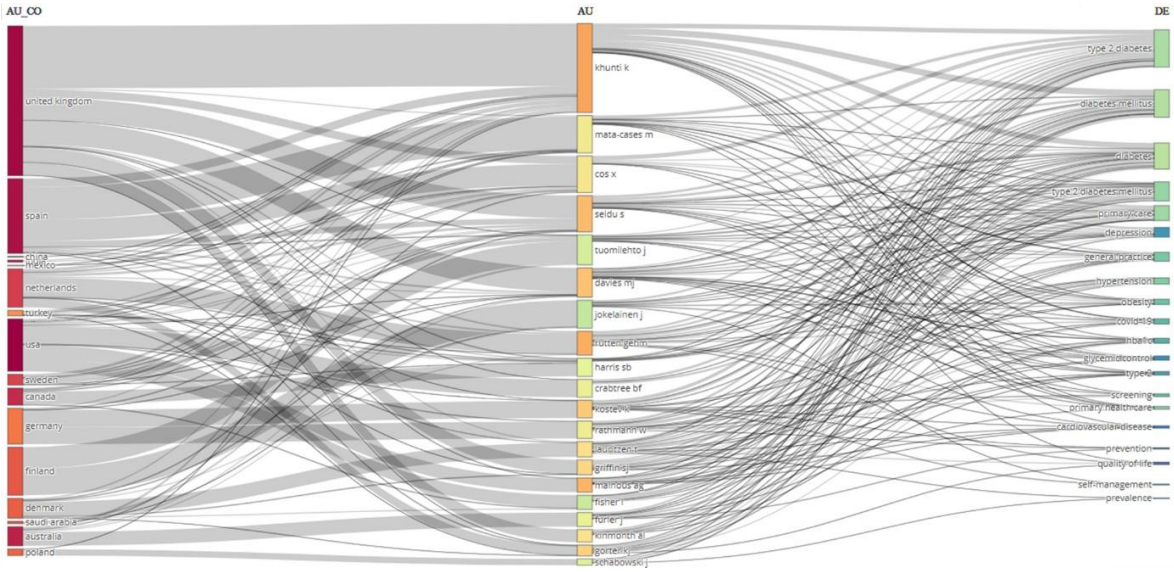

Figure 3. Three field of author country, author, and author keywords for diabates articles

Analysis of documents and references

A total of 3.355 documents were published in 62 different journals, with a total of 41.866 citations. The average citation count was 12.48, and the h-index was 78. The top five journals with the most publications in this field were as follows: Primary Care Diabetes (ACPI=10.28, C=9.970, n=970, 28.91%), Journal of Family Medicine and Primary Care (ACPI=3.07, C=864, n=281, 8.37%), BMC Family Practice (ACPI=20.96, C=3.584, n=171, 5.09%), Family Practice (ACPI=25.30, C=3.593, n=142, 4.23%), and Journal of Family Practice (ACPI=12.93, C=1.668, n=129, 3.84%) (Table 1).

Table 1. Top 20 Most published journals on diabetes in the PHC research area

Rank	Journal	JIF	Research Domain	SCIE/ SSCI/ ESCI	ACPI	C	N	%
1	Primary Care Diabetes	2.90	Endocrinology & Metabolism; Primary Health Care	SCIE, SSCI	10.28	9.970	970	28.91
2	Journal of Family Medicine And Primary Care	1.40	Primary Health Care	ESCI	3.07	864	281	8.37
3	BMC Family Practice	2.90	Medicine, General & Internal; Primary Health Care	SCIE, SSCI	20.96	3.584	171	5.09
4	Family Practice	2.20	Medicine, General & Internal; Primary Health Care	SCIE, SSCI	25.30	3.593	142	4.23
5	Journal of Family Practice	0.60	Medicine, General & Internal; Primary Health Care	SCIE, SSCI	12.93	1.668	129	3.84
6	Family Medicine and Primary Care Review	0.70	Primary Health Care	ESCI	0.90	111	123	3.66
7	American Family Physician	4.00	Medicine, General & Internal; Primary Health Care	SCIE, SSCI	20.92	2.510	120	3.57
8	British Journal of General Practice	5.90	Medicine, General & Internal; Primary Health Care	SCIE, SSCI	25.49	2.855	112	3.33
9	Journal of The American Board of Family Medicine	2.90	Medicine, General & Internal; Primary Health Care	SCIE, SSCI	19.84	2.182	110	3.27
10	Journal of Primary Care and Community Health	3.60	Primary Health Care	ESCI	4.41	428	97	2.89
11	Primary Care	1.90	Medicine, General & Internal; Primary Health Care	SCIE, SSCI	11.11	1.067	96	2.86

Table 1. Top 20 most published journals on diabetes in the PHC research area (continued)

Rank	Journal	JIF	Research Domain	SCIE/ SSCI/ ESCI	ACPI	C	N	%
12	Canadian Family Physician	3.10	Medicine, General & Internal; Primary Health Care	SCIE, SSCI	13.64	1.241	91	2.71
13	Annals of Family Medicine	4.40	Medicine, General & Internal; Primary Health Care	SCIE, SSCI	58.61	5.275	90	2.68
14	Scandinavian Journal of Primary Health Care	2.10	Health Care Sciences & Services; Medicine, General & Internal; Primary Health Care	SCIE, SSCI	18.30	1.647	90	2.68
15	Atencion Primaria	2.50	Medicine, General & Internal; Primary Health Care	SCIE, SSCI	4.66	340	73	2.17
16	Australian Family Physician	1.22	Medicine, General & Internal; Primary Health Care	SCIE	11.03	728	66	1.96
17	Australian Journal of Primary Health	1.30	Health Care Sciences & Services; Health Policy & Services; Primary Health Care; Public, Environmental & Occupational Health; Public, Environmental & Occupational Health	SCIE, SSCI	9.24	610	66	1.96
18	Korean Journal of Family Medicine	2.30	Primary Health Care	ESCI	6.47	401	62	1.84
19	African Journal of Primary Health Care Family Medicine	0.91	Primary Health Care	ESCI	6.86	391	56	1.66
20	Primary Health Care Research and Development	1.60	Primary Health Care	SCIE, SSCI	8.04	450	56	1.66

N: Record Count, JIF: Journal Impact Factor for 2022 years, C: Citation

Among the top 20 journals with the most diabetes-related publications in the PHC research area, it was observed that only five journals are indexed in ESCI, while the other 15 are indexed in SCIE. As seen in Table 1, the Annals of Family Medicine journal ranks 13th in terms of publication volume but has an exceptionally high average citation count of 58.61. This is also reflected in Appendix 3. Among the top 20 most cited papers in the relevant field, half of the journals were published in Annals of Family Medicine (Appendix 3). Additionally, Journal of The American Board of Family Medicine and Primary Care Diabetes each contributed two papers to the list.

Appendix 3. Top 20 Most Cited Studies on Diabetes in PHC Research Area

Rank	Title	Journal	JIF	Authors	Year	C
1	Development of a brief diabetes distress screening instrument	Annals of Family Medicine	4.4	Fisher, L; Glasgow, RE; (...); Polonsky, WH	2008	277
2	Impact of Peer Health Coaching on Glycemic Control in Low-Income Patients With Diabetes: A Randomized Controlled Trial	Annals of Family Medicine	4.4	Thom, DH; Ghorob, A; (...); Bodenheimer, TA	2013	215
3	Lay understanding of familial risk of common chronic diseases: A systematic review and synthesis of qualitative research	Annals of Family Medicine	4.4	Walter, FM; Emery, J; (...); Marteau, TM	2004	200
4	A systematic review of chronic disease management interventions in primary care	BMC Family Practice	2.9	Reynolds, R; Dennis, S; (...); Zwar, N	2018	191
5	Changes in age at diagnosis of type 2 diabetes mellitus in the United States, 1988 to 2000	Annals of Family Medicine	4.4	Koopman, RJ; Mainous, AG; (...); Geesey, ME	2005	184
6	Quality of life of patients with diabetes mellitus -: An overview of research in primary health care in the Nordic countries	Scandinavian Journal of Primary Health Care	2.1	Wändell, PE	2005	182
7	Diabetic foot ulcers: Prevention, diagnosis and classification	American Family Physician	4.0	Armstrong, DG and Lavery, LA	2005	180
8	Participatory Decision Making, Patient Activation, Medication Adherence, and Intermediate Clinical Outcomes in Type 2 Diabetes: A STARNet Study	Annals of Family Medicine	4.4	Parchman, ML; Zeber, JE and Palmer, RF	2010	174
9	Dietary Fiber for the Treatment of Type 2 Diabetes Mellitus: A Meta-Analysis	Journal of The American Board of Family Medicine	2.9	Post, RE; Mainous, AG; (...); Simpson, KN	2012	170
10	Depression and Increased Mortality in Diabetes: Unexpected Causes of Death	Annals of Family Medicine	4.4	Lin, EHB; Heckbert, SR; (...); Von Korff, M	2009	168
11	Impact of Electronic Health Record Clinical Decision Support on Diabetes Care: A Randomized Trial	Annals of Family Medicine	4.4	O'Connor, PJ; Sperl-Hillen, JM; (...); Gilmer, TP	2011	162
12	Competing demands or clinical inertia: The case of elevated glycosylated hemoglobin	Annals of Family Medicine	4.4	Parchman, ML; Pugh, JA; (...); Bowers, KW	2007	160
13	Effectiveness of Cinnamon for Lowering Hemoglobin A1C in Patients with Type 2 Diabetes: A Randomized, Controlled Trial	Journal of The American Board of Family Medicine	2.9	Crawford, P	2009	152
14	Management of Blood Glucose in Type 2 Diabetes Mellitus	Annals of Family Medicine	4.4	Ripsin, CM; Kang, H and Urban, RJ	2009	150

Appendix 3. Top 20 most cited studies on diabetes in PHC research area (continued)

Rank	Tirle	Journal	JIF	Authors	Year	C
15	Integrated Management of Type 2 Diabetes Mellitus and Depression Treatment to Improve Medication Adherence: A Randomized Controlled Trial	Annals of Family Medicine	4.4	Bogner, HR; Morales, KH; (...); Cappola, AR	2012	147
16	Type 2 diabetes and cardiovascular disease in South Asians	Primary Care Diabetes	2.9	Gholap, N; Davies, M; (...); Khunti, K	2011	145
17	Barriers to effective management of type 2 diabetes in primary care: qualitative systematic review	British Journal of General Practice	5.9	Rushforth, B; McCrorie, C; (...); Foy, R	2016	144
18	Cinnamon Use in Type 2 Diabetes: An Updated Systematic Review and Meta-Analysis	Annals of Family Medicine	4.4	Allen, RW; Schwartzman, E; (...); Phung, OJ	2013	144
19	Clinical inertia to insulin initiation and intensification in the UK: A focused literature review	Primary Care Diabetes	2.9	Khunti, K and Millar-Jones, D	2017	143
20	Diabetic Retinopathy	Primary Care	1.9	Hendrick, AM; Gibson, MV and Kulshreshtha, A	2015	142

JIF: Journal Impact Factor for 2022 years, C: Citation

According to Bradford's Law, the journals in the first zone are Primary Care Diabetes and Journal of Family Medicine and Primary Care. The second zone includes the following journals: BMC Family Practice, Family Practice, Journal of Family Practice, Family Medicine and Primary Care Review, American Family Physician, British Journal of General Practice, Journal of The American Board of Family Medicine, and Journal of Primary Care and Community Health. These journals in the first zone can be considered core journals that researchers in the PHC research area, specifically those focused on diabetes, should prioritize. The increasing number of publications in these journals over the years further supports this conclusion.

The ten journals with the most citations in diabetes research are as follows: Diabetes Care (C=10.131), The New England Journal of Medicine (C=2.845), Diabetic Medicine (C=2.477), Lancet (C=2.269), Journal of the American Medical Association (C=1.773), Diabetologia (C=1.755), Diabetes Research and Clinical Practice (C=1.652), British Medical Journal (C=1.390), Diabetes (C=1.367), and Annals of Internal Medicine (C=1.073).

Emerging trends and research focus

In the 3.335 diabetes-related articles conducted in the PHC research area, a total of 4.568 author keywords, 3.559 keyword plus terms were used, and these articles were associated with 21 research areas outside of PHC. The ten most frequently associated research areas, in order of frequency, are as follows: medicine general and internal (n=1.393), endocrinology and metabolism (n=972), health care sciences and services (n=157), public environmental and occupational health (n=70), health policy and services (n=67), orthopedics (n=44), sport sciences (n=44), ophthalmology (n=19), respiratory system (n=5), and emergency medicine (n=4). The prominent topics in diabetes research within PHC over the years are shown in Figure 4a and Figure 4b based on the author's keywords.

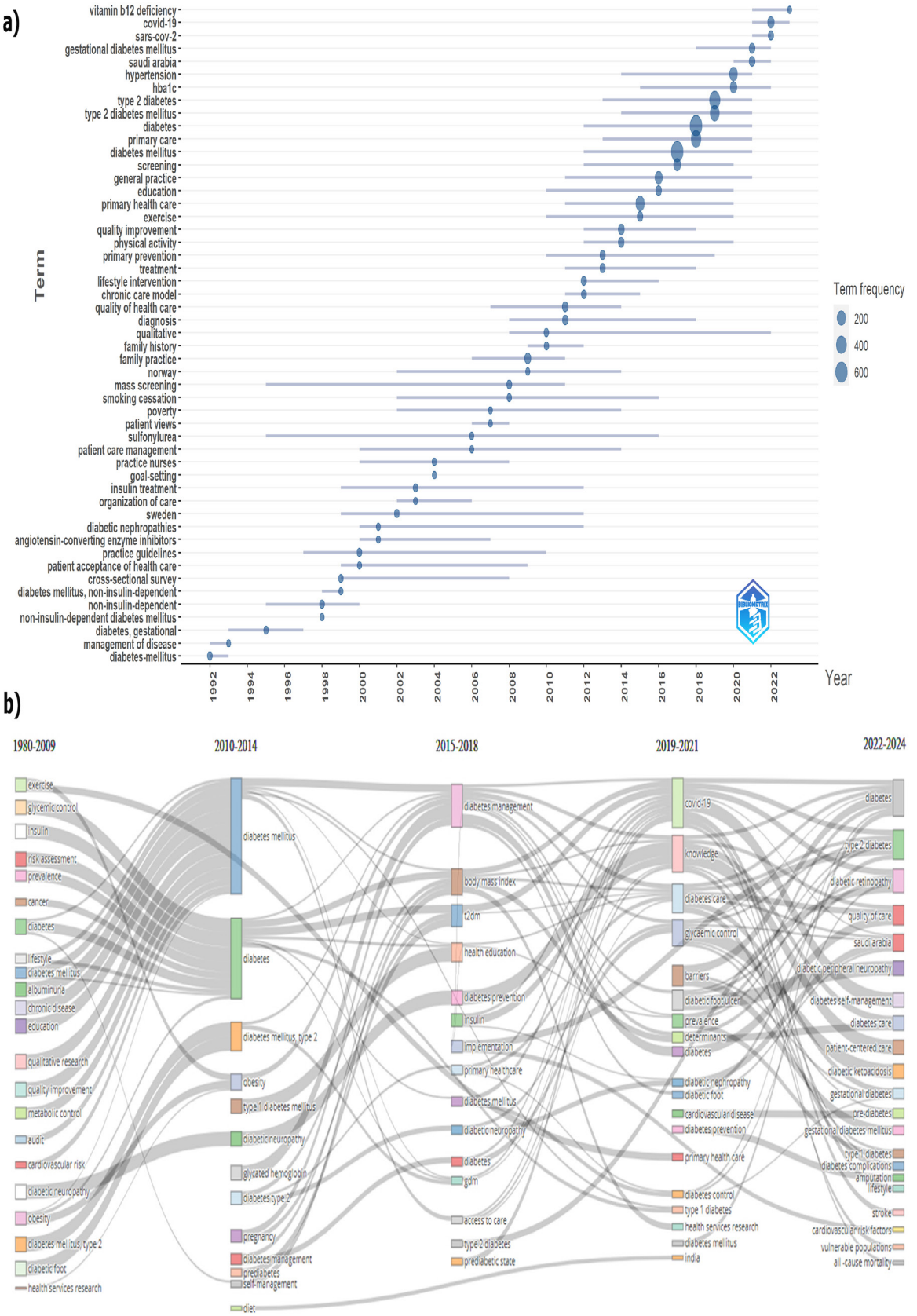

Figure 4. Author's keywords trend topic analyses (4a) and thematic evaluation for author's keywords (4b)

In our study, the eight topics obtained from the LDA topic modeling analysis conducted on the abstracts and titles of the relevant articles are presented in Table 2.

Table 2. Cluster Title and Description of LDA Topic Modeling Findings for Abstracts and Titles

Rank	Cluster Title	Narrative Description of Content
1	Diabetes management at PHC	Studies evaluating diabetes management in primary care are grouped under this heading.
2	Risk factors and management of diabetes in PHC	The words in this cluster describe the risk factors of diabetes and issues related to the management of risk factors (obesity, lifestyle, diet, body mass index) and are grouped under this heading.
3	Acute and chronic complications of diabetes in PHC	Topics such as retinopathy, nephropathy, neuropathy, diabetic foot ulcers, amputation, cardiovascular risk factors are grouped under this heading.
4	Gestational diabetes	Under this heading, the definition of diabetes in pregnant women, risk factors, diagnostic methods, the effect of drugs used during pregnancy on diabetes and research on referral situations are grouped.
5	Prediabetes, Type 1 diabetes	The definition of prediabetes and Type 1 diabetes are grouped under this heading.
6	COVID-19 and diabetes	Studies conducted to learn the relationship between the prevalence of diabetes and the interaction with diseases in patients with COVID-19 pneumonia are grouped under this heading.
7	Quality of life, awareness and health education	Studies evaluating the relationship between behavioral risk factors for diabetes and awareness are grouped under this heading.
8	Current treatment methods and guiding resources	Approaches to hypertension treatment, health management, types of medication, PHC guidelines, and hypertension control are grouped under this heading.

Discussion

In this study, a comprehensive bibliometric analysis and LDA topic modeling were conducted to visualize and evaluate the temporal dynamics and research trends of diabetes-related studies in the primary healthcare (PHC) research field. This study represents the first bibliometric assessment of research on diabetes and primary healthcare services and provides crucial data for future researchers in this area. A thorough analysis of the literature on diabetes mellitus and primary healthcare services was performed through bibliometric analysis. Furthermore, for diabetes, its complications, and clinicians and scientists working in primary care, the findings of this study will not only provide insights into research in this field but also offer a comprehensive overview and present key research directions. In this way, knowledge gaps will be identified, guiding future research directions, and acting as a catalyst to promote informed decision-making in research and clinical practice. The focus of our study was on the existing and emerging research in the field, addressing discussions on diabetes management within PHC. Although many bibliometric analyses have been conducted on diabetes, it has been noted that no specific analysis has been conducted in the critical area of PHC, which plays a vital role in diabetes management. With the increasing life expectancy worldwide, diabetes will continue to be a significant health issue in primary care settings. In fact, the observed increase in the number of publications related to diabetes in PHC over the years supports this finding.

In recent years, there has been a growing interest in topics such as obesity, hypertension, chronic diseases, exercise, and physical activity within the PHC field.

Primary healthcare services play a crucial role in the fight against diabetes by implementing various effective strategies. These include strengthening diagnostic and screening programs, expanding the use of digital health and telemedicine, providing personalized diabetes education, promoting healthy lifestyle programs and community engagement, utilizing motivational interviewing techniques, and effectively integrating community health centers into diabetes management. Through these initiatives, primary care can significantly enhance the effectiveness of preventive, protective, and therapeutic services for diabetes. The effectiveness of chronic disease management, particularly in primary care settings, is closely linked to a decrease in hospitalization rates [19]. As diabetes rates increase and hospital readmission rates remain high, hospitals will face challenges in assessing and addressing the educational needs of diabetic patients. This highlights the growing importance of PHC services. In the United States, 52.3% of all medical office visits were primary care visits in 2013, with diabetes ranking as the fifth most common primary diagnosis among these visits [20]. The prevalence of diabetes is expected to increase by 54% between 2015 and 2030, reaching over 54.9 million Americans. Annual deaths attributed to diabetes will rise by 38%, and the total annual medical and societal costs related to diabetes are projected to increase by 53%, surpassing $622 billion by 2030 [21]. The United States alone spends a staggering $294.5 billion annually on the diagnosis and treatment of diabetes and its complications, making it the country with the highest number of diabetes cases, followed by the People's Republic of China [22]. In Southeast Asia, it has been reported that 8.4% of total health expenditure is spent solely on diabetes treatment and medical care. India, as the hub of diabetes in the region, accounts for 87.8% of the diabetes patients in Southeast Asia. Health spending on diabetes in India ranks fourth, after the United States, China, and Germany [23].

In our study, we examined chronic disease management topics in PHC settings across various countries. Socioeconomic and demographic changes, along with the aging population, are factors influencing the prevalence of diabetes and other related health conditions [24]. Furthermore, urbanization and access to starch-based foods, coupled with sedentary lifestyles, have a significant impact on diabetes statistics in these countries [25]. Germany follows an integrated approach to disease management through its German Disease Management Program, which emphasizes coordination of care based on general practice and has reported positive outcomes [26]. In Korea, cardiovascular disease (CVD) is among the leading causes of mortality, and the government has implemented various policies since 2007 to facilitate the management of hypertension and diabetes and to encourage doctors and patients [27]. The burden created by the rise of non-communicable chronic conditions is expected to be more significant in low- and middle-income countries, where resources are limited [28].

Among the findings from our topic analyses, the management and risk factors of diabetes have emerged as a major focus. The challenge here lies in the increasing complexity of diabetes management, which results from the need to avoid hyperglycemia and hypoglycemia, the availability of multiple medication combinations, the variety of medical device options for diabetes management, and the need to facilitate lifestyle changes for patients. In Australia, significant budgets are allocated for the coordination and management of chronic diseases in primary care settings [29]. It is known that the development of an integrated clinical decision support system for the early detection and management of chronic diseases, which focuses on regular screening of at-risk individuals, early interventions, timely referrals to specialist services, and adherence to evidence-based guidelines, improves diabetes management. More importantly, the inclusion of care in primary health services and patient participation is crucial [30].

Acute and chronic complications of diabetes in PHC have been heavily discussed in the literature. Globally, it is reported that 6.7 million people aged 20-79 died due to diabetes and diabetes-related causes in 2021. This represents 12.2% of all deaths in this age group [4]. Previous studies have highlighted the high

prevalence of comorbidities in diabetic patients and the challenges in managing these multiple conditions [31]. Elevated blood glucose levels can lead to acute complications and chronic macrovascular and microvascular complications. These may include retinopathy, nephropathy, neuropathy, cardiovascular events, diabetic foot, cerebrovascular events, and cancer [32]. Diabetes is also the leading cause of vision loss and kidney failure in adults, as well as the most common cause of amputations [33]. Among patients with acute coronary syndrome, 20-30% have type 2 diabetes, and 40% of these have impaired glucose tolerance. Evidence suggests that mortality rates following acute myocardial infarction are twice as high in diabetic patients [34]. Diabetic foot ulcers are among the most common complications in poorly controlled diabetic patients. They are typically the result of poor glycemic control, underlying neuropathy, peripheral vascular disease, or inadequate foot care. Diabetic foot ulcers account for more healthcare visits than other diabetes-related complications. The annual incidence of diabetic foot ulcers worldwide ranges from 9.1 million to 26.1 million [35]. Studies show that the prevalence of foot ulcers in diabetic patients ranges from 4% to 10%, with a lifetime occurrence rate as high as 25% [36].

Diabetic retinopathy is one of the significant neurovascular complications of diabetes and is a leading cause of blindness in working-age adults. Worldwide, 93 million people are affected by diabetic retinopathy [37]. Diabetic kidney disease, especially in developed countries like the USA, is a leading cause of end-stage renal disease, and 30-40% of diabetes mellitus patients develop diabetic nephropathy [38]. The most common type of diabetic neuropathy, peripheral neuropathy, primarily affects the nerves in the extremities, especially the feet [39]. This condition primarily disrupts sensory function, leading to gradual numbness, which increases the risk of ulcers due to external injuries [40]. Other systemic disorders, such as hypertension, dyslipidemia, hypoproteinemia, anemia, nephropathy, neuropathy, and heart conditions, also need to be treated through appropriate medications and interdisciplinary collaboration. Therefore, the interdisciplinary clinical team is crucial in reducing risk factors, maintaining glycemic control, and mitigating complication risks [41]. Educating the patient about complications and the need for proper medical care can reduce complication risks and improve treatment adherence.

Gestational diabetes (GDM), which is defined as the onset of diabetes during the second or third trimester of pregnancy, is a common pregnancy-related condition [42]. In 2020, it was estimated that 20.9 million pregnant women and their newborns worldwide were affected by GDM. GDM is significantly associated with an increased risk of various adverse outcomes, including postpartum hemorrhage, preeclampsia, neonatal hypoglycemia, macrosomia, cesarean delivery, and maternal and neonatal mortality. Furthermore, GDM increases the likelihood of type II diabetes, obesity, and metabolic syndrome in both the women and their children later in life.

Our study reveals that prediabetes and type 1 diabetes are heavily researched topics in PHC studies on diabetes. The prevalence of prediabetes is increasing worldwide, and it is predicted that by 2030, more than 470 million people will have prediabetes. Lifestyle changes are a cornerstone in preventing diabetes for prediabetic individuals, potentially reducing relative risk by 40-70% [43]. To slow the growth of new diabetes cases, efforts to increase awareness of prediabetes, encourage healthy behaviors, and improve the availability of evidence-based lifestyle programs are essential [44]. Type 1 diabetes mellitus is one of the most common chronic diseases in children but can occur at any age [45]. Monitoring prediabetes and type 1 diabetes is critical in preventing complications, which is effectively managed through strong PHC services.

Another important topic addressed in our study is the relationship between the COVID-19 pandemic and diabetes. Diabetes Mellitus not only predisposes individuals to more severe disease but also doubles the risk of death from COVID-19 due to lung and heart involvement [46]. The treatment of COVID-19 in diabetic patients requires an integrated team approach to minimize medical complications and the risk of death. Numerous studies suggest an increased risk of Type 1 diabetes mellitus after COVID-19 infection, although there is no conclusive evidence linking causality [47].

Diabetic patients often live with a reduced quality of life due to complications. Emerging epidemiological studies have shown that patient education on lifestyle changes, such as balanced nutrition, weight loss, smoking cessation, and regular exercise, is effective in controlling glucose balance and improving quality of life [48]. Today, the PHC system plays a significant role in disease prevention, especially by promoting a healthy lifestyle that includes physical activity. There is a need to increase awareness of complications among primary care physicians at the forefront of diabetes care [49]. The treatment of chronic diseases like Type 2 diabetes is challenging because it requires continuous screening and follow-up. Despite new guidelines, clinical inertia from doctors, patients, or the healthcare system can lead to ineffective management of Type 2 diabetes [50]. Therefore, in the healthcare system, updates should be made through in-service training plans and the creation of working groups to keep information up to date. For future studies, it may be suggested to focus on more specific topics within PHC research, such as diabetic retinopathy and diabetic wounds. Additionally, it is recommended that health policymakers implement public awareness campaigns, develop healthy nutrition policies, and revise existing regulations. Strengthening primary healthcare services and promoting policies that encourage urban planning initiatives to facilitate physical activity should also be prioritized. Public health experts should support diabetes prevention by implementing healthy lifestyle programs in schools, workplaces, and communities. Furthermore, primary care physicians are advised to enhance counseling and educational services while closely monitoring patients' metabolic risk factors.

This study represents the first bibliometric analysis conducted in the PHC research area on diabetes, and it is the first bibliometric analysis in PHC using machine learning techniques. In our research, we identified the current state of the field, created visual maps, and used LDA topic modeling analysis to describe the general situation of the field under eight different topics. By examining trends and current issues, we provided references for future research. However, there are some limitations in our study. Firstly, all data was obtained from the WoS Core Collection. Although WoS indexes the most important journals in the field, some journals may not be indexed. Secondly, our study only analyzed articles and review articles. The primary reason for this is that these types of documents are the most suitable for capturing the essence of the field and provide the richest bibliometric data in terms of metrics such as abstracts, titles, keywords, and references. Thirdly, LDA cannot directly assign meaning to the topics it identifies; the interpretation of these topics relies on the researcher's judgment. Consequently, the generated topics may sometimes appear ambiguous or meaningless. However, this limitation has been mitigated through the expertise of primary care physicians specializing in diabetes. Additionally, LDA performs better with longer documents, as a larger sample of words enhances the model's ability to identify topics more accurately. In this regard, our study benefits from an ideal dataset.

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S.A.: Materials, Data Collection and/or Processing, Analysis and/or Interpretation.

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The effect of medical drug reminder mobile application on treatment compliance in women with breast cancer under adjuvant hormone treatment

Adjuvan hormon tedavisi gören meme kanserli kadınlarda, ilaç hatırlatma mobil uygulamasının tedaviye uyum üzerine etkisi

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Abstract

Purpose: This study was planned to investigate the effects of a nurse-led medication reminder mobile application on treatment adherence in women with breast cancer receiving adjuvant hormone therapy.

Materials and methods: The research was planned as a prospective quasi-experimental study with pretest, posttest, and control groups, in which the simple randomization method was employed. It consisted of 52 women with breast cancer receiving adjuvant hormone therapy, including 26 in the experimental group and 26 in the control group. The medication reminder mobile application developed by the researchers was utilized in the experimental group to determine its effect on treatment adherence. Data collection measures included a personal information form and the Medication Adherence Self-Efficacy Scale (MASES). After the baseline data of the experimental and control groups were collected, the experimental group used the "Medication Reminder" mobile application for eight weeks. At the end of the eight weeks, the MASES was applied to both groups again.

Results: A statistically significant difference was found between the pre-test and post-test MASES total scores of the experimental group (p<0.05). The post-test MASES total scores of the experimental group were significantly higher than their pre-test scores. The inter-group comparisons indicated that the post-test MASES total scores of the experimental group were statistically significantly higher than those of the control group (p<0.05).

Conclusion: It was concluded that the "Medication Reminder" mobile application was an effective tool in increasing treatment adherence in women with breast cancer under adjuvant hormone therapy.

Keywords: Adjuvant hormone therapy, breast cancer, medication adherence, mobile application, women.

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Öz

Amaç: Bu araştırma, adjuvan hormon tedavisi almakta olan meme kanseri kadınlarda, hemşire öncülüğünde hazırlanmış ilaç hatırlatma mobil uygulamasının tedaviye uyum üzerine etkisinin incelenmesi amacıyla planlanmıştır.

Gereç ve yöntemler: Bu araştırma ön test-son test kontrol gruplu, prospektif, basit randomizasyon yöntemi kullanılan, yarı deneysel bir çalışma olarak planlanmış olup, 26 deney ve 26 kontrol grubu olmak üzere toplam 52 adjuvan hormon tedavisi gören meme kanserli kadın ile yürütülmüştür. Araştırmacılar tarafından geliştirilen ilaç alarmı mobil uygulaması deney grubuna tedaviye uyum üzerine etkisini belirlemek amacıyla uygulanmıştır. Verilerin toplanmasında Kişisel Bilgi Formu ve İlaç Tedavisine Bağlılık/Uyum Öz-Etkililik Ölçeği (MASES) kullanılmıştır. Deney ve kontrol grubunun ilk verilerinin toplanmasının ardından deney grubu 8 hafta süre ile "İlaç Alarmı" mobil uygulamasını kullanmıştır. 8 haftalık sürecin sonunda her iki gruba tekrar MASES uygulanmıştır.

Bulgular: Deney grubundakilerin ön test – son test MASES toplam puanları arasında istatistiksel olarak anlamlı farklılık saptanmıştır (p<0,05). Deney grubunun son test MASES toplam puanları ön teste göre anlamlı derecede daha yüksek olduğu tespit edilmiştir. Gruplar arasında ise son test MASES toplam puanları açısından deney grubunun son test toplam puanları istatistiksel olarak anlamlı derece yüksek olduğu saptanmıştır (p<0,05).

Sonuç: Adjuvan hormon tedavisi alan meme kanserli hastalarda "İlaç Alarmı" mobil uygulamasının tedaviye uyumu arttırma konusunda etkili bir araç olduğu sonucuna ulaşılmıştır.

Anahtar kelimeler: Adjuvan hormon tedavisi, ilaç uyumu, meme kanseri, mobil uygulama, kadın.

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Introduction

Cancer is among the leading causes of mortality worldwide [1]. Breast cancer (BC) ranks first globally among cancer types in women, with the prevalence being 30.3% as shown by Globocan 2020 data [2]. A woman's lifetime likelihood of receiving a breast cancer diagnosis is 1 in 8. Hormone-receptor-positive (HR+) tumors are observed in >80% of the cases [3].

Adjuvant hormone treatments (AHT), such as tamoxifen and aromatase inhibitors (AI), have proven clinical benefits in reducing the risk for BC recurrence when taken for five to ten years for women with estrogen-receptor-positive (ER+) BC [4, 5]. Despite all known positive effects of AHTs in the clinical course, there is current evidence that AHT adherence problems occur. In a descriptive study that was conducted to investigate factors associated with non-adherence to AHT following breast cancer but was not intended to support women, develop interventions to promote their adherence, and inform them, Brett et al. [6] found that of those prescribed AHT, approximately 20% within two years and 50% within five years discontinued treatment.

Poor adaptation and lack of AHT adherence are linked to a higher risk of death. McCowan et al. [7] did a retrospective cohort research in the Tayside area of Scotland to investigate whether women with breast cancer used tamoxifen following breast cancer surgery and whether adherence affected survival, and found the mean duration of use as 2.42 years. The mortality rate over the 2.4-year period was 0.85. The mean adherence to tamoxifen was calculated as 93%. Adherence of less than 80% was associated with poorer survival and a mortality rate of 1.10. Only 49% of those who had a moderate level of tamoxifen adherence and were followed for 5 years or longer continued it for five years. Extending the time of tamoxifen use lowers mortality risk, but half of the women fail to fulfill the suggested five-year-long treatment. A significant proportion of women exhibit poor tamoxifen adherence and face higher mortality risk [7].

Many factors affect treatment adherence. Type of AHT drugs used, adverse effects experienced during treatment, early age/advanced age, cost of treatment, history of changing AHT type, being married, having undergone mastectomy/lumpectomy, presence of comorbidities, negative beliefs about the effectiveness of treatment, and receiving chemotherapy or radiotherapy are among the factors that affect treatment adherence [8-10]. Conditions linked to non-compliance should be named and new ways of promoting and sustaining compliance with AHT should be discovered. With the development of technology, the use of mobile applications in the health field is growing [11]. Technology-based interventions can be used as a new way of enhancing AHT non-compliance. The number of smartphone users has grown lately, which has offered an opportunity to utilize these devices to practice evidence-supported healthcare interventions [12, 13].

Mobile health applications are used in many areas, such as the management of an individual's health status (hospital appointment system, treatment adherence, etc.), monitoring of health status (patient monitoring devices, etc.), and keeping health records. Mobile health approaches that are easy to use, allow free access, provide symptom management, make recommendations on symptom management, and support communication with healthcare professionals are preferred by patients [14, 15].

There are many mobile applications developed for cancer treatment. Patients and healthcare staff can benefit from them. They have a critical role in the management of cancer treatment, i.e., handling adversities, backing up medication adherence, organizing and monitoring cancer treatment, offering information about cancer, and spotting and diagnosing disorders [16]. Mobile applications designed for women with breast cancer have positive effects in promoting patient health and have been shown to be used as an information source. They can also be utilized to manage disease and treatment-related symptoms, report treatment-related adverse events, and promote self-care [17].

Thakkar et al. [13] carried out a meta-analysis and found that 15 mobile-based messaging apps nearly multiplied the likelihood of medication compliance among patients with chronic diseases. A descriptive study by Ali et al. [18] showed that 66% of participants showed attention to the mobile application used in the study for AHT compliance and that the entirety of them were interested in its content. However, further research is needed to examine possible changes over time in social backing, AHT adherence and symptoms experienced, quality of life related to health, and further elements impacting AHT compliance [18].

The impact of a nurse-led medication reminder mobile application on treatment adherence in women with BC on adjuvant hormone therapy was studied in this research.

H_1=A mobile application that supports treatment adherence will affect the adherence in the short term in women with BC on adjuvant hormone therapy.

Materials and methods

Type of research

This research was planned as a prospective quasi-experimental study with pre-test, post-test, and control groups, in which the simple randomization method was used. Patients who had breast cancer and were on adjuvant hormone therapy were randomized to experimental and control groups.

Population and sample

Data were collected from women who presented to the Medical Oncology Unit of the Dışkapı Yıldırım Beyazıt Training and Research Hospital of the Health Sciences University, Ministry of Health, the Republic of Türkiye between September 2021 and November 2022, were diagnosed with breast cancer, and continued adjuvant hormone therapy at home. Sample size estimation was done with a power analysis by utilizing the data of a study conducted using the Medication Adherence Self-Efficacy Scale (MASES) to evaluate medication adherence and self-efficacy in cancer patients who had previously used oral chemotherapy drugs [19]. Accordingly, the sample size was estimated as 36 individuals, a minimum of 18 subjects per group (power: 90%; confidence interval: 95%; d=1.0; α=0.05). Considering that there might be some attrition and parametric tests could be used, it was decided to include 60 individuals in the study, 30 in each group. Eight patients with no regular attendance were excluded, with the eventual sample size being 52. The inclusion criteria for the participants in the study were being aged 18 and over, having been diagnosed with hormone receptor-positive breast cancer (stages 0-III), receiving adjuvant hormone therapy, having a smartphone, agreeing to use a mobile application on the smartphone for 2 months, and speaking Turkish for the experimental group. The control group consisted of participants aged 18 and over, having been diagnosed with hormone receptor-positive breast cancer (stages 0-III), receiving adjuvant hormone therapy, and speaking Turkish.

Randomization

Since it was planned to have an equal number of participants in the experimental and control groups in this study, the “simple randomization method” was employed. Patients who met the inclusion criteria for participation in the study were randomly assigned to the experimental (30 subjects) and control groups (30 subjects) according to their registration number given at the time of admission by numbering them from 1 to 60 and starting from the last admission. The randomization process was carried out on the https://www.randomizer.org/ website.

Measures

Face-to-face and telephone interviews were held to gather data. A three-section and 21-item form that the researcher designed was utilized to gather socio-demographic data from women with BC who volunteered to participate in the study, information about their disease and treatment process, and their technology use [20-22]. Dependent variables were measured with the Medication Adherence Self-Efficacy Scale (MASES), which Gözüm and Hacıhasanoğlu [23] adapted into Turkish. The scale was modified in a thesis study that was conducted by Tokdemir [24] and in which oral chemotherapy medication adherence used by cancer patients was evaluated. Nine experts (three specialist nurses in the fields of Nursing, Hematology, and Oncology, five faculty members, and one clinical nurse) were consulted in the process [24]. Since

the sample of our study included subjects with BC receiving AHT, the version of the MASES scale modified by Tokdemir [24] was preferred. The necessary permission was obtained from the author via e-mail to use the scale in our research. An eight-week intervention was planned to evaluate adherence in the short term as the study was conducted within the scope of a thesis process and a previous study by Graetz et al. [20] was taken as a reference.

This study was approved by the Pamukkale University Non-Interventional Clinical Ethics Committee (approved date: 02.02.2021 and approved number: 60116787-020/14394; issue: 03). Written permission was obtained from the Dışkapı Yıldırım Beyazıt Training and Research Hospital, Health Sciences University, Republic of Türkiye.

Design and implementation process of the mobile application

The design process of the medication reminder mobile application for women with BC on AHT was completed in four stages.

Stage 1: At this stage, studies on treatment adherence in women with BC receiving AHT, factors affecting adherence, problems with adherence, mobile applications supporting treatment adherence, and software development costs were examined.

After the design process of the mobile application was completed, a contract was drawn up with a company that met the design criteria by receiving financial support from the Pamukkale University Scientific Research Project (project number 2021SABE011). The mobile application provided access to the system by creating a username and password.

Stage 2: At this stage, the logo and design plans of the mobile application were handled by the researchers. Pink and white colors symbolizing breast cancer were taken as the basis for the user interface and general design of the application. The logo (Figure 1) and interface (Figure 2) of the mobile application are shown below.

Stage 3: The mobile application was tested at this stage. It was tested first by the researchers and then by five women with BC receiving AHT. Errors were detected and necessary modifications were made.

Stage 4: After the technical problems of the application were resolved, some changes considered by the researchers were made in the application, and it was finalized.

Participants registered in the system by the admin could log in to the application with their usernames and passwords. After logging in to the application, the person's name appeared on the screen. The names of the drugs used by the participant and their times and doses were entered into the system by the admin. The purpose of the application, a calendar, a medication prescription page, and user information were included in the interface content.

Figure 1. "MedicationReminder" mobile application logo

Figure 2. "Medication Reminder" mobile application interface

The application automatically sent a notification reading "medication time" with the user's name and the name of the drug to be taken at the time specified by the participant. Then, another message reading, "Hello, have you taken your medication?" was automatically sent to the user as a reminder an hour later. Patients could confirm this notification by responding "yes" or "no."

Experimental group

The researcher contacted patients who volunteered to participate in the study via phone, and the purpose of use and instructions for the "Medication Reminder" mobile application were explained. Afterward, participants were informed about how to download the mobile application from the virtual market and were requested to download it. A personal username and password were created for the participant who downloaded the mobile application via the admin page. The identification of the user who logged into the application was checked through the system, and the medication used by the patient and the time of use were defined for each patient. Then, the "Personal Information Form" and the "Medication Adherence Self-Efficacy Scale (MASES)" were filled in by the researcher via phone calls.

After the interview was completed, the participants started using the application. At the time specified by the participant, a reminder reading "It's time for medication" was automatically sent to the participant with the username and the name of the medication used. Then, an hour later, another reminder reading "Hello, have you taken your medication?" with the name of the medication was automatically sent to the participant. Patients answered this reminder with a "yes" or "no" response. At the end of the eight weeks, the "Medication Adherence Self-Efficacy Scale (MASES)" was re-administered via phone calls. Participants were requested not to use an additional reminder method during this period.

Control group

The control group participants were contacted via phone, and the "Personal Information Form" and the "Medication Adherence Self-Efficacy Scale (MASES)" were administered. No intervention was applied to the control group participants. The "Medication Adherence Self-Efficacy Scale (MASES)" was re-administered eight weeks after the first application. After the data collection phase, the control group participants were also allowed to use the mobile application.

Statistical analysis

Data were evaluated on the SPSS software. The 'Shapiro-Wilk test' was used to test the normality of the variables. As the data had no normal distribution, the Mann-Whitney U test (Z-table values) was used to compare the measurement values of two independent groups. The Wilcoxon test (Z-table values) was utilized to compare the measurement values of two dependent groups. In data with normal distribution, the independent samples t-test (t-table values) was used to compare the measurement values of two independent groups. Pearson-χ2 cross tables were employed to examine the relationships between two qualitative variables. Statistical significance was specified as $p<0.05$*.

Results

In the experimental group, the mean age was 46.03, with the standard deviation being 8.63, and varying between 33 and 59, 17 (56.7%) were high school graduates, 19 (63.3%) were married, 21 (70.0%) had an equal income and expenses, and 14 (46.7%) had no jobs. In the control group, the mean age was 47.63, the standard deviation was 7.24, varying between 30 and 58, 18 of the subjects (60.0%) had high school education, 16 (53.3%) were married, 20 (66.7%) had equal income and expenses, and 16 (53.3%) were unemployed. The group had no statistically meaningful association with education ($p=0.803$), age (years) ($p=0.137$), marital status ($p=0.728$), employment ($p=0.796$), and income ($p=0.836$). Table 1 presents participants' socio-demographic data.

Table 1. Comparison between sociodemographic variables and groups (n:60)

Variables	Experimental (n:30)		Control (n:30)		Statistical Analysis*
	n	%	n	%	Probability
Education					
Primary school	7	23.3	5	16.7	
High school	17	56.7	18	60.0	$X^2=0.439$ $p=0.803$
Undergraduate	6	20.0	7	23.3	
Marital status					
Married	19	63.3	16	53.3	
Single	5	16.7	6	20.0	$X^2=0.634$ $p=0.728$
Divorced/widowed	6	20.0	8	26.7	
Income					
Income<expenses	8	26.7	8	26.6	
Income=expenses	21	70.0	30	66.7	$X^2=0.353$ $p=0.836$
Income>expenses	1	3.3	2	6.7	
Employment status					
Yes	16	53.3	14	46.3	$X^2=0.067$ $p=0.796$
No	14	46.7	16	53.3	
	x̄ ± S.D.	**Median (Min.- Max.)**	**x̄ ± S.D.**	**Median (Min.- Max.)**	
Age (year)	46.03±8.63	45.0 (33.0-59.0)	47.63±7.24	50.0 (40.0-58.0)	$Z=1.488$ $p=0.137$

"Pearson-X^2" cross tables were used to examine the relationships between two qualitative variables. In data that did not have a normal distribution, "Mann-Whitney U" test (Z-table values) was used to compare the measurement values of two independent groups

Sixteen individuals (53.3%) in the experimental group had stage 1 disease, 26 (86.7%) had the disease for 7-23 months, 19 (63.3%) used tamoxifen, 16 (53.3%) had a planned treatment of 5-10 years, 14 (77.8%) received concurrent radiotherapy treatment, and 19 (34.6%) had received chemotherapy before AHT. Of the participants in the control group, 14 (46.7%) had stage 2 disease, 23 (76.7%) had the disease for 7-23 months, 13 (43.3%) used tamoxifen, 19 (63.3%) had a planned treatment for 5-10 years, 18 (82.4%) received concurrent radiotherapy treatment, and 26 (44.1%) had received chemotherapy before AHT. It was determined that 23 people (76.7%) in the experimental group had received information about the drugs they used, 23 (63.9%) said that the person who provided drug information was a physician, and that 15 (76.7%) found the information adequate. It was determined that 18 people (60.0%) in this group thought that cancer would not recur if the drugs were used regularly. Of the participants in the control group, 21 (70.0%) had received information about the drug they used, 21 (63.6%) stated that the person who provided information about the drugs was a physician, and 10 (47.6%) thought that the information they received was adequate. Seventeen participants (56.7%) in this group thought that cancer would not recur with regular use of drugs. The group did not have a statistically meaningful association with stage of disease (p=0.102), length of treatment (month) (p=0.092), duration of disease (p=0.453), drugs used (p=0.190), planned treatment time (p=0.517), status of receiving concurrent treatment (p=0.752), treatments received before AHT (p=0.256), status of receiving information about the drugs used (p=0.559), person giving information about the drugs (p=0.625), adequacy of the information (p=0.083), and belief that cancer would not recur with regular use of the drugs (p=0.965). Information about the disease and treatment process of the participants is shown in Table 2.

Table 2. Comparisons between the groups and information regarding the disease and treatment process (n:60)

Variables	Experimental (n:30)		Control (n:30)		Statistical Analysis*
	n	%	n	%	Probability
Stage of disease					
Stage 0	2	6.7	2	6.7	
Stage I	16	53.3	7	23.3	X^2=6.209 p=0.102
Stage II	9	30.0	14	46.7	
Stage III	3	10.0	7	23.3	
Length of disease					
0-6 months	4	13.3	6	20.0	
7- 23 months	26	86.7	23	76.7	X^2=1.584 p=0.453
2- 5 years	-	-	1	3.3	
Drugs used					
Tamoxifen	19	63.3	13	43.3	
Letrozole	9	30.0	11	46.7	X^2=3.325 p=0.190
Anastrozole	2	6.7	6	20.0	
Planned treatment time					
Unknown	1	3.4	2	6.7	
3-5 years	13	43.3	9	30.0	X^2=1.318 p=0.517
5-10 years	16	53.3	19	63.3	

Table 2. Comparisons between the groups and information regarding the disease and treatment process (n:60) (continued)

Variables	Experimental (n:30)		Control (n:30)		Statistical Analysis*
	n	%	n	%	Probability
Concurrent treatment**					
Chemotherapy	4	22.2	4	17.6	X^2=0.101 *p*=0.752
Radiotherapy	14	77.8	18	82.4	
Treatments before AHT **					
Chemotherapy	19	34.4	26	44.1	
Radiotherapy	7	12.7	3	5.1	X^2=4.052 *p*=0.256
Mastectomy	18	32.7	23	39.0	
Lumpectomy	11	20.0	7	11.8	
Receiving information about the drugs used					
Yes	23	76.7	21	70.0	X^2=0.341 *p*=0.559
No	7	23.3	9	20.0	
Source of the drug information*					
Physician	23	63.9	21	63.6	
Nurse	3	8.3	2	6.1	
Other patients	1	2.8	4	12.1	X^2=2.608 *p*=0.625
Pharmacy	4	11.1	3	9.1	
Book/the Internet	5	13.9	3	9.1	
Adequacy of the information					
Yes	15	65.2	10	47.6	
No	-	-	4	19.0	X^2=4.986 *p*=0.083
Undecided	8	34.8	7	33.3	
The belief that cancer will not recur with regular use of the drugs					
Yes	18	60.0	17	56.7	
No	1	3.3	1	3.3	X^2=0.072 *p*=0.965
Undecided	11	36.7	12	40.0	
	x̄ ± S.D.	**Median (Min.- Max.)**	**x̄ ± S.D.**	**Median (Min.- Max.)**	
Length of treatment (mont)	5.60±2.81	5.5 (1.0-12.0)	4.47±2.50	4.0 (1.0-12.0)	Z=1.687 *p*=0.0.92

"Pearson-X^2" cross tables were used to examine the relationships between two qualitative variables. In non-normally distributed data, "Mann-Whitney U" test (Z-table values) was used to compare the measurement values of two independent groups.
More than one answer was given to the question and percentages were calculated according to the increasing number of cases

It was determined that 30 participants (26.5%) in the experimental group used their mobile phones for communication, 14 (46.7%) had a moderate level of technology use skills, and that 17 (56.7%) used Android-based phones. Of the participants in the control group, 30 (25.5%) used their mobile phones for communication, 13 (43.3%) had a good level of technology use skills, and 16 (53.3%) used Android-based phones. The group had no statistically marked association with daily screen time (hours) (*p*=0.185), the purpose of mobile phone use (*p*=0.703), the model used (*p*=0.185), duration of smartphone use (years) (*p*=0.122), technology use skills (*p*=0.252). Table 3 reflects subjects' technology use features.

Table 3. Comparisons between technology use characteristics and groups (n:60)

Variables	Experimental (n:30)		Control (n:30)		Statistical Analysis*
	n	%	n	%	Probability
Purpose of using a mobile phone**					
Communication	30	26.5	30	25.4	X^2=3.803 *p*=0.703
Messaging	28	24.8	29	24.6	
Taking photographs	11	9.7	15	12.6	
Games	15	13.3	12	10.2	
Music	1	0.9	4	3.4	
The İnternet	16	14.2	12	10.2	
Social media	12	10.6	16	13.6	
Technology use skills					
Very good	3	10.0	6	20.0	X^2=4.087 *p*=0.252
Good	13	43.3	13	43.3	
Moderate	14	46.7	9	30.0	
Poor	-	-	2	6.7	
The model of the device					
IPhone	13	43.3	14	46.7	X^2=0.067 *p*=0.185
Android-based phones	17	56.7	16	53.3	
	x̄ ± S.D.	**Median (Min.- Max.)**	**x̄ ± S.D.**	**Median (Min.- Max.)**	
How long the person had the phone (year)	7.20±2.39	7.0 (4.0-15.0)	6.20±1.27	6.0 (4.0-9.0)	Z=-1.546 *p*=0.122
Daily screen time (hour)	3.07±1.8	3.0 (1.0-6.0)	2.60±0.97	2.5 (1.0-5.)	Z=-1.326 *p*=0.185

"Pearson-X^2" cross tables were used to examine the relationships between two qualitative variables. In non-normally distributed data, "Mann-Whitney U" test (Z-table values) was used to compare the measurement values of two independent groups.
More than one answer was given to the question and percentages were determined according to the increasing number of cases

The pre-test MASES total scores did not yield statistically meaningful variances between the groups (*p*=0.054). Table 4 displays a summary of scores on the pre-test.

According to an inter-group comparison, post-test total MASES scores yielded a statistically meaningful variance with the experimental group having markedly higher scores (*p*=0.002*). Table 5 presents the post-test MASES total scores of both groups.

An intra-group comparison indicated that the pre-and post-test scores of the experimental group on the MASES differed statistically meaningfully with scores obtained from the post-test application being markedly higher (*p*=0.001*). A similar comparison yielded no variance for the control group (*p*=0.113). Tables 6 and 7 display total MASES figures from the pre-and post-test applications for the groups, respectively.

Table 4. Inter-group comparison of pre-test MASES total scores (n:60)

Variable	Experimental (n:30)		Control (n:30)		Statistical Analysis*
	x̄ ± S.D.	Median (Min.- Max.)	x̄ ± S.D.	Median (Min.- Max.)	Probability
MASES total score					
Pre-test	59.83±5.99	60.5 (48.0-71.0)	56.30±7.82	57.0 (42.0-67.0)	t=1.964 *p*=0.054

In data with normal distribution, the independent samples t-test (t-table values) was used to compare the measurement values of two independent groups

Table 5. Comparison of post-test MASES total scores by groups (n:52)

Variable	Experimental (n:30)		Control (n:30)		Statistical Analysis*
	x̄ ± S.D.	Median (Min.- Max.)	x̄ ± S.D.	Median (Min.- Max.)	Probability
MASES total score					
Pre-test	62.07±4.68	62.5 (54.0-72.0)	56.0±6.96	54.5 (44.0-67.0)	Z=-3.171 **p*=0.002

In data with no normal distribution, the "Mann-Whitney U" test (Z-table values) was used to compare the measurement values of two independent groups, **p*<0.05 statisticallysignificant

Table 6. Comparison of the pre-test-post-test MASES total scores of the experimental group (n:26)

Variables	Experimental (n:26)	
	x̄ ± S.D.	Median (Min.- Max.)
MASES total score		
Pre-test	60.88±5.51	60.0 (50.0-71.0)
Post-test	62.07±4.68	62.5 (54.0-72.0)
Analysis Probability	Z=-3.379 **p*=0.001	

In data with no normal distribution, the "Wilcoxon" test (Z-table values) was used to compare two dependent groups, **p*<0.05 statisticallysignificant

Table 7. Comparison of the pre-test-post-test MASES total scores of the control group (n:26)

Variables	Control (n:26)	
	x̄ ± S.D.	Median (Min.- Max.)
MASES total score		
Pre-test	55.23±7.79	55.5 (42.0-67.0)
Post-test	56.00±6.96	54.5 (44.0-67.0)
Analysis Probability	Z=-1.586 *p*=0.113	

In data with no normal distribution, the "Wilcoxon" test (Z-table values) was used to compare two dependent groups

Discussion

There are many descriptive and qualitative studies in the literature into the factors affecting treatment adherence [8-10, 25-32]. As the rate and frequency of mobile phone use have increased in recent years, their use in healthcare services has also increased [12]. However, there is little research into the evidence-based effectiveness of mobile applications that include personalized, treatment-specific medication reminder goals [12, 27]. Our study is thought to make a significant contribution to the literature because it includes the evaluation of the evidence-based effectiveness of the "Medication Reminder" mobile application.

Graetz et al. [20] designed a RCT (a pilot study) to investigate application use one with and the other without a weekly reminder that supported treatment-related side effects and aromatase inhibitor use to reduce symptom burden and improve medication treatment adherence. The researchers compared the BApp+ reminder group in terms of using the application to report aromatase inhibitor adherence and symptoms (via text messages and/or email, as preferred) and the BApp group accessing the application but with no reminders delivered monthly. After the AHT, groups were followed for the first 6-8 weeks. The web-based mobile application with weekly reminders for AI adherence and real-time reporting of treatment-related adverse symptoms was reported to be feasible and effective for improving short-term AI adherence among women with HR+ breast cancer [20]. The study conducted by Graetz et al. [20], despite having a different design, aimed to remind people to take their medications. It is possible to say that the web-based intervention with a medication reminder feature had a positive effect on aromatase inhibitor treatment adherence in the short term, which was consistent with our study.

In a pilot study of 2019, Krok Schoen et al. [21] examined the effects of a text-based intervention application on treatment adherence in women receiving breast cancer treatment in the postmenopausal period. Text messages were delivered to participants' phones as a reminder for AHT medications every day, which continued for ninety days. The study results showed that the application not only supported adherence to AHT but also improved the well-being parameters of the patients. The levels of stress perceived fell markedly throughout the study. Self-report data and blood samples revealed that participants had AHT medication adherence. In the study conducted by Krok Schoen et al. [21], there was no randomization and no control group, and only the pre-test and post-test scores of the experimental group were compared. The study had a different intervention as a study design but addressed a goal and produced results similar to those of our study.

In a prospective randomized controlled study, Tan and colleagues [33] looked into how SMS reminders impacted medication adherence and serum hormone levels in patients with BC taking aromatase inhibitors. They compared SMS reminders with standard care. SMS reminders were sent to all patients participating in the study at the same time on a predetermined day and time weekly. Medication adherence was assessed with the Simplified Medication Adherence Questionnaire (SMAQ) in the sixth and twelfth months. According to the research by Tan et al. [33], the group receiving SMS reminders showed significant adherence compared to the group receiving standard care in the sixth month, with the inter-group variance being unmeaningful in the twelfth month. The inter-group comparison of serum hormone levels indicated no marked variance in the twelfth month. It was concluded that weekly SMS reminders improved medication adherence in the short term, but it had no effect on serum hormone levels in the long run [33]. The data from this study showed that the medication reminder intervention with daily text messages increased treatment adherence in the short term, consistent with the present research. The main distinction was that reminders were sent once a week on a day and time determined by the researchers.

In another randomized controlled study conducted by Hershman et al. [34] to determine whether a one-way SMS reminder could improve long-term adherence, patients received a text message twice a week, one on a randomly selected day during the week and another on a randomly selected day during the weekend, for three years. According to the data of the study,

the one-way text messaging intervention twice a week did not significantly affect AI adherence in women with BC. The study conducted by Hershman et al. [34] did not obtain a result similar to ours because the effects of a one-way text message reminder on long-term treatment adherence had been examined, and another distinction was that the frequency of reminders was twice a week, while it was every day in our study.

When other studies producing results similar to our research were examined [20, 21, 33, 34], it can be said that the scale used in our study was a short, intelligible, and effective tool in assessing treatment adherence in the short term. According to the research data, the medication reminder mobile application was an efficient tool in increasing treatment adherence in the short term in women with BC on AHT.

In conclusion, it was found that the medication reminder mobile application, which aimed to improve treatment adherence in patients with BC under AHT, offered an alternative to technological developments, was an evidence-based and effective method, and came up with a new approach that supported patients' treatment adherence. In future studies, longer-term follow-up of mobile medication reminder applications and evaluation of their results will reveal the importance of mobile applications such as "Medication Reminder," which is a personalized, treatment-specific smartphone application.

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Authors contributions: O.B., S.O. constructed the main idea and hypothesis of the study. O.B., S.O. They developed the theory and arranged/ edited the material and method section. O.B., S.O. have done the evaluation of the data in the results section. Discussion section of the article was written by O.B., S.O. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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Research Article

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The role of depression in obesity and the relationship between cognitive functions, leptin, ghrelin, and neuropeptide Y

Obezitede depresyonun rolü ve bilişsel işlevler, leptin, ghrelin, nöropeptid Y ilişkisi

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Abstract

Purpose: It is known that there is a two-way relationship in the etiopathogenesis of depression and obesity. This study aimed to investigate serum ghrelin, leptin, neuropeptide Y levels, cognitive functions, and atypical depressive features in obese and depressed patients. It is expected that obese and depressed patients will show similar features in terms of biochemical parameters and cognitive functions, and atypical depressive features may be high in obese individuals.

Materials and methods: The study included 56 obese individuals, 60 patients with major depressive disorder (MDD), and 53 healthy controls (HC). The questionnaires administered included socio-demographic data form, Hamilton Depression Rating Scale, Hamilton Anxiety Scale, Dutch Eating Behaviour Questionnaire, Structured Interview Guide for the Hamilton Depression Rating Scale-Seasonal Affective Disorder Version (SIGH-SAD). Cognitive functions were assessed with short computer version of Wisconsin Card Sorting Test (WCST), Berg's WCST, Stroop Colour-Word Test-Victoria version. Serum ghrelin, leptin, and neuropeptide Y levels were measured.

Results: The depression scores were found higher MDD group than obesity and HC groups (p<0.001) and anxiety scores similar in MDD and obesity groups (p=0.064). The groups had similar mean SIGH-SAD scores (p=0.989). There was no difference between groups in WCST scores (p>0.05). Differences were detected between the groups in the Stroop test. Leptin levels were higher (p<0.001), ghrelin (p=0.038) and neuropeptide Y (p<0.001) levels lower in obesity group compared to MDD and HC. Ghrelin levels negatively correlated with total number of incorrect responses in terms of cognitive functions in obese individuals (r=-0.259 p=0.049).

Conclusion: In our study, it was determined that although depressive symptoms were high in obesity, there were no atypical depressive features, executive functions were similar between the groups, and neurochemical marker levels were not similar in obesity and depression. Our results do not support the relationship between obesity and atypical depression.

Keywords: Obesity, depression, leptin, ghrelin, neuropeptide Y.

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Öz

Amaç: Depresyon ve obezite etyopatogenezinde karşılıklı iki yönlü bir ilişkinin olduğu bilinmektedir. Bu çalışmada, obezite ve depresyon hastalarında serum ghrelin, leptin, nöropeptid Y düzeylerinin, bilişsel işlevlerin ve atipik depresif özelliklerin araştırılması amaçlanmıştır. Nörokimyasal parametreler ve bilişsel işlevler açısından obezite ve depresyon hastalarının benzer özellikler göstereceği ve obez bireylerde atipik depresif özelliklerin yüksek olabileceği beklenmektedir.

Gereç ve yöntem: Çalışmaya 56 obez birey, 60 major depresif bozukluklu (MDB) hasta ve 53 sağlıklı kontrol alınmıştır. Sosyodemografik veri formu, Hamilton Depresyon Derecelendirme Ölçeği (HAM-D), Hamilton Anksiyete Ölçeği (HAS), Hollanda Yeme Davranışı Anketi, Yapılandırılmış Görüşme Kılavuzu Mevsimsel Duygudurum Bozukluğu Versiyonu (SIGH-SAD) uygulanmıştır. Bilişsel işlevler Wisconsin Kart Eşleme Testi'nin (WCST) kısa bilgisayar versiyonu Berg's WCST, Stroop testinin Victoria formu olan Victoria Stroop Test ile değerlendirilmiştir. Serum ghrelin, leptin, nöropeptid Y düzeyleri belirlenmiştir.

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Bulgular: MDB grubunda depresyon puanları obezite ve sağlıklı kontrol grubuna göre yüksek (p<0,001), MDB ve obezite gruplarında anksiyete puanları benzer (p=0,064) bulundu. SIGH-SAD puan ortalamaları açısından gruplar arasında fark bulunmadı (p=0,989). Gruplar WCST puanları açısından da benzerdi (p>0,05). Stroop testinde gruplar arasında farklılık tespit edildi. Leptin düzeyleri obezite grubunda diğer gruplardan yüksekti (p<0,001), ghrelin (p=0,038) ve nöropeptid Y (p<0,001) düzeyleri ise düşük bulundu. Obez bireylerde ghrelin düzeyleri ile bilişsel işlevler açısından toplam yanlış cevap sayısı arasında negative yönde zayıf düzeyde korelasyon saptandı (r=-0.259 p=0,049).
Sonuç: Çalışmamızda obezitede depresif belirtilerin yüksek olmasına karşın atipik depresif özellikler bulunmadığı, gruplar arasında yürütücü işlevlerin benzer olduğu, obezite ve depresyonda nörokimyasal belirtileç düzeylerinin benzer olmadığı belirlenmiştir. Sonuçlarımız obeziteyi atipik depresyon ilişkisini desteklememektedir.

Anahtar kelimeler: Obezite, depresyon, leptin, ghrelin, nöropeptid Y.

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Introduction

The most common psychiatric disorder is depression and it is known that obesity is high in depression, and depression and the prevalence of depressive symptoms are high in obesity [1, 2]. Increased appetite and reduced physical activity due to depression, the appetite-boosting side effects of medications used, and binge eating during depression facilitate the development of obesity [3]. While loss of weight and appetite is observed in classical depression resulting in decreased body weight, there is increased appetite and weight in “atypical depression”, which is a subtype of depression. The atypical subtype of depression is diagnosed in the presence of mood reactivity accompanied by two of the following characteristics: hypersomnia, leaden paralysis, increased appetite/weight gain, and long-lasting interpersonal rejection sensitivity. It has been argued that obesity can be a clinical manifestation of atypical depression [3-5]. There are studies suggesting that obesity is a clinical manifestation of atypical depression progressing with increased appetite and finding an association between atypical depression and a high BMI [6-8].

The role of parameters such as ghrelin, leptin, and neuropeptide Y in both obesity and depression has attracted more attention in recent years. Having appetising and adipogenous properties, ghrelin increases anxiety and depression-like behaviours [9]. Leptin increases energy consumption and diminishes appetite. Ghrelin antagonises the anorexigenic effect of leptin by means of hypothalamic neuropeptide Y/Y1 (NPY) receptor. Thus, there is a metabolic antagonism between leptin and ghrelin with respect to their functions in the body [9]. Leptin shows a negative correlation with depression and anxiety independent of body fat and weight; it is argued that the anti-depressive effects of leptin vanish due to leptin resistance in obese individuals [9]. NPY is the major peptide stimulating food intake and has an anxiolytic effect [10]. Known to play an important role in responding to stress and psychiatric disorders, NPY is also a major mediator of emotional eating [11, 12]. The relationships of these three parameters with obesity are clear, but their relationships with depression are not so apparent.

Depression and obesity have a negative impact on cognitive functions. Depression is known to involve attention and memory problems and impairment of executive functions [13, 14]. It has been reported that in obesity there is frontal/subcortical function deficiency; with increased Body Mass Index (BMI), cognitive functions deteriorate and impairment occurs in cognitive flexibility, inhibition capacity, working memory, decision making, verbal fluency, and planning [15]. It is not clear whether increased fat is a cause or result of impairment in cognitive functions. Worsened cognitive skills, increased impulsiveness, and decreased inhibition capacity are likely explanations for excessive eating, binge eating, and loss of eating control [16, 17]. Conversely, a recent meta-analysis has concluded that there is no cognitive function impairment in obesity [18]. It can be thought that cognitive functions may provide guidance in understanding the relationship between obesity and depression.

This study aimed to investigate the relationship between obesity and depression in a multifaceted manner. In this context serum

ghrelin, leptin, neuropeptide Y levels, and executive functions were examined in obese and depressed patients, and the two groups were compared in terms of atypical depressive features. It is expected that the two groups will be similar in terms of biochemical parameters and cognitive functions, and atypical depressive features will be high in obesity.

Materials and methods

Participants

The study included 60 patients who presented to the psychiatry outpatient clinic of Pamukkale University Medical School Hospital between December 2018 and September 2019 and who were diagnosed with MDD according to the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5) and 56 patients who presented to the endocrinology outpatient clinic of the Endocrinology and Metabolism Department of the University Hospital and were diagnosed with obesity with a BMI of 30 kg/m^2 and above. The healthy control group consisted of 53 individuals who had no MDD or obesity diagnosis and had similar characteristics to the patient groups in terms of age and gender. The healthy control group was formed from the hospital staff who volunteered upon announcement of the study. The ages of participants ranged between 18 and 65. Patients with other psychiatric disorders (schizophrenia, mental retardation, bipolar disorder) and those with a neurological disease leading to a cognitive function disorder (cerebrovascular disease, dementia) were excluded from the study. The subjects were informed about the study, and their verbal and written consents were obtained before inclusion.

This research project was approved by the Ethics Committee of Pamukkale University with their decision dated 14/11/2018 and numbered 60116787-020/77483. The study was also supported by the Scientific Research Projects Coordination Unit of Pamukkale University with their decision numbered 2018TIPF047.

Procedures

The participants were administered a psychiatric examination to explore the presence of a psychiatric disorder according to the DSM-5 criteria. Weight, height, waist circumference, and blood pressure measurements were carried out to explore presence of metabolic syndrome according to the NCEP-ATP III diagnostic criteria [19]. Abdominal obesity (waist circumference ≥102 cm in males and ≥88 cm in females), triglyceride (TG) (≥150 mg/dl or receiving pharmacological treatment for high TG), high-density lipoprotein (HDL) (<50 mg/dl in females and <40 mg/dl in males or receiving pharmacological treatment for low HDL), blood pressure (≥130/85 mmHg or receiving anti-hypertensive treatment), and fasting blood sugar (≥100 mg/dl or receiving treatment for high blood sugar) were considered for a metabolic syndrome diagnosis. The fasting blood sugar, TG, and HDL values were obtained from the endocrinology outpatient clinic records. A questionnaire prepared by the investigator questioning socio-demographic data and clinical characteristics was filled out and neuropsychological assessments were completed.

Psychometric assessment

Hamilton Depression Rating Scale (HAM-D)

It is a 17-item scale developed to assess the severity of depression [20]. A total score between 0 and 53 is obtained with higher scores indicating increased severity of depression. Scores 0-7 indicate no depression, 8-15 mild, 16-28 moderate and 29 and above severe depression. The scale was tested for validity and reliability in Turkish [21].

Hamilton Anxiety Rating Scale (HAM-A)

It was prepared to find the level of anxiety and symptom distribution and to measure changes in severity [22]. It consists of 14 items questioning mental and physical symptoms. A total score of 17 and less is rated as mild, between 18 and 24 as moderate, and 25 and above as severe anxiety. It was tested for validity and reliability in Turkish [23].

Structured Interview Guide for Hamilton Depression Rating Scale-Seasonal Affective Disorders Version (SIGH-SAD)

It is a structured interview guide designed to standardize the use of HAM-D and to cover the entire symptoms of depression [24]. This interview guide was constructed by adding 8 items prepared by Rosenthal for atypical depression to the 21-item form of HAM-D.

The atypical balancing score is shown as a percentage by dividing the scale score by the total 29-item SIGH-SAD score and multiplying it by 100. Its Turkish version was tested for validity and reliability [25].

Dutch Eating Behaviour Questionnaire (DEBQ)

Besides internal eating behaviours such as hunger, the questionnaire was developed also to reveal other external factors that affect eating. It consists of 3 subscales: restrained eating, emotional eating, and external eating. It is a 5-point Likert-type self-reporting scale consisting of 33 items [26]. The scale was tested for validity and reliability in Turkish [27].

Neuropsychological assessment

Wisconsin Card Sorting Test

The Psychology Experiment Building Language (PEBL)-Berg's "Wisconsin" Card Sorting Test (WCST), which is the short computer version of the Wisconsin Card Sorting Test, was administered to measure frontal lobe functions and executive functions [28]. This test evaluates an individual's problem-solving ability and ability to change his/her problem-solving strategy according to changing circumstances. WCST measures cognitive processes such as complex (executive) attention, perseveration, working memory, executive functions, concept formation, and abstract reasoning. When the classical application was compared to the short computer version involving 64 response cards, the results of both applications were reported to be similar [29]. The responses given by the subject until the application ends are recorded by the computer and the subject's test performance score is calculated by the existing program at the end of the test.

Stroop test

The Psychology Experiment Building Language (PEBL)-Victoria Stroop Test, which is the Victoria form of the Stroop Test, was administered to assess selective attention, focused attention, response inhibition, interference control, cognitive flexibility, and information processing rate [28]. The Stroop test is said to reflect three basic processes: selective attention, reading, and colour telling [30]. The scoring method in the Stroop Test Victoria Form involves simple measurements of time used to complete the parts. The time it took to complete each part and the number of errors were recorded automatically in a PEBL file with a participant code. While the first and second parts of the test were used to measure cognitive rate, the third part was used to measure response inhibition. The computer program (PEBL)-based management of the test required the participant, in the case of an error, to correct that error before continuing with the next item and this was to be reflected in the completion time of the test. Longer times indicate impairment of attention.

Biochemical assessment

After 12 hours of fasting, 10 cc of venous blood was taken from the antecubital vein of each participant in the case and control groups into a biochemistry tube. After the samples were kept at room temperature for approximately 20 minutes, they were centrifuged at 5000 rpm for 10 minutes and the samples obtained were stored at -80°C for biochemical analysis to determine leptin, ghrelin and neuropeptide Y levels. Biochemical parameters were measured by the ELISA method.

Statistical analysis

The data were analysed using the SPSS 25.0 (IBM SPSS Statistics 25 software Armonk, NY: IBM Corp.) package program. The continuous variables were expressed as means ± standard deviations and medians (smallest-largest values) and the categorical variables as numbers and percentages. Compliance of the data with a normal distribution was explored with the Kolmogorov Smirnov and Shapiro Wilk tests. When the parametric test assumptions were met, the differences in independent groups were compared using independent samples t test and One-Way Variance Analysis (Post hoc: "Tukey Test"). When the parametric test assumptions were not met, the independent group differences were compared using the Mann Whitney U test and Kruskal Wallis Test (Post hoc: "Bonferroni-Corrected Mann Whitney U test"). The relationship between the numeric variables were assessed with the Spearman correlation analysis. The differences between the categoric variables were analysed with the Chi-square Analysis. In all analyses, $p<0.05$ at 95% confidence interval was considered statistically significant.

Results

Socio-demographic data and scale results

The study included 56 subjects diagnosed with obesity, 60 patients diagnosed with MDD and 53 healthy controls. The participants' socio-demographic data and scale scores are shown in Table 1 and their health indicators in Table 2. Depression scores were higher in the MDD group than in the obesity and healthy control groups, but no difference was found between the anxiety scores of the MDD and obesity groups.

No difference was found between the MDD and obesity groups with respect to their mean SIGH-SAD scores, which measure atypical depression (Mann-Whitney U test p=0.945, z=-0.069). The emotional eating score was higher in the obesity group than in the other two groups. Thirty individuals (53.5%) in the obesity group were satisfying the MDD diagnostic criteria when they joined the study. No difference was found between the depressive patients in the obesity group and the MDD group with respect to their SIGH-SAD scores (p=0.456, z=-0.745, Mann-Whitney U test).

Table 1. Socio-demographic data and scale scores of the groups

	Obesity Mean±SD	MDD Mean±SD	Control Mean±SD	p^a	$p1^b$	$p2^b$	$p3^b$	χ^2
BMI	35.26±4.88	23.75±3.42	24.01±3.49	<0.001*	<0.001*	<0.001*	1.000	111.76
Age	39.02±8.41	36.57±12.12	35.6±7.65	0.080	-	-	-	5.057
Education Year	10.8±5.46	11.82±5.11	16.02±5.3	<0.001*	0.904	<0.001*	<0.001*	26.814
HAM-D	10.23±8.07	16.33±7.46	2.26±2.04	<0.001*	<0.001*	<0.001*	<0.001*	88.609
HAM-A	17.2±13.28	21.55±12.18	7.47±6.45	<0.001*	0.064	<0.001*	<0.001*	42.780
SIGH-SAD	23.65±23.53	20.87±11.15	28.36±33.1	0.989	-	-	-	0.022
DEBQ-Restrained	14.32±9.71	13.53±11.6	13.09±11.59	0.608	-	-	-	0.996
DEBQ Emotional	18.55±17.77	8.88±12.26	10.06±12.18	0.005*	0.005*	0.056	1.000	10.596
DEBQ-External	13.89±7.47	11.2±9.57	14.13±8.91	0.159	-	-	-	3.676

HAM-D: The Hamilton Rating Scale for Depression, HAM-A: Hamilton Anxiety Rating Scale, DEBQ: The Dutch Eating Behaviour Questionnaire
SIGH-SAD: Structured Interview Guide for Hamilton Depression Rating Scale Seasonal Affective Disorder
p: Obesity-MDD-Control, *p1*: Obesity-MDD, *p2*: Obesity-Control, *p3*: MDD-Control, MDD: Major depressive disorder, BMI: Body-Mass Index
SD: Standard Deviation, χ^2: Chi-squared test, a: Kruskal Wallis Test, b: Mann Whitney U test

Table 2. Clinical characteristics of the groups

Variables		Obesity n (%)	MDD n (%)	Control n (%)	p^a	χ^2
Gender	Female	38 (67.9%)	40 (66.7%)	36 (67.9%)	0.987	0.026
	Male	18 (32.1%)	20 (33.3%)	17 (32.1)		
Psychiatric History	Yes	32 (57.1%)	37 (61.7%)	23 (43.4%)	0.133	4.034
	No	24(42.9%)	23 (38.3%)	30 (56.6%)		
Number of MDD Episodes	None	22(39.3%)	0	35 (66.0%)	<0.001*	64.788
	Single	14(25.0%)	34(56.7%)	17 (32.1%)		
	Multiple	20 (35.7%)	26 (43.3%)	1 (1.9%)		
Family History of Psychiatric Disorder	Yes	18 (32.1%)	23 (38.3%)	14 (26.4%)	0.401	1.827
	No	38 (67.9%)	37(61.7%)	39(73.6%)		
Metabolic Syndrome	Yes	30 (54.6%)	12 (20.0%)	7 (13.2%)	<0.001*	25.204
	No	26 (46.4%)	48 (80%)	46 (86.8%)		
Comorbid Medical Condition	Yes	19 (33.9%)	20 (33.3%)	14 (26.4%)	0.643	0.882
	No	37 (66.1%)	40 (66.7%)	39 (73.6%)		

a: Chi-squared test, MDD: Major Depressive Disorder

Neuropsychological assessment

While the groups were found similar with respect to their WCST scores, the obesity group showed a poorer performance than the control group in the Stroop test (Table 3). No significant difference was found in the cognitive functions of the individuals in the obesity group with and without a metabolic syndrome diagnosis (p>0.05, Mann-Whitney U test).

Table 3. Neuropsychological test comparisons between groups

	Tests	Obesity Mean±SD	MDD Mean±SD	Control Mean±SD	p^a	χ^2
WCST	**Categories Completed**	2.73±1.48	2.6±1.65	3.13±1.52	0.191	3.309
	Total Correct	41.91±11.43	42.38±11.84	45.08±11.29	0.171	3.527
	Total Errors	22.09±11.43	21.62±11.84	18.36±9.8	0.146	3.842
	Perseverative Responses	19.2±8.86	16.97±10.08	18.79±5.65	0.410	1.785
	Perseverative Errors	9.75±6.03	8.1±6.66	8.68±4.38	0.274	2.588
	Nonperseverative Errors	12.34±13.19	13.52±13.79	9.68±8.69	0.574	1.109
	Conceptual Level Response	35.3±14.71	35.85±15.53	39.57±14.1	0.130	4.076
	Learning to Learn	2.31±8.68	1.49±7.41	-0.79±5.17	0.187	3.348
Stroop Test	**Stroop Part D**	113.22±58.04	102.01±49.98	95.89±64.78	0.123	4.184
	Stroop Part W	84.29±54.09	75.1±37.25	60.25±26.08	0.041*	6.377
	Stroop Part C	109.09±75.92	89.82±50.49	76.65±53.92	0.043*	6.303

MDD: Major Depressive Disorder, WCST: Wisconsin Card Sorting Test, Stroop Part D: Dots, Stroop Part W: Neutral Words
Stroop Part C: Color Words, *p*: Obesity-MDD-Control, SD: Standard deviation, a: Kruskal Wallis test

Data on parameters of leptin, ghrelin and neuropeptide Y

The leptin levels were found higher and the ghrelin and neuropeptide Y levels lower in the obesity group compared to the other groups (Table 4). The relationships between the neuropsychological tests and the BMI, HAM-D, HAM-A, and biochemical parameters are presented in Table 5 and Table 6. A moderate positive correlation was found in the MDD group and a positive low correlation in the obesity group between the HAM-D, HAM-A scores and the Stroop Part D, Stroop Part W, Stroop Part C times (p>0.05 for all). In the control group, a moderate positive correlation was found between the mean BMI and the mean Stroop Part D, Stroop Part W, and Stroop Part C times.

Table 4. Comparison of serum leptin, ghrelin and neuropeptide Y levels of the groups

	Obese (N=56)	MDD (N=60)	Control (N=53)	p^a	$p1^b$	$p2^b$	$p3^b$	F
		Mean±SD						
Leptin	33.17±21.3	15.36±19.39	18.33±17	<0.001*	<0.001*	<0.001*	0.703	13.317
Ghrelin	168.63±68.67	204.65±78.84	187.47±72.76	0.038*	0.029*	0.392	0.446	3.341
NeuropeptideY	0.15±0.04	0.18±0.05	0.18±0.05	<0.001*	0.001*	0.001*	1.000	8.783

MDD: Major Depressive Disorder, a: ANOVA test, b: Tukey Test, *p*: Obesity-MDD-Control, *p1*: Obesity-MDD, *p2*: Obesity-Control, *p3*: MDD-Control

Table 5. The relationship between biochemical parameters with cognitive functions

			Total Correct	Total Errors	Perseverative Errors	Nonperseverative Errors	Learning to Learn	Stroop Part D	Stroop Part W	Stroop Part C
MDD	Leptin	r	0.041	-0.041	0.099	-0.027	-0.080	0.069	-0.012	0.101
		p	0.771	0.771	0.477	0.845	0.669	0.620	0.930	0.466
	Ghrelin	r	-0.090	0.090	0.008	0.146	-0.278	0.086	0.081	0.051
		p	0.519	0.519	0.953	0.291	0.130	0.537	0.563	0.716
	Neuropeptide Y	r	-0.213	0.213	0.073	0.294*	-0.287	0.234	0.331*	0.304*
		p	0.121	0.121	0.601	0.031*	0.118	0.088	0.014*	0.025*
Obesity	Leptin	r	-0.046	0.046	0.003	0.067	-0.084	0.079	0.092	-0.007
		p	0.732	0.732	0.980	0.616	0.630	0.553	0.494	0.960
	Ghrelin	r	0.259*	-0.259*	0.064	-0.255	0.274	-0.209	-0.139	-0.187
		p	0.049*	0.049*	0.633	0.053	0.111	0.115	0.299	0.159
	Neuropeptide Y	r	0.085	-0.085	0.095	-0.064	0.123	0.016	0.177	0.113
		p	0.526	0.526	0.478	0.631	0.482	0.903	0.183	0.398
		p	0.146	0.146	0.339	0.309	0.279	0.290	0.254	0.399
Control	Leptin	r	0.109	-0.111	0.040	-0.111	0.083	0.016	0.062	0.102
		p	0.448	0.437	0.783	0.437	0.648	0.910	0.665	0.478
	Ghrelin	r	-0.027	0.039	0.060	0.056	0.374*	-0.131	-0.161	-0.049
		p	0.852	0.787	0.675	0.696	0.032*	0.358	0.258	0.732
	Neuropeptide Y	r	0.024	-0.035	0.064	-0.019	0.403*	-0.156	-0.161	-0.108
		p	0.870	0.805	0.654	0.894	0.020*	0.275	0.260	0.451

Spearman Correlation Analysis, Stroop Part D: Dots, Stroop Part W: Neutral Words, Stroop Part C: Color Words

Table 6. The relationship between scale scores and BMI with cognitive functions

			Total Correct	Total Errors	Perseverative Errors	Nonperseverative Errors	Learning to Learn	Stroop Part D	Stroop Part W	Stroop Part C
MDD	HAM-D	r	-0.017	0.017	-0.359*	0.214	-0.480*	0.427**	0.447*	0.395*
		p	0.899	0.899	0.007*	0.114	0.005*	0.001*	0.001*	0.003*
	HAM-A	r	-0.111	0.111	-0.343*	0.261	-0.566*	0.372**	0.295*	0.284*
		p	0.417	0.417	0.010*	0.052	0.001*	0.005*	0.027*	0.034*
	BMI	r	-0.063	0.063	-0.013	0.098	-0.009	0.127	0.111	0.111
		p	0.647	0.647	0.924	0.471	0.961	0.350	0.417	0.415
Obesity	HAM-D	r	-0.094	0.094	-0.060	0.029	0.140	0.416*	0.402*	0.416*
		p	0.474	0.474	0.651	0.823	0.416	0.001*	0.001*	0.001*
	HAM-A	r	-0.035	0.035	-0.092	-0.010	0.116	0.287*	0.334*	0.340*
		p	0.791	0.791	0.485	0.942	0.502	0.026*	0.009	0.008*
	BMI	r	-0.190	0.190	-0.126	0.134	-0.185	0.139	0.150	0.111
		p	0.146	0.146	0.339	0.309	0.279	0.290	0.254	0.399
Control	HAM-D	r	-0.026	0.014	0.150	-0.059	0.114	0.041	0.126	0.114
		p	0.852	0.923	0.282	0.674	0.520	0.771	0.369	0.418
	HAM-A	r	-0.187	0.200	0.245	0.107	0.011	0.158	0.134	0.128
		p	0.180	0.151	0.077	0.448	0.952	0.258	0.339	0.360
	BMI	r	-0.340*	0.329*	0.206	0.255	-0.216	0.559*	0.592*	0.495*
		p	0.013*	0.016*	0.139	0.065	0.221	<0.001*	<0.001*	<0.001*

Spearman Correlation analysis. BMI: Body Mass Index, HAM-D: The Hamilton Rating Scale for Depression
HAM-A: Hamilton Anxiety Rating Scale, Stroop Part D: Dots, Stroop Part W: Neutral Words, Stroop Part C: Color Words

Discussion

Our study aimed to examine the two-way relationship between obesity and major depressive disorder and to seek an answer to the question of whether a subgroup of obesity patients has undiagnosed depression (especially atypical depression). The MDD ratio was 53.5% in the obesity group, but it did not differ from the depression group with respect to the characteristics of atypical depression. The prevalence of MDD was higher in the obesity group than in the control group. Our results strongly support the comorbidity of obesity and depression as in the previous studies [2, 31]. A meta-analysis has found that BMI was 2.55 times higher in atypical depression subtype than in melancholic subtype. It has been stressed that obesity seen in depression may be associated with an atypical subtype of depression and it deserves clinical investigation [32]. Atypical depression was found to be accompanied by female gender, unhealthy behaviours (smoking, social isolation, decreased physical activity, etc.), and psychiatric comorbidities as well as obesity, cardiovascular diseases, and metabolic syndrome [33]. A study exploring the causal relationship between obesity and depression found a correlation between BMI and increased appetite, but no causal relationship was found when the other atypical symptoms were investigated as a whole [34]. Our results also suggest that the comorbidity of depression is high in obesity, but it does not qualify as a characteristic for the atypical depression subtype. This subject requires long-term follow-up studies with larger patient groups.

Emotional eating scores

Emotional eating scores were found higher in the obesity group than in other groups. The high rate of emotional eating in the obesity group may be related to the high rate of

depressive symptoms in this group. Obesity was found higher in individuals who have a higher level of emotional eating and find it difficult to control themselves [7]. It has been argued that emotional eating is associated with body weight; obese individuals eat more in negative emotional states than those with a low or normal weight, and eating increases the level of anxiety [35]. A meta-analysis found that the restrained, uncontrolled and emotional eating scores of obese people were higher than those of individuals with a normal weight and BMI showed a positive correlation with emotional and uncontrolled eating [36]. Available data suggest that learning methods to express negative emotions and to cope with these emotions would be beneficial in fighting obesity in individuals who exhibit emotional eating behaviours.

Cognitive functions

In our study, no difference was found between the groups with respect to executive functions. While the healthy control group showed the best performance in selective attention, focused attention, response inhibition, interference control, and information processing rate, the performance of the obesity group was found the lowest. It was determined that as BMI increased, cognitive function performance decreased in the control group. As depression and anxiety symptoms increased, their performance in the attention test declined in the obesity and MDD groups. Our findings indicate that executive function and attention performances are similar between obesity and depression and do not differ from the healthy control group. In the healthy control group, cognitive performance decreases as weight increases. These findings are similar to previous studies [37-40]. New studies are needed to understand the relationship between weight gain and cognitive functions.

Biochemical parameters

In our study, the highest serum leptin value was found in the obesity group and the lowest in the MDD group. Studies have found a strong correlation between high leptin levels and the atypical subtype of MDD (in the present and recovered patients). In patients who were currently in an episode, high leptin levels were found associated with hyperphagia, weight gain, and leaden paralysis, which are characteristic features of atypical depression. This relationship was found to indicate a leptin resistance caused by increased fatty tissue. This relationship has not been observed with the other subtypes [41]. Plasma and cerebrospinal fluid leptin levels were found to decrease in patients with anorexia nervosa (AN). It has been shown that the changes in leptin levels disappear when the body weight returns to normal and the plasma and CSF leptin levels of recovered AN patients are similar to the control values in the long run [42]. Hippocampal leptin deficiency is argued to cause obesity-induced depression and leptin has antidepressant effects [43]. In a study made with women with eating spectrum disorder, serum leptin levels showed a negative correlation with depression and anxiety independent of body fat and weight, and leptin's antidepressant effects disappeared due to leptin resistance in obese people [9]. Severely obese individuals can be resistant to the effects of leptin; the increased serum leptin levels in these individuals are often seen as an indication of leptin resistance [44]. Previous studies support weight gain associated with atypical depressive features and leptin resistance. In our study, leptin levels may have been low in the depression group due to the lack of atypical depressive features in the obesity and depression groups. Follow-up studies on both obesity and depression and comparison studies after weight loss and depression recovery are needed in this area.

The highest mean ghrelin value was found in the MDD group and the lowest in the obesity group. Ghrelin has appetising and adipogenous effects [45]. While ghrelin levels have been found low in obese individuals, they have been found high in those diagnosed with AN [46]. Ghrelin has been found to inhibit hypothalamic serotonin release and to activate the hypothalamic pituitary adrenal axis, which can aggravate anxiety and depression symptoms [9, 47]. The high level of ghrelin found in the MDD group in our study is consistent with the studies in the literature reporting depressive symptom-increasing properties of ghrelin. Some studies have linked better cognitive functions to high serum ghrelin levels and found that the plasma concentration of this hormone decreases in older individuals and Alzheimer's patients [44]. Our results are consistent with those of the studies that link better cognitive functions to high serum ghrelin levels [44, 48]. A borderline

positive relationship was found between ghrelin and cognitive functions in the obesity and control groups.

The obesity group had the lowest mean neuropeptide Y value. It is known to play an important role in the response to stress and psychiatric disorders; it is potentially a major mediator of “emotional eating” [49]. NPY is known to be the strongest endogenous substance showing an antagonist effect on the behavioural outcomes of anxiety and stress [11]. The risk of anxiety and depressive disorder was found high in individuals whose NPY levels were low in their peripheral NPY measurements [12]. NPY has been shown to enhance neuroprotection, stimulate neurogenesis, and alleviate neuroinflammation [50]. No correlation was found in our study between serum NPY levels and depression or anxiety. However, a positive correlation was found between the serum neuropeptide Y levels and the WCST learning-to-learn scores in the control group.

The study may contribute to the literature by examining the two-way relationship between obesity and depression in terms of emotional eating and cognitive functions and by trying to predict this with biochemical parameters associated with obesity. One of the limitations of this study is that the education levels of the healthy subjects included in the study were statistically significantly higher compared to the patient group. Neurocognitive tests are influenced by education. It can be said that weight control is achieved better as the level of education goes up. The prevalence of metabolic syndrome was higher in the obesity group than in the other groups due to the accompanying physical diseases. Not excluding these diseases is another limitation of our study; accompanying physical diseases may have influenced the levels of depression and anxiety. In addition, the fact that the effects of the antidepressants used on neurochemical parameters were not examined is a limitation of our study and should be taken into consideration when evaluating our results.

Our results show that emotional eating behavior scores are higher in the obesity group, executive dysfunction is not seen in the obesity group, but executive dysfunction may be seen as depression and anxiety symptoms increase, selective and sustained attention is impaired in the obesity group, this impairment may increase as depression and anxiety symptoms increase, leptin resistance is present in the obesity group, serum ghrelin has the feature of increasing depressive symptoms, and ghrelin may be associated with good cognitive function level. Our study indicates that the obesity group does not show a significant feature in terms of atypical depression subtype, but obesity and depression comorbidity are high, and these individuals should definitely be evaluated for depression.

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Research Article

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Mitochondrial dysfunction in children with chronic kidney disease

Kronik böbrek hastalığı olan çocuklarda mitokondriyal disfonksiyon

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Abstract

Purpose: We aimed to determine serum mitochondrial open reading frame 12S rRNA-c (MOTS-C) levels as an indicator of mitochondrial dysfunction in childhood chronic kidney disease patients and to investigate the relationship of this parameter, which is a metabolic regulatory factor, with renal anemia, hypertension metabolic acidosis and renal osteodystrophy.

Materials and methods: The study included 46 children with chronic kidney disease and 46 healthy children of similar age and gender. The patient group was divided into G1-G5 subgroups according to glomerular filtration rate, etiology, renal replacement therapies and the presence of anemia, hypertension, hyperparathyroidism and metabolic acidosis. Data were analyzed using SPSS 25.0 package program.

Results: The mean MOTS-C level was 60.47±11.1 ng/ml in patients with chronic kidney disease and 105.2±54.7 ng/ml in healthy children (p=0.001). The MOTS-C level was significantly lower in children with chronic kidney disease. In addition, there was no significant difference between patients who underwent renal transplantation and patients on chronic dialysis or predialysis. MOTS-C levels were significantly lower in patients with hyperparathyroidism and anemia compared to those without.

Conclusion: In our study, we demonstrated that mitochondrial damage in children with chronic kidney disease begins even in the early stages, renal osteodystrophy and anemia contribute to this condition, and mitochondrial inflammation persists even after kidney transplantation in these patients.

Keywords: Mitochondria, MOTS-C, children, chronic kidney disease.

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Öz

Amaç: Çocukluk çağı kronik böbrek hastalarında mitokondriyal disfonksiyonun bir göstergesi olarak serum MOTS-C düzeylerini belirlemeyi ve metabolik düzenleyici bir faktör olan bu parametrenin renal anemi, hipertansiyon, metabolik asidoz ve renal osteodistrofi ile ilişkisini araştırmayı amaçladık.

Gereç ve yöntem: Çalışmaya kronik böbrek hastalığı olan 46 çocuk ve benzer yaş ve cinsiyette 46 sağlıklı çocuk dahil edildi. Hasta grubu glomerüler filtrasyon hızına göre G1-G5, etiyoloji, renal replasman tedavileri ve anemi, hipertansiyon, hiperparatiroidizm ve metabolik asidoz varlığına göre alt gruplarına ayrıldı. Veriler SPSS 25.0 paket programı kullanılarak analiz edildi.

Bulgular: Ortalama MOTS-C düzeyi kronik böbrek hastalığı olanlarda 60,47±11,1 ng/ml iken sağlıklı çocuklarda 105,2±54,7 ng/ml idi (p=0,001). MOTS-C düzeyi kronik böbrek hastalığı olan çocuklarda anlamlı derecede düşüktü. Ayrıca, böbrek nakli yapılan hastalar ile kronik diyaliz veya prediyaliz hastaları arasında anlamlı bir fark yoktu. MOTS-C düzeyleri hiperparatiroidizm ve anemisi olan hastalarda olmayanlara kıyasla anlamlı derecede düşüktü.

Sonuç: Çalışmamızda, kronik böbrek hastalığı olan çocuklarda mitokondriyal hasarın erken evrelerde bile başladığını, renal osteodistrofi ve aneminin bu duruma katkıda bulunduğunu ve mitokondriyal inflamasyonun bu hastalarda böbrek naklinden sonra bile devam ettiğini gösterdik.

Anahtar kelimeler: Mitokondri, MOTS-C, çocuklar, kronik böbrek hastalığı.

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Introduction

Mitochondrial dysfunction is associated with increased oxidative stress (OS) and metabolic disorders and is known to contribute to the pathophysiology and progression of chronic kidney disease (CKD) [1-4]. Uremia in CKD leads to the release of proinflammatory cytokines (IL-1, IL-6, TNF). These cytokines are toxic to mitochondria, affect mitochondrial function and trigger cellular aging. OS, defined as disturbances in the pro-/antioxidant balance, is highly damaging to cells due to excessive formation of reactive oxygen (ROS) and nitrogen species [5]. Chronic inflammation and mitochondrial dysfunction are increasingly recognised as contributors to kidney fibrosis and end-stage renal disease [4, 5]. The kidney is a mitochondria-rich organ and mitochondrial superoxide production leads to oxidative damage, which in turn damages mitochondrial DNA and the electron transport chain. In patients with advanced stages of CKD, increased OS is associated with complications such as hypertension, atherosclerosis, inflammation, and anemia [5]. Persistence of oxidative stress and mitochondrial dysfunction leads to transition from acute kidney injury to chronic kidney disease [5].

Mitochondrial-derived peptides (MDPs) humanin and mitochondrial open reading frame 12S rRNA-c (MOTS-c) are known to play a role in cell survival, apoptosis suppression and glucose control [4]. In response to an increase in oxidative stress, the MOTS-c protein translocates to the nucleus. MOTS-c has been demonstrated to interact with Nrf2 in the nucleus, thereby regulating the expression of antioxidant response element genes [4]. In the only study of MDPs in patients with CKD, MOTS-C levels were found to be low in serum and muscle, while humanin levels were found to be low in muscle and normal in serum. The situation in childhood remains unclear. In our study, we aimed to determine serum MOTS-C levels as an indicator of mitochondrial dysfunction in childhood CKD patients and to investigate the relationship of this parameter, which is a metabolic regulatory factor, with renal anemia, hypertension, metabolic acidosis and renal osteodystrophy.

Materials and methods

A prospective cross sectional study involving children with CKD and healthy children of similar age and sex was conducted at a tertiary care referral hospital. Weight (kg), height (cm) and manual blood pressure were measured. Clinical data including age, gender, CKD duration, etiology, and treatment were collected from patients' medical records.

Glomerular filtration rate (GFR) was calculated using the Schwartz formula (height (cm) x 0.413 /plasma creatinine (mg/dl)) [6]. CKD is defined as abnormalities of kidney structure or function, present for a minimum of 3 months, with implications for health [7]. CKD is classified based on GFR category (G1–G5) [8]. Patients with CKD were divided into predialysis, hemodialysis, peritoneal dialysis and renal transplant groups according to their treatment. Additionally, patients with CKD were divided into subgroups according to the presence of anemia, hypertension, renal osteodystrophy, and metabolic acidosis.

Anemia in children was defined using age-specific thresholds, namely, for 0.5 to 4 years, an Hb <11 g/dl; for 5 to 11 years, an Hb <11.5 g/dl; and for 12 to 14 years, an Hb <12 g/dl [9]. Hypertension was defined as systolic and/or diastolic blood pressure above the 95th percentile for age, gender, and height [10]. The target range for PTH is 35-70 pg/mL in CKD stages 2-3 and 70-110 pg/mL in CKD stage 4 and <300 pg/mL in pediatric patients with CKD stage 5 [11]. Patients with a blood pH of less than 7.35 and an HCO3 of less than 22 mmol/l were defined as having metabolic acidosis [7].

Urea, creatinine, sodium, potassium, calcium, phosphorus, alkaline phosphatase, vitamin D and PTH were evaluated in routine laboratory tests in serum samples taken in the morning. Serum calcium, phosphorus and alkaline phosphatase levels were measured by photometric method, and vitamin D and PTH levels were measured by electrochemiluminescence immunologic method. MOTS-C levels were analyzed from patient venous blood samples collected by enzyme-linked immunosorbent assay (ELISA) in the Medical Biochemistry research laboratory.

Data were analyzed using SPSS 25.0 (IBM SPSS Statistics 25 software) package program. Continuous variables were analyzed as mean ± standard deviation, median (IQR: Interquartile range) and categorical variables are given as numbers and percentages. When parametric test assumptions were met Independent samples t test was used in the comparison of independent group differences test; when parametric test assumptions were not met, independent group differences Mann Whitney U test was used for comparison. According to the reference study results [4], they had a strong effect size (d=1.43) for MOTSC results. Assuming we can achieve a lower effect size (d=0.7), when at least 90 participants (at least 45 participants per group) were included in the study, that would result in 80% power with a 95% confidence level (5% type 1 error rate).

Permission was obtained from Pamukkale University Non-Interventional Clinical Research Ethics Committee for the study (approved date: 04.03.2025 and approved number: E-60116787-020-665941).

Results

The study encompassed 46 patients diagnosed with chronic kidney disease (20 female and 26 male) and an equivalent number of patients from the control group (21 female and 25 male). The mean age of patients with CKD was 13.7±5.6 years, while the mean age of patients from the control group was 12.5±2.8 years. Age and gender of the patient and healthy control groups were similar.

The mean MOTS-C level was 60.47±11.1 ng/ml in patients with CKD and 105.2±54.7 ng/ml in healthy children (p=0.001). The MOTS-C level was significantly lower in children with CKD. Among the patients with CKD, 10 were in chronic peritoneal dialysis, 3 were in a chronic hemodialysis program, 11 had received kidney transplantation, 22 were in the pre-dialysis stage and were not yet on renal replacement therapy. In addition, there was no significant difference between patients who underwent renal transplantation and patients on chronic dialysis or predialysis (Table 1). Among the predialysis patients, 1 had stage 1, 3 had stage 2, 9 had stage 3, and 9 had stage 4 CKD. MOTS-C levels were found to be non-statistically lower in grade 4 compared to the other groups (Figure 1). When the etiologies of patients with CKD were analyzed, 42% had glomerular disease, 43% had tubular disease and 15% had unknown etiology; there was no significant difference between the groups in terms of MOTS-C levels (Table 2).

Anemia was present in 37% of patients with CKD, hyperphosphatemia in 43%, vitamin D deficiency in 57%, secondary hyperparathyroidism in 56%, metabolic acidosis in 43%, and hypertension in 58%. MOTS-C levels were significantly lower in patients with hyperparathyroidism and anemia compared to those without (Table 3). MOTS-C levels were similar in patients with metabolic acidosis, hyperphosphatemia or hypertension compared to those without. There was a negative correlation between serum MOTS-C and PTH levels in children with chronic kidney disease (Figure 2).

Table 1. MOTS-C levels in healthy and chronic kidney disease children

	MOTS-C (ng/ml)		MOTS-C (ng/ml)		
	Chronic Kidney Disease (n=46)	**Healthy group (n=46)**	**Pre-dialysis (n=22)**	**Kidney transplanted (n=11)**	**Dialysis (n=13)**
Mean±SD	60.4±11.1	105.2±54.7	62.1±10.7	61.2±13.2	56.5±9.8
Median	60.09	102.1	62.8	59.7	57.4
IQR	(53.1-66.8)	(49.9-61.4)	(55.7-67.3)	(52.6-71.5)	(49.9-61.4)
p	0.0001* (t=-5.15)		0.360[a] (t=0.92)	0.607[b] (t=-0.51)	0.72[c] (t=1.34)

SD: Standard Deviation; IQR: Interquartile Range (25th – 75th percentiles), *p<0.05 statistically significant
Comparison of the CKD and healthy groups, [a-b-c] Independent t test was used to compare groups
[a] Comparison of the predialysis group with other groups in patients with CKD
[b] Comparison of the kidney transplanted group with other groups in patients with CKD
[c] Comparison of the dialysis group with other groups in patients with CKD

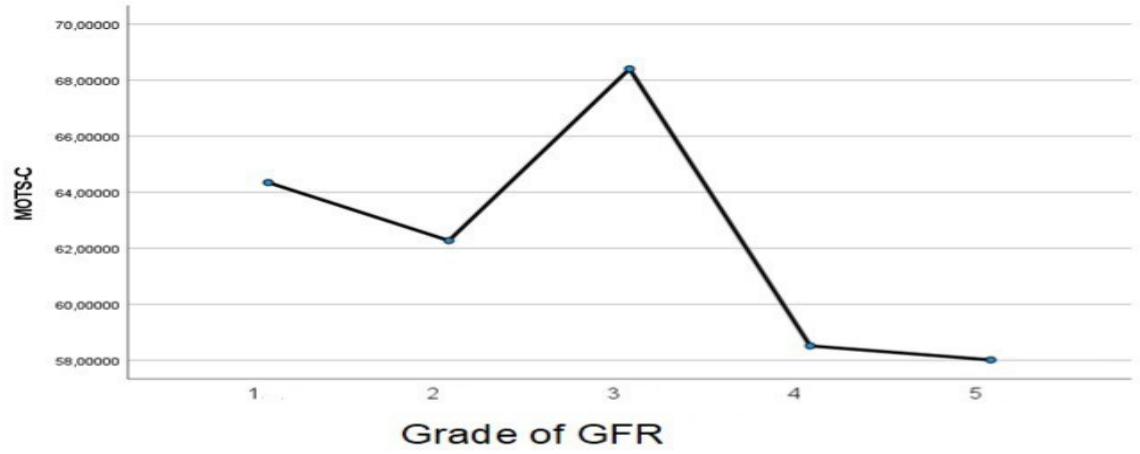

Figure 1. Serum MOTS-C levels according in stages of GFR in CKD

Table 2. MOTS-C levels according to the etiology of childhood chronic kidney disease

Classification of Chronic Kidney Disease	Etiology of Chronic Kidney Disease	n (46)	MOTS-C (ng/ml) Mean±SD	*p*
Glomerular (n=19)	Focal Segmental Glomerulosclerosis	9		
Hemolytic Uremic Syndrome	2			
Crescentic Glomerulonephritis	1			
IGA nephropathy	1		58.8±11.8	
Congenital Nephrotic Syndrome	2			
Lupus Nephritis	2			
Chronic Tubulointerstitial Nephritis	2			0.268* t=-1.12
Tubular (n=20)	Cystic Kidney Disease	2		
Neurogenic Bladder	6			
Vur Nephropathy	5		62.5±9.9	
Cystinosis	3			
Other Urological Anomalies	4			
Unknown Cause (n=7)**	Chronic Kidney Disease of Unknown Cause	7	57.9±14.7	

SD: Standard Deviation, t= independent samples t test
*=Comparison of glomerular and tubular CKD patients; **Unknown Cause group was not included in the comparison due to lack of numbers

Table 3. Comparison of MOTS-C levels with the presence of anemia, hyperparathyroidism, hyperphosphatemia in Chronic Kidney Disease group

MOTSC (ng/ml)	Anemia		Hyperparathyroidism		Hyperphosphatemia	
	Yes	No	Yes	No	Yes	No
Mean±SD	54.8±9.2	63.7±10.9	57.6±10.3	64.2±11.3	56.9±10.8	63.3±10.8
Median	56.6	64.3	57.0	64.0	57.0	64.3
IQR	50.6-59.2	56.7-70.0	50.6-65	57.7-71.1	50.4-60.8	55.7-69.8
p	0.007* (t=2.8)		0.045* (t=2.05)		0.053 (t=1.9)	

SD: Standard Deviation; IQR: Interquartile Range (25^{th} – 75^{th} percentiles), *p<0.05 statistically significant, t= independent samples t test

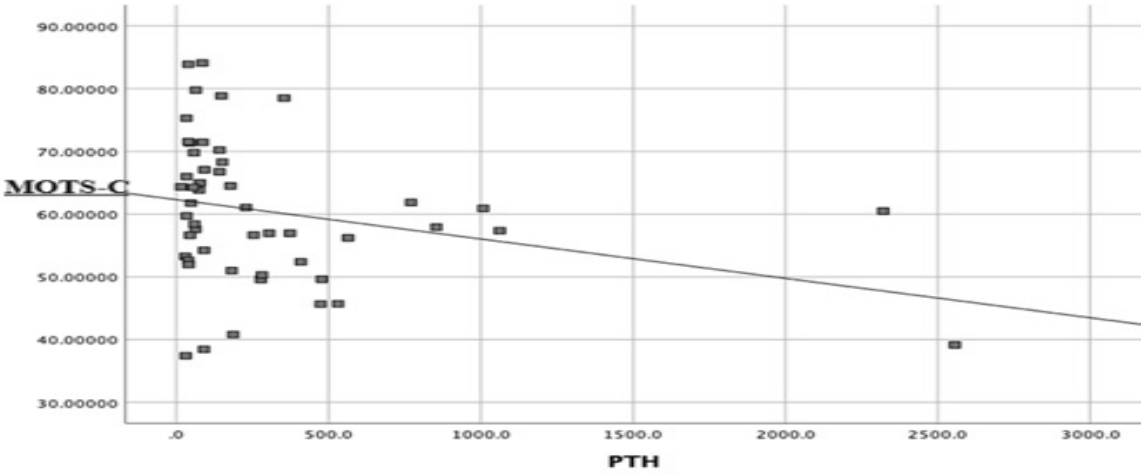

Figure 2. Negative correlation of MOTS-C with parathormone (PTH) levels in children with Chronic Kidney Disease (r:-0.311 *p*=0.036)

Discussion

In the present study, it was demonstrated that mitochondrial dysfunction is present in patients with childhood CKD, independent of staging, etiology, and the type of renal replacement therapy received. Furthermore, we established that anemia and secondary hyperparathyroidism also increase mitochondrial dysfunction. While only one study in the literature showed that mitochondrial-derived peptides are decreased in CKD patients in adulthood, this study is the first to show that MOTS-C, one of the mitochondrial-derived peptides, is decreased in children with CKD.

The MDPs, humanin and MOTS-c are involved in cell survival, apoptosis suppression and glucose metabolism; there is only one study in the literature on its role and levels in CKD [4]. In this study, MOTS-C levels were found to be low in serum and muscle, while humanin levels were found to be low in muscle and normal in serum.

The results of the study suggest that MDP levels are associated with evidence of systemic inflammation and oxidative stress in muscles, two hallmarks of premature aging and uremia [4]. In another study, muscle biopsies were performed in adult patients with CKD and it was found that the number of mitochondrial DNA decreased as the stage progressed [1]. In the present study, while MOTS-C levels were found to be significantly lower in paediatric patients with CKD compared to healthy children, no difference was observed between predialysis patients and patients receiving renal replacement therapy. Of particular interest was the observation that MOTS-C levels in patients with normal GFR post-renal transplantation, regarded as the optimal renal replacement therapy, were also significantly lower than those in the control group (*p*=0.002). This finding suggests that mitochondrial inflammation may persist in these patients, even in cases where GFR improves following renal transplantation. Regarding the etiology of CKD, MOTS-C levels were similar between the groups in terms of glomerular and tubular pathologies. In addition, the apparent decrease in MOTS-C as the grade 4-5 progressed in children with CKD was not statistically significant. These findings suggested the presence of mitochondrial inflammation in children with CKD from early stages and independent of etiology.

The mechanism by which high parathyroid hormone levels are a risk factor for cardiovascular diseases has been suggested to be that they cause oxidative damage by causing endothelial damage [12]. Two studies in the literature have demonstrated that oxidative stress markers are elevated in patients with hyperparathyroidism, and serum levels of these markers decrease following parathyroidectomy [13, 14]. In our study, we observed that MOTS-C levels were lower in CKD patients with hyperparathyroidism in comparison to those without hyperparathyroidism, thus indicating that the presence of hyperparathyroidism, in addition to CKD, is a contributing factor to mitochondrial dysfunction.

Hypertension is known to increase ROS production, leading to endothelial dysfunction and mitochondrial dysfunction. In hypertension, an excess of ROS generation cannot be counterbalanced by endogenous mitochondrial protective antioxidant mechanisms, leading to an increased state of mitochondrial oxidative stress [15]. While it is known that high blood pressure causes oxidative damage, our study did not show that high blood pressure also causes damage to the mitochondria in patients with CKD.

In a study investigating the relationship between anemia and oxidative damage, it was shown that both oxidative stress and DNA damage were increased in patients with iron deficiency anemia. It was interpreted that increased oxidative stress is an important factor causing DNA damage in patients with iron deficiency anemia [16]. In a study evaluating oxidative damage and renal function in children with iron deficiency anemia, markers of renal damage and oxidative damage, such as urinary microalbumin, were found to be high in children with iron deficiency anemia. At the end of the study, they suggested that oxidative damage contributed to the pathogenesis of renal function in these patients [17]. In the present study, MOTS-C levels were found to be significantly lower in CKD patients with anaemia in comparison to children without anaemia. This finding indicates that the presence of anemia contributes to the exacerbation of mitochondrial dysfunction in patients with CKD.

One of the causes of chronic inflammation and oxidative damage in CKD is metabolic acidosis [18]. Metabolic acidosis was found in 43% of our patients and MOTS-C levels in these patients were similar to those in patients without acidosis.

It is known that the presence of mitochondrial dysfunction in CKD and concomitant pathologies such as uremia, metabolic acidosis, hypertension and anemia increases dysfunction and that mitochondrial dysfunction contributes to CKD progression. In our study, which is the first of its kind in childhood, we have shown that mitochondrial damage begins even in the early stages in children with CKD, renal osteodystrophy and anemia contribute to this condition, and mitochondrial inflammation continues in these patients even after kidney transplantation.

The single-centre, modest sample size and cross-sectional nature of our study are limitations.

MOTS-c has recently attracted attention as a potential prevention or therapeutic option for obesity and T2DM [19]. We believe that our small study on MOTS-c, a mitochondrial polypeptide, in childhood CKD patients will shed light on similar studies on a larger scale. In addition, our study may pave the way for studies in which mitochondrial peptides can be used in therapeutic treatment to prevent mitochondrial dysfunction, which plays a role in the progression from acute kidney injury to chronic kidney injury.

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Authors contributions: I.G. has constructed the main idea and hypothesis of the study. She developed the theory and edited the material and method section. E.A. has done the evaluation of the data in the results section. Discussion section of the article was written by I.G. and E.A. and they reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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Research Article

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Is the CONUT score a prognostic index in multiple myeloma?

CONUT skoru multipl myelomda prognostik bir gösterge midir?

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Abstract

Purpose: We aimed to evaluate the impact of the Controlling Nutritional Status (CONUT) score on prognosis in patients with multiple myeloma (MM).

Materials and methods: Our study was designed retrospectively. We calculated the CONUT score based on serum albumin, total cholesterol and lymphocytes. The study included 213 patients; 99 (46.5%) were female and 114 (53.5%) were male. The median follow-up period was 38 months (1-161).

Results: The median age was 64 years. We participated the patients into four groups. It was defined as CONUT scores: normal (0-1), low (2-4), moderate (5-8), and high (9-12). We found significant differences between overall survival (OS) and progression-free survival (PFS) with regard to CONUT score, respectively, as high (OS:12, PFS:1 months), moderate (OS:27, PFS:13 months) and low (OS:54, PFS:28 months) (p<0.001 and p=0.001). In the multivariate analysis for OS, having moderate CONUT score (HR: 2.21, p=0.005) and high CONUT score (HR: 2.38, p=0.033) were increased the risk of mortality. In the multivariate analysis for PFS, compared to a normal CONUT score, a moderate CONUT score (HR: 1.85, p=0.007), and a high CONUT score (HR: 2.01, p=0.043) were found to increase the risk of progression.

Conclusion: We found that a high CONUT score is related to decreased OS and PFS. In our study, we showed that the CONUT score is an independent, useful and strong prognostic index in MM.

Keywords: CONUT score, multiple myeloma, survival, prognosis.

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Öz

Amaç: Multiple Myelom (MM) hastalarında CONUT skorunun prognoza etkisini değerlendirmeyi amaçladık.

Gereç ve yöntem: Çalışmamız retrospektif bir çalışma olarak tasarlandı. CONUT skorunu serum albumin, total kolesterol ve lenfosit değerlerine göre hesapladık. Bu çalışmaya 99'u (%46,5) kadın, 114'ü (%53,5) erkek olmak üzere 213 hasta dahil edildi. Median takip süresi 38 ay idi (1-161).

Bulgular: Median yaş 64 idi. Hastaları CONUT skoruna göre dört gruba ayırdık: normal (0-1), düşük (2-4), orta (5-8) ve yüksek (9-12). CONUT skoruna göre genel sağkalım (OS) ve progresyonsuz sağkalım (PFS) arasında sırasıyla yüksek (OS:12, PFS:1 ay), orta (OS:27, PFS:13 ay) ve düşük (OS:54, PFS:28 ay) anlamlı farklar bulduk (p<0,001 ve p=0,001). OS için yapılan çok değişkenli analizde CONUT skorunun orta düzeyde olması 2,21 kat (HR:2,21, p=0,005), CONUT skorunun yüksek olması 2,38 kat (HR:2,38, p=0,033) mortalite riskini arttırıyordu. PFS için yapılan çok değişkenli analizde normal CONUT düzeyiyle karşılaştırıldığında orta derecede CONUT skorunun (HR:1,85, p=0,007), yüksek CONUT skorunun (HR:2,01, p=0,043) ilerleme riskini arttırdığı belirlendi.

Sonuç: Yüksek CONUT skorunun OS ve PFS'de azalma ile ilişkili olduğunu belirledik. Çalışmamızda CONUT skorunun MM'da bağımsız, güçlü bir prognostik indeks olduğunu gösterdik.

Anahtar kelimeler: CONUT skor, multipl myelom, sağkalım, prognoz.

Coşkun EN, Akgün Çağlıyan G. CONUT skoru multipl myelomda prognostik bir gösterge midir? Pam Tıp Derg 2025;18:628-636.

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Introduction

Multiple myeloma (MM) is a disease caused by the uncontrolled proliferation of plasma cells that secrete monoclonal antibodies [1]. Multiple myeloma is a disorder that accounts for 1% of cancers and 10% of hematologic malignancies [2]. The incidence of MM is increased with age; it is more common in men [3]. Over the years, numerous staging systems have been developed to estimate prognosis in MM. Although the International Staging System (ISS) is the most widely accepted, it has been updated to the Revised ISS (R-ISS) to include lactate dehydrogenase (LDH) levels and cytogenetic characteristics. According to R-ISS, a stage is defined as stage I, II and III. When the stage increases, OS and PFS will decrease. R-ISS is a system that is more predictive of treatment in newly diagnosed transplant-eligible myeloma patients, but it only works with short-term studies that include patients under 65 years of age. For this reason, there are geriatric system-based care needs such as age, performance status and comorbidities. MM is a plasma cell dyscrasia with clinical findings and features of multiple organ involvement. Firstly, patients with MM apply to non-hematological medical departments such as nephrology, physical therapy, and neurosurgery. Pathological fractures due to osteolytic lesions are the most common symptoms. Particularly painful vertebral fractures and radicular back and waist pain are caused. Other clinical findings include anemia, infections, osteolytic lesions, neuropathy and renal involvement. Pneumonia, urinary system diseases and sepsis can be observed. Renal involvement is related to hyperuricemia, hypercalcemia, infections and tubulopathy. Fatigue, constipation, nausea and confusion are clinical findings due to hypercalcemia. Due to hyperviscosity syndrome, thrombosis and bleeding are rare. However, it is an important clinical condition needing plasmapheresis. Systemic therapy is usually inevitable at the time of diagnosis of MM. The decision of ASCT is still the most important parameter in therapy of MM. Initial therapy should be determined according to patients' eligibility for ASCT. Patients who are eligible for ASCT are <65-70 years old, have few comorbidities and fit. Despite the emergence of new-generation therapeutic agents, MM remains an incurable malignancy [4-6].

Malnutrition has occured as a widespread problem in patients with cancer. It has been attempted to be defined by components such as inadequate nutrition, weight loss, immobility, and sarcopenia. A more objective method is Controlling Nutritional Status (CONUT score), which is calculated according to serum albumin, total cholesterol levels and lymphocyte values. It has recently gained much attention and provides valuable insights into the immuno-nutritional status [7, 8]. The CONUT score has been demonstrated in various cancer types and in cardiovascular diseases by correlating nutritional and immune status with disease severity and adverse clinical outcomes [8-11]. Thus, elucidating the effect of the CONUT score in hematological malignancies has gained impetus in recent years. In our study, we examined the prognostic significance of the CONUT score in patients diagnosed with MM.

Materials and methods

Patients

The study included 213 patients newly diagnosed with MM who presented to the hematology clinic between 2008 and 2023. It was a retrospective cross-sectional study. The study protocol was approved by the Pamukkale University Faculty of Medicine Ethics Committee (date: 05.09.2023, issue: 60116787-020-415620). Due to the retrospective design, no interventions or procedures were performed on the patients. Patients with unavailable clinical or laboratory data at diagnosis and those receiving lipid-lowering therapy were excluded. Data collected at diagnosis included immunoglobulin subtypes, R-ISS stages, CONUT score, OS, and PFS. The R-ISS was evaluated based on ISS and cytogenetic characteristics.

The patients had received chemotherapy as bortezomib, thalidomide, lenalidomide, daratumumab, carfilzomib, ixazomib, and pomalidomide. The first therapy was bortezomib ± cyclophosphamide and steroid due to the payment order in our country. Patients were categorized based on ASCT status as having undergone one, two, or no transplants. Some patients received immunomodulatory, proteasome inhibitor, and monoclonal antibody treatments at an earlier stage, which was related to availability and drug payment instructions at

different periods in our country. Some patients had received treatments earlier with off-label approval.

CONUT score

The CONUT score is a method that provides insight into the nutritional status and is calculated with points as follows: Serum albumin: ≥3.5 g/dL, 3.0-3.49 g/dL, 2.5-2.99 g/dL, <2.5 g/dL (0, 2, 4 ,6 points). Lymphocyte count: ≥1600/mm^3, 1200-1599/mm^3, 800-1199/mm^3, <800/mm^3 (0, 1, 2, 3 points). Total cholesterol: ≥180 mg/dL, 140-179 mg/dL, 100-139 mg/dL, <100 mg/dL (0, 1, 2, 3 points) respectively. The sum of the scores categorizes nutritional status as follows: Score 0-1: Normal; 2-4: Low; 5-8: Moderate; and 9-12: High malnutrition.

Statistical analysis

We analyzed data using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA). We presented descriptive statistics as counts and percentages for categorical variables and as means and medians (with minimum and maximum). We defined OS as the time from diagnosis to the last follow-up or death. We defined PFS as the time from diagnosis to the last follow-up, disease progression, relapse, or death. We conducted comparisons of OS and PFS using the Kaplan-Meier method. Finally, we performed multivariate Cox regression analyses to evaluate the influence of various clinical variables on mortality and progression risk. We considered a *p*-value of <0.05 statistically significant.

Results

Patient characteristics

We analyzed 213 patients with MM, comprising 114 men (53.5%) and 99 women (46.5%). The median age was 64 years (min: 40-max: 89). According to the R-ISS, 29 (13.6%) were classified as Stage I, 58 (27.2%) as Stage II, and 126 (59.2%) as Stage III in all patients. In terms of immunoglobulin subtypes, 76 patients (35.7%) had immunoglobulin (Ig) G kappa, 49 patients (23.0%) had Ig G lambda, 17 patients (8.0%) had Ig A kappa, 19 patients (8.9%) had Ig A lambda, 31 patients (14.6%) had kappa light chain, and 21 patients (9.9%) had lambda light chain. Renal dysfunction was present in 131 patients (61.5%). Regarding autologous stem cell transplantation (ASCT), 90 patients (42.3%) did not undergo ASCT, 110 patients (51.6%) underwent one ASCT, and 13 patients (6.1%) underwent ASCT twice. Based on the CONUT score, 55 (25.8%) had a normal score, 91 (42.7%) had a low score, 54 (25.4%) had a moderate score, and 13 patients (6.1%) had a high score. The median follow-up duration was 38.0 months (1-161). The median OS was 50 months (5-year OS, 41.6%; 95% CI, 40.38%-59.61%) (Figure 1a and 1b). The median PFS was 22 months (5-year PFS, 18.6%; 95% CI, 17.17%-26.82%) (Figure 2a and 2b). Demographic characteristics and laboratory data, OS and PFS are summarized in Tables 1a-1d.

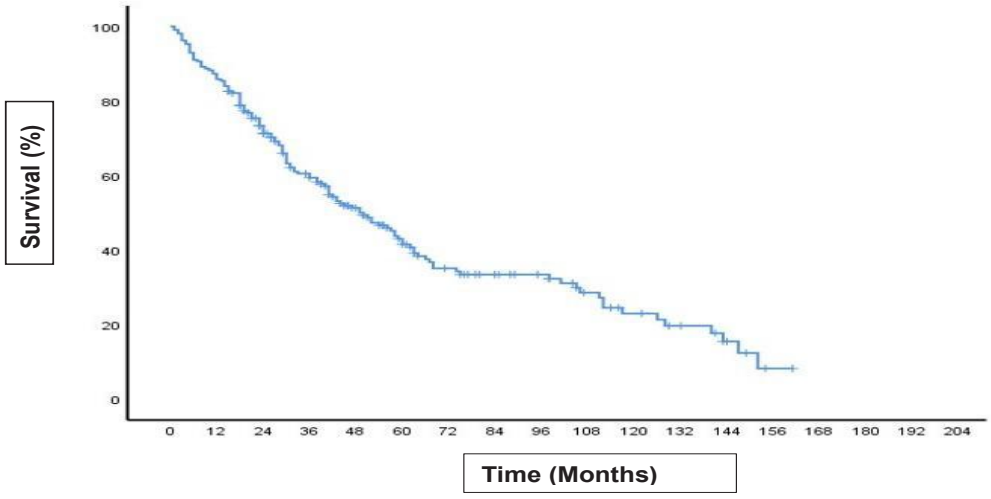

Figure 1a. Survival (%); Time (months)

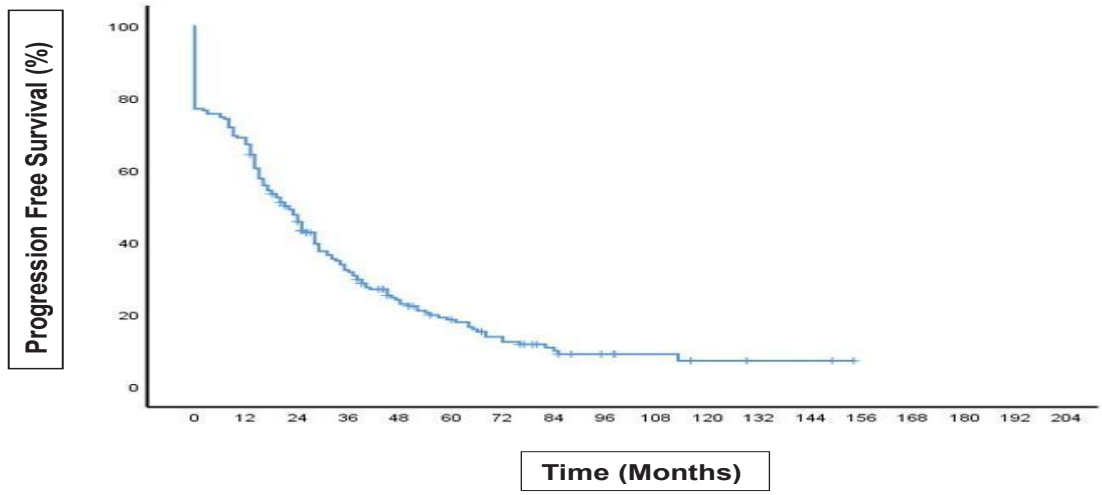

Figure 1b. PFS (%); Time (months)

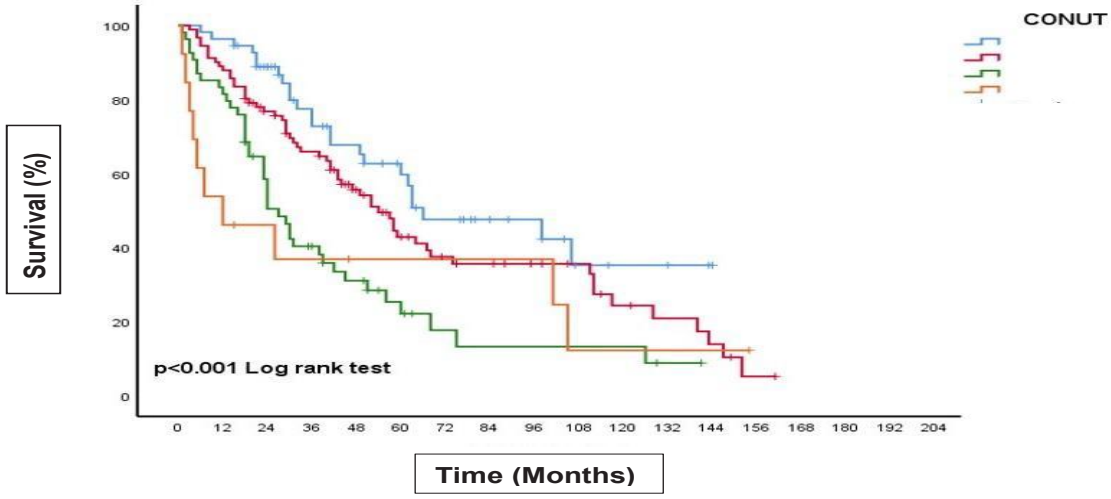

Figure 2a. Survival (%); Time (months); CONUT (normal, low, moderate, high)

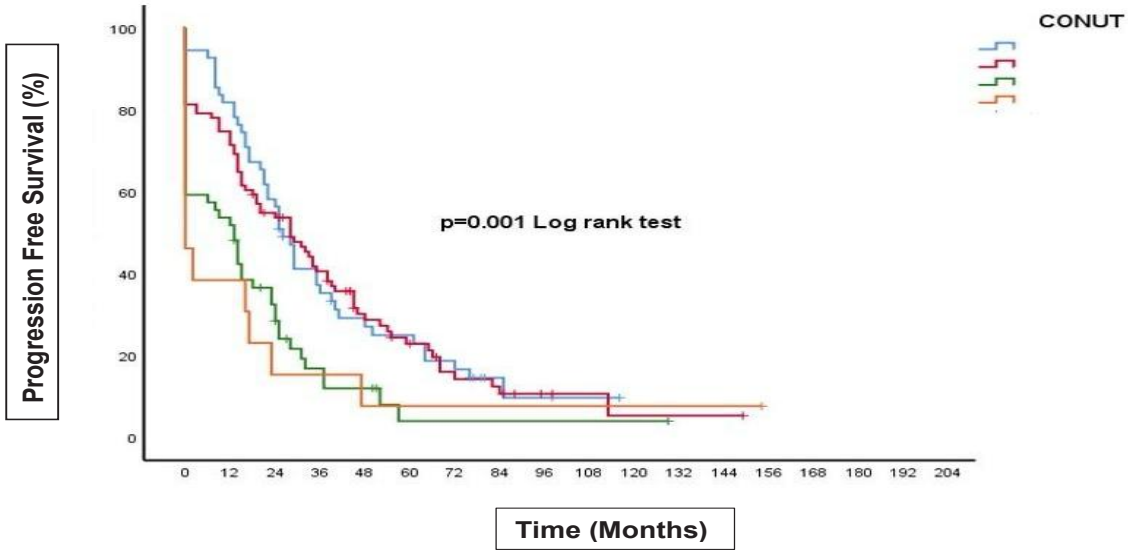

Figure 2b. PFS (%); Time (months); CONUT (normal, low, moderate, high)

Table 1a. Patient characteristics

	Median	Min-Max
Age (years)	64.0	40-89
WBC (K/uL)	6270.0	1870.0-14220.0
Lymphocytes (K/uL)	1650.0	220.0-7600.0
Hb (g/dL)	9.8	(5.5-15.8)
Albumin (g/dL)	3.47	(1.8-4.8)
LDH (U/L)	178.0	58.0-1105.0
Total cholesterol (mg/dL)	169.0	39.0-2831.0
ß2M (mg/L)	6.5	1.9-29.5

* WBC: white cell count, Hb: Hemoglobin, LDH: lactate dehydrogenase, ß2M: β2-microglobulin

Table 1b. Patient characteristics and laboratory findings

Gender	Male, 114; Female, 99
Renal dysfunction	Yes, 131; No, 82
Ig subtype	IgG Kappa, 76; IgG Lambda, 49; IgA Kappa, 17; IgA Lambda, 19; Kappa, 31; Lambda 21
R-ISS	I, 29; II, 58; III, 126
ASCT	None, 90; Once, 110; Twice, 13
CONUT	Normal, 55; Low, 91; Moderate, 54; High, 13

*R-ISS: Revised International Staging System, ASCT: Autologous stem cell transplant, CONUT: Controlling Nutritional Status

Table 1c. OS comparison according to LDH and Hemoglobin levels

Variable	2 years %	5 years %	Median (%95 CI)	*p*
Hemoglobin (gr/dl)				
<8.5	65.3	28.7	33.00 (21.78-44.21)	0.044*
>8.5	73.1	45.6	57.00 (42.71-71.28)	
Lactate dehydrogenase (U/L)				
<220	72.8	42.9	56.00 (47.36-64.63)	0.048*
≥220	67.4	36.6	30.00 (27.05-32.95)	

Kaplan Meier curve, Long rank test, *p<0.05 statistically significant

Table 1d. PFS comparison according to LDH and Hemoglobin levels

Variable	2 years %	5 years %	Median (%95 CI)	*p*
Hemoglobin (gr/dl)				
<8.5	31.3	9.0	14.00 (11.68-16.33)	0.018*
>8.5	50.3	21.7	25.00 (20.10-29.90)	
Lactate dehydrogenase (U/L)				
<220	49.2	22.2	24.00 (18.70-29.26)	0.035*
≥220	36.2	8.5	18.00 (13.96-22.03)	

Kaplan Meier curve, Long rank test, *p<0.05 statistically significant

Univariate and multivariate analysis of OS and PFS

In the univariate analysis of OS, age, renal dysfunction, R-ISS stage, ASCT status, and CONUT score (Figure 1b and Figure 2b) were found to be significant (Table 2). Variables that were significant in the univariate analyses were included in the multivariate Cox regression model. According to the model results, having a moderate CONUT score increased the risk of death by 2.21-fold (HR: 2.21, 95% CI: 1.27-3.84, *p*=0.005), while having high CONUT score increased the risk by 2.38-fold (HR: 2.38, 95% CI: 1.07-5.31, *p*=0.033). Additionally, undergoing ASCT once (HR: 0.37, 95% CI: 0.23-0.60, *p*<0.001) and twice (HR: 0.34, 95% CI: 0.15-0.78, *p*=0.012) was related to reduced risk of mortality (Table 2).

Table 2. Univariate and multivariate analysis of OS

		Univariate analysis				Multivariate analysis		
Variables		**n (%)**	**Median**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Age	**≤65**	121 (56.8%)	60.0	48.43-71.56	<0.001*	Ref.		
	>65	92 (43.2%)	31.0	20.73-41.26		0.95	0.63-1.45	0.838
Renal Dysfunction	**No**	82 (38.5%)	74.0	34.63-113.36	<0.001*	Ref.		
	Yes	131 (61.5%)	33.0	34.9-41.09		1.29	0.78-2.14	0.308
R-ISS Stage	**I**	29 (13.6%)	98.0	26,37-169,62		Ref.		
	II	58 (27.2%)	63.0	50.89-75.10	0.002*	1.08	0.52-2.24	0.824
	III	126 (59.2%)	31.0	21.43-40.56		1.21	0.56-2.52	0.613
ASCT	**None**	90 (42.3%)	23.0	18.52-27.48		Ref.		
	Once	110 (51.6%)	68.0	29.48-106.51	<0.001*	0.37	0.23-0.60	<0.001*
	Twice	13 (6.1%)	74.0	49.06-98.93		0.34	0.15-.078	0.012*
CONUT	**Normal**	55 (25.8%)	66.0	26.4-105.59		Ref.		
	Low	91 (42.7%)	54.0	43.03-64.96	<0.001*	1.22	0.71-2.09	0.456
	Moderate	54 (25.4%)	27.0	21.08-32.91		2.21	1.27-3.84	0.005*
	High	13 (6.1%)	12.0	0.0-35.11		2.38	1.07-5.31	0.033*

R-ISS: Revised International Staging System, ASCT: Autologous stem cell transplant, CONUT: Controlling Nutritional Status
Kaplan Meier curve, Long rank test, cox regression, *p<0.05 statistically significant

In the univariate analysis of PFS, age, renal dysfunction, R-ISS stage, ASCT status, and CONUT score were identified as significant factors (Table 3). Variables that were significant in the univariate analyses were included in the multivariate Cox regression model. The model results indicated that having a moderate CONUT score increased the risk of progression by 1.85-fold (HR: 1.85, 95% CI: 1.18-2.89, *p*=0.007), while a high CONUT score increased the risk by 2.01-fold (HR: 2.01, 95% CI: 1.02-3.96, *p*=0.043). Moreover, undergoing ASCT once (HR: 0.41, 95% CI: 0.26-0.61, *p*<0.001) and twice (HR: 0.51, 95% CI: 0.25-0.99, *p*=0.048) was associated with a reduced risk of progression (Table 3).

Table 3. Univariate and multivariate analysis of PFS

		Univariate analysis			Multivariate analysis		
Variables		**Median**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Age	**≤65**	28.0	22.17-33.82	0.001*	Ref.		
	>65	13.0	7.99-18.00		1.05	0.71-1.53	0.796
Renal Dysfunction	**No**	34.0	26.72-41.27	<0.001*	Ref.		
	Yes	15.0	12.53-17.46		1.53	1.00-2.34	0.047*
R-ISS Stage	**I**	26.0	14.18-37.82		Ref.		
	II	29.0	19.79-38.20	0.030*	0.65	0.38-1.11	0.119
	III	15.0	11.12-18.87		0.63	0.35-1.15	0.135
ASCT	**None**	1.00	-		Ref.		
	Once	32.00	25.43-38.56	<0.001*	0.41	0.26-0.61	<0.001*
	Twice	35.00	16.21-53.78		0.51	0.25-0.99	0.048*
CONUT	**Normal**	26.0	21.64-30.35		Ref.		
	Low	28.0	16.77-39.22	0.001*	0.94	0.63-1.39	0.764
	Moderate	13.0	6.93-19.06		1.85	1.18-2.89	0.007*
	High	1.0	-		2.01	1.02-3.96	0.043*

* R-ISS: Revised International Staging System, ASCT: Autologous stem cell transplant, CONUT: Controlling Nutritional Status
Kaplan Meier curve, Long rank test, cox regression, *p<0.05 statistically significant

Discussion

The CONUT score is a recent immunonutritional marker used to designate patients with malnutrition [12]. It is expressed that the CONUT score is successful in predicting poor prognosis and postoperative complications in cancer. These parameters that constitute the CONUT score are routinely measured during blood collection in daily clinical practice. The CONUT score is related to progression and mortality in patients with cancer. We studied the relationship of CONUT score and survival in patients with MM. We found that patients with high CONUT scores had reduced OS and PFS; we showed that a high CONUT score is an independent and robust prognostic index in patients with MM in our study.

The prognosis of MM, like that of other cancers, is related to some factors in the way that patient characteristics, stage of disease, cytogenetic features, and response to treatment [13]. Malnutrition is a common issue among cancer patients. It contributes not only to physical and functional impairment but also to a poorer response to therapy. The CONUT score provides valuable insight into the nutritional and immunological status of patients. Using these parameters, patients are assigned scores and categorized accordingly. The utility of the CONUT score in nutritional status, determining severity, and predicting adverse clinical outcomes has been demonstrated [7]. Furthermore, the prognostic significance of the CONUT score is known in solid organ cancers, cardiovascular diseases, and renal diseases [9, 10].

Thus, elucidating the impact of the CONUT score in hematological malignancies has recently gained impetus. Nagata et al. [14] retrospectively evaluated 476 cases diagnosed with diffuse large cell B-cell lymphoma (DLCBL), and the patient group with a CONUT score ≥4 had lower OS and PFS. Akgün Çağlıyan et al. [15] reported that a high CONUT score (≥2) pointed to lower OS and PFS in 266 patients with DLCBL. Ureshino et al. [16] noted that a low CONUT score in adult T-cell leukemia was related to better survival and may predict a favorable prognosis for transplantation. Senjo et al. [17] evaluated 174 patients with acute myeloid leukemia by omitting the lymphocyte count parameter to adapt the CONUT score and showed that the simplified CONUT score was

successful in predicting prognosis. Okamato et al. [18] evaluated the CONUT score in 64 MM patients and found that, particularly among younger patients eligible for transplantation. They expressed that the CONUT score was a prognostic index with patients having a high CONUT score (>4) showing a shorter median OS. Furthermore, Zhou et al. [19] retrospectively assessed 245 MM patients, stratifying them into three groups. They found that 5-year OS was 65.1% with a low CONUT score (≤3), 38.9% with a moderate CONUT score (4-9), and 16.6% with a high CONUT score (>9). Results confirmed that a high CONUT score was an independent risk factor for OS.

In our study, we examined 213 patients with MM in four groups according to the CONUT score: normal, low, medium and high. In our study, the median hemoglobin value was found to be 9.8 (5.5-15.8) g/dL. We performed the analysis according to hemoglobin level; median OS was 57.00 (min: 42.71-max: 71.28) months in those with hemoglobin>8.5 g/dL, and median OS was found 33.00 (min: 21.78-max: 44.21) months in those with hemoglobin <8.5. Our study has once again shown that the level of anemia is an indicator of prognosis and survival at the time of diagnosis. We performed according to LDH level; median OS was 30.00 (min: 27.05-max: 32.95) months in those with LDH ≥220, and median OS was 56.00 (min: 47.36-max: 64.63) months in those with LDH <220. We detected that the median PFS in patients with LDH ≥220 was 18.00 (min: 13.96-max: 22.03) months, and with LDH<220, it was 24.00 (min: 18.70-max: 29.26) months. In our study, we detected the median OS in the patient group with R-ISS stage I was 98 (min: 26.37-max: 269.62) months, with stage II the median OS was 63 (min: 50.89-max: 75.10) months, and with stage III the median OS was 31 (min: 21.43-max: 40.56) months, consistent with the literature. In the survival analyses, we detected median OS with a normal CONUT score was 66.00 (min: 26.40-max: 105.59) months, median OS with a low CONUT score was 54.00 (min: 43.03-max: 64.96) months, median OS with a moderate CONUT score was 27.00 (min: 21.08-max: 32.91) months, and median OS with a high CONUT score was 12.00 (min: 0.00-max: 35.11) months. We observed that a high CONUT score was related to decreasing OS. We found that the risk of mortality with a moderate CONUT score increased by 2.21 fold and with a high CONUT score increased by 2.38 fold. We found that 5-year OS was 41.6% and 5-year PFS was 18.6% in our study with 213 MM patients. The results indicated that having a moderate CONUT score increased the risk of progression by 1.85-fold, while a high CONUT score increased the risk by 2.01-fold. We detected a negative correlation between the CONUT score and both OS and PFS. Moreover, undergoing ASCT was associated with a reduced risk of progression. We showed that ASCT remains a beneficial therapy in patients with MM. Furthermore, significant differences were noted between high CONUT scores and factors in the way that age, renal dysfunction, and R-ISS stage. We found a difference between ≤65 and >65 years old in terms of OS and PFS. We showed that if the patients had renal dysfunction and advanced stage, they had reduced OS and PFS and a high CONUT score. Overall, our results noted that the CONUT score is a strong index of poor prognosis in MM.

The CONUT score is an easy method to calculate; it can be implemented during routine blood collection in MM patients at diagnosis. A high CONUT score is related to reducing survival. We proved that the CONUT score is an independent, useful, and poor prognostic index in MM. However, we hope for prospective studies with larger patient groups to further validate the long-term reliability and validity of the CONUT score.

Limitation

We designed our study as a retrospective and single centre. There was no record about calorie and diet uptake. We did not evaluate body mass index at during the diagnosis. There were some differences between the types of therapies. Some patients were applied therapies at an earlier line. This condition was related to accessibility and drug payment instructions and off-label approval at different periods.

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Research Article

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Demographic and microbiological characteristics and mortality status of patients diagnosed with tuberculosis and treated between 2018 and 2023

2018 ve 2023 yılları arasında tüberküloz tanısı ile tedavi uygulanan hastaların demografik ve mikrobiyolojik özellikleri ve mortalite durumları

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Abstract

Purpose: We aimed to determine the demographic data, radiological findings, comorbid conditions, antituberculosis drug resistance rates, and tuberculosis-related mortality of patients with pulmonary and pleural tuberculosis where mycobacterium tuberculosis growth was detected in culture.

Materials and methods: Data from 439 patients diagnosed with pulmonary and pleural tuberculosis in the chest diseases hospital clinic between January 2018 and December 2023 were retrospectively evaluated. Patients whose sputum, bronchial lavage and pleural fluid samples were positive for Mycobacterium tuberculosis complex in BACTEC 460 TB system medium were included in the study. We recorded the patients' demographic data, nationality, concomitant diseases, chest X-ray findings, drug resistance, and mortality status. We recorded the single, double, triple, and quadruple drug resistance rates against four main antituberculosis drugs using the Mycobacterium tuberculosis complex susceptibility tests.

Results: We tested sputum from 338 patients, bronchial lavage from 87 patients, and pleural fluid culture from 14 patients. EZN staining showed that acid-resistant bacillus was present in 224 (51%). Comorbidities were Chronic Obstructive Pulmonary Disease (36.4%), hypertension (26.4%), and bronchiectasis (18.7%). The evaluation of the patients' chest x-rays revealed that 45.1% had infiltration, 33.3% had cavitation and infiltration, and 7.1% had cavitation. Exitus occured in 7.7% of the patients due to tuberculosis. Drug resistances were isoniazid 47 (10.7%), rifampin 15 (3.4%), ethambutol 4 (0.9%), streptomycin 37 (8.4%), and multidrug-resistant 15 (14 + RR (1)) (3.4%). We detected single drug resistance in 44 patients, double drug resistance in 18 patients, triple drug resistance in 2 patients, and quadruple drug resistance in 4 patients.

Conclusion: Tuberculosis incidence and mortality are decreasing; it is still an important public health problem. It can be mortal, especially in patients with advanced age and comorbidities.

Keywords: Tuberculosis, drug resistance, comorbidities.

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Öz

Amaç: Mycobacterium tuberculosis üremesi halinde saptanan akciğer ve plevra tüberkülozlu olguların demografik verileri, radyolojik bulguları, komorbid hastalıkları, antituberküloz ilaç direnç oranları ve tüberküloza bağlı mortalite oranlarının belirlenmesi amaçlandı.

Gereç ve yöntem: Ocak 2018 ile Aralık 2023 arasında göğüs hastalıkları hastanesi kliniğinde akciğer ve plevra tüberkülozu tanısı konulan 439 hastanın verileri retrospektif olarak değerlendirildi. Balgam, bronş lavaj ve plevral sıvıdan alınan örneklerde, BACTEC 460 TB system besi yerinde mycobacterium tuberculosis kompleks üremesi saptanan hastalar çalışmaya alındı. Hastaların demografik verileri, uyruğu, eşlik eden hastalıkları, göğüs röntgen bulguları, ilaç direnci ve mortalite durumu kaydedildi. Mycobacterium tuberculosis kompleks duyarlılık testlerinde dört ana antitüberküloz ilacına karşı tek, çift, üçlü ve dörtlü ilaç direnci oranları kaydedildi.

Bulgular: 338 hastada balgam, 87 hastada bronş lavajı, 14 hastadan plevra sıvısı kültürü gönderilmiş ve 224 hastada (%51) EZN boyama ile aside dirençli basil tespit edilmiş. Eşlik eden hastalıklar Kronik Obstrüktif Akciğer Hastalığı (%36,4), hipertansiyon (%26,4) ve bronşektazi (%18,7) olarak tespit edilmiştir. Hastaların PA Akciğer grafileri değerlendirildiğinde %45,1'inde bilateral üst zonlarda infiltrasyon, %33,3'ünde kavitasyon ve infiltrasyon, %7,1'inde ise kavitasyon mevcuttu. Hastaların %7,7'sin de tüberküloz nedeni ile exitus geliştiği belirlendi. İlaç dirençleri izoniazid 47 (%10,7), rifampin 15 (%3,4), etambutol 4 (%0,9), streptomisin 37 (%8,4) ve çoklu ilaca dirençli 15 (14 + RR (1)) (%3,4) idi. 44 hastada tek ilaç direnci, 18 hastada çift ilaç direnci, 2 hastada üçlü ilaç direnci ve 4 hastada dörtlü ilaç direnci tespit edildi.

Sonuç: Tüberküloz insidansı ve mortalitesi azalmakla birlikte, hala önemli bir halk sağlığı sorunudur. Özellikle ileri yaş ve eşlik eden hastalıkların da hastalarda mortaliteyi artırdığı görülmüştür.

Anahtar kelimeler: Tüberküloz, ilaç direnci, eşlik eden hastalıklar.

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Introduction

Tuberculosis (TB) is an important public health problem caused by Mycobacterium tuberculosis, which causes mortality and morbidity in Türkiye as well as all over the world. According to the World Health Organization (WHO) data for 2022, it was determined that 10.6 million people were infected with tuberculosis bacilli, and 1.13 million people lost their lives due to tuberculosis disease. Similarly, in our country, it was determined that the incidence of TB has decreased significantly in the data collected from 2005 to the present. In 2020, the incidence was determined as 10.6 per hundred thousand [1, 2].

Tuberculosis, a treatable and preventable disease, typically affects the lungs but can also affect the other organs. The disease is more common in adults and men. In the diagnosis of pulmonary TB, the disease is suspected based on history, physical examination findings, and PA chest X-rays and can be diagnosed using bacteriological or histopathological methods. Risk factors and comorbidities are very important in the development of TB. There is an increased risk of developing tuberculosis in advanced age, HIV infection, immunosuppressive therapy, tumor necrosis factor alpha (TNF-α) antagonists, steroid use, close contact with infected individuals, chronic renal failure, dialysis, leukemia, lymphoma, head and neck malignancies, diabetes, cachexia, smoking, and alcohol abuse [3].

Drug resistance is still one of the important problems in tuberculosis treatment. Among people diagnosed with TB, it includes all patients with rifampin-resistant TB (RR-TB) and multidrug-resistant TB (MDR-TB), defined as resistance to both rifampin (R) and isoniazid (H) (MDR/RR-TB). WHO data indicates that the number of MDR patients has declined over time, reaching 410 thousand in 2020 (1). Türkiye is not among the 30 countries with a high disease burden in terms of MDR-TB. In the Global TB 2020 Report, the estimated number of MDR cases in Türkiye was 2.2% in new cases in 2020 and 8.6% in previously treated patients; the RD/MDR-TB rate was given as 2.4% in new cases and 9.7% in previously treated cases [2].

In this study, we aimed to determine the demographic data, radiological findings, comorbid conditions, antituberculosis drug resistance rates, and tuberculosis-related mortality of patients with pulmonary and pleural tuberculosis where mycobacterium tuberculosis growth was detected in culture between 2018 and 2023. We present our data to contribute national data about tuberculosis.

Materials and methods

Approval was obtained from the Samsun University Non-Interventional Clinical Research Ethics Committee for the study (approval date: 11/09/2024, approval number: 2024/16/3).

We retrospectively evaluated the data of 452 patients diagnosed with pulmonary and pleural tuberculosis at the chest diseases hospital clinic between January 2018 and December 2023. The study excluded 13 patients whose data were not accessible. Patients with *mycobacterium tuberculosis complex* growth in sputum, bronchial lavage and pleural fluid were included in the study, while patients with non-tuberculosis *mycobacterium tuberculosis* were excluded from the study.

We recorded the patients' demographic information, nationality, comorbid conditions, chest X-ray findings, drug resistance, and mortality status.

Two separate specialist physicians evaluated the chest X-ray findings. The chest X-ray findings of the patients were categorized as infiltration, cavitation and infiltration, cavitation, mass, and pleural fluid.

Samples of sputum, bronchial lavage, and pleural fluid from the patients were stained with the EZN method to look for acid-resistant bacillus. These were then grown in the BACTEC 460 TB system. In the Mycobacterium tuberculosis complex susceptibility tests, the rates of single, double, triple, and quadruple drug resistance to four major antituberculosis drugs (H, R, streptomycin (S), and ethambutol (E)) were investigated using the BACTEC 460 TB system recommended by the National Committee Clinic Laboratory Standards (NCCLS).

Patients who exitus while under tuberculosis treatment were considered tuberculosis-related deaths.

The SPSS 23.0 (SPSS Inc., Chicago, IL, USA) program was used for statistical evaluation. We calculated the frequencies and percentages of categorical variables, as well as the mean and standard deviation values of numerical variables. We applied the chi-square for categorical variables and the independent sample t-test for numerical data.

Results

We detected Mycobacterium tuberculosis growth in 439 patients with pulmonary tuberculosis, of whom 100 were female and 339 were male. The mean age of the patients was 52.7±18.4 (F: 50.1±21.9, M: 53.4±17.2). 5.2% (n=23) of the patients were foreign nationals. We tested sputum from 338 patients, bronchial lavage from 87 patients, and pleural fluid culture from 14 patients. EZN staining showed that acid-resistant bacillus was present in 224 (51%). 62.6% of the patients had comorbidities. The most common comorbidities were Chronic Obstructive Pulmonary Disease (COPD) (36.4%), hypertension (26.4%), and bronchiectasis (18.7%). Evaluation of the patients' chest x-rays revealed 45.1% with infiltration, 33.3% with cavitation and infiltration, and 7.1% with cavitation only. We determined that tuberculosis claimed the lives of 7.7% of the patients (Table 1). No statistically significant difference was found between foreign patients and the local population in terms of multidrug resistance, widespread drug resistance and tuberculosis-related mortality.

Table 1. Demographic characteristics

Age	
Female	52.7±18.4 50.1±21.9
Male	53.4±17.2
Gender	
Female n (%)	100 (22.8)
Male n (%)	339 (77.2)
Nationality	
Turkish citizen (%)	416 (94.8)
Foreign national (%)	23 (5.2)
Culture	
Sputum	338 (77)
Bronchial lavage	87 (19.8)
Pleural fluid	14 (3.2)
Comorbidity and risk factors n (%)	275 (62.6)
COPD	160 (36.4)
Hypertension	116 (26.4)
Bronchiectasis	82 (18.7)
İschemic heart disease	82 (18.7)
Diabetes	72 (16.4)
Malignite	24 (5.4)
History of chemotherapy	20 (4.6)
Chronic kidney failure	19 (4.3)
Cerebrovascular disease	12 (2.7)
Corticosteroid treatment history	12 (2.7)

Table 1. Demographic characteristics (continued)

Asthma	11 (2.5)
HIV infected	6 (1.4)
Chest deformity	6 (1.4)
DIAH	5 (1.1)
Dialysis	3 (0.7)
Previous history of TB	2 (0.5)
AntiTNF treatment history	2 (0.5)
Chest x-ray	
Infiltration	198 (45.1)
Cavitation and infilration	146 (33.3)
Cavitation	31 (7.1)
Mass	24 (4.8)
Pleural effusion	14 (3.2)
Missing data	29 (6.6)
Exitus from TB	34 (7.7)

COPD: Chronic obstructive pulmonary disease, DIAH: Diffuse interstitial lung disease, HIV: Human Immunodeficiency Virus
TNF: tumor necrosis factor, TB: Tuberculosis

When we looked at the patients who died during tuberculosis treatment, it was found that the ages and underlying diseases of the deceased patients were statistically higher and that deaths occurred in patients with COPD, DIAH, and cerebrovascular disease (Table 2). The comorbidity and drug resistance status of patients who exited are shown in Table 3.

Evaluation of the patients' culture results revealed that 68 (15.5%) patients were resistant to at least one tuberculosis drug. H was detected in 47 (10.7%), R in 15 (3.4%), E in 4 (0.9%), S in 37 (8.4%), and MDR in 15 (14 + RR (1)) (3.4%) (Figure 1). Single drug resistance was detected in 44 patients, double in 18 patients, triple in 2 patients, and quadruple drug resistance was detected in 4 patients (Table 4). Previous history of TB (n=1), bronchiectasis (n=1), malignancy (n=1), HIV infection (n=1), and diabetes (n=1) were detected as risk factors in 5 (35.7%) of the MDR patients. Antituberculosis resistance rates between 2018 and 2023 are given in Table 5.

Table 2. Mortality, age, gender, and comorbidity rates

	Exitus (n, %)	**Alive (n, %)**	**χ^2**	**t**	***p***
Age	69.8±12.2	48.4±17.5	-	-10.2	0.000*
Gender					
Male	28 (6.4)	307 (70.5)	0.59	-	0.441
Female	6 (1.3)	94 (21.6)			
Comorbidity and risk factors n (%)	31 (91.2)	243 (60.6)	36.57	-	0.000*

*= $p<0.05$, Independent samples t test was conducted on independent groups,
The Pearson Chi-Square test was used for the categorical variables, χ^2= Chi-square test and t = Independent samples t-test

Table 3. Comorbidity and drug resistance status in patients with exitus

Co-morbidity n (%)	34
COPD	23 (67.6%)
Bronchiectasis	9 (26.5%)
Hypertension	6 (17.6%)
İschemic heart disease	6 (17.6%)
Diabetes	6 (17.6%)
Cerebrovascular disease	4 (11.8%)
Chronic kidney failure	3 (8.8%)
DIAH	3 (8.8%)
Malignite	2 (5.9%)
Chest deformity	2 (5.9%)
History of chemotherapy	1 (1.9%)
Corticosteroid treatment history	1 (2.9%)
Dialysis	1 (2.9%)
AntiTNF treatment history	1 (2.9%)
Drug resistance n (%)	
Single drug resistance	
H	2 (%5.9)
S	1 (2.9)
Double drug resistance	
HS	2 (%5.9)
Quadruple drugs resistance	
HRSE	1 (%2.9)

H: isoniazid, R: rifampicin, E: ethambutol, S; streptomycin, MDR: multidrug resistance, COPD: Chronic obstructive pulmonary disease DIAH: Diffuse interstitial lung disease, TNF: tumor necrosis factor

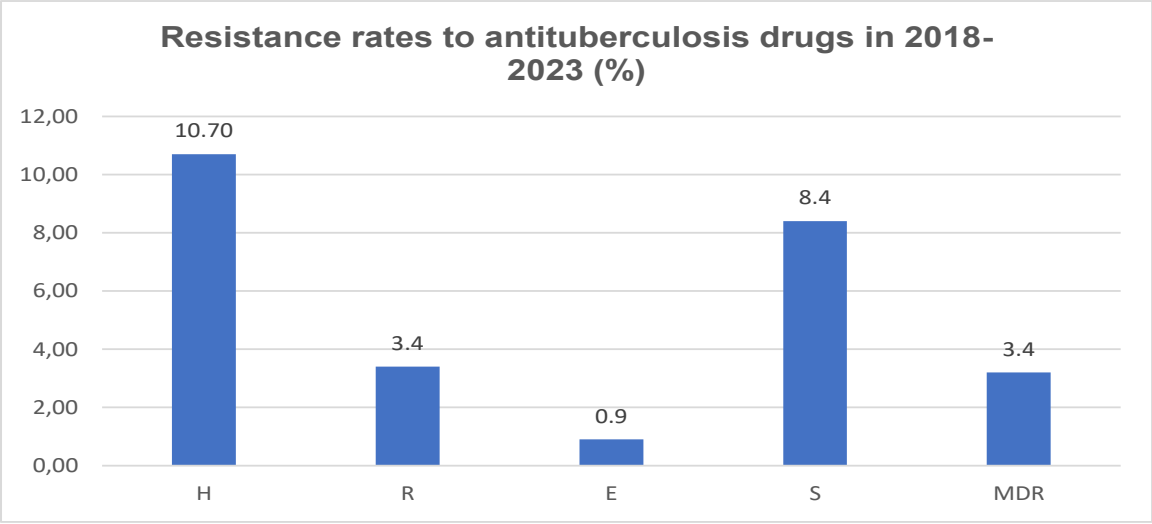

Figure 1. Resistance rates to antituberculosis drugs in 2018-2023 (%)

H: isoniazid, R: rifampicin, E: ethambutol, S; streptomycin, MDR: multidrug resistance, The BACTEC 460 TB system recommended by the National Committee for Clinical Laboratory Standards (NCCLS) was used in Mycobacterium tuberculosis complex susceptibility testing

Table 4. Drug resistance rates

	n (%)
Single drug resistance	44 (9.9)
H	23 (5.2)
R	1 (0.2)
E	0
S	20 (4.5)
Double drug resistance	18 (4.1)
HR	8 (1.8)
HS	10 (2.3)
Triple drugs resistance	
HRS	2 (0.5)
Quadruple drugs resistance	
HRES	4 (0.9)

H: isoniazid, R: rifampicin, E: ethambutol, S; streptomycin, These findings were compared with WHO global resistance rates (see Discussion)

Table 5. Drug resistance rates between 2018-2023

	H (%)	R (%)	E (%)	S (%)	MDR (%)
2018	14 (15.1)	9 (9.7)	2 (2.2)	10 (10.8)	9 (9.7)
2019	7 (9.2)	3 (3.9)	0	7 (9.2)	3 (3.9)
2020	8 (11.9)	1 (1.5)	1 (1.5)	3 (4.5)	1 (1.5)
2021	6 (9.8)	0	0	3 (4.9)	0
2022	10 (13.9)	2 (2.8)	1 (1.4)	8 (11.1)	2 (2.8)
2023	2 (2.9)	0	0	6 (8.6)	0
Total	47 (10.7)	15 (3.4)	4 (0.9)	37 (8.4)	15 (3.4)

H: Isoniazid, R: Rifampicin, E: Ethambutol, S; Streptomycin, MDR: Multidrug Resistance

Discussion

In our study, we found the mortality rate due to tuberculosis to be 7.7%. We observed that mortality was higher in patients with advanced age and those with comorbidities such as COPD, DIAH, and cerebrovascular disease. In addition, while MDR was found to be 3.4%, we also found that MDR was higher in individuals with a history of TB, bronchiectasis, DM, malignancy, and HIV infection as risk factors. Risk factors and comorbidities are quite important in the development of pulmonary TB. Studies have found different rates of diseases accompanying TB. In studies conducted by Sağıroğlu et al. [4], Dolla et al. [5], Hase et al. [6], Giridharan et al. [7], and Liu et al. [8], TB-accompanying diseases were found at different rates. The different study results suggest that the relationship between comorbidity and TB has not been fully determined, and its interaction with living conditions is unknown. In studies, DM, hypertension, COPD, bronchiectasis, and malignancies were the most frequently detected comorbid diseases. In our study, COPD, hypertension, bronchiectasis, and diabetes were the most frequently detected diseases, similar to other studies.

Chest radiographs can provide important clues in patients with TB symptoms. The sensitivity of chest radiographs in the diagnosis

of active TB is 70-80%, and the specificity is 60-70% [3]. Di Gennaro et al. [9], Alavi et al. [10], Oriekot et al. [11], Hase et al. [6], Piccazzo et al. [12], and Xie et al. [13] found infiltration and cavitary lesions in the bilateral upper zones and these findings were the most common chest radiograph findings. In our study, similar to other studies, the most common chest radiograph findings were 45.1% infiltration and 33.3% cavitation and infiltration.

Mortality due to tuberculosis in the world varies between 7% and 35% depending on the tuberculosis burden of the countries, socioeconomic status, non-infectious concomitant diseases, presence of HIV infection, and MDR. Mortality rates were found to be 8% in the study of Di Gennaro et al. [9], less than 10% in the study of Suhairi et al. [14], 6.5% in the study of Abedi et al. [15], and 4.6% in the study of Xie et al. [13]. The study by Sağıroğlu et al. [4] reported our national data at 6.83%. Studies have determined that DM, HIV, and malignancy are important in mortality. Alavi et al. [10] found that the elderly population had a higher mortality rate (26.5%) compared to the young population (4.1%). In the study conducted by Zahar et al. [16] in patients who developed respiratory failure due to TB, a mortality rate was 26.2%. In the study conducted by Elhisdi et al. [17], the mortality rate was 47.7%, and comorbidities were COPD, hypertension, DM, and bronchiectasis. In the study conducted by Hase et al. [6], mortality independent of age was found to be 11.3%, while in terms of early mortality, the rate was found to be 28.1%, especially in patients over the age of 84. Other studies evaluating early mortality found the rates of 19.6% and 14.4%, respectively [18]. In most of the studies, risk factors for mortality were identified as low socio-economic level, severe malnutrition, recurrent infection, smear-positive pulmonary TB, MDR cases, high average age, DM, COPD, and malignancy [8-18]. We found that our study's TB-related mortality rate was comparable to studies conducted in our own country. The common characteristics of the patients were having comorbidities and being older (average age 69.8±12.2).

Drug resistance surveillance in tuberculosis is an important component of the tuberculosis control program. Drug resistance is the most important factor determining the type of treatment, duration, and prognosis of the disease in TB treatment. The estimated rate of new TB cases with MDR/RR-TB worldwide was 4% in 2015, decreasing to 3.3% in 2022; the estimated rate of previously treated MDR/RR-TB cases was 25% in 2015, decreasing to 17% in 2022 [19]. In the Global TB 2020 Report, the estimated number of MDR cases in Türkiye was 2.2% for new cases in 2020, 8.6% for previously treated patients, and the RR/MDR-TB rate was 2.4% for new cases and 9.7% for previously treated cases [2]. The most comprehensive article reflecting the resistance status in our country includes data from a meta-analysis study covering the results of 21 studies and 27,959 strains conducted between 1984-1989 and 1990-1995. In 1984-1989, H was 27.8%, S was 22.5%, E was 7.8%, R was 22.3%, and PRZ was 1.6%, while in 1990-1995, H was 23.8%, S was 17.9%, E was 7.7%, R was 22.1%, and PRZ was 6.5% [20]. When the resistance studies conducted in our country were examined, Tarhan et al. [21] H 6.5%, R 6.5%, E 4.3%, S 10.8%, MDR 2.17%, Perincek et al. [22] H 10.4%, R 3%, E 0.7%, S 0.7%, MDR 2.2%, Aslan et al. [23] H 7.1%, R 2.6%, S 6.6%, E 0.5%, MDR 3.5%, Porsuk et al. [24] H 9.9%, R 1.8%, E 1.2%, and S 4.7%, MDR 3.5%, Sağıroğlu et al. [4] H 7.1%, R 2%, E 5.6%, S 5.1%, MDR 2.5%, Yakupoğlu Y. et al. [25] H 8%, R %1.2, S 4.1%, E 0.2%. According to the data of the Tuberculosis Control Department of the Ministry of Health, antituberculosis drug resistance rates in all cases (2010-2020) are summarized in Table 6. From 2010 to 2020, there was a significant decrease in all drug resistances. In 2020, H was found to be 10.7%, R to be 2.9%, E to be 2.8%, S to be 9.1%, and MDR to be 2.6% [2]. In studies conducted in our country, resistance rates are similar to the data from the Tuberculosis Control Department of the Ministry of Health, according to the years in which they were conducted. A study conducted in Iran observed that drug resistance rates in relapse cases were higher than the world average. Any resistance 68%, mono-resistance 19%, multi-drug resistance 28% [26]. In our study, while the H (10.7%), R (3.4), and S (8.4%) rates were similar, the E (0.9%) rate was low, and our MDR (3.4) rates were high. The high rate of MDR was thought to be due to regional differences.

Table 6. According to the data of the Tuberculosis Control Department of the Ministry of Health, antituberculosis drug resistance rates in all cases (2010-2020)

	Drug Resistans				
	H (%)	**R (%)**	**E (%)**	**S (%)**	**MDR (%)**
2010	13.3	6.8	5.2	9.1	5
2011	13.7	6.7	5	10.3	5.4
2012	15.4	6.6	5.4	11.3	5.4
2013	13.6	5.1	4.2	9.4	4.1
2014	13.8	6.4	5	10.6	4.6
2015	13.7	5.4	4.4	11.3	4.1
2016	11.9	4.2	3.7	10.6	3.3
2017	12.5	4.2	4	10.5	3.2
2018	12.6	4.1	3.1	10.4	3.2
2019	10.8	1.4	2.3	9.3	2.3
2020	10.7	2.9	2.8	9.1	2.6

H: Isoniazid, R: Rifampicin, E: Ethambutol, S; Streptomycin, MDR: Multidrug Resistance

Patients with MDR are special patients in TB treatment and follow-up. Therefore, it is crucial to evaluate risk factors thoroughly. Results from studies by Xie et al. [13], Blöndal et al. [27], and Pradipra et al. [28] showed that MDR developed more in patients who had TB in the past, HIV, diabetes, or COPD. Similarly, in our MDR cases, there were previous TB, HIV infection, malignancy, diabetes, and bronchiectasis.

The retrospective nature of our study and the small number of patients were limitations.

As a result, although TB incidence and mortality are decreasing, it is still an important public health problem. It can be mortal, especially in patients with advanced age and comorbidities.

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Research Article

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Evaluation of preanalytical error processes in the microbiology laboratory and effect of training on these processes

Tıbbi mikrobiyoloji laboratuvarında preanalitik hata süreçlerinin değerlendirilmesi ve eğitimin bu süreçlere etkisi

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Abstract

Purpose: We aimed to calculate preanalytical error rates in the Medical Microbiology Laboratory of our hospital by the six-sigma method and examine the effect of training on error rates by comparing performances of processes before and after training.

Materials and methods: All samples evaluated between 2016-2021 were retrospectively examined. Rejected samples, blood culture contamination rate and urine culture contamination rate were evaluated via Laboratory Error Classification System. The staff obtaining laboratory samples were trained by means of live classes during 2017, 2018 and 2019, and with online classes during 2021. Error rates and sigma levels were calculated before and after training.

Results: 685591 samples were accepted by our laboratory; 1175 (0.2%) were rejected. The most frequent cause of rejection (53.4%) was hemolysis of sample. The sigma levels showed hemolysis of the sample as the most frequent cause of rejection, with a value of 4.7 (good performance). Among other quality indicators, the rate of urinary culture contamination was 11.4%, and the rate of blood culture contamination was 3.5%. The total sigma level of urine culture contamination was 2.9 (unacceptable performance), and the total blood culture contamination was 3.5 (minimal performance). Error rates had generally decreased after training, while an increase in performance at the sigma level was detected at all three indicators.

Conclusion: In order to minimize preanalytical errors in the medical laboratory, the preanalytical process should be regularly surveyed by quality and performance indicators, and continuing education should provide current information.

Keywords: Microbiology laboratory, preanalytical error, training.

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Öz

Amaç: Bu çalışmada, hastanemiz Tıbbi Mikrobiyoloji Laboratuvarında preanalitik hata oranlarının altı sigma yöntemi ile hesaplanması, eğitim öncesi ve sonrası süreç performansını karşılaştırarak eğitimin hata oranlarına etkisinin incelenmesi amaçlanmıştır.

Gereç ve yöntem: 2016-2021 yılları arasında değerlendirilen tüm numuneler retrospektif olarak incelendi. Laboratuvar hata sınıflama sistemi (LHSS) üzerinden reddedilen numuneler, kan kültürü kontaminasyon oranı ve idrar kültürü kontaminasyon oranı gözden geçirildi. Numune alan personele 2017, 2018 ve 2019 yılında yüzyüze, 2021 yılında çevirimiçi eğitimler verildi, eğitim öncesi ve sonrası hata oranları ve sigma düzeyleri hesaplandı.

Bulgular: Laboratuvarımıza 685591 numune kabul edilmiş, 1175'i (%0,2) reddedilmiştir. En sık ret nedeni hemolizli numunedir (%53,4). Sigma düzeylerine bakıldığında en sık ret nedeni olan hemolizli numunede 4,7 (iyi performans) olarak saptanmıştır. Diğer kalite göstergelerinden idrar kültürü kontaminasyon oranı %11,4, kan kültürü kontaminasyon oranı %3,5 olarak bulunmuştur. İdrar kültürü kontaminasyonunun sigma düzeyine bakıldığında 2,9 (kabul edilemez performans); kan kültürü kontaminasyonunun toplamda 3,5 (minimum performans) olduğu görülmüştür. Eğitim sonrası hata oranlarının genel olarak azaldığı görülmüş, sigma düzeyinde performans artışı her üç göstergede de tespit edilmiştir.

Sonuç: Tıbbi laboratuvarlarda preanalitik hataları en aza indirebilmek için preanalitik süreç kalite ve performans göstergeleri ile düzenli olarak takip edilmeli, sürekli eğitimlerle de bilgilerin güncel kalması sağlanmalıdır.

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Anahtar kelimeler: Mikrobiyoloji laboratuvari, preanalitik hata, eğitim.

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Introduction

Medical laboratories play a critical role in the diagnosis, prevention and treatment of diseases. Good laboratory practice is based on producing an accurate result for the appropriate patient in an appropriate time frame. For this reason, the laboratory testing process is analyzed in three phases including the preanalytical, analytical and postanalytical processes. Most of the errors (approximately 70%) are found in the preanalytical phase. Preanalytical phase errors include those that occur between the ordering of the test by the clinician and the start of biochemical analysis, most of which are preventable [1, 2].

ISO 15189:2012 Quality Standards of Medical Laboratories require recording, surveillance and improving all errors happening during laboratory processes. All laboratories should regularly detect and follow-up these errors. For this, standard methods such as quality indicators are used [3-6].

The Safety Reporting System is a national database in which medical errors occurring in state hospitals belonging to the Ministry of Health are recorded. This database includes the Laboratory Error Classification System (LECS), which is in common use by all laboratories, to choose a cause of error and reject a sample. Microbiology laboratories follow these recorded rates of rejected samples and causes of rejection in accordance with Health Quality Standards. Although not included in this system, blood culture and urine culture contamination rates are other quality indicators, which are regularly followed up by microbiology laboratories [7, 8].

The six-sigma method has become a preferred method in recent years in the detection and evaluation of process performance. The six-sigma method includes a set of rules based on statistical calculations. First, the process sigma level is calculated by transforming the number of errors to errors in one million and a scale between 0-6 is used. According to this scale, 6 reflects a fewer number of errors, and values nearing 0 reflect increasing rates of error [5, 8].

Errors occurring in the preanalytical phase generally happen outside the laboratory, for which reason tracking and controlling these errors are harder than those occurring in other phases. The importance of training is emphasized in the effort to decrease preanalytical errors, and a significant decrease in errors achieved by training is reported [4, 9].

We aimed to calculate preanalytical error rates in the Medical Microbiology Laboratory of our hospital by six-sigma method and examine the effect of training on error rates by comparing performances of processes before and after training in this study.

Materials and methods

All samples evaluated in our Medical Microbiology Laboratory of Balıkesir State Hospital and those that were rejected due to inappropriateness for analysis between 2016-2021 were retrospectively examined. The total numbers of samples received by our laboratory each month were obtained from the Laboratory Data Administration System.

Permission was obtained from the Ethics Committee of Clinical Investigations of Balıkesir University for the study (permission date: 10.08.2022, permission number: 2022/85).

Rejection of samples is done via LECS in our laboratory. The preanalytical testing process of the Medical Microbiology Laboratory was evaluated by reviewing rates of rejection, blood culture contamination rates, and urine culture contamination rates from LECS.

Determination of error rates and process sigma level

The total number of samples and rejected samples were used to calculate error rates in one million (using the formula “Error in 1 million = error number*1000000/total number of test orders”). Sigma levels were calculated by entering the value of error in 1 million at http://www.westgard.com/calculators/SixSigCalc.htm and performance evaluations were done.

These values were classified as:

1. 'Very good' if ≥5.0

2. 'Good' if between 4.0-5.0

3. Minimal performance if between 3.0-4.0

4. Unacceptable performance if <3.0

Evaluation of the effect of training

Starting in 2017, a face-to-face training on "Techniques of Appropriate Sampling" was provided every year to all staff members employed in sampling (midwives, nurses, health technicians, emergency medical technicians, and physicians) by a Medical Microbiology Specialist during March and April. The COVID-19 pandemic prevented this training in 2020, which was restarted again in 2021 on an "online" basis. First a "pre-test" and an "end-test" were performed to evaluate the efficacy of training. Rates of laboratory rejection and sigma levels were examined in 3 time periods (before training in January and February-1st analysis period; the first month after training in May and June-the 2nd analysis period; and the sixth month after training in September and October-the 3rd analysis period and the differences were compared statistically.

Statistical analysis

The data obtained in the study were entered in SPSS 22.0 (SPSS INC, Chicago, IL, USA) software and statistical analyses were performed. Since all variables in the study were categorical (expressed as presence/absence or yes/no), the distribution of the data was expressed as percentages and numbers (n). The Chi-square test was used to compare independent groups for categorical variables. A *p*-value of <0.05 was accepted as statistically significant.

Results

A total of 685591 samples were accepted by our laboratory in six years; 1175'i (0.2%) were rejected after selecting an appropriate cause of rejection from LECS. The rate of rejection was highest in 2016, after which it decreased in the following years, but there were no significant differences between the yearly rejection rates (*p*=0.483) (Table 1).

When we look at the distribution of rejected samples via LECS according to clinics, it was determined that 638 (54.3%) of 1175 rejections were from outpatient clinics, 303 (25.8%) were from inpatient clinics and 234 (19.9%) were from intensive care units (ICU), and the rejection rate detected in outpatient clinics was found to be statistically significant (*p*=0.001). The highest rejection rate was seen in outpatient clinics for six years, while the rate in ICU was 23.7% in 2016 and decreased to 7.8% in 2021, and the rejection rate in the inpatient clinics was 22.1% in 2016 and increased to 32.8% in 2021.

Table 1. The distribution of samples rejected by the Microbiology Laboratory via LECS according to years (n/%)

Year	Number of Samples Arriving at the Laboratory (n)	Number of Rejected Samples (n)	Rejection rate (%)	*p* value
2016	159249	611	0.4	0.483* $X^2$0.045
2017	150957	234	0.2	
2018	135568	122	0.1	
2019	96406	72	0.07	
2020	71344	72	0.1	
2021	72067	64	0.09	
Total	685591	1175	0.2	

LECS: Laboratory Error Classification System. *The Chi-square test was used

In the evaluation of preanalytical error causes in LECS, the most common (53.4%) cause was hemolysis of the sample, followed by inappropriate sample material (18.5%) and use of an inappropriate container (17.4%). When the causes of rejection were evaluated according to years, the most common cause was hemolysis of the sample in 2016 and 2017, while inappropriate container use was most common in 2018 and inappropriate sample material was the most common cause in 2019-2021. In the evaluation of the sigma values of errors, 4.7 (good performance) was found for hemolysis of the sample, while it was 5 and higher (very good performance) for all other causes of errors. The sigma levels of errors according to years showed the lowest value (4.3; good performance) for hemolysis of samples in 2016 and 2017, with very good performance for all other years (Table 2).

Among other indicators of quality that which Medical Microbiology Laboratories regularly evaluate, the contamination rate of urine cultures was 11.4%, and the contamination rate of blood cultures was 3.5%. The highest contamination rate of urine culture (16.5%) was in 2016, the lowest rate (8.1%) in 2021, the highest contamination rate of blood culture (4.7%) in 2018 and the lowest (2.2%) in 2020. These decreases in both urine culture and blood culture were not found to be statistically significant (p=0.403 for urine culture, p=0.716 for blood culture) (Table 3). Of the 521 blood cultures evaluated as contamination, 325 (62.4%) were from ICU patients, 196 (37.6%) were from inpatient clinics, and the difference was found to be statistically significant (p=0.001). Of the 3775 urine cultures in which contamination was detected, 2955 (78.3%) were from outpatient clinics, 435 (22%) were from ICU patients, and 385 (10.2%) were from inpatient clinics, and the difference was found to be statistically significant (p=0.028). Over the years, blood culture contamination was detected more in the ward only in 2021, while in all other years, it was detected higher in samples from ICU, and urine culture contamination was always detected higher in outpatients for six years.

The total sigma levels of urine culture contamination was 2.9 (unacceptable performance), and this level was <3.0 throughout the study duration; while total sigma levels of blood culture contamination was 3.5 (minimal performance), which remained between 3.0-4.0 (minimal performance) throughout the study duration (Table 4).

In evaluation of preanalytic error rates and their relationship with training, the rate of rejection of samples via LECS was found to decrease or remain stable with training. The decrease in 2019 was statistically significant (p=0.043) and the sigma levels had increased. The contamination rates of urine cultures had increased in 2017 in spite of training, decreased in 2018 one month after training, increased six months later, showed a similar course in 2019, increased a little one month after training in 2021, and decreased six months after training. No statistical significance was found in any of these increase or decreases (2017 p=0.737, 2018 p=0.422, 2019 p=0.970, 2021 p=0.719). Sigma levels showed an increase with training in 2018 and 2021. Blood culture contamination rates had decreased one month after training in 2017, 2018 and 2019, increased six months later, had increased in 2021 in comparison with before training but no statistical significance was found (2017 p=0.357, 2018 p=0.285, 2019 p=0.570, 2021 p=0.557). Sigma levels had shown an increase with training (except 2021), after which they had decreased (Table 5).

Table 2. Causes of microbiology laboratory preanalytical errors via LECS and sigma levels according to years

Preanalytical Error Causes	Sigma Levels According to Years													
	2016		2017		2018		2019		2020		2021		Total	
	Number of errors (n)	Sigma level (DPM)	Number of errors (n)	Sigma level (DPM)	Number of errors (n)	Sigma level (DPM)	Number of errors (n)	Sigma level (DPM)	Number of errors (n)	Sigma level (DPM)	Number of errors (n)	Sigma level (DPM)	Number of errors (n)	Sigma level (DPM)
Hemolyzed sample	426	4.3 (2675)	142	4.7 (941)	21	5.2 (155)	7	5.3 (73)	20	5.0 (280)	11	5.2 (153)	627	4.7 (915)
Inappropriate material	59	5.2 (144)	40	5.1 (199)	36	5.0 (243)	29	5.5 (41)	25	5.4 (56)	28	5.6 (28)	217	5.0 (317)
Use of inappropriate container	86	4.8 (540)	31	5.1 (205)	37	5.0 (273)	21	5.1 (218)	12	5.1 (168)	18	5.0 (250)	205	5.0 (299)
Insufficient amount of sample	22	5.2 (138)	10	5.4 (66)	13	5.3 (96)	6	5.4 (62)	7	5.3 (98)	4	5.4 (56)	62	5.3 (90)
Faulty barcoding	10	5.4 (63)	5	5.5 (33)	14	5.3 (103)	9	5.3 (93)	8	5.2 (112)	2	5.6 (28)	48	5.4 (70)
Lypemic sample	4	5.6 (25)	6	5.5 (40)	1	5.9 (7)	0	0	0	0	1	5.7 (14)	12	5.7 (18)
Clotted sample	4	5.6 (25)	0	0	0	0	0	0	0	0	0	0	4	5.9 (6)
Total	611	4.2 (3837)	234	4.5 (1550)	122	4.7 (900)	72	4.7 (747)	72	4.6 (1009)	64	4.7 (888)	1175	4.5 (1714)

LECS: Laboratory Error Classification System, DPM: Error rate in one million

Table 3. Causes of preanalytical errors in the bacteriology laboratory and distribution according to years (n/%)

Sample	Year																					
	2016			2017			2018			2019			2020			2021			Total			*p* value
	C	T	%	C	T	%	C	T	%	C	T	%	C	T	%	C	T	%	C	T	%	
Urine culture	990	5996	16.5	488	5552	8.8	836	7257	11.5	705	6065	11.6	424	4133	10.3	332	4100	8.1	3775	33103	11.4	0.403* $X^2$0.037
Blood culture	157	3488	4.5	92	2895	3.2	119	2507	4.7	65	1991	3.3	45	2014	2.2	43	1837	2.3	521	14732	3.5	0.716* $X^2$0.095

C: Number of contaminations, T: Total number of samples. *The Chi-square test was used

Table 4. Bacteriology laboratory preanalytical error causes and sigma levels according to years

Causes of Preanalytical Error	Sigma Levels According to Years											
	2016		2017		2018		2019		2020		2021	
	Error number (n)	Sigma level (DPM)	Error number (n)	Sigma level (DPM)	Error number (n)	Sigma level (DPM)	Error number (n)	Sigma level (DPM)	Error number (n)	Sigma level (DPM)	Error number (n)	Sigma level (DPM)
Urine culture cont.	990	2.5 (165110)	488	2.9 (87896)	836	2.7 (115199)	705	2.7 (116241)	424	2.8 (102589)	332	2.9 (80976)
Blood culture cont.	157	3.2 (45011)	92	3.4 (31779)	119	3.2 (47467)	65	3.4 (32647)	45	3.6 (22344)	43	3.5 (23408)

DPM: Error rate in one million, cont: Contamination

Table 5. Comparison of microbiology laboratory preanalytical error rates and sigma levels before and after training

			Causes of Preanalytical Errors					
			LECS system	*p** value	Urine Culture Contamination	*p** value	Blood Culture Contamination	*p** value
2017	Error rate % (r/t)	Before training	0.3 (75/29500)	0.443 $X^2$0.046	6.9 (80/1156)	0.737 $X^2$2.465	3.9 (29/750)	0.357 $X^2$0.087
		Just after training	0.1 (39/26222)		8.1 (68/837)		1.4 (8/576)	
		6 months after training	0.1 (17/24401)		10.2 (71/695)		4.1 (10/244)	
	Sigma level (DPM)	Before training	4.4 (2542)		3.0 (69204)		3.3 (38667)	
		Just after training	4.5 (1487)		2.9 (81243)		3.8 (13889)	
		6 months after training	4.7 (697)		2.8 (102158)		3.3 (40984)	
2018	Error rate % (r/t)	Before training	0.1 (18/25066)	-	13.3 (168/1266)	0.422 $X^2$0.041	7.9 (26/331)	0.285 $X^2$5.550
		Just after training	0.1 (20/23018)		9.4 (108/1149)		3.1 (13/415)	
		6 months after training	0.1 (24/21862)		15.2 (172/1129)		5.3 (22/412)	
	Sigma level (DPM)	Before training	4.7 (718)		2.7 (132701)		3.0 (78550)	
		Just after training	4.7 (869)		2.9 (93995)		3.4 (31325)	
		6 months after training	4.6 (1098)		2.6 (152347)		3.2 (53398)	
2019	Error rate % (r/t)	Before training	0.1 (16/16522)	0.043 $X^2$5.561	12.6 (139/1104)	0.970 $X^2$0.007	4.4 (16/363)	0.570 $X^2$0.026
		Just after training	0.1 (14/12597)		12.6 (120/951)		2.9 (9/314)	
		6 months after training	0.02 (5/17727)		12.0 (117/973)		5.7 15/265	
	Sigma level (DPM)	Before training	4.6 (968)		2.7 (125906)		3.3 (44077)	
		Just after training	4.6 (1111)		2.7 (126183)		3.5 (28662)	
		6 months after training	5.0 (282)		2.7 (120247)		3.1 (56604)	
2021	Error rate % (r/t)	Before training	0.1 (12/10014)	-	8.2 (49/599)	0.719 $X^2$1.284	0.4 (1/235)	0.557 $X^2$0.021
		Just after training	0.1 (11/9554)		8.7 (59/678)		2.7 (11/408)	
		6 months after training	0.1 (14/14354)		5.7 (43/759)		2.5 (7/283)	
	Sigma level (DPM)	Before training	4.6 (1198)		2.9 (81803)		4.2 (4255)	
		Just after training	4.6 (1151)		2.9 (87021)		3.5 (26961)	
		6 months after training	4.6 (975)		3.1 (56653)		3.5 (24735)	

LECS: Laboratory Error Classification System, r: Rejected samples, t: Total samples, DPM: Error rate in one million
*The Chi-square test was used

Discussion

Preanalytical phase errors are important, as they constitute approximately 70% of all errors observed during the laboratory process and many are preventable [1, 10, 11]. The most frequently reported errors were laboratory errors in the 2017 report of the Türkiye National Safety Reporting System, and nine out of ten errors were from the preanalytical phase [9].

Most of the studies on causes of errors detected during the preanalytical phase include data from Medical Biochemistry Laboratory, while data such as presented here, from Medical Microbiology Laboratory are very scarce. Oğuz et al. [12] have found a sample rejection rate of 0.8% in pediatric patients in the preanalytical phase. Koçer et al. [13] have detected a total sample rejection rate of 0.8% in the Hematology Laboratory and also found that the rate of rejected samples was higher for inpatients. Erdem et al. [14] have found a sample rejection rate of 0.2% in their study evaluating 1307013 blood samples. Lee [15] has found a preanalytical error rate of 0.4% in the clinical laboratory of a Korean university hospital and have reported a more frequent sample rejection rate in outpatients in comparison to inpatients. We have detected a sample rejection rate of 0.2% via LECS, with higher rates in outpatients than all other inpatients in all the years, and we found a significant decrease in sample rejection rates from the ICU. The highest rate of rejection was found for 2016, while a non-significant decrease was observed for the duration of the study. While this may show an improvement in process management for the preanalytical phase in our hospital, it also reflects a requirement for more elaborate studies on efforts for decreasing sample rejection rates in outpatients.

Hemolysis of the sample is frequently is the most frequent cause of preanalytical errors in medical laboratories. Among preanalytical error types in the GRS 2017 report, the most frequent (29.4%) cause of error was hemolysis of the sample [9]. In the questionnaire of International Clinical Chemistry and Laboratory Medicine Federation (IFCC) on 391 laboratories, the rate of hemolysis was reported between 1-5% [16]. Arıcı [17] has detected hemolysis of the sample, clotting of the sample and an inappropriate amount of the sample as the most frequent causes of rejection of samples in medical biochemistry laboratories. Zorbozan et al. [18] have found the most frequent cause of preanalytical rejection via LECS system in the Parasitology Laboratory as insufficient amount of sample (47.3%), followed by an inappropriate test order (16.8%). We found the most frequent preanalytical causes of error via the LECS as hemolysis of sample, followed by inappropriate sample material and use of inappropriate container. Although the sigma level never fell below 4 during these years, causes of rejection seem to be preventable errors in sampling. It should not be forgotten that a high quality of health services can be achieved only by a team-work; thus, a regular surveillance of indices of quality in parallel with a close coordination and cooperation with all units is required to decrease test rejection.

In the study by Veranyurt et al. [19] studying preanalytical errors in the Microbiology Laboratory between 2016-2018, rates of rejection via LECS were found as 1.1%, 0.9%, and 1.2% according to years, and the most frequent cause of error was insufficient sample amount, followed by clotted sample and hemolysis of sample. Blood culture contamination rates were found 4.4%, 4.1%, and 4.3% from 2016 to 2018. Çeken et al. [8] have found the most frequent cause of rejection via LECS in the Microbiology Laboratory as hemolysis of the sample in 2016, while the most frequent cause was contamination of the urine culture. The accepted target value for blood culture contamination rate is 3% in Türkiye, while each center determines their own target value for the rate of urine culture contamination, as there is not a universally accepted level in Türkiye [20, 21]. In studies conducted in Türkiye, the contamination rate of blood culture is reported between 5.4-8.2% [22-25] and the contamination rate of urine culture is reported between 5.5-46.2%, which is a wide range [7, 26, 27]. We found the blood culture contamination rate as 3.5% and the urine culture contamination rate as 11.4% in our study. The contamination rate of blood culture has reached 4.8% as the highest value in this six-year period and fell below the target value during 2020-2021.

The sigma value was above 3 during the whole process, showing “minimal performance”. The urine culture contamination rate was

highest in 2016, undulating during 2017 and 2018 as it decreased-increased, and continued to decrease in 2019 and afterwards. The sigma value was below 3 during the six years, which was “unacceptable performance”. From this data, we may assume that things are getting better in decreasing blood culture contamination, while the process of decreasing errors is not easy due to the fact that samples are provided by patients. In this respect, additional informative brochures, such as a directive for providing a urine culture sample given to the patients or posted on WC doors may provide a positive contribution.

While the fact that many of the errors during the preanalytical process are preventable implies that administration of the preanalytical process should be easy, the other aspect that most errors are related to staff not working at the laboratory actually makes the process administration harder. Regular analysis by the laboratory specialist is not sufficient, and additional correctional or preventive measures are needed. Many studies have stressed that education is indispensable in decreasing errors, regular in-service education, sustainability of training, and practical field training are important, and error rates have significantly decreased after training [16, 28-30]. The effect of training aiming to decrease preanalytical error rates was analyzed both statistically and by evaluating sigma levels. Also, analysis was made one month and six months after training in order to better evaluate the short- and long-term effects of education. While decreases in error rates were observed after training, a statistically significant difference was not found. We feel that the cause of this is small numerical values of differences between % rates. Generally, performance increase in sigma level was detected in all three parameters. Rejection via LECS has decreased during these years, and it decreased in 2017 after training in comparison to 2016 and has maintained this level. Especially, while sample numbers are similar in 2017 and 2018, error rates have decreased by half in comparison to the preceding year. The decrease one month after training in contamination rates in blood culture shows the positive effect of training, while the increase in contamination rates six months later shows that important information is forgotten in time, and the effect of training decreases. The lowest blood culture contamination rate was detected in the beginning of 2021, which may be due to a more meticulous approach in sampling by the staff during the COVID-19 pandemic. Urine culture contamination rates have shown an increase-decrease independent of training, but while this rate was 18% in the first analysis phase of 2016, it has shown a gradual decrease over the years to nearly 6% at the last analysis phase of 2021. Similarly, the decrease in error rates over the years was also observed in the other two parameters. We believe this to be a cumulative effect of training. In light of all this data, training may be considered as a fundamental step in decreasing errors. On the other hand, the effect of training decreases in time, and all that was told is forgotten. In our hospital, in order to increase the efficiency of training, we are increasing the frequency of education and using of additional administrative activities that support practical knowledge along with theoretical knowledge, such as “practical training in the field with small groups”.

Studies investigating the preanalytical error rate by both sigma level and statistical analysis, including the fundamental indicators of the preanalytical phase of Medical Microbiology Laboratory, and also covering a large time period are very scarce. In this respect, our study is valuable, and we believe that it will contribute to the medical literature. Limitations of our study include its retrospective design, decreasing number of samples evaluated in the laboratory in recent years, absence of training in 2020, and use of online training in 2021 due to the COVID-19 pandemic.

In conclusion, most of the errors in medical laboratories occur during the preanalytical phase. In order to minimize these errors, the preanalytical phase should be kept under close surveillance regularly via quality and performance indicators, and this information should be kept up-to-date by continuous training. We found that causes of rejection in LECS are frequently simple and preventable errors such as hemolysis of the sample, inappropriate material or inappropriate container. The sigma level of LECS rejection reasons was good and better in all parameters, the sigma level of blood culture contamination rate was good, and the sigma level of urine culture contamination was unacceptable performance. A decrease in error rates in all three indicators was observed with training, followed by an increase of error rates

again in some parameters after a duration of six months following training. But in the long run, training was observed to exert a positive overall effect and decrease the error rates. In light of these results, we believe that efforts to pursue the current quality goals should be strengthened by providing continuous training in our hospital, but different additional precautions may be required in order to decrease the urine culture contamination rate.

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Research Article

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Trends in osteochondral lesions of talus in twenty years and most cited twenty-five articles: a web-based bibliometric analysis

Talusun osteokondral lezyonlarında son yirmi yıllık eğilimler ve en çok atıf alan 25 makale: web tabanlı bibliyometrik bir analiz

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Abstract

Purpose: This study analyzes published research on osteochondral lesions of the talus (OLT) to identify surgical trends and highlight key findings. The focus is evaluating publication types, themes, and citation impact from 2003 to 2023.

Material and methods: To identify surgical trends and research focus, this study analyzed 553 publications from the Thomson Reuters Web of Knowledge database on OLT, indexed between 2003 and 2023. These publications were categorized by themes such as general topics, surgical strategies, autograft and allograft techniques, microfracture, pediatrics, sports, stem cell therapies, cartilage/chondrocyte implantation, AMIC, outcomes, complications, radiology, grafts, and scaffolds. The study also identified the top 25 cited articles over the period.

Results: The analysis revealed that 427 were original articles, and 56 were reviews. General topics (94 articles) and microfracture (73) were the most frequently covered themes. Findings indicate a significant increase in publications over the past five years, with microfracture and OATS being prevalent topics. The number of publications peaked in 2021, with the Hospital for Special Surgery contributing the most. Most articles were published in "Foot and Ankle International" and the "American Journal of Sports Medicine." The USA led in the number of publications, followed by South Korea. The most cited article was "Treatment of osteochondral lesions of the talus: a systematic review" by Zengerink Maartje et al., with 370 citations.

Conclusion: The study highlights a growing interest in OLT, particularly among orthopedic surgeons, with increased publications over recent years. There is a preference for autograft techniques, and AMIC has emerged as a promising treatment. This study highlights evolving trends in OLT management, emphasizing the need for continued research to optimize patient outcomes.

Keywords: Talus, osteochondral, bibliometric, web.

Aydemir AN, Yücens M. Trends in osteochondral lesions of talus in twenty years and most cited twenty-five articles: a web-based bibliometric analysis. Pam Med J 2025;18:660-671.

Öz

Amaç: Bu çalışma, talusun osteokondral lezyonları (TOL) üzerine yayımlanmış araştırmaları analiz ederek cerrahi eğilimleri belirlemeyi ve önemli bulguları vurgulamayı amaçlamaktadır. Çalışmanın odak noktası, 2003-2023 yılları arasında yayın türlerini, temalarını ve atıf etkilerini değerlendirmektir.

Gereç ve yöntem: Bu çalışma kapsamında, 2003-2023 yılları arasında Thomson Reuters Web of Knowledge veri tabanından indekslenmiş olan talusun osteokondral lezyonları ile ilgili 553 yayın analiz edilmiştir. Bu yayınlar genel konular, cerrahi stratejiler, otogreft ve allogreft teknikleri, mikrofraktür, pediatri, spor, kök hücre tedavileri, kıkırdak/kondrosit implantasyonu, AMIC tekniği, sonuçlar, komplikasyonlar, radyoloji, greftler ve iskele sistemleri gibi temalara göre kategorize edilmiştir. Ayrıca belirtilen dönemde en fazla atıf alan 25 makale belirlenmiştir.

Bulgular: Yapılan analizde, 427 yayının orijinal makale, 56 yayının ise derleme olduğu tespit edilmiştir. Genel konular (94 makale) ve mikrofraktür (73 makale) en sık işlenen temalardır. Bulgular son beş yılda yayın sayısında önemli bir artış olduğunu ve mikrofraktür ile OATS tekniklerinin sıkça ele alınan konular olduğunu göstermektedir. Yayın sayısı 2021 yılında zirve yapmış olup, Hospital for Special Surgery en çok katkı sağlayan kurum olarak öne çıkmıştır. Makalelerin çoğu "Foot and Ankle International" ve "American Journal of Sports Medicine" dergilerinde yayımlanmıştır. Ülke bazında en fazla yayın Amerika Birleşik Devletleri'nden yapılırken, Güney Kore ikinci sırada yer almıştır. En çok atıf alan makale, 370 atıfla Zengerink Maartje ve arkadaşları tarafından yazılan "Treatment of osteochondral lesions of the talus: a systematic review" başlıklı makaledir.

Sonuç: Bu çalışma, TOL konusundaki ilginin özellikle ortopedi cerrahları arasında giderek arttığını ve son yıllarda yayın sayısının yükseldiğini ortaya koymaktadır. Otogreft tekniklerinin tercih edildiği, AMIC tekniğinin ise umut verici bir tedavi yöntemi olarak öne çıktığı görülmektedir. Çalışma, TOL tedavisindeki gelişen eğilimleri vurgulayarak hasta sonuçlarını optimize etmek adına sürekli araştırmaya ihtiyaç duyulduğunu belirtmektedir.

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Anahtar kelimeler: Talus, osteokondral, bibliometrik, web.

Aydemir AN, Yücens M. Talusun osteokondral lezyonlarında son yirmi yıllık eğilimler ve en çok atıf alan 25 makale: web tabanlı bibliyometrik bir analiz. Pam Tıp Derg 2025;18:660-671.

Introduction

Osteochondral lesions of the talus (OLT) represent a challenging subset of ankle injuries, encompassing a spectrum of pathological changes involving both articular cartilage and subchondral bone [1]. These lesions can result from acute trauma, such as ankle sprains or fractures, or from chronic overuse, leading to degenerative changes over time [2]. Clinical presentation varies widely, from mild discomfort and swelling to severe pain and joint instability, depending on the lesion's size, location, and chronicity [3]. Recent improvements in imaging—most notably high-resolution MRI—have markedly enhanced our capacity to detect and characterize these lesions, thereby enabling more tailored treatment planning [4]. Despite these advances, the optimal management of talar osteochondral lesions remains controversial, with various surgical and non-surgical options available. Beyond symptom relief, these treatments focus on restoring the biomechanical stability of the ankle joint and minimizing the risk of long-term complications, particularly osteoarthritis [5].

Various surgical options are employed, tailored to each lesion's specific size, location, and severity. Arthroscopic debridement and microfracture: Successful for small to moderate lesions (<1.5 cm^2), typically in the talar dome. A minimally invasive approach in which the surgeon uses an arthroscope to access the ankle joint. The damaged cartilage and underlying bone are debrided [cleaned] to remove loose fragments and damaged tissue. Microfracture involves making small holes in the subchondral bone to stimulate the formation of fibrocartilage to replace the damaged cartilage [6]. Osteochondral Autograft Transfer (OATS): This is suitable for larger lesions (>1.5 cm^2) or for lesions that have not responded to conservative treatment. A cylindrical segment of intact cartilage and underlying bone is collected from a minimally loaded portion of the patient's knee joint. This plug is then transferred to the lesion site on the talus and fixed in place, restoring the articular surface [7]. Recently, studies have used periosteal bone as a graft instead of knee cartilage [8]. Autologous chondrocyte implantation (ACI): Reserved for larger lesions or when other techniques have failed. In the first stage, healthy cartilage cells are collected from a low-stress zone within the patient's knee. These cells are then cultured in a laboratory. This increases their number. To encourage proper integration and cartilage formation, the cultured chondrocytes are implanted into the talus defect under a periosteal patch [9]. Osteochondral allograft transplantation: Used for large lesions where autografting is not feasible. It involves the transplantation of donor osteochondral tissue [usually from a cadaveric source] to replace the damaged area of the talus. The procedure aims to restore the joint surface and functionality [10]. Autologous Matrix Induced Chondrogenesis (AMIC) is a minimally invasive surgical technique designed to promote articular cartilage regeneration in osteochondral lesions of the talus. Suitable for small to medium-sized lesions, typically less than 2 cm^2, it combines the benefits of microfracture with a collagen matrix to enhance the healing process. Ankle arthroscopy visualizes the osteochondral lesion and assesses the surrounding cartilage and bone. Microfracture is applied, and a collagen-based graft is positioned over the microfractured area. The collagen matrix is secured with fibrin glue or sutures [11]. Mesenchymal stem cells (MSCs), with their intrinsic regenerative abilities, make stem cell therapy an up-and-coming method for addressing cartilage and bone injuries associated with osteochondral lesions of the talus. MSCs are multipotent cells capable of differentiating into different cell types, including chondrocytes [cartilage cells] and osteoblasts [bone-forming cells], making them ideal candidates for tissue repair [12].

Bone marrow mesenchymal stem cells (BM-MSCs) are derived from the patient's bone marrow, usually from the iliac crest. Adipose-derived stem cells (ADSCs) are derived from the patient's adipose tissue through minimally invasive liposuction, peripheral blood-derived stem cells, obtained from peripheral blood

through specialised techniques such as apheresis [13]. Stem cell therapy holds great promise as a regenerative treatment option for talar osteochondral lesions, offering potential benefits in cartilage repair, joint preservation, and functional restoration [14].

This research identifies shifts and patterns in the surgical treatment of OLT by systematically analyzing studies from the past two decades. The study seeks to categorize the types of publications, focus areas, and citation impacts within OLT research, providing insights into the evolving preferences and techniques in treatment strategies and highlighting key findings that contribute to optimizing patient outcomes.

Materials and methods

553 OLT research articles, letters, case reports, reviews, and meeting abstracts published between January 2003 and December 2023 and indexed by title and abstract in Thomson Reuters Web of Knowledge were analysed. The publications reviewed were those with osteochondral lesions of the talus, osteochondritis dissecans of the talus, chondral defects of the talus, and chondrocyte implantation of the talus in the title. Articles with an English abstract and title were included in the study. They were categorised according to the type of publication. The top 25 most-cited articles were also documented between 2000 and 2023.

The topic was the question of publications on OLT. Surgical strategies were the main topic, followed by general topics: OATS, Allograft Osteochondral Transplantation (AOT), microfracture, paediatrics, sports, technique, stem cells, adjuvant therapies, additional injury, cartilage/chondrocytes, AMIC, outcome, complications, radiology, graft, and scaffold were the titles of the most relevant articles, which were examined by title and abstract where published documents on the same study were available.

This article did not require ethics committee approval as there was no animal model or human tissue evaluation. The title Osteochondral lesions of the talus was analysed as the main topic of the papers. General topics include ethology, anatomical localisation, biomechanical or kinematic analysis. OATS encompasses studies investigating the application of osteochondral and osteoperiosteal autografts in managing talar osteochondral lesions. AOT includes papers on osteochondral allografts and osteoperiosteal allografts. Microfracture includes documents on the treatment of OLT as a method of subchondral stimulation with microfracture. Paediatric groups include skeletally immature patients. Sports include all sporting activities such as athletics, football, and basketball. Stem cells include stem cell derivatives. Adjuvant therapies include platelet-rich plasma and hyaluronic acid injections. Additional injury describes injuries such as lateral ligament injuries. Cartilage/chondrocytes includes articles on cartilage autograft transfer, chondrocyte implantation, EMCA, and allograft transplantation. AMIC includes articles about autologous matrix-induced chondrogenesis. The outcome includes articles written as a result of medium- and long-term follow-up. Complications include articles written about complications and their therapies, subchondral cysts, and their complications. Radiology includes articles on radiological imaging techniques such as MRI, CT scans, X-rays, and scintigraphy. Grafting includes articles using bone grafts other than AMIC. Scaffolds include articles using scaffolds, such as hyaluronic acid.

Results

A total of 553 articles on OLT were reviewed. Four hundred twenty-seven were original articles, and 56 were review articles. The distribution of the articles according to the titles chosen by the majority of topics is shown in Table 1. The most popular titles are "general topics" with 94 articles and "microfracture" with 73 articles. Publication years and citations are shown in Figure 1. The year with the most published articles is 2021, with 59 articles, followed by 2020 with 52 articles. The centre with the most published articles is the Hospital for Special Surgery, which has 41 articles, followed by the University of Amsterdam, which has 27 articles. The distribution of centres is shown in Figure 2. Most [n=56, 11%] articles were published in the Foot and Ankle International, followed by the American Journal of Sports Medicine [n=48, 9.5%] (Figure 3).

Table 1. Quantitive distrubutions of Publications

	2023	2022	2021	2020	2019	2018	2017	2016	2015	2014	2013	2012	2011	2010	2009	2008	2007-2006	2005-2004-2003	Total
General topics	5	10	12	6	2	6	7	3	2	3	9	9	5	6	4	3	2	-	94
OATS	2	7	6	7	2	5	1	6	5	4	6	2	3	-	3	1	4	2	66
AOT	-	3	3	-	4	3	3	-	1	2	1	1	1	3	-	-	-	-	25
Microfracture	7	6	12	6	7	3	5	4	6	2	3	4	1	2	1	1	1	2	73
Pediatric	5	4	5	4	-	3	2	1	1	1	2	1	-	-	-	-	2	1	32
Sports	1	2	2	2	2	-	2	2	1	1	-	-	-	-	-	-	-	-	15
Technics	5	4	9	5	4	-	2	4	2	3	3	1	1	3	1	4	2	1	54
Stem cell	2	5	-	2	2	2	4	4	2	4	2	-	3	1	-	-	-	-	33
Adjuvant therapies	2	1	1	4	4	1	2	4	3	-	-	4	1	-	-	-	-	-	27
Additional injury	2	4	4	5	1	1	-	2	1	1	-	-	-	-	1	-	-	-	22
Cartilage / chondrocyte	2	1	6	6	1	-	2	-	-	5	3	3	3	3	2	1	-	3	41
AMIC	1	5	5	4	5	2	6	2	3	-	4	1	2	-	-	-	-	-	40
Outcome	4	2	8	3	9	4	2	4	3	2	6	1	1	2	3	1	1	1	57
Complication	4	3	4	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	13
Radiology	6	3	5	7	6	5	3	5	2	2	1	5	5	2	-	2	2	1	62
Graft	7	4	2	2	-	1	2	-	1	-	1	-	1	-	-	-	1	-	22
Scafold	4	2	2	1	1	-	6	2	-	-	-	-	1	-	-	-	1	-	20

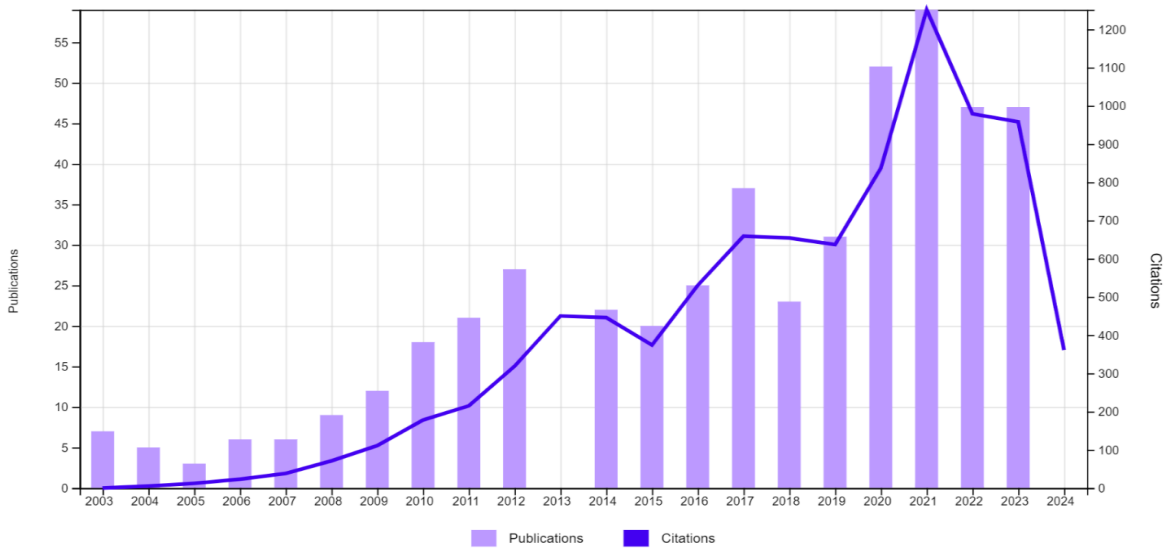

Figure 1. Publication years and citiations

Figure 2. Centers with the most articles published

Figure 3. Journals with the most articles published

The most prolific author was Kennedy JG, who had 94 publications, followed by Dahmen J. and Kerkhoffs GMMJ, who had 20 publications (Figure 4). Only 24 published articles had a primary author who was not an orthopaedic or podiatric surgeon. The United States published the most papers, 166, followed by South Korea with 55 (Figure 5). The 25 most cited articles, years of publication, journals, total citations, and average citations per year are shown in Table 2. The most cited article was ‘’Treatment of osteochondral lesions of the talus: a systematic review’’ by Zengerink et al. [5] in 2010 with 370 citations. The mean number of citations per year is shown in Table 3.

Figure 4. Authors who published the most articles

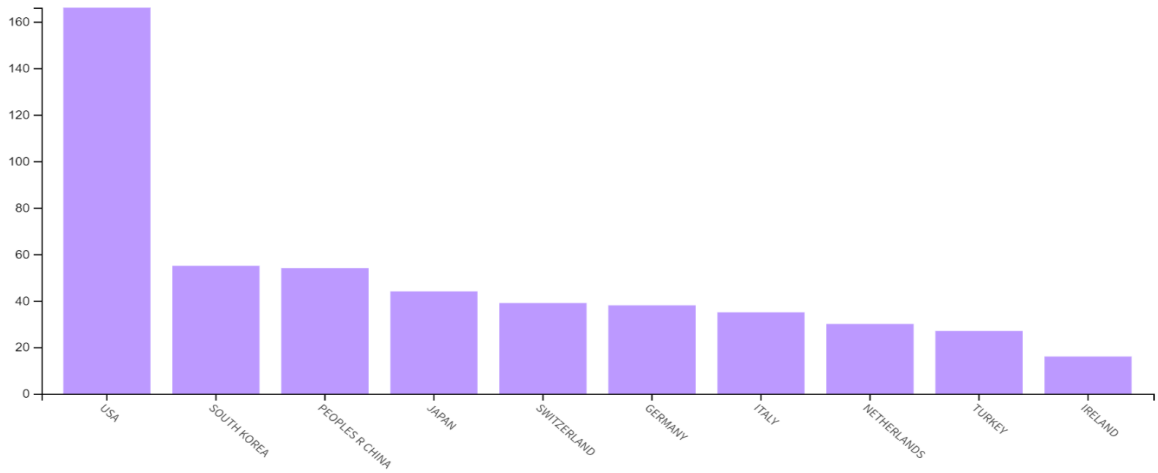

Figure 5. Countries with the most articles published

Table 2. Most cited 25 articles between 2003-2023

	Title	Authors	Source Title	Year	Total Citations	Average Per Year
1	Treatment of osteochondral lesions of the talus: a systematic review	Zengerink, Maartje et al.	Knee Surgery Sports Traumatology Arthroscopy	2010	370	24.67
2	Osteochondral Lesion of the Talus Is There a Critical Defect Size for Poor Outcome?	Choi, Woo Jin et al.	American Journal of Sports Medicine	2009	275	17.19
3	Arthroscopic treatment of chronic osteochondral lesions of the talus - Long-term results	Ferkel, Richard D et al.	American Journal of Sports Medicine	2008	273	16.06
4	Osteochondral lesions of the talus: Localization and morphologic data from 424 patients using a novel anatomical grid scheme	Raikin, Steven M. et al.	Foot & Ankle International	2007	267	14.83
5	Osteochondral lesions of the talus: Randomized controlled trial comparing chondroplasty, microfracture, and osteochondral autograft transplantation	Gobbi, Alberto et al.	Arthroscopy-The Journal of Arthroscopic and Related Surgery	2006	217	11.42
6	The morbidity associated with osteochondral harvest from asymptomatic knees for the treatment of osteochondral lesions of the Talus	Reddy, Sudheer et al.	American Journal of Sports Medicine	2007	181	10.06
7	Platelet-Rich Plasma or Hyaluronate in the Management of Osteochondral Lesions of the Talus	Mei-Dan, Omer et al.	American Journal of Sports Medicine	2012	162	12.46
8	Lesion Size Is a Predictor of Clinical Outcomes After Bone Marrow Stimulation for Osteochondral Lesions of the Talus: A Systematic Review	Ramponi, Laura et al.	American Journal of Sports Medicine	2017	147	18.38
9	Outcome of osteochondral autograft transplantation for type-V cystic osteochondral lesions of the talus	Scranton, PF et al.	Journal of Bone and Joint Surgery-British Volume	2006	145	7.63
10	Arthroscopic autologous chondrocyte implantation in osteochondral lesions of the talus - Surgical technique and results	Giannini, Sandro et al.	American Journal of Sports Medicine	2008	142	8.35
11	Prospective study on diagnostic strategies in osteochondral lesions of the talus - Is MRI superior to helical CT?	Verhagen, RAW et al.	Journal of Bone and Joint Surgery-British Volume	2005	142	7.1
12	Arthroscopic treatment of osteochondral lesions of the talus	Robinson, DE et al.	Journal of Bone and Joint Surgery-British Volume	2003	138	6.27
13	Osteochondral lesions of the talus: A new magnetic resonance grading system with arthroscopic correlation	Mintz, DN et al.	Arthroscopy-The Journal of Arthroscopic and Related Surgery	2003	131	5.95
14	Cartilage repair evolution in post-traumatic osteochondral lesions of the talus: From open field autologous chondrocyte to bone-marrow-derived cells transplantation	Giannini, Sandro et al.	Injury-International Journal of the Care of the Injured	2010	122	8.13

Table 2. Most cited 25 articles between 2003-2023 (continued)

	Title	Authors	Source Title	Year	Total Citations	Average Per Year
15	Donor-Site Morbidity After Osteochondral Autologous Transplantation for Lesions of the Talus	Paul, J. et al.	Journal of Bone and Joint Surgery-American Volume	2009	117	7.31
16	The Treatment of Osteochondral Lesions of the Talus with Autologous Osteochondral Transplantation and Bone Marrow Aspirate Concentrate: Surgical Technique	Kennedy, John G. et al.	Cartilage	2011	104	7.43
17	Intermediate Outcomes of Fresh Talar Osteochondral Allografts for Treatment of Large Osteochondral Lesions of the Talus	Haene, Roger et al.	Journal of Bone and Joint Surgery-American Volume	2012	102	7.85
18	Osteochondral lesions of the talus aspects of current management	Hannon, C. P. et al.	Bone & Joint Journal	2014	99	9
19	Second-Look Arthroscopic Findings and Clinical Outcomes After Microfracture for Osteochondral Lesions of the Talus	Lee, Keun-Bae et al.	American Journal of Sports Medicine	2009	99	6.19
20	Surgical Treatment of Osteochondral Lesions of the Talus by Open-Field Autologuous Chondrocyte Implantation A 10-Year Follow-up Clinical and Magnetic Resonance Imaging T2-Mapping Evaluation	Giannini, Sandro et al.	American Journal of Sports Medicine	2009	96	6
21	Mosaicplasty with autogenous talar autograft for osteochondral lesions of the talus after failed primary arthroscopic management - A prospective study with a 4-year follow-up	Kreuz, PC et al.	American Journal of Sports Medicine	2006	94	4.95
22	Current Concept Review: Osteochondral Lesions of the Talus	McGahan, Patrick et al.	Foot & Ankle International	2010	85	5.67
23	Arthroscopic microfracture for osteochondral lesions of the talus	Lee, Keun-Bae et al.	Knee Surgery Sports Traumatology Arthroscopy	2010	81	5.4
24	Evaluation and Management of Osteochondral Lesions of the Talus	Looze, Christopher A. et al.	Cartilage	2017	80	10
25	Clinical outcomes of platelet rich plasma (PRP) as an adjunct to microfracture surgery in osteochondral lesions of the talus	Guney, Ahmet et al.	Knee Surgery Sports Traumatology Arthroscopy	2015	76	7.6

Table 3. Citataion reports among years

Year	Citiation Per Article	Total Citation	Total Article	Citiation Per Year
2023	0.71	33	46	0.71
2022	2.77	133	48	1.38
2021	5.93	374	63	1.97
2020	8.48	416	49	2.12
2019	12.78	409	32	2.55
2018	16.68	417	25	2.78
2017	19.19	691	36	2.74
2016	24.03	625	26	3
2015	20.43	470	23	2.27
2014	15.66	376	24	1.5
2013	30.25	968	32	2.75
2012	29.22	906	31	2.43
2011	22.14	465	21	1.7
2010	57.47	1207	21	4.1
2009	61.5	738	12	4.07
2008	54.4	544	10	3.4
2007/06	93.25	1119	12	5.32
2005/04/03	48.5	970	20	2.42

Discussion

The number of publications on OLT has increased in recent years. The year with the most articles published is 2021, with 59, and approximately 50% of the articles were published in the last five years. This result shows that there is a trend towards OLT, especially among orthopaedic surgeons. As mentioned in the results section, only 24 first authors were not orthopaedic surgeons in publications on OLT.

The most commonly written titles are general topics. For example, the etiology, anatomy, lesion size, depth, and location, additional injury, type of injury, and predisposing factors of OLT have been described in many articles [15, 16]. Microfracture is still one of the less invasive treatment methods that has been used for many years in the surgical treatment of OLT, with published long-term results [6]. This is supported by the fact that microfracture is the second most widely written topic in this study, with 77 articles. Most of the articles on microfracture were published in 2021, with 12 articles. Surgical strategies may be modified depending on the size, depth, and localisation of the lesion [17]. The use of osteochondral grafts is one of these options [18]. Studies have shown that reconstructions with autograft in talar osteochondral lesions are more successful in the medium and long term than reconstructions with allograft [19]. This study found 66 articles on autograft and 25 articles on allograft. This could be an indication that the use of autograft is more practical and convenient. According to the results of this study, OATS is the most common surgical approach after microfracture in OLT treatment. Recently, AMIC treatment has been an increasing treatment protocol in OLT surgery. The first articles described this method with medial malleolus osteotomy, but nowadays, newer articles use AMIC procedures without malleolus osteotomy, direct visualisation of the ankle [20]. Almost all articles on AMIC have been published in the last decade. There seems to be a trend towards this procedure.

Cartilage/chondrocyte articles were concentrated in 2009-2015, decreased significantly in the next five years, and peaked in 2020-2021. Treatments based on chondrocytes usually require two-stage surgical procedures [21]. Sometimes it wasn't cost-effective. Articles on stem cell and adjuvant therapies show a relatively homogeneous distribution after 2011. They are used in the literature to accelerate postoperative recovery or as an adjunct to conservative treatment [22, 23]. A certain number of radiological studies were published in all years. However, as expected for outcome studies, there has been an increase in the last decade [24-27]. Although the 25 most cited articles cover almost all topics, more outcome studies exist.

In 2017, a systematic review study, ''Lesion Size Is a Predictor of Clinical Outcomes After Bone Marrow Stimulation for Osteochondral Lesions of the Talus'', had an average citation per year of 18.38. It should be considered a remarkable and promising study [3]. The study by Looze et al. [28] has an average of 10 citations per year, more than the articles further up the list. This study showed that articles on complications were published mainly in the last 5 years. This finding can be interpreted as understanding the importance of medium- and long-term outcomes and unsuccessful surgical management [29].

Analysis of the literature on OLT from 2003 to 2023 reveals significant trends and insights into the evolving surgical management of these complex ankle injuries. The study highlights a marked increase in research publications in recent years, reflecting growing interest and advances in treatment options, particularly among orthopaedic surgeons. General topics and microfracture remain prominent areas of focus, highlighting their continued relevance in the management of OLT. There is a clear preference for autograft techniques over allografts, with autografts demonstrating superior mid- and long-term results. In addition, the emergence of AMIC as a treatment strategy signals a shift towards innovative and minimally invasive approaches. With the increasing adoption of AMIC, it is anticipated that the use of mosaicplasty may decline in the coming years. Despite these advances, continued research is essential to understand further and improve long-term surgical efficacy, outcomes, and complications. This study highlights the need for ongoing investigation and evaluation of treatment strategies to improve patient care and optimise outcomes in the management of OLT.

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Authors' contributions: M.Y. developed the study's main idea and hypothesis. A.N.A. formulated the theory and structured the materials and methods section. M.Y. conducted the data analysis in the results section. M.Y. wrote the discussion section, which was later reviewed, revised, and approved by A.N.A. Additionally, all authors actively contributed to discussions throughout the study and approved the final version of the manuscript.

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

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Research Article

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Determination of sex with occipital condyle measurements on three-dimensional computed tomography images

Üç boyutlu bilgisayarlı tomografi görüntülerinde oksipital kondil ölçümleri ile cinsiyetin değerlendirilmesi

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Abstract

Purpose: Morphometric measurements of cranial bones in skeletal remains are important for sex determination. Nowadays, radiological imaging techniques such as computed tomography are very useful in obtaining population-specific data. The aim of this study was to evaluate the usefulness of morphometric measurements of the occipital condyles in 3D cranial CT modeling for sex estimation in a Turkish population.

Materials and methods: In this study, three-dimensional images of the occipital condyles were obtained by retrospectively using the carotid CT angiography images from the radiology department between 2019 and 2021. In these images, the length, width and height of both occipital condyles, distances between the basion with and the anterior and posterior ends of the occipital condyles, distances between the opisthion the anterior and posterior ends of the occipital condyles, anterior, posterior, minimum and maximum intercondylar distances, maximum bicondylar distance, right and left sagittal condylar angles, sagittal intercondylar angle, were measured.

Results: A statistically significant sexual dimorphism was found for all parameters except the left condylar angle measured in the occipital condyles. According to multivariate discriminant analysis models, sex could be estimated with an accuracy of 81.2% to 84.6% for males and 52.9% to 68.8% for females.

Conclusion: It was determined that three-dimensional computed tomography images of the occipital condyles can be used in sex estimation.

Keywords: Sex estimation, forensic anthropology, identification, occipital condyle, 3D modelling.

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Öz

Amaç: İskelet kalıntılarındaki kranial kemiklerin morfometrik ölçümleri cinsiyetin belirlenmesinde önemlidir. Günümüzde, bilgisayarlı tomografi gibi radyolojik görüntüleme teknikleri popülasyona özgü veriler elde etmede oldukça faydalıdır. Bu çalışmanın amacı, Türk popülasyonunda cinsiyet tahmini için 3B kranial BT modellemesinde oksipital kondillerin morfometrik ölçümlerinin yararlılığını değerlendirmektir.

Gereç ve yöntem: Bu amaçla, radyoloji anabilim dalı tarafından 2019-2021 yılları arasında çekilmiş karotis anjio BT görüntüleri retrospektif olarak kullanılarak oksipital kondillerin üç boyutlu görüntüleri elde edilmiştir. Bu görüntülerde her iki oksipital kondilin uzunluğu, genişliği ve yüksekliği, bazion ile oksipital kondilin ön ve arka uçları arasındaki mesafeler, opistion ile oksipital kondilin ön ve arka uçları arasındaki mesafeler, ön, arka, minimum ve maksimum interkondiler mesafeler, maksimum bikondiler mesafe, sağ ve sol sagital kondiler açılar, sagital interkondiler açı ölçülmüştür.

Bulgular: Sol kondiler açı dışındaki tüm parametrelerde cinsiyete göre istatistiksel olarak anlamlı farklılık olduğu ve oksipital kondillerde cinsel dimorfizm bulunduğu saptanmıştır. Diskriminant analizi ile oluşturulan çok değişkenli modellere göre erkeklerde %81,2 ile %84,6, kadınlarda ise %52,9 ile %68,8 doğrulukta cinsiyet tahmini yapılabileceği gösterilmiştir.

Sonuç: Oksipital kondillerin üç boyutlu bilgisayarlı tomografi görüntülerinin cinsiyet tahmininde kullanılabileceği saptanmıştır.

Anahtar kelimeler: Cinsiyet tahmini, adli antropoloji, kimliklendirme, oksipital kondil, 3B modelleme.

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Introduction

Human skeletal remains identification is one of the fundamental issues that require technical and expert knowledge covering many disciplines such as anatomy, radiology, archaeology, dentistry and genetics, which are of interest to physical anthropology as well as forensic medicine [1, 2]. The determination of the sex is one of the most important steps in the process of identification. This is because the methods to be used to determine other characteristics, such as age and height estimation depend on the correct estimation of sex. It also eliminates half of the options in determining to whom the remains belong to [3, 4]. In cases of mass deaths such as natural disasters, wars, and major accidents, sex estimation becomes difficult due to fragmented and incomplete skeletal remains [5]. In this case, the reliability and accuracy of sex determination from skeletal remains depends on the anatomical region available [6].

The skull is the second-best region for predicting sex after the pelvis, according to morphometric and morphological studies of the skull [7]. It is important to examine the base of the skull for sex estimation because the base of the skull may remain intact compared to other parts of the skull in various violent events such as fires, natural disasters and terrorist incidents due to its thickness and protected anatomical position [8, 9].

Computed tomography (CT) is increasingly used in forensic medicine [10]. It is stated that the use of CT images in the analysis of skeletal remains allows for faster evaluation, reduces the risks of deterioration, loss and similar risks of existing skeletal remains during transport, and allows for an opinion to be obtained by transmitting them to an expert anywhere in the world using information technologies [11].

This study aimed to establish a standard for the morphometry of the occipital condyles (OC) on three-dimensional computed tomography (3DCT) models of the skull for sex estimation. Furthermore, the aim was to develop discriminant functions to investigate the potential use of occipital condyles in sex estimation in the contemporary Turkish population and to contribute to the occipital condyle database.

Material and methods

This study was initiated following the granting of approval by the Ethics Committee for Non-Interventional Clinical Research of Pamukkale University (2021- E-60116787-020-49028). All procedures performed in studies were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Carotid CT angiography images taken by the Department of Radiology between April 2019 and March 2021 were used retrospectively. A total of 481 (292 males and 189 females) images of the carotid CT angiography of individuals 18 years of age and older were included in the study. Scans with motion artefacts and cases with head trauma or joint pathology, such as arthrosis, were excluded. The carotid CT angiography investigations were made with the Philips Ingenuity 128 CT device and scans were obtained in 1 mm slices. The archived images were reloaded onto a standard work station (IntelliSpace Portal; release 10.1; Philips Medical Systems), and skull base images including the occipital condyles were obtained after a three-dimensional (3D) reconstruction was performed through bone window adjustments.

Landmarks used in present study

The basion (B), which is the point where the anterior edge of the foramen magnum (FM) meets the mid-sagittal line; the opisthion (O), which is the point where the posterior edge of FM meets the mid-sagittal line; and the anterior and posterior ends of the right and left OC determined in the horizontal plane are the landmarks determined for the evaluation (Figure 1).

Morphometric measurement points of the occipital condyle on CCT images

- **Occipital condyle lenght (OCL):** Maximum length along the long axis of the joint surfaces of the OCL (right and left)

- **Occipital condyle width (OCW):** Maximum width along a line vertical to the longitudinal axis of the articular surface of the OC (right and left)

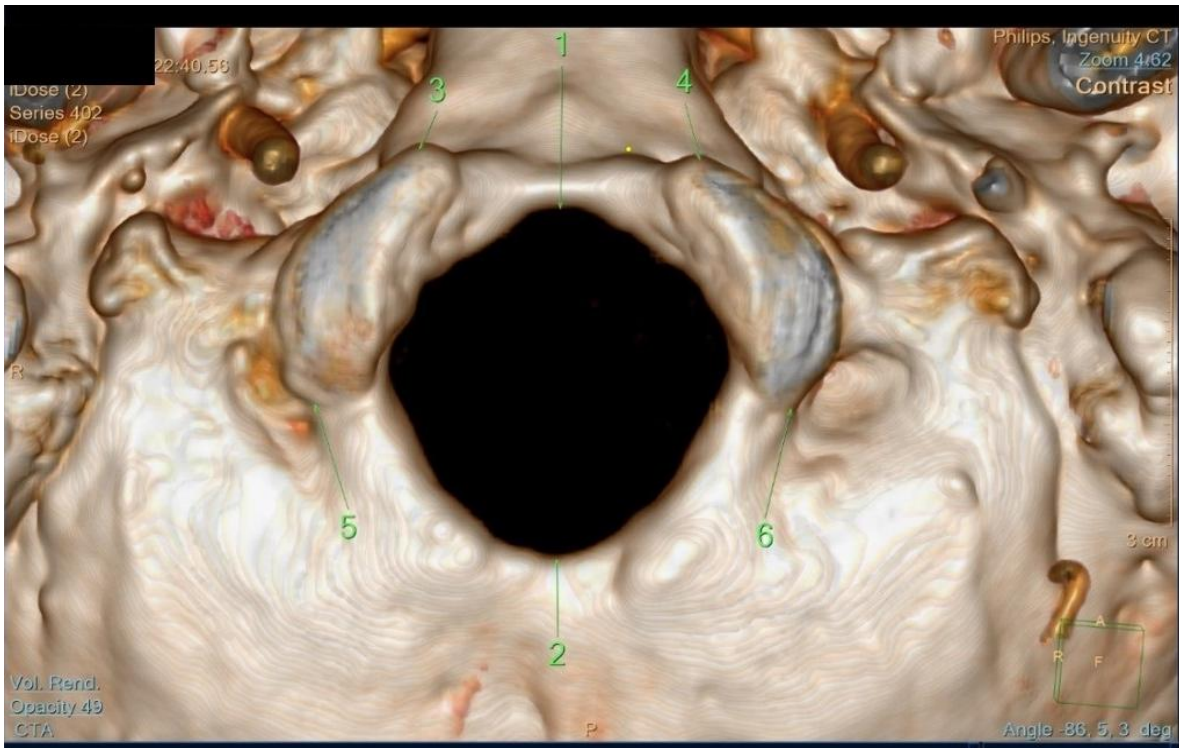

Figure 1. Landmarks used in present study

1. Basion (B), 2. Opisthion (O), 3. The anterior tip of the right occipital condyle (right OCAT)
4. The anterior tip of the left occipital condyle (left OCAT), 5. The posterior tip of the right occipital condyle (right OCPT), 6. The posterior tip of the left occipital condyle (left OCPT)

- **Condylar index (Baudoin condylar index, BCI):** OCW / OCLx100 (right and left)
- **Occipital condyle height (OCH):** The thickness at the intersection of the lines used to measure OCL and OCW (right-left)
- **Anterior intercondylar distance (AID):** Distance between the anterior ends of the joint surfaces of the right and left OC
- **Posterior intercondylar distance (PID):** Distance between the posterior ends of the joint surfaces of the right and left OC
- **Minimum intercondylar distance (MinID):** Minimum distance between medial joint surface edges of both OCs
- **Maximum intercondylar distance (MaxID):** Maximum distance between medial edges of joint surfaces of both OCs
- **Maximum bicondylar distance (MaxBD):** Maximum distance between lateral joint surface edges of both OCs
- **Sagittal intercondylar angle (total SICA):** Angle between the long axes of the right and left OCs
- **Right and left sagittal condylar angle (right SCA – left SCA):** Angle between condylar long axis and sagittal midline
- Distance between anterior tip of right OC and B (right OCAT-B)
- Distance between anterior tip of left OC and B (left OCAT-B)
- Distance between posterior tip of right OC and B (right OCPT-B)
- Distance between posterior tip of left OC and B (left OCPT-B)
- Distance between anterior tip of right OC and O (right OCAT-O)
- Distance between anterior tip of left OC and O (left OCAT-O)
- Distance between posterior tip of right OC and O (right OCPT-O)
- Distance between posterior tip of left OC and O (left OCPT-O)

Statistical analysis

Data were evaluated using SPSS 25.0 [IBM SPSS Statistics 25 software (Armonk, NY: IBM Corporation)]. Continuous variables are presented as mean ± standard deviation, median (25^{th}-75^{th} percentile: IQR), min-max, and categorical variables are presented as numbers and percentages. To test the suitability of the data for normal distribution, the Kolmogorov-Smirnov test was used. In examining the differences between groups, the independent samples t test was used when parametric test assumptions were met, and the Mann Whitney U test was used when parametric test assumptions were not met. Differences between categorical variables were examined with Chi square analysis. In dependent group comparisons, a paired samples t test was used when parametric test assumptions were met. Intraclass correlation coefficient (ICC-ICC) was used to examine the reliability of the measurements taken. The Discriminant Analysis method was used to determine sex differences. In discriminant analysis models, canonical correlation coefficients (CCC), Wilks lambda' values and explained variance values were examined. As a result of the univariate examinations, different multivariate models were used, both clinically and statistically, and model equations were created using the results of the models with the highest discrimination values. The same observer re-evaluated 10 randomly selected carotid angio-CT images to evaluate the intra-observer agreement. Intra-observer agreement was analysed using intraclass correlation coefficient (ICC) scores with a 95% confidence interval (ICC score range: 0.91-1). In all analyses, $p<0.05$ was considered statistically significant.

Results

The study included 481 cases, 189 females (39.3%) and 292 males (60.7%). Mean age was 56.64±14.22 (min-max: 19-87) in females and 58.84±14.26 (min-max: 19-89) in males. With the exception of the left SCA, all measurements of the right and left OCs were significantly different between the male and female cases ($p<0.05$). Right OCL, left OCL, right OCW, left OCW, right OCH, left OCH, AID, PID, MaxID, MinID, MaxBD, right OCAT-B, left OCAT-B, right OCPT-B, left OCPT-B, right OCAT-O, left OCAT-O, right OCPT-O, left OCPT-O measurements were greater in the male cases than in the female cases. Right BCI, left BCI, right SCA, total SICA measurements were greater in the female cases than in the male cases ($p<0.05$) (Table 1).

Table 1. Descriptive statistical analysis results

	Males			Females			*p*
	Mean±SD	**Med (IQR)**	**Min-max**	**Mean±SD**	**Med (IQR)**	**Min-max**	
Right OCL	25.55±2.24	25.8 (24.13-27.1)	17.7-31.5	23.29±1.97	23.3 (22-24.65)	18.2-30.7	0.0001* (t=11.294)
Left OCL	25.51±2.46	25.6 (23.83-27.18)	17.8-31.1	23.22±1.93	23.1 (21.85-24.6)	18.1-28.3	0.0001* (t=11.408)
Right OCW	11.69±1.38	11.6 (10.7-12.6)	8.6-15.9	11.11±1.25	11 (10.3-11.75)	8.3-15	0.0001* (z=-4.753)
Left OCW	11.9±1.33	11.8 (11-12.8)	8.9-16.2	11.19±1.41	11 (10.2-11.95)	8.4-16	0.0001* (z=-5.893)
Right BCI	46.14±7.04	45.13 (41.21-50.15)	30.94-77.18	48.09±7.19	47.3 (43.06-52.28)	35.43-72.5	0.003* (z=-3.022)
Left BCI	47.14±7.6	46.23 (41.9-51.21)	31.94-78.09	48.58±7.64	47.11 (43.69-52.95)	35.11-77.78	0.033* (z=-2.13)
Right OCH	10.08±1.46	9.9 (9.03-11.08)	6.9-14.9	9.66±1.22	9.6 (8.8-10.5)	6-13.2	0.003* (z=-2.954)

Table 1. Descriptive statistical analysis results (continued)

	Males			Females			*p*
	Mean±SD	**Med (IQR)**	**Min-max**	**Mean±SD**	**Med (IQR)**	**Min-max**	
Left OCH	10.08±1.46	9.9 (9-11)	6.9-14.6	9.5±1.24	9.6 (8.6-10.4)	6-12.1	0.0001* (z=-3.496)
AID	22.77±2.75	22.7 (21.2-24.4)	15.1-33.5	21.09±2.45	21 (19.4-22.6)	12.1-29.3	0.0001* (t=6.821)
PID	45.08±3.46	45.2 (42.6-47.5)	35.9-54.2	42.29±3.59	42.1 (39.9-44.5)	34.8-52.2	0.0001* (z=-8.041)
MaxID	33.92±2.37	33.9 (32.23-35.4)	25.6-40.1	32.01±2.31	32 (30.4-33.6)	26.9-39.3	0.0001* (t=8.731)
MinID	21.35±2.48	21.3 (19.8-22.8)	13.6-28.6	19.91±2.19	19.9 (18.4-21.45)	14.1-27.3	0.0001* (t=6.529)
MaxBD	51.25±3.13	50.9 (48.93-53.28)	42.3-59.5	48.13±3.18	48.2 (45.7-50.15)	39.9-55.8	0.0001* (t=10.607)
Right SCA	26.56±5.74	26 (23-30)	10-48	28.13±6.1	27 (24-32)	11-49	0.004* (z=-2.915)
Left SCA	27.5±6.34	27 (23-31)	5-47	28.17±5.81	28 (24-31)	15-48	0.169 (z=-1.376)
Total SICA	54.06±10.6	53 (47-60)	22-89	56.3±10.69	55 (49-62)	34-94	0.016* (z=-2.405)
Right OCAT-B	13.28±1.65	13.15 (12.1-14.2)	9-22	12.19±1.29	12.2 (11.3-13)	8.5-16.2	0.0001* (t=8.104)
Left OCAT-B	13.35±1.71	13.2 (12.23-14.4)	9-22.4	12.26±1.35	12.2 (11.25-13.2)	9.4-17.7	0.0001* (z=-7.237)
Right OCPT-B	29.44±1.93	29.3 (28.3-30.68)	28.3-30.6	27.49±1.91	27.4 (26.2-29)	20.1-33.3	0.0001* (z=-9.73)
Left OCPT-B	29.42±1.98	29.5 (28.03-30.6)	23.8-34.6	27.5±2.08	27.4 (26.1-28.8)	22.5-39.8	0.0001* (t=10.152)
Right OCAT-O	41.12±3.14	41.3 (39.4-43.1)	26.1-51.2	38.3±2.7	38.4 (36.55-40.3)	25.9-43.5	0.0001* (t=10.16)
Left OCAT-O	41.4±2.96	41.5 (39.7-43.1)	26.8-51.6	38.45±2.43	38.5 (36.5-40.3)	32.4-44.1	0.0001* (t=11.446)
Right OCPT-O	28.41±2.76	28.25 (26.7-29.6)	21.7-42.2	27.03±2.58	26.8 (25.15-29.1)	19.6-34	0.0001* (z=-4.984)
Left OCPT-O	28.89±2.71	28.8 (27.2-30.58)	21.1-42.2	27.09±2.47	26.9 (25.4-28.85)	21.3-36.1	0.0001* (t=7.381)

*$p<0.05$ statistical significance difference; SD: Standard deviation; Med (IQR): Median (25^{th}-75^{th} percentile); Min: Minimum max: Maximum; t: Independent Sample T test; z: Mann Whitney U test

The results of the univariate discriminant function analysis used to analyse sex differences are shown in Table 2. The accuracy of sex classification of the variables with the highest variance explaining value was 85.3% in males and 53.4% in females in left OCAT-O; 82.5% in males and 55.6% in females in right OCL; 80.8% in males and 56.1% in females in left OCL; and 79.8% in males and 52.4% in females in maxBD (Table 2).

Table 2. Univariate discriminant function analysis results

Variables	Sexing Accuracy Rates			CCC	Wilks' Lambda	*p*	Explained Variance (%)
	Male n=292)	Female n=189)	Total				
Right OCL	241 (82.5%)	105 (55.6%)	346 (71.9%)	0.459	0.79	0.0001*	21.1
Left OCL	236 (80.8%)	106 (56.1%)	342 (71.1%)	0.444	0.803	0.0001*	19.7
Right OCW	259 (88.7%)	36 (19%)	295 (61.3%)	0.208	0.957	0.0001*	4.3
Left OCW	250 (85.6%)	59 (31.2%)	309 (64.2%)	0.246	0.94	0.0001*	6.1
Right BCI	275 (94.2%)	17 (9%)	292 (60.7%)	0.134	0.982	0.003*	1.8
Left BCI	285 (97.6%)	7 (3.7%)	292 (60.7%)	0.092	0.992	0.043*	0.8
Right OCH	274 (93.8%)	11 (5.8%)	285 (59.2%)	0.146	0.979	0.001*	2.1
Left OCH	267 (91.4%)	31 (16.4%)	298 (61.9%)	0.201	0.96	0.0001*	4
AID	246 (84.2%)	63 (33.3%)	309 (64.2%)	0.298	0.911	0.0001*	8.9
PID	241 (82.5%)	89 (47.1%)	330 (68.6%)	0.363	0.869	0.0001*	13.2
MaxID	246 (84.2%)	91 (48.1%)	337 (70.0%)	0.371	0.863	0.0001*	13.8
MinID	244 (83.6%)	61 (32.3%)	305 (63.4%)	0.286	0.918	0.0001*	8.2
MaxBD	233 (79.8%)	99 (52.4%)	332 (69.0%)	0.436	0.81	0.0001*	19
Right SCA	278 (95.2%)	17 (9%)	295 (61.3%)	0.13	0.983	0.004*	1.7
Left SCA	292 (100%)	189 (0%)	292 (0.6%)	0.054	0.997	0.241	0.3
Total SICA	283 (96.9%)	9 (4.8%)	292 (60.7%)	0.103	0.989	0.024*	1.1
Right OCAT-B	242 (82.9%)	73 (38.6%)	315 (65.4%)	0.332	0.89	0.0001*	11
Left OCAT-B	247 (84.6%)	74 (39.2%)	321 (66.7%)	0.319	0.898	0.0001*	10.2
Right OCPT-B	247 (84.6%)	106 (56.1%)	353 (73.3%)	0.444	0.803	0.0001*	19.7
Left OCPT-B	242 (82.9%)	98 (51.9%)	340 (70.6%)	0.421	0.823	0.0001*	17.7
Right OCAT-O	252 (86.3%)	93 (49.2%)	345 (71.7%)	0.421	0.823	0.0001*	17.7
Left OCAT-O	249 (85.3%)	101 (53.4%)	350 (72.7%)	0.463	0.785	0.0001*	21.4
Right OCPT-O	261 (89.4%)	52 (27.5%)	313 (65.0%)	0.244	0.94	0.0001*	6
Left OCPT-O	246 (84.2%)	77 (40.7%)	323 (67.1%)	0.32	0.898	0.0001*	10.2

*$p<0.05$ statistical significance difference; CCC: Canonical Correlation Coefficient, Wilk's Lambda coefficient

With the exception of the right SCA, the left SCA, total SICA, the right BCI and the left BCI, the multivariate discriminant function analysis was performed by creating different models with variables (Table 3).

Table 3. Multivariate discriminant function analysis

Functions	Variables	Sexing Accuracy Rates		CCC	Wilks' Lambda	*p*	Explained Variance (%)
		Male (n=292) n (%)	Female (n=189) n (%)				
Function 1	right OCL, left OCL, right OCW, left OCW	240 (82.2%)	113 (59.8%)	0.52	0.729	0.0001*	27.1
Function 2	right OCL, left OCL, right OCW, left OCW, right OCH, left OCH	242 (82.9%)	112 (59.3%)	0.523	0.726	0.0001*	27.4
Function 3	AID, PID, MaxID, MinID, MaxBD	244 (83.6%)	100 (52.9%)	0.474	0.775	0.0001*	22.5
Function 4	right OCAT-B, left OCAT-B, right OCPT-B, left OCPT-B	237 (81.2%)	115 (60.8%)	0.5	0.75	0.0001*	25
Function 5	right OCAT-O, left OCAT-O, right OCPT-O, left OCPT-O	246 (84.2%)	102 (54%)	0.481	0.769	0.0001*	23.1
Function 6	right OCAT-B, left OCAT-B, right OCPT-B, left OCPT-B, right OCAT-O, left OCAT-O, right OCPT-O, left OCPT-O	241 (82.5%)	123 (65.1%)	0.562	0.684	0.0001*	31.6
Function 7	right OCL, right OCW, right OCH	240 (82.2%)	118 (62.4%)	0.493	0.757	0.0001*	24.3
Function 8	left OCL, left OCW, left OCH	239 (81.8%)	114 (60.3%)	0.501	0.749	0.0001*	25.1
Function 9	right OCAT-B, right OCPT-B, right OCAT-O, right OCPT-O	243 (83.2%)	108 (57.1%)	0.537	0.711	0.0001*	28.9
Function 10	left OCAT-B, left OCPT-B, left OCAT-O, left OCPT-O	238 (81.5%)	119 (63%)	0.541	0.708	0.0001*	29.2
Function 11	right OCL, right OCW, right OCH, right OCAT-B, right OCPT-B, right OCAT-O, right OCPT-O	244 (83.6%)	127 (67.2%)	0.576	0.668	0.0001*	33.2
Function 12	left OCL, left OCW, left OCH, left OCAT-B, left OCPT-B, left OCAT-O, left OCPT-O	245 (83.9%)	130 (68.8%)	0.598	0.643	0.0001*	35.7
Function 13	right OCL, right OCW, right OCH, AID, PID, MaxID, MinID, MaxBD, right OCAT-B, right OCPT-B, right OCAT-O, right OCPT-O	246 (84.2%)	127 (67.2%)	0.587	0.655	0.0001*	34.5

Table 3. Multivariate discriminant function analysis (continued)

Functions	Variables	Sexing Accuracy Rates		CCC	Wilks' Lambda	*p*	Explained Variance (%)
		Male (n=292) n (%)	Female (n=189) n (%)				
Function 14	left OCL, left OCW, left OCH, AID, PID, MaxID, MinID, MaxBD, left OCAT-B, left OCPT-B, left OCAT-O, left OCPT-O	243 (83.2%)	130 (68.8%)	0.61	0.628	0.0001*	37.2
Function 15	AID, PID, MaxID, MinID, MaxBD, left OCL, left OCW, left OCH, left OCAT-B, left OCPT-B, left OCAT-O, left OCPT-O, right OCL, right OCW, right OCH, right OCAT-B, right OCPT-B, right OCAT-O, right OCPT-O	247 (84.6%)	130 (68.8%)	0.622	0.613	0.0001*	38.7

*p<0.05 statistical significance difference; CCC: Canonical Correlation Coefficient, Wilk's Lambda coefficient

The multivariate analysis showed an accuracy percentage ranging from 81.2% to 84.6% in males and from 52.9% to 68.8% in females. Function 15 had the highest accuracy in classifying sex (males: 84.6%; females: 68.8%), followed by functions 14, 13, 12 and 11, respectively. The constants and variable coefficients of these functions are shown in Table 4.

Table 4. The discriminant equations coefficients for functions 11-15

	Function 11		Function 12		Function 13		Function 14		Function 15	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
AID	-	-	-	-	-0.752	-0.845	-0.061	-0.233	-1.063	-1.190
PID	-	-	-	-	-0.825	-0.872	-0.505	-0.475	-2.231	-2.181
MaxID	-	-	-	-	2.842	2.728	3.121	2.980	3.257	3.100
MinID	-	-	-	-	1.749	1.719	1.132	1.140	1.941	1.912
MaxBD	-	-	-	-	0.945	0.934	0.633	0.659	0.378	0.433
Left OCL	-	-	0.659	0.206	-	-	1.171	0.618	1.385	0.948
Left OCW	-	-	5.961	5.590	-	-	6.790	6.367	5.012	4.646
Left OCH	-	-	3.116	3.072	-	-	2.888	2.866	-0.659	-0.574
Left OCAT-B	-	-	1.755	1.337	-	-	0.089	-0.144	0.684	0.474

Table 4. The discriminant equations coefficients for functions 11-15 (continued)

	Function 11		Function 12		Function 13		Function 15		Function 15	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Left OCPT-B	-	-	3.807	3.902	-	-	2.294	2.476	1.069	1.312
Left OCAT-O	-	-	3.759	3.627	-	-	3.086	3.008	0.316	0.312
Left OCPT-O	-	-	1.220	1.035	-	-	0.775	0.566	1.931	1.630
Right OCL	0.585	0.218	-	-	0.995	0.564	-	-	0.114	-0.078
Right OCW	5.502	5.235	-	-	6.144	5.856	-	-	3.307	3.201
Right OCH	4.662	4.580	-	-	4.781	4.703	-	-	5.165	5.066
Right OCAT-B	2.078	1.695	-	-	0.459	0.233	-	-	0.002	-0.068
Right OCPT-B	4.809	4.718	-	-	3.319	3.384	-	-	3.910	3.781
Right OCAT-O	2.973	2.847	-	-	2.389	2.292	-	-	1.886	1.843
Right OCPT-O	1.461	1.384	-	-	1.243	1.208	-	-	0.732	0.821
Constant	-230.074	-203.096	-223.198	-194.799	-255.805	-226.806	-248.955	-218.424	-276.174	-243.940

When comparing the results obtained by calculating the values of the relevant variables by substituting them into the two equations given separately for male and female, the larger result indicates the estimated sex. The discriminant function equation for function 15 with the highest value of explanatory variance:

Male= -276.174 – 1.063(AID) - 2.231(PID) + 3.257(MaxID) + 1.941(MinID) – 0.378(MaxBD) + 1.385(left OCL) + 5.012(left OCW) – 0.659(left OCH) + 0.684(left OCAT-B) + 1.069(left OCPT-B) + 0.316(left OCAT-O) + 1.931(left OCPT-O) + 0.114(right OCL) + 3.307(right OCW) + 5.165(right OCH) + 0.002(right OCAT-B) + 3.910(right OCPT-B) + 1.886(right OCAT-O) + 0.732(right OCPT-O)

Female= -243.940 – 1.190(AID) - 2.181(PID) + 3.100(MaxID) + 1.912(MinID) – 0.433(MaxBD) + 0.948(left OCL) + 4.646(left OCW) – 0.574(left OCH) + 0.474(left OCAT-B) + 1.312(left OCPT-B) + 0.312(left OCAT-O) + 1.630(left OCPT-O) – 0.078(right OCL) + 3.201(right OCW) + 5.066(right OCH) – 0.068(right OCAT-B) + 3.781(right OCPT-B) + 1.843(right OCAT-O) + 0.821 (right OCPT-O)

Discussion

There are many studies that have performed morphometrical analysis of the occipital condyles for anatomical, clinical and forensic purposes [8, 12, 13-24]. In the literature, morphometric evaluation of the occipital condyle has been performed on the skull [12, 13, 15-20], on CT images of the skull [21, 24] and only in a few studies on 3D CT models [8, 14]. In the study by Avcı et al. [25], measurements were taken from both bone tissue and 3D CT images of the dry skull and the results were compared. In this study, occipital condyles were analysed for sex differences in 3D models of carotid angio-CT images of living subjects.

In this study, sexual dimorphism was followed in the parameters of the OC, except for the left SCA. The measurement values of all parameters with sexual dimorphism (except right SCA, total SICA, right and left BCI) were higher in males than in females (p<0.05). These results were found to be similar to most studies in the literature [8, 12, 13-18, 20-22, 24]. However, in the study by Natsis et al. [19], there was no statistically significant difference between sex and right and left occipital condyle width. There was no statistically significant difference between left OCW and sex in the study by Abo El Atta et al. [23]. Çiçekçibaşı et al. [13] found no statistically significant difference in mean total SICA between sexes.

Comparison of occipital condyle measurements in different populations is shown in Table 5. When the mean values of the occipital condyle measurements are examined, it can be seen that they are similar to many population studies [8, 12, 13, 15, 17, 18-21]. However, our results also differed from those of some populations [14, 16, 22-24]. It has been suggested that the differences between the mean values of the same variables in different studies may be due to reasons such as methodological differences and population differences.

In our study, the right OCPT-B was the best measure for sex estimation in univariate discriminant function analyses with an accuracy rate of 73.3%. In the multivariate discriminant function analyses, the correct classification percentages of the models with the highest variance explanation value were between 77.1% and 78.4%, ranging from 81.2% to 84.6% for males and 52.9% to 68.8% for females. Aljarrah et al. [24] reported that multivariate analysis including all eight variables of FM and OC predicted sex with an accuracy of 71.6% (73.3% for males and 69.9% for females). In a study by Gapert et al. (2009) [12], the most successful single variable for predicting sex was the maximum bicondylar distance with 69.2%. In a multivariate discriminant function analysis, the highest accuracy rate (76.7%, male: 72%, female: 81.7%) was obtained in the model including the variables left OC length, right OC width and minimum intercondylar distance [12]. In the study by Macaluso et al. [15], the most effective single variable for sex prediction was the maximum bicondylar distance with 67.6% (male: 61.1%, female: 75%). A stepwise analysis using the maximum length of the left OC and the minimum distance between OCs showed the highest classification accuracy of 67.7% (male: 68.6% - female: 66.7%) [15]. In El Barrany et al. [21], discriminant function analyses showed that the minimum intercondylar distance, right OC length and foramen magnum width were the main variables in sex prediction. In the function including these variables, the correct prediction rate was found to be 84.3% in all cases, 81% in males and 87.5% in females [21]. Abo El Atta et al. [23] reported that the most significant discriminating variables in the prediction of sex were right OC length and foramen magnum width. They found that the sex prediction accuracy rate was 66.5% (male: 63.2%- female: 69.1%) for right OC length. In a multivariate discriminant function analysis, they achieved a correct classification rate of 70.9% (male: 59.3%- female: 80.2%) [23]. In Madadin et al. [22], correct sex classification in univariate and multivariate discriminant function analysis ranged from 51% to 71%. The highest correct sex classification rate (male 70%, female 72%, total 71%) was obtained in the model that included the variables of right and left OC length and width and maximum bicondylar distance [22]. In the study by Singh and Talwar [26], the accuracy of predicting sex on the basis of discriminant function analysis was reported to be between 66% and 70%. Uysal et al. [27] reported an accuracy rate of 81% for sex determination using the model including right OCL and OCW and FM width.

In conclusion, in the present study, morphometric analysis of OCs in the contemporary Turkish population revealed sexual dimorphism in all parameters except the left sagittal condylar angle. In the multivariate functional analysis, the correct sex prediction rate of the occipital condyles was 77.1% to 78.4% (81.2% to 84.6% in males and 52.9% to 68.8% in females). This study showed that measurements from 3D CT images of occipital condyles can be used for sex estimation. Thus, 3D modelling of cranial CT images will help to create databases to study population-specific differences in contemporary societies and to use these data for sex determination in forensic investigations, as it provides the opportunity to examine the skulls of living individuals with known sex, age and medical history.

Table 5. Comparison of the measurements of the occipital condyles in the present study with the results of other studies

Studies	Population	Material	Right OCL		Left OCL		Right OCW		Left OCW		Right OCH		Left OCH	
			M	F	M	F	M	F	M	F	M	F	M	F
Present study	Turkish	3DCT	25.55±2.24	23.29±1.97	25.51±2.46	23.22±1.93	11.69±1.38	11.11±1.25	11.9±1.33	11.19±1.41	10.0±1.46	9.66±1.22	10.08±1.46	9.50±1.24
Çiçekçibaşı et al. [13]	Turkish	Dry skull	25.13±2.36	23.44±1.90	24.82±2.43	23.17±1.89	-	-	-	-	-	-	-	-
Gapert et al. [12]	British	Dry skull	24.95±2.53	23.30±2.28	25.16±2.51	23.74±2.44	12.01±1.41	11.42±1.21	12.05±1.69	11.57±1.16	-	-	-	-
Macaluso et al. [15]	France	Dry skull	24.62±2.65	22.99±2.28	24.99±3.09	22.88±2.69	12.30±1.27	11.59±1.03	12.25±1.51	11.57±1.09	-	-	-	-
Oliviera et al. [18]	Brazil	Dry skull	26.74±2.96	25.45±3.21	26.85±2.97	24.65±3.23	13.51±1.38	12.68±1.56	13.79±1.39	12.71±1.75	-	-	-	-
Natsis et al. [19]	Greek	Dry skull	26.30±2.92	24.70±2.66	26.48±2.80	24.57±2.13	13.13±2.01	13.04±1.99	13.24±2.20	12.74±1.63	-	-	-	-
Lyrtzis et al. [20]	Greek	Dry skull	24.33±2.57	22.95±2.96	24.07±2.59	23.23±2.71	12.10±1.50	11.43±1.47	12.21±1.66	11.46±1.51	-	-	-	-
Kumar et al. [17]	Indian	Dry skull	23.88±1.50	22.60±1.30	24.99±1.82	24.20±1.62	12.97±1.43	12.65±1.33	14.11±1.01	13.85±1.02	8.64±0.74	6.92±0.72	9.32±0.78	9.21±0.76
Kalthur et al. [16]	Indian	Dry skull	22.8±2.5	21.4±2.9	22.9±2.4	21.6±2.6	10.5±1.8	12.0±2.3	10.8±2.4	12.2±2.6	-	-	-	-
Singh and Talwar [26]	Indian	Dry skull	-	-	-	-	-	-	-	-	-	-	-	-
El- Barrany et al. [21]	Sudanese	CT	25.52±2.68	21.41±2.05	25.40±3.04	21.50±2.19	11.39±1.51	10.62±1.34	11.33±1.71	10.38±1.33	-	-	-	-
Madadin et al. [22]	Saudi Arabian	CT	21.10±1.55	19.94±1.81	21.11±1.72	20.05±1.82	10.58±1.08	10.27±1.30	10.72±1.16	10.48±1.31	-	-	-	-
Abo El-Atta et al. [23]	Egyptian	CT	20.8±2.4	18.9±2.5	20.7±2.4	19.7±2.2	11.6±1.3	11.1±1.4	11.2±1.3	11.3±1.2	-	-	-	-
Aljarrah et al. [24]	Saudi Arabian	CT	22.02±2.2	20.9±2	21.3±2	20.2±1.9	11.3±1.40	10.99±1.41	11.43±1.36	11.2±1.37	-	-	-	-
Gümüşsoy et al. [14]	Turkish	CT -3DCT	21.0±1.8	18.8±1.7	20.5±2.0	18.9±1.6	10.7±1.2	9.9±1.1	10.9±1.4	10.2±1.2	9.5±2.0	8.9±1.2	9.4±1.4	8.7±1.0
Abdel-Karim et al. [8]	Egyptian	3DCT	26.91±2.41	24±1.33	27.09±2.55	23.67±1.43	12.22±1.33	11.13±1.11	11.91±1.20	10.75±1.18	-	-	-	-

OCL: Occipital condyle lenght, OCW: Occipital condyle width, OCH: Occipital condyle height, AID: Anterior intercondylar distance, PID: Posterior intercondylar distance, MaxID: Maximum intercondylar distance MinID: Minimum intercondylar distance, MaxBD: Maximum bicondylar distance, M: male, F: female

Table 5. Comparison of the measurements of the occipital condyles in the present study with the results of other studies (continued)

Studies	Population	Material	AID		PID		MaxID		MinID		MaxBD	
			M	F	M	F	M	F	M	F	M	F
Present study	Turkish	3DCT	22.77±2.75	21.09±2.45	45.08±3.46	42.29±3.59	33.92±2.37	32.01±2.31	21.35±2.48	19.91±2.19	51.25±3.13	48.13±3.18
Çiçekçibaşı et al. [13]	Turkish	Dry skull	16.09±1.93	14.68±1.80	-	-	-	-	-	-	-	-
Gapert et al. [12]	British	Dry skull	-	-	-	-	36.82±3.10	35.12±3.09	21.12±3.18	19.0±2.40	51.29±2.97	48.67±3.17
Macaluso et al. [15]	France	Dry skull	-	-	-	-	37.46±3.54	36.78±3.69	20.63±3.18	19.07±2.14	51.32±3.70	48.73±3.27
Oliviera et al. [18]	Brazil	Dry skull	-	-	-	-	-	-	-	-	-	-
Natsis et al. [19]	Greek	Dry skull	19.82±3.19	18.77±3.26	52.8±4.93	50.13±4.71	-	-	-	-	-	-
Lyrtzis et al. [20]	Greek	Dry skull	21.17±2.71	20.05±2.45	43.36±3.35	41.23±3.30	-	-	-	-	-	-
Kumar et al. [17]	Indian	Dry skull	17.63	17.30	-	-	-	-	-	-	-	-
Kalthur et al. [16]	Indian	Dry skull	21±03	22±03	38±3	39±3	26±3	25±2	-	-	45±4	46±3
Singh and Talwar [26]	Indian	Dry skull	-	-	--	-	26.15±3.31	24.71±4.57	14.88±2.26	14.33±2.56	46.73±2.79	44.29±2.34
El- Barrany et al. [21]	Sudanese	CT	-	-	-	-	27.20±2.74	25.46±2.50	10.49±2.63	9.62±2.15	48.90±4.73	46.89±4.02
Madadin et al. [22]	Saudi Arabian	CT	-	-	-	-	-	-	-	-	43.67±2.93	43.45±3.77
Abo El-Atta et al. [23]	Egyptian	CT	-	-	-	-	28.9±5.9	27.2±4.9	15.4±9.0	13.7±3.1	-	-
Aljarrah et al. [24]	Saudi Arabian	CT	-	-	-	-	-	-	-	-	-	-
Gümüşsoy et al. [14]	Turkish	CT -3DCT	21.6± 1.1	20.3±1.8	44.6±1.7	43.4±2.2	-	-	-	-	-	-
Abdel-Karim et al. [8]	Egyptian	3DCT	-	-	-	-	31.57±3.27	30.42±2.10	7.22±1.33	6.83±0.86	51.09±3.35	49.54±3.03

OCL: Occipital condyle lenght, OCW: Occipital condyle width, OCH: Occipital condyle height, AID: Anterior intercondylar distance, PID: Posterior intercondylar distance
MaxID: Maximum intercondylar distance, MinID: Minimum intercondylar distance, MaxBD: Maximum bicondylar distance, M: male, F: female

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Illuminating the thymus mystery in pediatric CT studies

Pediatrik BT çalışmalarında timusun gizemini aydınlatmak

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Abstract

Purpose: This study aims to provide a comprehensive assessment of thymic morphology, attenuation, and anatomical variations in pediatric patients using modern multi-detector computed tomography (MDCT). Additionally, it seeks to establish reference values and analyze the factors influencing thymic density.

Materials and methods: A retrospective analysis was conducted on 622 pediatric chest CT scans obtained between 2017 and 2024. Thymic shape, contour, density, and size parameters were evaluated. Thymic attenuation was graded using a standardized scoring system, and measurements of thymic dimensions were recorded. Statistical analyses were performed to assess correlations between thymic density, age, gender, and morphological characteristics.

Results: Thymic morphology exhibited significant variation, with quadrilateral shape being the most common (42.1%), followed by round-oval (29.7%) and triangular (28.1%). Thymic attenuation showed no significant correlation with age (p=0.156) or gender (p=0.191). Regression analysis revealed a negative association between anteroposterior diameter and thymic density (β=-0.4019, p=0.015), while transverse diameter was positively correlated with thymic density (β=0.5465, p<0.001). No significant association was found between thymic shape, contour, localization, and thymic attenuation.

Conclusion: This study provides a detailed evaluation of normal thymic imaging characteristics in pediatric patients, offering reference values for radiologists. Recognizing thymic variability across different age groups is essential to avoid misdiagnosis and unnecessary interventions. Future research should focus on longitudinal studies and advanced imaging techniques to refine diagnostic criteria.

Keywords: Thymus, pediatric imaging, MDCT, thymic density, thymic morphology.

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Öz

Amaç: Bu çalışma, modern çok kesitli bilgisayarlı tomografi (MDCT) kullanarak pediatrik hastalarda timusun morfolojisi, dansitesi ve anatomik varyasyonlarını kapsamlı bir şekilde değerlendirmeyi amaçlamaktadır. Ayrıca, referans değerler belirlemeyi ve timus dansitesini etkileyen faktörleri analiz etmeyi hedeflemektedir.

Gereç ve yöntem: 2017 ile 2024 yılları arasında elde edilen 622 pediatrik göğüs BT taraması retrospektif olarak analiz edildi. Timusun şekli, konturu, dansitesi ve boyut parametreleri değerlendirildi. Timus dansitesi standart bir skorlama sistemi kullanılarak sınıflandırıldı ve timusun boyutsal ölçümleri kaydedildi. Timus dansitesi ile yaş, cinsiyet ve morfolojik özellikler arasındaki ilişkileri değerlendirmek için istatistiksel analizler yapıldı.

Bulgular: Timus morfolojisinde belirgin farklılıklar gözlendi; en yaygın şekil dörtgen (%42,1) olup, bunu yuvarlak-oval (%29,7) ve üçgen (%28,1) şekiller takip etti. Timus dansitesi ile yaş (p=0,156) veya cinsiyet (p=0,191) arasında anlamlı bir korelasyon saptanmadı. Regresyon analizinde, ön-arka çap ile timus dansitesi arasında negatif bir ilişki (β=-0,4019, p=0,015) bulunurken, transvers çap ile timus dansitesi arasında pozitif bir ilişki (β=0,5465, p<0,001) gözlendi. Timus şekli, konturu ve lokalizasyonu ile timus dansitesi arasında anlamlı bir ilişki bulunmadı.

Sonuç: Bu çalışma, pediatrik hastalarda normal timus görüntüleme özelliklerini ayrıntılı olarak değerlendirerek radyologlar için referans değerler sunmaktadır. Farklı yaş gruplarındaki timus varyasyonlarının tanınması, yanlış tanıların ve gereksiz girişimlerin önlenmesi açısından kritiktir. Gelecekteki araştırmaların, uzunlamasına çalışmalar ve ileri görüntüleme tekniklerini içerecek şekilde tanı kriterlerini daha da geliştirmeye odaklanması gerekmektedir.

Anahtar kelimeler: Timus, pediatrik görüntüleme, MDCT, timus dansitesi, timus morfolojisi.

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Introduction

The thymus is a key endocrine organ located in the mediastinum, primarily involved in T-cell development and immune function. Its appearance on computed tomography (CT) varies significantly with age, posing a challenge in distinguishing normal from pathological thymic characteristics. However, evaluating a normal thymus via CT can be challenging, as its morphology and size can vary significantly, even among children of the same age. The thymus is a bilobed structure encapsulated in connective tissue and positioned anterior to the ascending aorta, pulmonary outflow tract, and superior vena cava, while posterior to the sternum [1-4]. Initially presenting as a soft-tissue density with quadrilateral contours, it gradually transitions into a triangular configuration with concave or straight borders as age progresses [5].

It is the first lymphoid organ to form and grows significantly during infancy [6]. The thymus reaches its peak size during adolescence, weighing between 20-50 grams, before undergoing physiological involution, where fat replaces thymic parenchyma, reducing its size to 5-15 grams in adulthood [7]. The density of the thymus on computed tomography (CT), measured in Hounsfield Units (HU), has also been noted to decrease with age, from 80 HU to 56 HU, probably as a result of fat infiltration and cellular regression [8]. This involution is more pronounced in males than females but remains a dynamic process, as the thymus retains its regenerative capacity across all ages [9, 10]. Stress-related factors, such as chemotherapy, can lead to thymic rebound hyperplasia, where the gland temporarily enlarges beyond expected limits [11, 12].

The thymic shape shows significant variation even within the same age group [10]. Due to the dynamic changes the thymus undergoes in pediatric patients, a thorough understanding of its anatomy is crucial for radiologists to achieve accurate diagnoses [5]. Despite advancements in imaging, radiologists often misinterpret normal thymic variations, leading to unnecessary interventions such as biopsies or thymectomy [13, 14].

Currently, no standardized guidelines exist for pediatric thymic evaluation via CT, leaving interpretation reliant on expert consensus. Previous studies have examined thymic morphology and attenuation using earlier-generation CT scanners or limited sample sizes [8, 15-17]. Our study, leveraging modern multi-detector CT (MDCT) and a larger cohort, provides a comprehensive assessment of thymic imaging characteristics in children.

Methods

Study population and data collection

Permission was obtained from Pamukkale University Non-Interventional Clinical Research Ethics Committee for the study (approval dated: 21.01.2025, approval number: E-60116787-020-643305).

Pediatric chest CT scans from January 2017 to October 2024 were reviewed using the hospital's electronic Picture Archiving and Communication System (PACS).

Among 1666 initial chest CT scans, 570 were non-contrast, 42 were repeat scans, and 99 were excluded due to poor image quality. After excluding cases with congenital or acquired thoracic pathology, malignancies, or trauma, 622 patients (226 girls, mean age 9.97±5.16 years; 396 boys, mean age 10.86±5.16 years) were included in the final analysis. Patients were categorized into six age groups: 0-12 months, 1-3 years, 4-6 years, 7-10 years, 11-14 years, and 15-18 years.

CT protocol

All CT scans were acquired using a 64-slice MDCT scanner (Philips Ingenuity 128, Philips Healthcare, Amsterdam, the Netherlands) with parameters optimized for pediatric imaging, including the following: matrix, 512x512; field of view, 250 mm; gantry rotation time, 0.625 s; slice collimation, 0.625 mm; pitch, 1; interslice gap, 0 mm. Scans were performed at 80-120 kV, tube currents of 20-150 mA, and a slice thickness of 0.5 mm. Intravenous contrast media (1–2mL/kg) was administered in applicable cases. Two radiologists (G.G. and N.P., with 13 and 2 years of experience) independently reviewed the scans on PACS.

Evaluation of the imaging findings of thymus

Thymic fat content was graded using the Ackman et al. [18] scoring system (0-3 scale), and CT attenuation values were measured using a region of interest (ROI) covering the maximum thymic area. In this system, grade 0 represents complete fatty replacement of the thymus, with no visible soft tissue. Grade 1 indicates a predominantly fatty thymus. Grade 2 signifies a thymus with approximately equal amounts of fatty and soft tissue attenuation. Grade 3 is characterized by the thymus having an attenuation similar to that of muscle and soft tissue. Efforts were made to avoid measurements from areas with streak or beam hardening artifacts or other tissues such as surrounding mediastinal fat or large blood vessels to prevent a false decrease or increase in the ROI.

The thymus shape was classified as round-oval, quadrilateral, or triangular, while its lateral borders were described as convex, concave, or straight. When the thymic border showed a combination of these contours, it was labeled as “mixed. Thymic lateral dominance was also recorded as right-sided, left-sided, or midline (Figure 1).

The thymus’s maximum anteroposterior (AP) diameter was recorded at the thickest section along the midline, marking the division between the right and left lobes. The maximum thickness of each thymus lobe at the level of the mid-gland was measured as previously recommended by Baron et al. [1] The thymus’s maximum transverse diameter was calculated by measuring the greatest distance between the external boundaries of its lobes. Each lobe’s maximum thickness was assessed by taking a perpendicular measurement from its long axis to the most lateral edge of the gland (Figure 2).

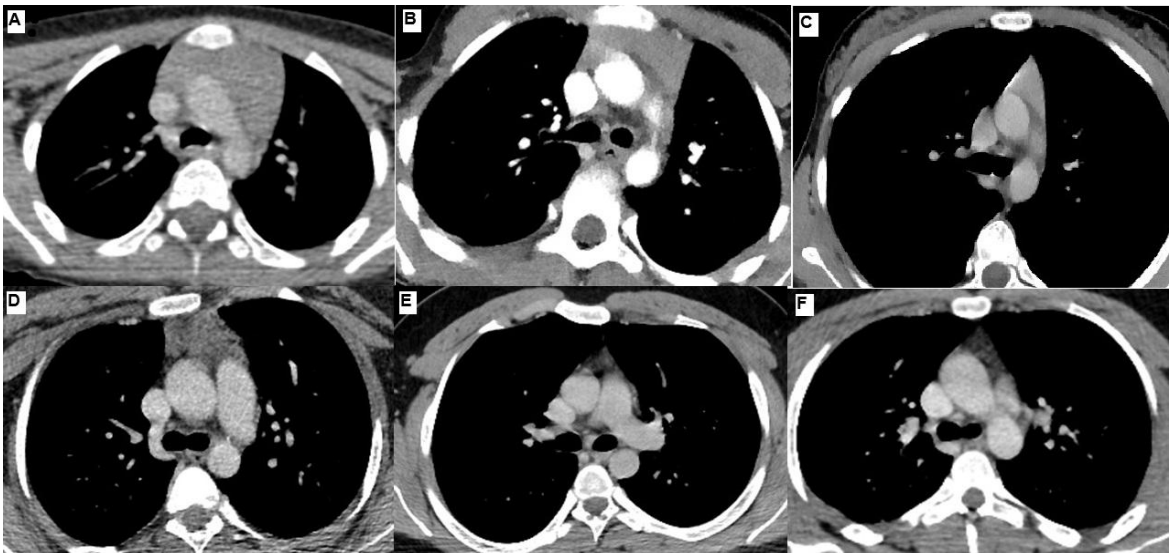

Figure 1. Axial contrast-enhanced thoracic CT images demonstrating different thymic morphologies in pediatric patients. (A) A 1-year-old girl with a round-oval thymus, biconvex margins, is predominantly left-sided; Score 3 indicates a primarily soft-tissue attenuated thymus. (B) A 7-year-old boy presented with a quadrilateral thymic shape, straight margins, and central positioning; Score 3, reflecting a soft-tissue dominant thymic composition. (C) 11-year-old girl with a triangular thymic structure, straight contours, predominantly left-sided placement; Score 3, representing a mostly soft-tissue attenuated thymus. (D) 14-year-old girl exhibiting a quadrilateral thymus with mixt margins (right straight, left convex), centrally located; Score 2, displaying an approximately equal mix of fatty and soft-tissue attenuation. (E) 16-year-old boy with a triangular thymus, straight margins, centrally positioned; Score 1, characterized by a predominantly fatty thymus. (F) 17-year-old boy featuring a triangular thymus, straight contours, centrally located; Score 0, denoting complete fatty replacement of the thymus

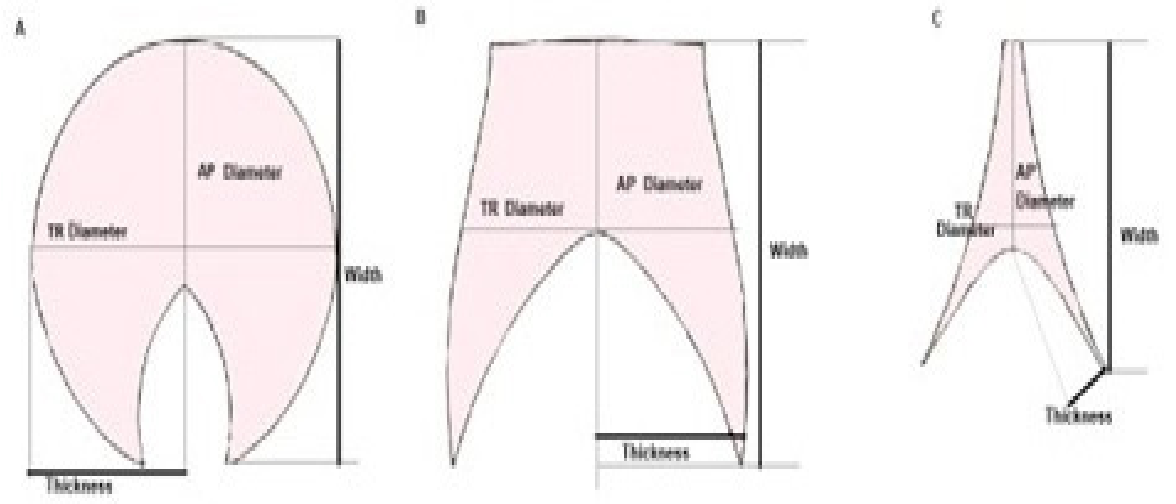

Figure 2. The diagram illustrates the measurements of various thymic configurations, including (A) round-oval, (B) quadrilateral, and (C) triangular shapes. It highlights the maximum anteroposterior (AP) and transverse (TR) diameters, as well as the greatest width and thickness of the thymic lobes for each morphological variant

Statistical analysis

A priori power analysis was conducted to determine the adequacy of the sample size. The required sample sizes for different effect sizes were calculated based on an alpha level of 0.05 and a desired power of 0.80. For an independent samples t-test, the necessary sample sizes were 788 for a small effect (Cohen's d=0.2), 128 for a medium effect (Cohen's d=0.5), and 52 for a large effect (Cohen's d=0.8). For ANOVA comparisons, the required sample sizes were 392 for a small effect, 78 for a medium effect, and 32 for a large effect. Given that our study included 622 participants, the sample size was sufficient to detect medium and large effect sizes with a power of at least 0.80.

All statistical analyses were performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA). The normality of continuous variables was assessed using the Kolmogorov-Smirnov test. Normally distributed data were presented as mean ± standard deviation (SD) and compared using the independent samples t-test for two-group comparisons and one-way analysis of variance (ANOVA) for multiple-group comparisons, followed by post hoc analyses when necessary. Non-normally distributed data were expressed as median [interquartile range (IQR)] and analyzed using the Mann-Whitney U test for two-group comparisons and the Kruskal-Wallis test for multiple-group comparisons.

Correlations between continuous variables were assessed using Spearman's rank correlation test. The association between categorical variables was evaluated using the Chi-square test and reported as frequencies and percentages. To identify independent predictors of thymic density, multiple linear regression analysis was performed, adjusting for age, sex, and thymic size parameters. A *p*-value of less than 0.05 was considered statistically significant.

Results

A total of 622 pediatric patients were included in the study, consisting of 322 males (51.8%) and 300 females (48.2%). The mean age of the study population was 8.4±3.2 years.

Thymic density and visual scoring

There was no significant correlation between thymic density and visual thymic scoring (Spearman's rho=0.010, *p*=0.802). Although mean thymic density increased with higher thymic scores (Table 1), one-way ANOVA did not show a statistically significant difference among the groups (F=0.471, *p*=0.703).

Table 1. Thymic density and visual scoring

	Thymic score	Mean Density (HU)	Std Dev	ANOVA F-value	*p* value (ANOVA)
1	0	30.2	7.9	0.471	0.703
2	1	35.6	8.3	0.471	0.703
3	2	40.8	9.7	0.471	0.703
4	3	45.2	10.5	0.471	0.703

Spearman's correlation analysis was used to evaluate the relationship between thymic density and visual scoring (rho=0.010, p=0.802), while p-values in the table were derived from Spearman's correlation and one-way ANOVA

Age and gender-related variations

Thymic density was slightly higher in males (42.1±9.8 HU) than in females (40.3±8.9 HU), but this difference was not statistically significant (p=0.191, independent samples t-test). Age-related trends were also not significant (p=0.157, Kruskal-Wallis test), suggesting thymic density does not decline uniformly across pediatric populations (Table 2).

Table 2. Thymic density across age groups

	Age Group	Mean Density (HU)	Std Dev	H value	*p* value
1	0-12 months	59.93	32.68	7.986	0.157
2	1-3 years	64.39	27.14		
3	4-6 years	69.67	26.00		
4	7-10 years	68.28	29.57		
5	11-14 years	69.16	24.06		
6	15-18 years	62.86	26.98		

p-values are derived from the Kruskal-Wallis test. No statistically significant difference was found among the age groups (p=0.157)

Size-related parameters and their association with thymic density

Regression analysis demonstrated a significant association between thymic density and some anatomical dimensions of the gland. AP diameter was negatively correlated with thymic density (β=-0.4248, p=0.010). Transverse diameter showed a positive correlation with thymic density (β=0.5450, p=0.001). Right lobe thickness (β=0.5984, p=0.073) showed a trend towards significance but did not reach statistical significance. Left lobe thickness (β=-0.0731, p=0.847) was not significantly associated with thymic density (Table 3).

Thymic shape, contour, and localization

The thymic shape, contour, and localization were assessed for their potential impact on thymic density. No significant association was found between thymic shape and thymic density (χ^2=3.62, p=0.727). Although thymic contour types did not significantly affect thymic density (χ^2=19.61, p=0.075), the trend suggests a potential association that may warrant further investigation. Thymic localization was not significantly associated with thymic density (χ^2=15.06, p=0.238). These findings suggest that thymic density is primarily influenced by gland size rather than its morphological characteristics, age, or sex differences. Among these factors, thymic contour showed the closest trend toward significance, suggesting a potential relationship (Table 4).

Table 3. Regression analysis results

Variable	Beta Coefficient (β)	Standard Error	t-value	*p* value	95% CI Lower	95% CI Upper
AP diamater	-0.4248	0.1647	-2.5789	*0.015	-0.7483	-0.1013
Transverse diamater	0.5450	0.1559	3.4941	< 0.001	0.2387	0.8513
RL Thickness	0.5984	0.3329	1.7974	0.097	-0.0554	1.2522
LL Thickness	-0.0730	0.3780	-0.1933	0.876	-0.8154	0.6692

Multiple linear regression analysis was conducted to determine independent predictors of thymic density. AP: Anteroposterior, RL: Right lobe, LL: Left lobe

Table 4. Shape, contour, and localization analysis

	Variable	Chi-square (χ^2)	Degrees of Freedom	*p* value
1	**Thymic Shape** Rectangular: 262 (42.1%) Oval: 185 (29.7%) Triangular: 175 (28.1%)	3.62	6	0.727
2	**Thymic Contour** Biconvex: 191 (30.8%) Biconcave: 99 (15.9%) Straight: 133 (21.4%) Mixed: 198 (31.9%)	19.61	12	0.075
3	**Thymic Localization** Predominantly right: 276 (44.5%) Predominantly left: 226 (36.5%) Midline: 118 (19.0%)	15.06	12	0.238

Chi-square test was used to evaluate the association between thymic shape, contour, localization, and thymic density

Discussion

Our findings highlight considerable variability in thymic morphology and density across pediatric age groups.

Thymic morphology and age-related changes

The most common thymic shape was quadrilateral (42.1%), followed by round-oval (29.7%) and triangular (28.1%). Younger children predominantly exhibited round-oval and quadrilateral thymuses, whereas older children more frequently displayed triangular configurations. These findings align with previous research indicating progressive thymic involution with age. Thymic margins also showed notable variability. Mixed margins were the most common (31.9%), followed by biconvex (30.8%) and straight (21.4%) margins, whereas biconcave borders were relatively rare (15.9%). The presence of biconvex margins in younger children and the predominance of straight margins in older individuals are consistent with the established pattern of thymic involution leading to glandular flattening and contour changes during adolescence [19].

Thymic position and gender differences

The thymus was predominantly located in a right-sided position (44.5%), followed by a left-sided position (36.5%) and midline placement (19.0%). Younger children (0–12 months) were

more likely to have a right- or left-sided thymus, whereas older children, particularly those over 15 years, exhibited more frequent midline positioning. These findings emphasize the importance of recognizing positional variations to avoid misinterpreting normal anatomy as mediastinal pathology.

Contrary to some previous studies, our findings did not reveal a statistically significant difference in thymic attenuation between male and female participants (p=0.191). Although hormonal influences have been suggested as a factor in thymic involution, our results indicate that sex may not be a primary determinant of thymic attenuation values [20]. Additionally, thymic scoring results did not show a significant difference between sexes, contradicting earlier reports that proposed a higher thymic attenuation and slower fat infiltration in females. This discrepancy may be due to differences in study populations, imaging protocols, or interobserver variability in thymic scoring.

Thymic attenuation and involution

Although thymic involution is a well-documented phenomenon, our study did not demonstrate a significant correlation between age and thymic density (p=0.156, Spearman correlation). This finding contrasts with previous literature that describes a steady decline in thymic attenuation with increasing age [8, 17]. One possible explanation for this discrepancy is the relatively narrow age range of our study population, which may not fully capture the complete spectrum of thymic involution observed in older individuals. Additionally, external factors such as physiological stress, chronic illness, or hydration status may contribute to individual variability in thymic density.

Size-related variations and their impact on thymic density

Regression analysis showed significant associations between thymic density and anatomical dimensions: AP diameter was inversely correlated with thymic density (beta=-0.4019, p=0.015), suggesting that larger thymuses exhibit more fat infiltration. Transverse diameter was positively correlated (beta=0.5465, p<0.001), indicating that wider thymuses retain more soft tissue. Right and left lobe thicknesses showed no significant associations (p>0.05).

These results highlight the importance of considering thymic shape and volumetric changes in radiological assessments. While traditional metrics such as thymic scoring and attenuation values provide useful information, our findings suggest that size-related parameters may be more reliable indicators of thymic composition than previously assumed.

Clinical implications and comparison with previous studies

This study highlights the importance of recognizing normal thymic variations in pediatric imaging to avoid misdiagnosis and unnecessary interventions. Our findings provide a reference for radiologists in differentiating typical thymic morphology from pathology. Future research should explore longitudinal changes and the impact of physiological stressors on thymic development.

Limitations and future directions

Despite the strengths of a large sample size and advanced MDCT technology, this study has limitations. As a retrospective analysis, selection bias may be present. Additionally, while known thymic abnormalities were excluded, subclinical variations cannot be ruled out. The lack of longitudinal follow-up limits understanding of progressive thymic changes. Future studies should establish standardized reference values and explore advanced imaging techniques such as dual-energy CT or MRI for better characterization of thymic composition.

In conclusion, this study provides a comprehensive evaluation of thymic morphology and density in pediatric patients using MDCT. Age-related changes in thymic shape and attenuation were observed, with size-related parameters influencing density. These findings serve as a valuable reference for radiologists, aiding in accurate assessment and reducing unnecessary interventions. Understanding the expected variations in thymic appearance across pediatric age groups is essential to prevent diagnostic errors. Future studies should refine these findings with longitudinal data and advanced imaging approaches to improve clinical interpretations.

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

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HSPB7 and tetranectin levels are associated with severity of COVID-19

HSPB7 ve tetranektin düzeyleri COVID-19'un şiddeti ile ilişkilidir

Özgen Kılıç Erkek, Gülşah Gündoğdu, Davut Akın, Melek Bor Küçükatay

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Abstract

Purpose: COVID-19 may have acute and chronic adverse effects on the cardiovascular system. Heat shock protein beta-7 (HSPB7) is a cardiovascular heat-shock protein, and tetranectin is a type-C calcium (Ca)-binding lectin. The present study was conducted to investigate the relationship between HSPB7, tetranectin, disease severity and myocardial injury in COVID-19.

Materials and methods: This study included 26 COVID-19 patients and 26 age and sex-matched healthy controls. Demographic characteristics, routine hemograms, and biochemical parameters were recorded. COVID-19 patients were classified as having mild to moderate and severe COVID-19 using clinical and laboratory data. HSPB7 and tetranectin levels were measured using commercial ELISA kits.

Results: C-reactive protein, fasting glucose, ferritin, neutrophil-to-lymphocyte, monocyte-to-lymphocyte, and platelet-to-lymphocyte ratios were significantly elevated, whereas calcium, and albumin were decreased in COVID-19 patients (p<0.05). Respiratory rate, D-dimer, and ferritin were higher while SO2 and lymphocyte counts were lower in severe COVID-19 patients (p<0.05). Serum HSPB7 levels were higher in COVID-19 patients vs healthy controls (p<0.01), whereas tetranectin concentration was lower (p<0.001). When the cases were evaluated according to the severity of the disease it was observed that, HSPB7 level was increased in patients with severe COVID-19 and tetranectin was decreased parallel to the severity of the disease (p<0.001 and p<0.001, respectively). HSPB7 concentration was positively correlated with ferritin (p=0.002). Tetranectin was negatively correlated with HSPB7, ferritin and troponin (p=0.041, p<0.01, and p=0.005, respectively).

Conclusion: The consequence of the present study indicates tetranectin as a potential biomarker for an accurate and more comprehensive understanding severity of cardiac damage in COVID-19 patients.

Keywords: COVID-19, HSPB7, tetranectin, cardiac injury.

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Öz

Amaç: COVID-19'un kardiyovasküler sistem üzerinde akut ve kronik olumsuz etkileri olabilmektedir. Isı şoku proteini beta-7 (HSPB7), kardiyovasküler bir ısı şoku proteinidir ve tetranektin, tip-C kalsiyum (Ca) bağlayıcı bir lektindir. Bu çalışmanın amacı, COVID-19'da HSPB7, tetranektin, hastalık şiddeti ve miyokardiyal hasar arasındaki ilişkiyi tespit etmektir.

Gereç ve yöntem: Bu çalışmaya 26 COVID-19 hastası ve 26 yaşı ile cinsiyeti eşleştirilmiş sağlıklı kontrol dahil edildi. Demografik özellikler, rutin hemogramlar ve biyokimyasal parametreler kaydedildi. COVID-19 hastalarının klinik ve laboratuvar verileri kullanılarak hafif, orta ve şiddetli COVID-19 hastası olarak sınıflandırıldı. HSPB7 ve tetranektin seviyeleri ticari ELISA kitleri kullanılarak ölçüldü.

Bulgular: COVID-19 hastalarında C-reaktif protein, açlık glukozu, ferritin, nötrofil-lenfosit, monosit-lenfosit ve trombosit-lenfosit oranları anlamlı olarak artarken, kalsiyum ve albümin ise azaldı (p<0,05). Şiddetli COVID-19 hastalarında solunum sayısı, D-dimer ve ferritin daha yüksekken, SO2 ve lenfosit sayıları daha düşüktü (p<0,05). Serum HSPB7 düzeyleri COVID-19 hastalarında sağlıklı kontrollere göre daha yüksekti (p<0,01), tetranektin düzeyi ise daha düşüktü (p<0,001). Hastalığın şiddetine göre değerlendirildiğinde, HSPB7 düzeyinin şiddetli COVID-19 hastalarında arttığı, tetranektin düzeyinin ise hastalığın şiddetine paralel olarak azaldığı görüldü (sırasıyla p<0,001 ve p<0,001). HSPB7 konsantrasyonunun ferritin ile pozitif korelasyon gösterdiği görüldü (p=0,002). Tetranektin, HSPB7, ferritin ve troponin ile negatif korelasyon gösterdi (sırasıyla p=0,041, p<0,01 ve p=0,005).

Sonuç: Mevcut çalışmanın sonuçları, tetranektini COVID-19 hastalarında, kardiyak hasarın ciddiyetinin belirlenmesinde ve daha kapsamlı bir şekilde anlaşılması için potansiyel bir biyobelirteç olabileceğini göstermektedir.

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Anahtar kelimeler: COVID-19, HSPB7, tetranektin, kardiyak hasar.

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Introduction

The World Health Organization (WHO) reported that the world faced a new coronavirus, a potential pandemic agent, in the early days of 2020. The etiological agent is SARS-CoV-2, which is an RNA virus from the Coronaviridae family [1]. It can affect the cardiovascular system directly or indirectly. Therefore, people with cardiac pathologies (acute coronary syndrome, myocardial damage, myocarditis, arrhythmia, pulmonary embolism, etc.) are accepted as a risky group [2]. Studies have shown that biomarkers of myocardial damage, especially cardiac troponin I and T, increase in infected patients. Although the mechanisms of COVID-19 causing myocardial injury are not fully understood yet, systemic inflammation, interferon-mediated immune response, cytokine storm induced by T helper cells, hypoxia, direct damage to cardiomyocytes, myocardial interstitial fibrosis and stabilization of coronary plaque are considered to be responsible [3].

Currently, studies on the use of serum proteins as biomarkers in the early diagnosis or in determining the prognosis of various diseases are widely carried out. Heat shock protein beta-7 (HSPB7), also known as the cardiovascular heat-shock protein, is a member of the small heat shock protein family [4]. HSPB7 is necessary for the maintenance of myofibril structure in skeletal muscle and mutations of this protein have been linked to dilated cardiomyopathy as well as heart failure in humans [5]. HSPB7 was demonstrated to release from the damaged cardiomyocytes to the blood and elevated serum HSPB7 level was proposed as an independent risk factor for myocardial injury [6].

Tetranectin, whose gene is known as CLEC3B, is a type C calcium (Ca)-binding lectin that increases plasminogen activation [7]. Tetranectin is known to take part in tissue remodeling and development due to its ability to bind extracellular matrix (ECM) components by regulating ECM proteolysis. In addition, it stimulates proteolytic activation of proteases and growth factors. Circulating tetranectin levels were shown to be downregulated in cardiac pathologies [8]. Hence, it is also used as a biomarker for risk of heart injury [9].

Despite many studies demonstrating the relationship between HSPB7, tetranectin and cardiovascular disease, there is no study evaluating these protein levels in COVID-19 infection, which may also cause cardiac injury [10]. Therefore, we aimed to demonstrate HSPB7 and tetranectin levels in serum samples from hospitalized COVID-19 patients and their association with disease prognosis. We consider that the results of the current study may lead to important information about the relationship between serum levels of these two proteins and the severity of COVID-19 disease as well as cardiac involvement in the future.

Materials and methods

Permission was obtained from Pamukkale University Non-Interventional Clinical Research Ethics Committee for the study (permission date (03.08.2021), Number: E-60116787-020-90354 (NO: 14). Procedures were performed according to the Declaration of Helsinki. Written informed consent was obtained from the subjects before the study.

Participants

26 adult patients with COVID-19 and 26 age- and sex-matched healthy controls from the same geographic area (the city of Denizli in the central southern part of Türkiye) participated in the study. The age range in both groups was 40-60 years. Patients were confirmed as SARS-CoV-2 positive by RT-PCR (Rotor GENE, Qiagen, USA) using oro and nasopharyngeal swab samples (Bioseepdy SARS CoV-2 Double Gene RT-qPCR Kit) and admitted to the COVID-19 Department of Pamukkale University Hospital. Moreover, COVID-19 participants were distributed into two subgroups, including a mild group (n=13) and a severe group

(n=13). Severe COVID-19 is characterised by features of severe pneumonia such as dyspnea, respiratory frequency ≥28 breaths per minute and blood oxygen saturation ≤93%, lower lymphocyte count and elevated D-dimer, ferritin and CRP levels [11].

Patient exclusion criteria

Patients with chronic diseases such as DM, chronic kidney disease, COPD, hypertension, and malignant disorders, and those who did not want to participate in the study were not included.

Blood Collection and Processing

Blood was drawn on the first day of admission with symptoms from the forearm veins of PCR-positive patients. Plasma was collected into tubes with spray-coated dipotassium EDTA (1.5 mg/ml) and serum was collected into plain tubes without any anticoagulant. After centrifugation at 3500 rpm for 20 minutes (room temperature), serum and plasma were stored at -80 °C until the experimental procedure was performed. Routine hemogram and biochemistry values [white blood cell (WBC), C-reactive protein (CRP), Neutrophil-to-lymphocyte ratio (NLR), Platelet-to-lymphocyte ratio (PLR), Monocyte-to-lymphocyte ratio (MLR), fasting blood glucose, Creatine kinase (CK), Ca, Albumin, Urea, Ferritin, troponin) were recorded.

Measurements of HSPB7 and tetranectin levels

Serum HSPB7 (BT Lab, E5380Hu, China) and tetranectin (BT Lab, E6262Hu, China) levels were determined by enzyme-linked immunosorbent assay (ELISA) kits.

Statistical analysis

Power analyses were performed by the G-power program (version 3.1.9.2. Heinrich-Heine-Universitat, Duesseldorf, Germany). The effect size obtained from the reference studies was quite strong (d=0.957). For a strong effect size (dz=0.8), when at least 52 people (at least 26 for each group) were included in the study, it was calculated that 80% power could be obtained at the 95% statistical confidence level.

The analysis of the data was carried out using the SPSS 25.0 package program and given as “mean ± standard deviation”. For statistical comparison of biomarker levels in serum, an independent samples t test was used. The relationship between HSPB7 and tetranectin and other laboratory parameters was evaluated by using Pearson’s correlation analysis. The statistical significance was accepted as $p<0.05$.

Results

26 patients with COVID-19 disease (43.8% men, mean age=45.76±1.38) and 26 age–sex matched healthy controls (56.3% men, mean age=50.88±1.93) were included in this study. The demographic features and laboratory parameters of the participants are given in Table 1. Age and gender of COVID-19 patients were not different from healthy controls ($p>0.05$). Mean CRP, fasting blood glucose, ferritin levels, and neutrophil count were significantly increased in COVID-19 patients compared to healthy controls ($p<0.001$). Mean Ca, albumin and lymphocyte count were significantly lower in COVID-19 patients than in healthy individuals ($p<0.001$). There were no significant differences in hemoglobin (Hgb), CK, urea levels, white blood cell (WBC), platelet count and monocyte count between COVID-19 patients and healthy controls ($p>0.05$). Mean neutrophil count, neutrophil-to-lymphocyte ratio (NLR), Platelet-to-lymphocyte ratio (PLR), and Monocyte-to-lymphocyte ratio (MLR) were significantly higher in COVID-19 patients compared to healthy controls ($p<0.001$).

When COVID-19 patients were evaluated according to the severity of the disease (Table 2), respiratory rate, D-Dimer and ferritin levels were higher in severe COVID-19 patients compared to subjects with mild-moderate COVID-19 ($p<0.001$). Oxygen saturation (SO_2) and mean lymphocyte count were significantly lower in severe COVID-19 patients compared to mild-moderate ($p=0.015$ and $p=0.007$, respectively), whereas no statistically significant alterations were observed in mean pulse, CRP and troponin levels.

Table 1. The demographic characteristics and laboratory parameters of COVID-19 patients and healthy controls

	Healthy Controls n=26	COVID-19 n=26	*p* [a]
Age (years)	45.76±1.38	50.88±1.93	0.105 (t=-1.652)
Gender (men %)	43.8%	56.3%	0.254
CRP (mg/L)	1.23±0.17	79.11±11.06	0.000* (t=-7.035)
Hgb (g/dl)	13.85±0.41	12.96±0.47	0.170 (t=1.393)
Fasting Blood Glucose (mg/dl)	90.42±2.43	154.73±12.97	0.001* (t=-4.872)
CK (U/L)	0.81±0.03	3.67±2.77	0.308 (t=-1.030)
Ca (mg/dl)	9.35±0.09	8.34±0.14	0.001* (t=5.992)
Albumin (g/dl)	46.72±0.62	32.83±1.68	0.001* (t=7.740)
Urea (mmol/L)	24.57±1.52	34.88±4.89	0.050 (t=-2.012)
Ferritin (ng/ml)	52.35±8.96	901.49±130.47	0.001* (t=-6.235)
WBC (mm^3)	7.11±0.26	8.49±0.76	0.092 (t=-1.717)
Platelet count (K/μL)	249.00±11.25	254.27±24.79	0.847 (t=-0.194)
Neutrophil count (K/μL)	4.31±0.22	7.17±0.77	0.001* (t=-3.582)
Lymphocyte count (K/μL)	2.23±0.10	0.89±0.13	0.001* (t=8.089)
Monocyte count (K/μL)	0.42±0.02	0.44±0.06	0.815 (t=-0.235)
NLR	1.99±0.12	11.02±1.73	0.001* (t=-5.190)
MLR	0.20±0.01	0.56±0.06	0.001* (t=-5.671)
PLR	114.69±5.97	353.15±39.43	0.001* (t=-5.979)

Results are given in mean ± SD. [a]p-values were calculated using independent samples t test
CK: Creatin kinase, Ca: Calcium, PLR: Platelet-to-lymphocyte, NLR: Neutrophil-to-lymphocyte ratio, MLR: Monocyte-to-lymphocyte ratio
WBC: White blood cell, *: $p<0.05$ difference from healthy controls. CRP: C-reactive protein, Hgb: Hemoglobin

Table 2. Comparison of the clinical and laboratory parameters of mild-moderate and severe COVID-19 patients

	Mild-Moderate COVID-19 n=11	Severe COVID-19 n=15	*p* [a]
Age (years)	52.53±9.29	48.63±10.58	0.329 (t=0.996)
Pulse (beats per minute)	82.86±15.85	91.72±10.56	0.121 (t=-1.606)
Respiratory Rate (breaths per min)	20.46±2.32	28.90±4.67	0.001* (t=-6.070)
SO_2 (%)	94.80±2.11	91.81±3.65	0.015* (t=2.628)
D-Dimer (ng/ml)	559.53±526.96	2121.18±1474.89	0.001* (t=-3.727)
CRP (mg/dL)	65.64±48.38	97.49±63.58	0.159 (t=-1.453)
Ferritin (ng/ml)	250.59±195.15	1452.72±490.03	0.001* (t=-8.661)
Troponin (ng/ml)	10.22±8.33	16.70±15.54	0.182 (t=-1.374)
Lymphocyte count (K/μL)	1.18±0.76	0.49±0.17	0.007* (t=2.929)

Results are given in mean ± SD. [a]p-values were calculated using independent samples t test, SO_2: Oxygen saturation, CRP: C -reactive protein
*: $p<0.05$ differences from mild-moderate COVID-19 group

Serum HSPB7 level was significantly higher in COVID-19 patients than in healthy controls (the HSPB7 levels of COVID-19 patients and healthy controls were 6.04±0.82 and 5.05±0.80 ng/mL, respectively, p=0.006) (Figure 1A), and serum tetranectin concentration was lower in COVID-19 patients compared to controls (the tetranectin values of patients with COVID-19 and healthy controls were 87.85±33.54 and 54.69±16.05 ng/mL, respectively, p=0.001) (Figure 1B).

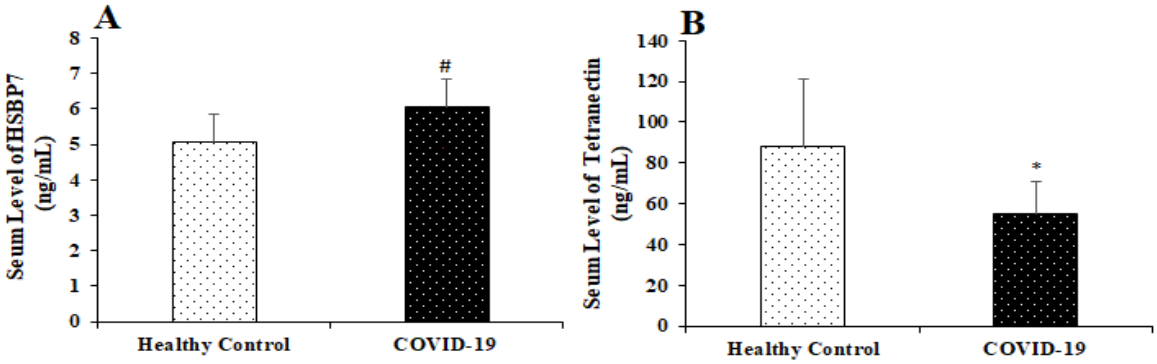

Figure 1. Serum levels of HSPB7

(A) and tetranectin, (B) in COVID-19 patients and healthy controls (Results are given in mean±SD
*: p<0.001 difference from healthy controls, #: p<0.01 difference from control)

When HSPB7 and tetranectin levels of COVID-19 patients were compared according to the severity of the disease, there were significant increases in HSPB7 (Figure 2A) and significant decreases in tetranectin levels (Figure 2B). Serum HSPB7 level was significantly higher in severe COVID-19 patients (7.76±1.6 ng/mL) compared to mild-moderate COVID-19 patients (5.18±0.59 ng/mL) and healthy controls (5.08±0.8 ng/mL) with p<0.001. However, serum HSPB7 levels of mild-moderate COVID-19 patients were not different from healthy controls. In addition, serum tetranectin levels were significantly lower in mild-moderate (65.06±16.92 ng/mL) and severe COVID-19 patients (45.86±2.98 ng/mL) compared to healthy controls (87.85±23.54 ng/mL) (p=0.035 and p=0.001, respectively). Serum tetranectin levels of mild-moderate COVID-19 patients were not different from patients with severe COVID-19.

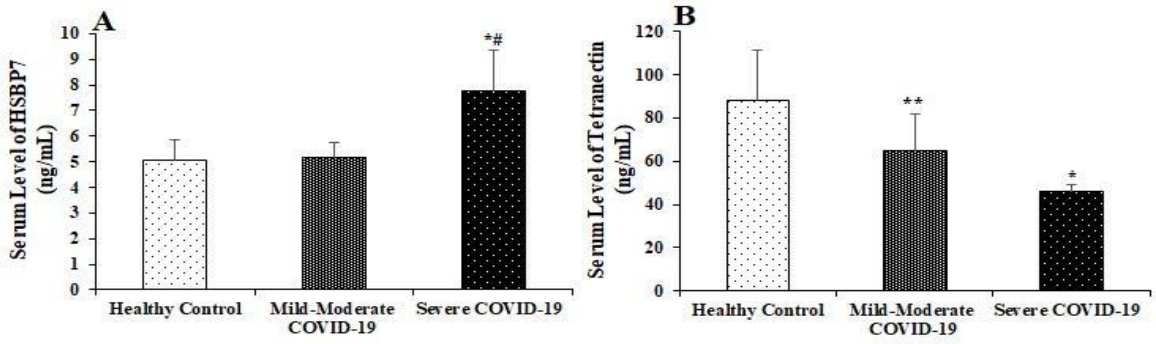

Figure 2. Serum HSPB7

(A) and tetranectin, (B) levels in healthy controls and COVID-19 patients according to severity of the disease (Results are given in mean±SD
*: p<0.001 difference from control, **: p<0.05 difference from control, #: p<0.001 difference from mild-moderate COVID-19

Figure 3 presents correlations of serum HSPB7, tetranectin, ferritin and troponin levels in COVID-19 patients. Serum HSPB7 level was positively correlated with ferritin (r=0.581, *p*=0.002) (Figure 3A), whereas tetranectin concentration was negatively correlated with ferritin (r=-0.529, *p*=0.005) in COVID-19 patients (Figure 3B). There was a statistically significant negative correlation between serum HSPB7 and tetranectin levels (r=-0.403, *p*=0.041) (Figure 3C). The positive correlation between serum HSPB7 and troponin levels was not statistically significant (r=0.364, *p*=0.068) (Figure 3D), while the negative correlation between serum tetranectin and troponin levels was found to be statistically significant (r=-0.605, *p*=0.001) in COVID-19 patients (Figure 3E).

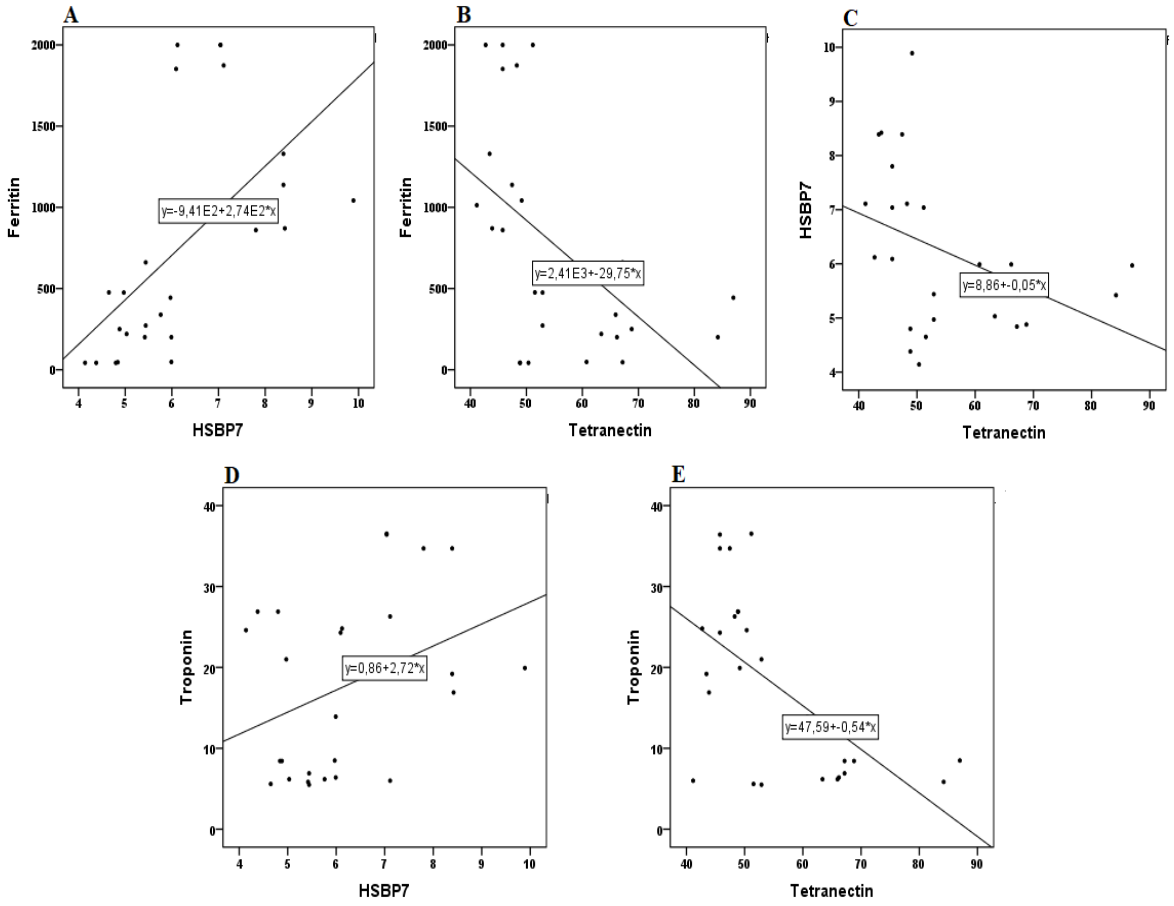

Figure 3. Pearson correlation scatter plot in COVID-19 patients

A: The correlation between circulating HSPB7 and ferritin levels (*p*=0.002)
B: The correlation between serum tetranectin and ferritin levels (*p*=0.005)
C: The correlation between circulating HSPB7 and tetranectin levels (*p*=0.041)
D: The correlation between serum HSPB7 and troponin levels (*p*=0.068)
E: The correlation between circulating tetranectin and troponin levels (*p*=0.001)

Discussion

There is growing interest in identifying reliable diagnostic biomarkers to predict disease severity and cardiac involvement in COVID-19. In this context, our study focused on evaluating the potential roles of HSPB7 and tetranectin as prognostic markers. The key findings demonstrated that serum tetranectin levels were significantly reduced in both mild-moderate and severe COVID-19 patients, whereas elevated circulating HSPB7 levels were observed only in those with severe disease. To explore the potential association with myocardial injury, we performed correlation analyses between these biomarkers and serum troponin levels. Notably, tetranectin levels showed a significant

negative correlation with troponin, while the correlation between HSPB7 and troponin was not statistically significant. These results suggest that tetranectin may serve as a more consistent and sensitive biomarker for both disease severity and cardiac involvement in COVID-19.

In some COVID-19 patients, a cytokine storm occurs with the immune system being affected [12]. An often hallmark of the immune response to various invaders is characterized by increased neutrophil and decreased lymphocyte counts. Monocytes are also important components of the innate immune response, acting as a link to the adaptive immune system by antigen presentation to lymphocytes [13]. NLR, MLR, and PLR may be considered as markers of inflammation [14], representing cell activation associated with increased mortality in cardiovascular disease [15]. We found that patients' neutrophil count, NLR, MLR, and PLR data were higher and lymphocyte count was lower compared to healthy subjects. Activated neutrophils, which migrate from blood to the immune organ to release large amounts of reactive oxygen species, can cause DNA damage and free the virus from the cells. The high NLR values of COVID-19 patients in our study may indicate that these patients were exposed to tissue damage caused by oxidative damage. Increased PLR associated with lung injury and pulmonary endothelial cells activates platelets in the lungs, causing microthrombus formation and may increase platelet consumption [16]. Higher NLR and MLR levels in discriminating between different patient groups hospitalized for fever due to Sars-COV-2 infection and those without Sars-COV-2 infection were investigated [17, 18]. In addition, vascular permeability and capillary leakage increase due to systemic inflammation, causing an albumin shift toward the extravascular space. In this case, hypoalbuminemia may become more profound with the disruption of albumin synthesis. Albumin may also downregulate ACE2 receptors for modulating COVID-19 disease [19]. Low levels of albumin may result in upregulation of ACE2 receptors and a rise in COVID-19 disease. Consistent with these results, we have found lower albumin levels in COVID-19 disease.

In the case of cytokine storm respiratory failure, hypoxemia, hypotension or shock induced by COVID-19 can cause insufficient oxygen supply to myocardial tissue, leading to damage [10, 20, 21]. As an acute-phase inflammatory mediator, elevated CRP has been linked to unfavorable aspects of COVID-19 disease, such as myocardial injury and death [22]. Accordingly, we observed that CRP, ferritin and fasting blood glucose were increased in our patient group. Analysis in the literature revealed that inflammatory parameters including CRP and ferritin were higher in patients with elevated fasting blood glucose, in line with our results. Meanwhile, the counts of lymphocytes were lower and neutrophils were higher in the highest fasting glucose group compared to healthy individuals [23]. These data indicated that raised fasting glucose levels were associated with infection and immunity in COVID-19 patients. Higher serum ferritin level were related to the development of acute respiratory distress syndrome (ARDS) [24] and death [25]. In concordance with these findings, we demonstrated that ferritin levels were increased the severity of COVID-19. As expected, respiratory rate was elevated and SO_2 was decreased with severity of the disease and these consequences may result in an insufficient oxygen supply to the myocardium and cause muscle damage. D-dimers are produced when plasmin cleaves fibrin to break down clots. Elevation of D-Dimer is common in patients with COVID-19 and is associated with severity of the disease and mortality [26].

HSPB7, also known as cardiovascular heat-shock protein, is a member of the small heat-shock protein family sharing a conserved α-crystallin domain in the C-terminal region [4, 27, 28]. It was suggested to have the potential to be a diagnostic marker for myocardial damage, heart failure and an independent risk factor for acute coronary syndrome [6, 29]. Tetranectin, a protein located in the heart, was selected due to its association with cardiac metabolic pathways [9]. Prior literature has demonstrated an anti-thrombotic and an anti-proliferative role for tetranectin [7]. Higher plasma tetranectin levels were inversely associated with cardiovascular risk factors [8]. McDonald et al. [30] reported that a decrease in circulating tetranectin

may indicate cardiac uptake to help conflict myocardial interstitial fibrosis, or a decrease in circulating tetranectin may predispose to the development of heart failure.

The risk of in-hospital death in severe COVID-19 patients can be predicted via myocardial damage biomarkers and is linked to inflammatory response as well as cardiovascular comorbidities [3, 31, 32]. The exact mechanism of COVID-19 leading to myocardial damage remains not fully understood yet [33]. Elevated circulating HSPB7 levels in severe COVID-19 patients and reduced serum tetranectin concentrations, associated with the severity of the disease, were demonstrated in the current study. These two proteins were negatively correlated with each other. Furthermore, not HSPB7, but serum tetranectin levels were negatively correlated with troponin levels. When evaluated together, our results may indicate a more prominent role for tetranectin as a marker of cardiac involvement and severity of COVID-19.

Ferritin was also demonstrated to have an emerging role as a marker in the prognosis of COVID-19. Previous studies revealed the association between serum ferritin levels and clinical characteristics of COVID-19 patients including severity of the disease, as well as mortality and comorbidities [25]. Ferritin increases in the circulation during viral infections and is consistent with a highly inflammatory state [34]. Elevated levels of ferritin due to cytokine storm have also been reported in COVID-19 patients [35]. In line with above-mentioned reports, serum ferritin levels were increased in COVID-19 patients with disease severity in the current study. We also observed that ferritin was correlated positively with HSPB7 and negatively with tetranectin.

Recent studies have focused on the effects of COVID-19 on heart damage. Among the long-term complications following COVID-19 are ischemic heart disease, heart failure, arrhythmias, and myocarditis [36]. Studies have consistently shown that underlying cardiovascular disease in patients with COVID-19 and the development of acute cardiac injury due to COVID-19 illness are associated with significantly worse outcomes.

Numerous mechanisms have been suggested to explain cardiac injury: damage mediated by cytokines, microvascular thrombi, and/or direct cardiomyocyte injury due to viral invasion of the myocardium [37], although the precise effects on heart muscle remain unclear. Acute myocardial injury has been linked to persistent symptoms even 12 months after the initial COVID-19 infection, with an increased hospital readmission rate [38]. This is likely because myocardial dysfunction persists after the initial infection. Tobler et al. [38] reported that new-onset hypertension and heart failure were present in 2% of patients who were more than one year out from their acute COVID-19 infection. A high number of patients have ongoing myocardial inflammation after a COVID-19 infection; however, this is not diagnostic. Although they are primarily seen in patients with underlying cardiac conditions and/or advanced age, they may also be seen in those without preexisting cardiac disease. HSPB7 and tetranectin proteins are cardiac damage specific proteins and the levels changed with COVID-19, and may be it is a molecular reason for tissue damage after COVID-19 infection. Understanding the significance of these conditions in association with COVID-19 illness is of critical importance in ensuring an accurate diagnosis and timely management.

Despite the valuable findings presented in this study, several limitations should be acknowledged. First, the study was conducted in a single center with a relatively small sample size, which may reduce the generalizability of the results and prevent assessment of population heterogeneity among COVID-19 patients. Second, imaging data such as thoracic CT or echocardiographic evaluations were not available, which limited our ability to objectively differentiate between mild and severe disease or to assess myocardial injury directly. Finally, the cross-sectional design did not allow for longitudinal monitoring of biomarker fluctuations throughout the illness.

In summary, our findings suggest that, while both HSPB7 and tetranectin are associated with COVID-19-related cardiac involvement, tetranectin appears to be a more consistent and sensitive biomarker. HSPB7 levels were significantly elevated only in cases of

severe disease. In contrast, tetranectin levels decreased progressively with increasing disease severity and demonstrated a significant negative correlation with serum troponin levels, a well-established indicator of myocardial injury. These findings suggest a potential role for tetranectin as a biomarker reflecting the severity of COVID-19 and its associated cardiac involvement. Nevertheless, the potential utility of HSPB7 and tetranectin as markers of cardiac damage in COVID-19 warrants further investigation. Future studies with larger cohorts and mechanistic insights are needed to clarify their roles in the pathogenesis of COVID-19-related myocardial injury.

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Diagnostic challenges in pulmonary hamartomas: a case series

Pulmoner hamartomlarda tanısal zorluklar: vaka serisi

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Abstract
Pulmonary hamartoma, despite its benign nature, can pose diagnostic challenges due to imaging findings that mimic malignancy. This case series discusses the diagnostic and therapeutic difficulties in pulmonary hamartomas. These three cases that are presented here were surgically managed under suspicion of malignancy and one case was diagnosed as a typical carcinoid tumor postoperatively. The study retrospectively evaluated four cases referred for surgery due to suspected malignancy. The cases were analyzed in terms of radiological, surgical, and pathological findings. Three cases showed benign features but were operated on due to suspected malignancy and history of malignancy, and were confirmed as hamartomas on pathology. One case, despite radiological features suggesting benignity, was identified as a typical carcinoid tumor postoperatively. Pulmonary hamartomas, despite their benign nature, can mimic malignancy and rarely undergo malignant transformation. Therefore, a multidisciplinary approach, surgical resection, and histopathological examination are essential for accurate diagnosis. Incorporating genetic analyses could enhance the diagnostic process further.

Keywords: Pulmonary hamartoma, malignancy, surgical resection, histopathology, multidisciplinary approach.

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Öz
Pulmoner hamartomlar, benign olmalarına rağmen maligniteyi taklit eden görüntüleme bulguları nedeniyle tanısal zorluklar yaratabilir. Bu çalışmada, malignite şüphesiyle cerrahiye alınan üç hamartom vakası ve cerrahi sonrası tipik karsinoid tümör tanısı alan bir vaka sunularak, pulmoner hamartomların tanı ve tedavisinde karşılaşılan güçlükler tartışılmıştır. Çalışma, malignite şüphesi nedeniyle cerrahiye yönlendirilen dört vakanın retrospektif olarak değerlendirilmesiyle gerçekleştirilmiştir. Vakalar radyolojik, cerrahi ve patolojik bulgular açısından incelenmiştir. Üç vaka, benign özellikler göstermesine rağmen malignite şüphesiyle opere edilmiş ve patoloji sonucunda hamartom olarak raporlanmıştır. Bir vaka ise, radyolojik olarak benign özellikler taşımasına rağmen cerrahi sonrası tipik karsinoid tümör olarak tanımlanmıştır. Pulmoner hamartomlar, benign görünümlerine rağmen maligniteyi taklit edebilir ve nadiren malign transformasyon gösterebilir. Bu nedenle, multidisipliner bir yaklaşım, cerrahi rezeksiyon ve histopatolojik inceleme, kesin tanı için vazgeçilmezdir. Genetik analizlerin eklenmesi, tanı sürecini daha kapsamlı hale getirebilir.

Anahtar kelimeler: Pulmoner hamartom, malignite, cerrahi rezeksiyon, histopatoloji, multidisipliner yaklaşım.

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Introduction

Pulmonary hamartomas are benign lesions that account for approximately 8% of lung tumors and represent 75-77% of this benign group [1, 2]. Although these lesions are often detected incidentally and rarely have malignant potential, they can pose significant diagnostic challenges by mimicking lung cancers, metastases, and tuberculosis in radiological imaging [3]. Imaging characteristics, particularly misleading parameters such as low FDG uptake values, may raise suspicions of malignancy [4].

The literature indicates that 91% of pulmonary hamartomas contain cartilaginous components and typically appear as well-defined nodules with clear borders [5]. However, in cases lacking lipomatous or calcified content, findings resembling malignancy or

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malignant transformation may be observed [6]. Consequently, a multidisciplinary approach and, when necessary, surgical intervention are critical for accurate diagnosis and management [7].

In this case series the diagnostic and therapeutic challenges of pulmonary hamartomas were discussed through the presentation of three cases surgically managed under suspicion of malignancy and one case diagnosed postoperatively as a typical carcinoid tumor.

Case presentations

Case 1

A male patient with a prior diagnosis of rectal adenocarcinoma, aged 59, was referred to our clinic for evaluation due to findings in the Computed Tomography (CT), which revealed a 16x13 mm nodular lesion in the right middle lobe (Figure 1). Radiological findings based on tomography were suspicious of metastasis; however, positron emission tomography (PET) showed no evidence of uptake. Consequently, right middle lobectomy was performed. Pathological examination identified the mass as a hamartoma. The patient remains under routine oncological follow-up.

Case 2

A male patient with a prior diagnosis of osteosarcoma in the right maxilla, aged 62, underwent PET-CT follow-up. A subsolid nodule in the right lower lobe showed an increased FDG uptake (SUV max: 2.09) (Figure 2). Secondary malignancy or metastasis could not be excluded; a right lower lobe superior segmentectomy was performed. Pathology confirmed the diagnosis of chondroid hamartoma. The patient is under oncological follow-up.

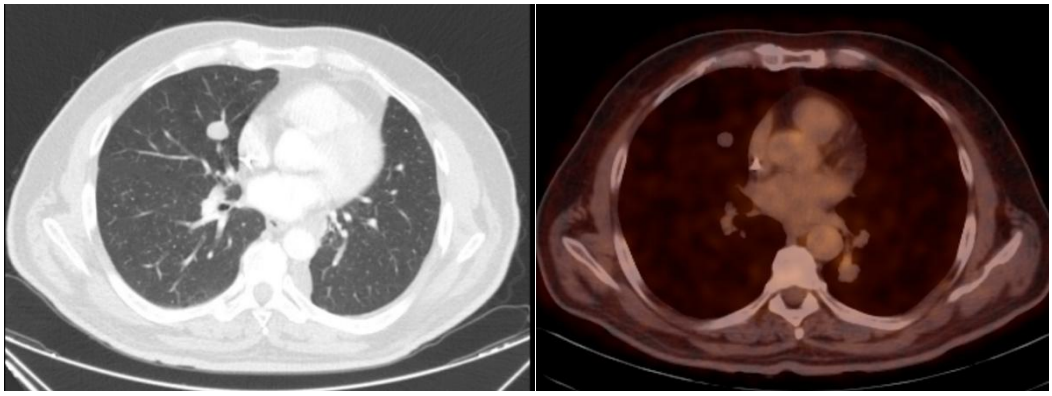

Figure 1. CT and PET-CT fusion images showing a nodular lesion in the right middle lobe

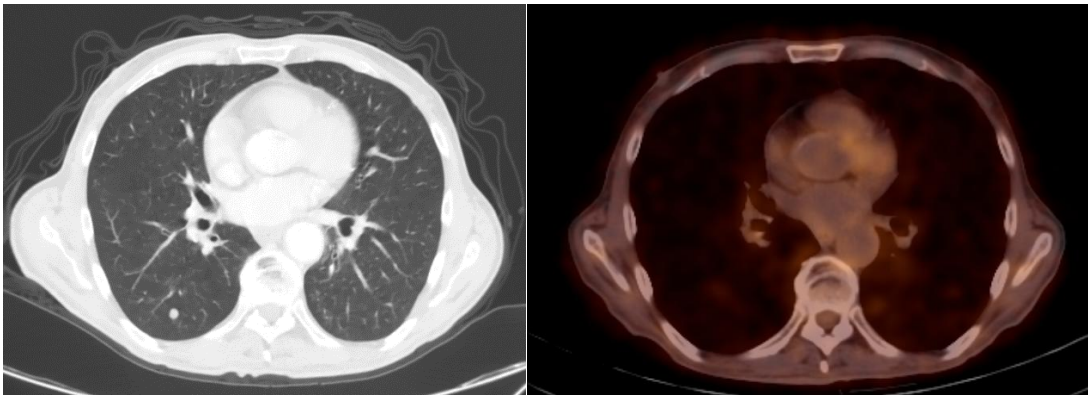

Figure 2. CT and PET-CT fusion images of a subsolid nodule in the right lower lobe

Case 3

A male patient with a previous diagnosis of laryngeal cancer, aged 64, was referred to our clinic with PET-CT, which revealed a spiculated, hypermetabolic nodule in the left upper lobe. Follow-up CT demonstrated an increase in the solid component; prompting surgical intervention, no SUV uptake was detected (Figure 3). A left upper lobe wedge resection revealed a hamartoma on pathology. Additional findings included bronchiectasis and fibrotic changes.

Case 4

A 65-year-old male presented with a nodular lesion in the right lower lobe with benign FDG enhancement on PET-CT (SUV max: 2.73). Due to its size, lobulated appearance and proximity to the inferior pulmonary vein, a right lower lobectomy was performed (Figure 4). Pathology identified the mass as a typical carcinoid tumor and cartilaginous osseous metaplasia secondary to bronchiectasis; surgical margins were reported as clear. The patient is under a regular follow-up period.

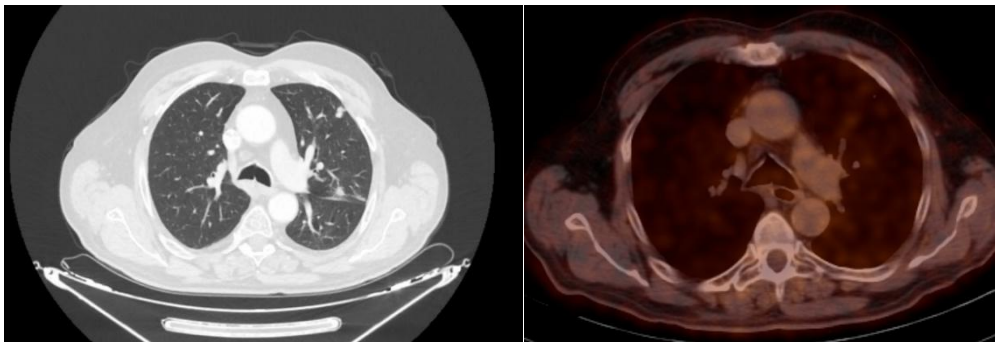

Figure 3. CT and PET-CT fusion images demonstrating a lobulated mass in the left upper lobe

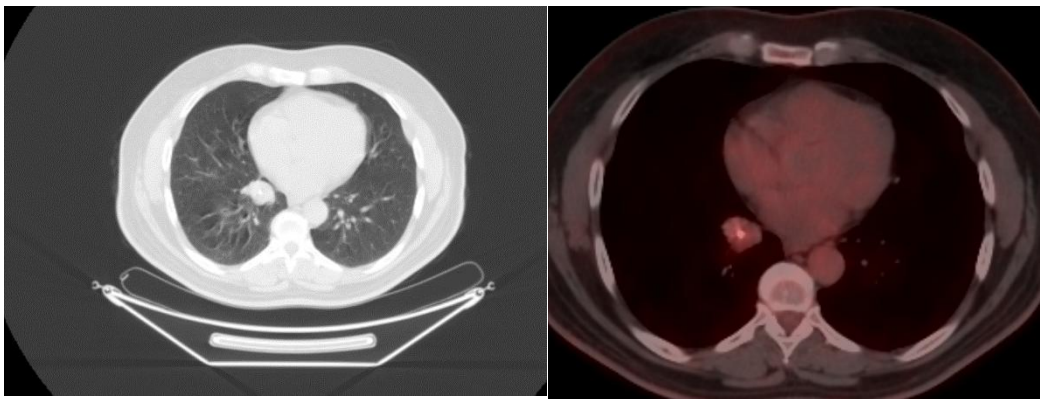

Figure 4. PET – CT scan showing a nodular lesion in the right lower lobe

Discussion

Pulmonary hamartomas can mimic malignancy on radiological imaging despite their benign nature, as evidenced by our cases. For instance, in Case 1, a lesion suspicious for malignancy on PET-CT was ultimately diagnosed as a hamartoma pathologically. While low SUV values generally suggest benignity, these parameters alone are insufficient to exclude malignancy [4]. This highlights the critical role of a multidisciplinary approach and surgical evaluation in clarifying diagnoses. Lung nodule, which was detected in patients, especially those who have a prior history of malignancy, yields high suspicion for metastasis. Particularly well-shaped and round lesions, such as hamartoma, may be interpreted as secondary malignancies of the lungs.

Case 4 underlines the rare but documented association between hamartomas and malignancy. In this case, a lesion considered benign on imaging was diagnosed as a typical carcinoid tumor on pathological examination after lobectomy. Similar findings have been

reported in the literature, where lesions resembling hamartomas were subsequently identified as malignant [3, 8, 9]. This emphasizes the importance of not neglecting the potential for malignant transformation in hamartomas which may result in significant diagnostic and therapeutic errors. This patient's pathological findings were reported as cartilaginous osseous metaplasia consistent with radiologic image characteristics, which is assumed to be hamartoma.

1. Surgical approaches and clinical findings

In our series, surgical resection was the main choice of diagnosis and treatment modality. In Case 2, a subsolid nodule suspected of malignancy on PET-CT necessitated a segmentectomy, leading to a diagnosis of chondroid hamartoma. Segmentectomy is a valuable surgical technique for achieving complete resection and also a diagnostic procedure while preserving lung function. VATS-based segmentectomies, in particular, are associated with lower complication rates and shorter recovery times [10]. Similarly, in Case 3, wedge resection provided both diagnostic clarity and symptomatic relief.

2. Radiological and pathological evaluation

Hamartomas are typically characterized by benign features such as calcification, fat content, and well-defined borders. However, these findings can sometimes resemble malignancy [2]. In Case 3, for instance, a spiculated, hypermetabolic nodule was initially interpreted as metastatic, yet pathology confirmed it as a hamartoma. This aligns with literature emphasizing that, despite their benign features, hamartomas have the potential to mimic malignancy [5]. On PET-CT, hamartomas typically show minimal or no FDG uptake, supporting their benign nature [11].

3. Molecular and genetic insights

Understanding the genetic basis of pulmonary hamartomas is essential for evaluating malignancy risk. Mutations in the HMGA2 gene are implicated in hamartoma pathogenesis and may indicate malignant transformation risk [1]. However, genetic analyses were not performed in our cases, highlighting a potential area for further evaluation.

4. Importance of a multidisciplinary approach

All cases in our series underline the value of a multidisciplinary approach. Cases were assessed by a thoracic oncology council before surgery, demonstrating the pivotal role of collaborative evaluation in achieving accurate diagnoses and emphasizing the importance of multidisciplinary assessment and surgical intervention for pulmonary lesions with suspected malignancy [9].

In conclusion, this case series illustrates that pulmonary hamartomas, despite their benign nature, can mimic malignancy and, in rare instances, undergo malignant transformation. Patients who have lung nodules with a history of prior malignancy or newly diagnosed lung cancer undergo a challenging process to discriminate between benign and malignant. Our findings highlight the critical role of multidisciplinary approaches and surgical pathology in the diagnostic and therapeutic process. In such cases, reliance solely on radiological findings is insufficient; surgical resection and histopathological examination remain indispensable. Additionally, integrating genetic analyses could provide a more comprehensive understanding of these lesions.

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