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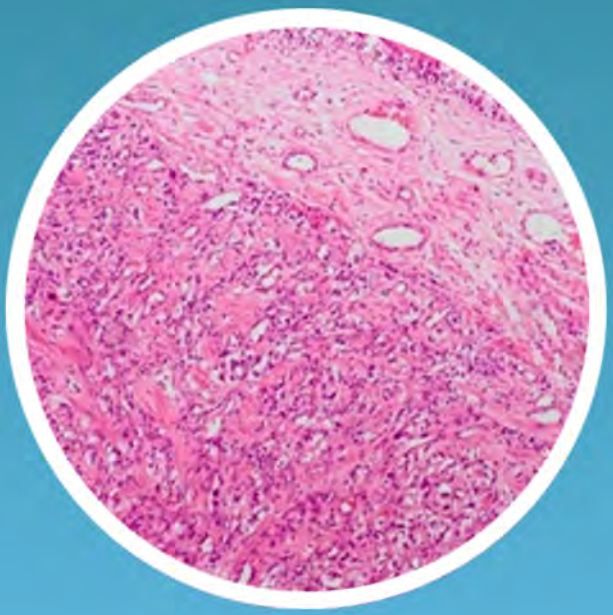

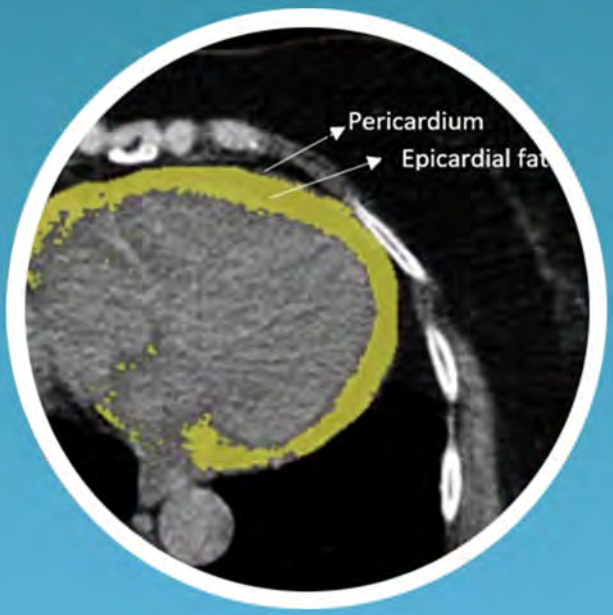

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Case Report

The European Research Journal 2023;9(4):618-629 Original Article

DOI: 10.18621/eurj.1028350 *Cell Biology*

Radiosensitivity of glioblastoma multiforme and astrocytic cell lines in cell signalling aspects

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ABSTRACT

Objectives: The aim of this study is to investigate the radiosensitivity of Glioblastoma multiforme (GBM; U87 MG) and astrocyte (SVG p12) cell lines in vitro through the signalling pathways.

Methods: GBM and astrocytes were treated with 2, 4, 6, and 8 gray of ionized radiation, followed by a clonogenic assay. The effective dose of radiation was determined as 2 gray. Immunofluorescence technics selected to analyse the macrophage migration inhibiting factor (MIF), nuclear factor of activated T-cells cytoplasmic 2 (NFATc2), osteopontin (OPN), mammalian target of rapamycin (mTOR) and stage-specific embryonic antigen-1 (SSEA-1). Additionally, p53 and cell cycle assays were performed.

Results: On day 1, astrocytes showed decreased expression of MIF, OPN and mTOR and increased expression of SSEA-1 in the test group after 2 gray radiation. GBM showed decreased expression of p53 and mTOR, but increased expression of NFATc2. The results of MIF expression were found higher in GBM compared to astrocytes on day 1. Interestingly, on day 12, increased expression of SSEA-1, OPN and p53 were observed in both cell lines' test groups. Further analysis showed that all control groups of GBM and astrocytes were significantly accumulated in the S phase. After radiotherapy application, percentage of GBM in G0/G1 phases and especially in G2/M phases increased; conversely, in the S phase it decreased. Moreover, percentage of astrocytes increased in the S phase and decreased in G0/G1 phases and in G2/M phases.

Conclusions: This combination of findings suggests that as a result of the radiotherapy effect, GBM started to accumulate on check points. The central question in this study focused on changes in molecular protein expression in cancer cells after radiotherapy, particularly key signalling pathways of tumorigenesis and a new possible point of view for treating such diseases.

Keywords: Glioblastoma multiforme, cell cycle, radiosensitivity, cell signalling

Gliomas are the most common primary intracranial tumour type in adults, representing 81% of malignant brain tumours. Glioblastoma multiforme (GBM) is the most aggressive type of glioma, with average of 14 months survival time even after surgical operation, chemotherapy and radiotherapy treatments [1].

Traditionally, GBM had been divided into two

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groups as “primary-de novo” and “secondary". However, a new molecular staging system was reported by WHO in 2016 and molecular .classification of the tumour characteristics. This molecular classification indicated that IDH wild type glioblastoma develops faster and more aggressively than IDH mutant variants, which are more common in elderly people. The definition of new GBM subtypes implicates that tumours differences are not only cytogenetics origin but also in histopathological differences [2].

The current standard treatment procedure for early-diagnosed GBM patients is surgical resection followed by adjuvant radiotherapy and concomitant temozolomide chemotherapy with 60 Gray (Gy) (conventionally divided into fractions) [3]. The use of radiation in the brain has significantly acute adverse effects such as permanent radiation damage, fatigue, hair loss and increased intracranial pressure [4]. Tumour is heterogeneous group which includes cells that have stem cell like character. These cells may be increase with differentiated phenotype. [5]. For that reason, current clinical treatments can develop the tumour initiator cell (TIC) subpopulation and may cause tumour recurrence due to self-renewal characteristics. TICs or stem cells in tumour tissue [6] also stimulate the cancer progression, consequently, altering signalling pathways and cell cycle regulation [7]. Since tumours have various types of molecular subgroups, cell identification of tumour initiator cells has always been the missing puzzle pieces in the approaches to cancer initiation. Identification of specific cell types by cell surface markers such as the stage-specific embryonic antigen-1 (SSEA-1) and osteopontin (OPN) is one of the most reliable ways of selection and isolation of these cells [8].

A number of researchers have reported that SSEA-1 / CD15 / Lewis X, a neural progenitor cell marker, is expressed in GBM cancer stem cells. In addition to their regeneration and differentiation ability, SSEA-1 positive cells give much higher rise to tumour initiation in mouse xenograft models compared to SSEA-1 negative cells [9]. The main target of radiotherapy and chemotherapy is to minimize the high proliferative cells, ending up with the slow-cell cycle and silent stem cells [10]. The cell cycle is the fundamental function for properly duplicating DNA [11]. Previous research has shown that p53, one of the basic proteins of the control points in the cell cycle, mediates transition from G1 phase to S phase and from G2 phase to mitotic division with other regulatory proteins in radiation-induced DNA damage [12]. Among all tumour suppressor genes, p53 plays an important role in the pathogenesis of many common malignancies including brain cancer. p53 has been shown to induce apoptosis, activate cell cycle, stimulate cell differentiation, and involve tumour suppressor activity, including in DNA repair pathways [13]. On the other hand, it has been shown that the increase of p53 expression is less in radiation-resistant cell lines and based on these observations, these investigators have argued about how function of p53 is essential for radiation sensitivity [14].

Mammalian target of rapamycin (mTOR) is a mediator protein with a key role in the phosphatidyl-inositol-3-kinase (PI3K) signalling pathway, has an important role in the regulation of biological processes such as cell growth, proliferation and cell survival. Abnormal signalling in mTOR / PI3K signal is marked in many types of cancer and may affect tumorigenesis and resistance treatment.

Macrophage migration inhibiting factor (MIF) is a mediator protein and effective as a cytokine, hormone and enzyme [15]. When MIF functions as a cytokine, it specifically induces angiogenesis and cell cycle, besides inhibits p53-induced apoptosis and plays a significant role in tumorigenesis by activating PI3K / Akt pathway [16].

Osteopontin (OPN or SPP1) is expressed in many cell types but especially in osteoblasts, osteocytes, chondrocytes, fibroblasts, macrophages and T cells. Furthermore, OPN is an early stage differentiation marker for osteoblasts and osteoclasts [17]. This protein is a pro-inflammatory and largely associated with cancer pathophysiology, cell adhesion, migration, tumour progression, metastasis development and resistance to treatment [18]. GBM patients have positive association between OPN expression and malignancy grade besides OPN serum level was a poor prognostic marker for GBM patients [19].

Nuclear Factor of Activated T-Cell (NFAT) family members, first described as a transcription activators of T cells, play roles in many biological processes such as inflammatory response, angiogenesis, cardiac valve formation, skeletal development, bone homeostasis, axonal orientation [20].

To achieve a better description of GBM tumour

cells and astrocytic cell line biology in the view of tumour response to radiation treatment, OPN, MIF, nuclear factor of activated T-cells cytoplasmic 2 (NFATc2) for inflammation, mTOR for autophagy, p53 for cell cycle, SSEA-1 for tumour initiating futures were investigated with a considered cell signalling approach.

METHODS

Cell Culture

GBM (U87 MG ATCC® HTB14™) and astrocyte (SVG p12 ATCC® CRL-8621™) cell lines were cultured in 10% Fetal Bovine Serum (Gibco-42F957/K) containing Eagle's Minimum Essential Medium (Sigma-RNBG0666). Cells were passaged every 2-3 days after confluence reached about 80%. Cells were cultured and used between passage numbers of 4-10. cell counting was performed with cell count and viability kit (Muse Cell count & viability kit Millipore-2932688). According to the viable cell number, solutions were diluted to 5×104 cells/mL and used in experimental culture technic. Cells were cultured on the 15 mm cover glasses. Each well of 6 well plates contained three 15 mm cover glasses.

Irradiation

For full scatter conditions, a special type of solid water phantom was designed, and 6 well-plates were placed in, along the central axis. 6 well plate was filled with culture medium and placed in the phantom. Total depth of cells was set to be 1.5 cm from the couch top. The set-up was scanned with a Toshiba Asteion (Japan) CT. For achieving the monitor units (MU) of prescribing doses (including attenuation of the couch), a RT plan which ensures uniform dose on cells was created by Xio TPS (v4.8, Elekta, Sweden); gantry angle of 180°, at 100 cm source to surface distance (SSD) to the couch top, using a 23×23 cm^2 field size at 1.5 cm depth.

Irradiation was performed using 6 MV Elekta Precise linac (Elekta, Sweden) at the conditions of RT plan setup described above. Dosimetry verification was evaluated by ion chamber for absolute dosimetry and by calibrated Gafchromic EBT3 (NJ, USA) films which were cut in the shape of flasks and placed at the bottom of them for ensuring uniform dose. Measurements showed that doses were accurate to within ±3%. Cells were then irradiated with various doses (2, 4, 6, 8 Gy) at a dose rate of 300 MU/min (Fig. 1). The control group was also transferred to Ege University Department of Radiation Oncology but left non-irradiated, to expose the whole cell groups to the same environmental conditions. The irradiated and control group then assayed for colony formation.

Clonogenic Survival Assays

Exponentially growing cells were cultured and plated in 6 well dishes. To plate the accurate number of cells is essential for obtaining the correct data for plating efficiency (PE). Cells were left to grow in humidified CO_2 incubator to form sufficiently large clones consisting of 50 or more cells. At 12th day of colonization, colonies were stained with crystal violet dye (Merck 42555) and colonies containing ≥ 50 cells were scored (Fig. 2). Each colony represented one cell surviving after irradiation or without irradiation for the control group. The whole procedures were repeated three times independently. Clonogenic survival curves were plotted as the log of the surviving fraction as a function of the dose.

Plating efficiency (PE) was given by Equation 1 [21].

Equation 1 PE (%) = Number of colonies counted × 100 / Number of cells seeded

The cell survival fraction (SF) was calculated by Equation 2 [21].

Equation 2 SF = Colonies counted / Cells seeded × PE.

After the clonogenic assay analysis, the slope of the survival curve by Do (the dose to reduce survival to 37% of its value at any point on the final near-exponential portion of the curve) was calculated. This dose (2 Gy for each cell line) was selected for further experiments.

Immunofluorescence

2×105 cells/mL cells were cultured on 15 mm cover glasses. After 24 hours incubation, cells fixated in 4% paraformaldehyde (Sigma P-6148) for 30 minutes and were permeabilised with 0.25% Triton X-100 (Bio Basic Canada Inc.-C34H62O11) for 15 min and blocked with 1% bovine serum albumin (BSA Chem Cruz sc-2323) in 1X phosphate buffered saline (PBS). Primary antibodies, OPN (Proteintech 22952-1-AP),

MIF (Santa Cruz sc-271631), NFATc2 (Proteintech 22023-1-AP), p53 (Leica Biosystems NCL-p53-CM5p), mTOR (Bioss BS-3494R) and SSEA-1 (Santa Cruz sc-101462) were diluted 1/100 and incubated at +4oC overnight. Secondary antibodies (Invitrogen Alexa Fluor 488 A11034 anti rabbit, Invitrogen Alexa Fluor 555 A32727 anti mouse) were diluted 1/200 and incubated for an hour. Samples were mounted with Fluoroshield Mounting Medium with DAPI (Abcam ab104139). Samples were observed by the appropriate fluorescent filter by Olympus CellSens Entry (Japan) and analysed by five individuals independently in ImageJ which is public domain open-source software.

Immunocytochemistry

Cells were cultured, fixed and permeabilised as the same way with immunocytochemistry procedure (above). Cells were treated with H_2O_2 (Merck Emprove exp.-K41544097)for 10 min and washed with PBS, blocked with 1% bovine serum albumin (BSA) in 1X phosphate buffered saline (PBS). Primary antibody p53 (Leica Biosystems) diluted at 1/100 ratio and cells incubated overnight in primary antibody. Biotinylated secondary antibody (ScyTek Laboratories SHP125) was diluted 1/200 and cells incubated in it for 40 min, then cells were treated with HRP streptomycin solution for 40 min and rinsed with PBS. DAB solution (ScyTek Laboratories ACK125) was applied for final colouring. Images were photographed by Avertv and analysed in ImageJ software. İmage J analysis made in 40× magnification. For every group min 100 cells were counted and evaluated by five different individuals.

Cell Cycle

2×105 cells/mL cells were cultured on 15 mm cover glasses. After 24 hours incubation, cells were fixed with 70% ice-cold ethanol overnight and Cell cycle kit (Muse Millipore-2941162) instructions were followed.

Statistical Analysis

To examine the association between radiation factors on cell cycle phase's results were evaluated by t-tests.

RESULTS

Clonogenic Assay

Control and radiated groups of GBM and Astrocytic cell lines were observed via Olympus BX50 (Japan) microscope for 12 days (Fig. 1) and at the end of the experiment, cell lines stained with crystal violet for quantitative analysis conducted by four independent expert individually (Fig. 2).

Immunofluorescence and Immunocytochemistry

SSEA-1

Control and experiment groups of GBMCs showed higher expression than ACs. All experiment groups shows higher expression of SSEA-1 on day 12 when compared to on day 1. Remarkably, GBMCs and ACs displayed increased expressions of SSEA-1 after radiotherapy treatment (Table 1) (Fig. 3).

p53

It has been shown that p53, one of the basic proteins of the control points of the cell cycle, mediates transition from G1 phase to S phase and from G2 phase to mitotic division with other regulatory proteins in radiation-induced DNA damage [22]. Among tu-

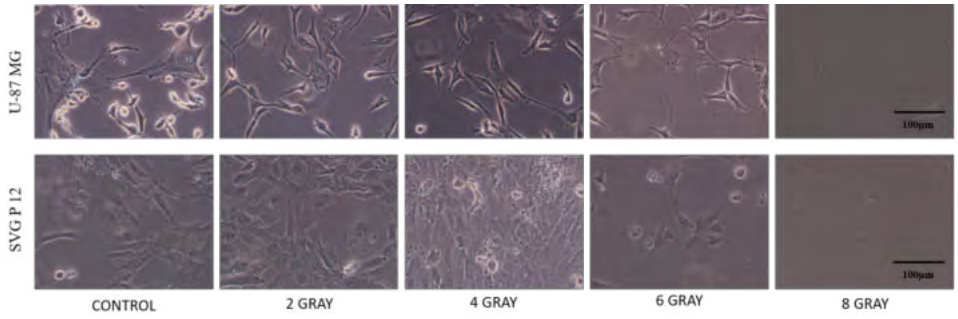

Fig. 1. Phase-contrast images of GBMCs and ACs cells after 12 days of culturing of control and test groups. Images are at the same magnification (Scale bar 100 µm).

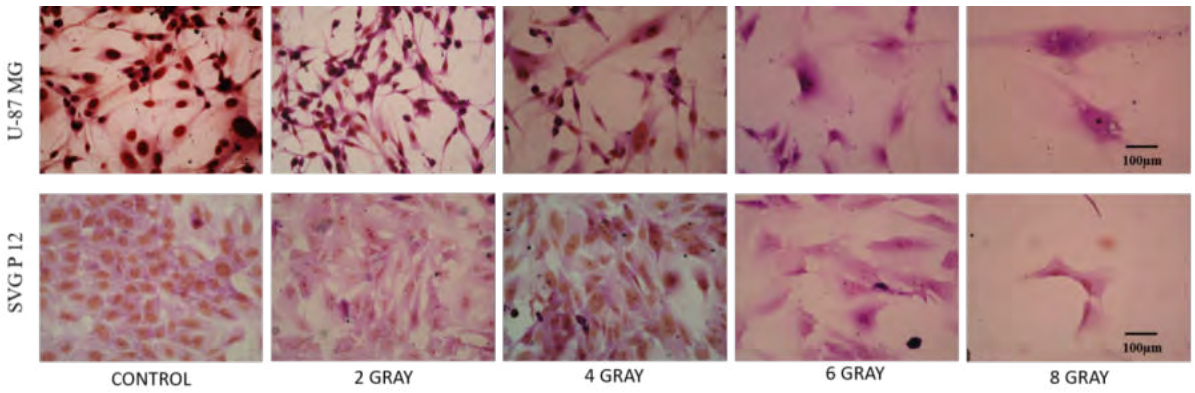

Fig. 2. Colonogenic assay; GBMCs and ACs cells crystal violet staining. Cells were cultured for 12 days after radiotherapy, after fixation stained with crystal violet dye, and counted for control and test groups (Scale bar 100 µm)).

Table 1. SSEA-1 day 1 and day 12 IF results

d1	GBMC control < GBMC test
	AC control < AC test
	GBMC control > AC control
	GBMC test > AC test
d12	GBMC control < GBMC test
	AC control < AC test
	GBMC control > AC control
	GBMC test > AC test
d1 vs d12	GBMC control d12 > GBMC control d1
	AC control d12 > AC control d1
	GBMC test d12 > GBMC test d1
	AC test d12 > AC test d1

SSEA-1 = stage-specific embryonic antigen-1, GBMC = glioblastoma multiforme cell, AC = astrocyte cell, d1 = day 1, d12 = days 12

mour suppressor genes, p53 plays an important role in the pathogenesis of many types of malignancies [13]. Radiation-sensitive cell lines exposed to 2 Gy radiation showed a significant increase in p53 within 8 hours [14]. According to our findings, on day 1 in GBMCs control group p53 expression was higher than GBMCs test group and on day 12 ACs test group p53 expression was higher than ACs control group. Both test groups of tumour cell line and astrocytic cell line showed increment in p53 expressions on day 12 which can indicate that in the long-term tissues or cells that suffering from ionized radiation effects, similarly up-regulates one of the key regulators of cell cycle and apoptosis (Table 2) (Fig. 4).

mTOR

GBMCs control group's mTOR expression was higher than ACs control group, the result demonstrates

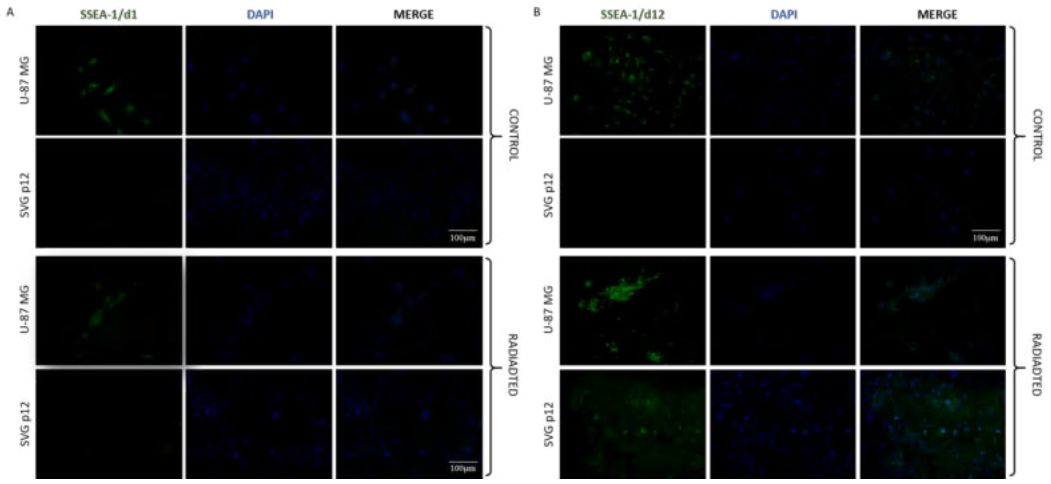

Fig. 3. SSEA - 1 expressions of GBMCs and ACs cells after (A) 1 day and (B) 12 day of culturing (Scale bar 100 µm).

Table 2. p53 day 1 and day 12 IF results; The results for p 53 shows that GBMCs control group's p53 expression was higher than GBMCs experiment group on day 1

d1	GBMC control > GBMC test
	AC control < AC test
	GBMC Control ~ AC Control
	GBMC test < AC Test
d12	GBMC control < GBMC test
	AC control < AC test
	GBMC Control < AC Control
	GBMC test ~ AC Test
d1 vs d12	GBMC control d1 ~ GBMC control d12
	AC control d1 ~ AC control d12
	GBMC test d1 < GBMC test d12
	AC test d1 < AC test d12

GBMC = glioblastoma multiforme cell, AC = astrocyte cell, d1 = day 1, d12 = days 12

that after radiotherapy treatment mTOR expressions are reduced (Table 3) (Fig. 5).

MIF

After radiotherapy, ACs and GBMCs showed increased expression of MIF than control and after radiotherapy GBMCs showed higher expressional level than ACs (Table 4) (Fig. 6).

OPN

According to our results in ACs after radiotherapy on day 1 low expression level observed in osteopontin and beside on day 12 test group ACs osteopontin high expression level observed compared to day 1 test group. On day 12 increasing of osteopontin expression observed in GBMCs test group compared to the GBMCs control group (Table 5) (Fig. 7).

NFATc2

Increasing in NFATc2 expression in GBMCs after radiation treatment compared to GBMCs control group (Table 6) (Fig. 8).

Cell Cycle

Both GBMCs and ACs control groups significantly accumulated in S phase. After radiotherapy application for GBMCs in G0/G1 and especially G2/M phase increasing and S phase decreasing observed. For ACs increasing S phase and decreasing in G0/G1 and G2/M phases observed (Fig. 9).

DISCUSSION

Due to the resistance of traditional cancer treatment approaches, development of targeting therapies for TICs can be the destination of new approaches to the cancer treatments. To choose the key regulator targets, understanding the nature and the response to the external impacts of these cells are initial and inevitable. It is important to classify and isolate these cells from the tumour tissue by using surface markers such as SSEA-1. Collection for SSEA-1+ cells enriches for glioma tumour TIC subpopulations in all of the

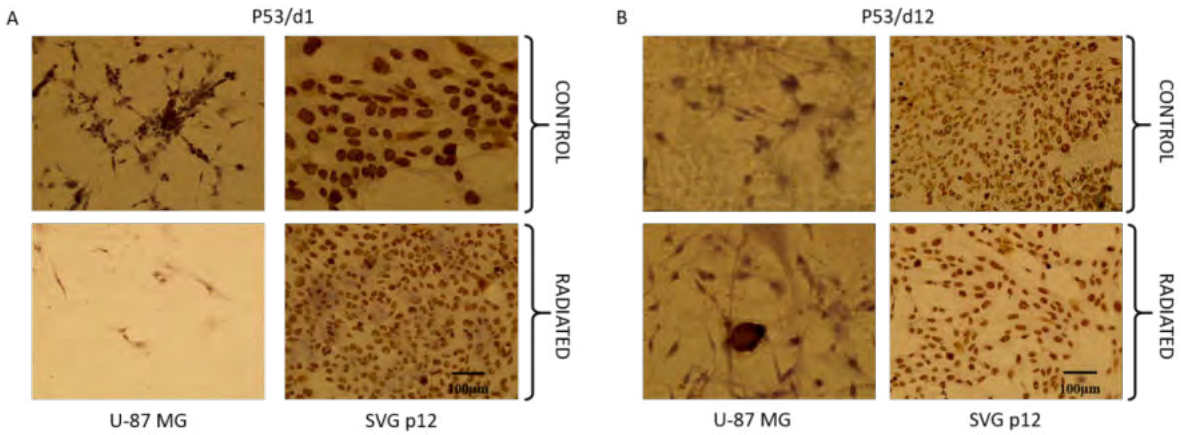

Fig. 4. p53 expressions of GBMCs and ACs cells after (A) 1 day and (B) 12 day of culturing (Scale bar 100 µm).

GBMCs [9].

In our experiments, we found that SSEA-1 expression of GBMCs both control and test group were higher than ACs control and test groups. However, every experimental groups presented increased expression of SSEA-1 on day 12 when compared with on day 1; in addition, after radiotherapy application, both GBMCs and ACs displayed increased expressions of SSEA-1 (Table 1) (Fig. 3). This might indicate that radiotherapy resistant and tumour initiating properties acquiring cells were survived.

Table 3. mTOR day 1 IF results

d1	GBMC control > GBMC test
	AC control ~ AC test
	GBMC control > AC control
	GBMC test > AC test

mTOR = mammalian target of rapamycin, GBMC = glioblastoma multiforme cell, AC = astrocyte cell, d1 = day 1

For tumour cells present SSEA-1 show tumour initiating capacities more than mature astrocytic cells; however, with radiation stimulation both astrocytic and tumour cell line SSEA-1 expression increase observed. Radiotherapy application clearly is not sufficient for tumour therapy alone and afterwards resistant cells present more SSEA-1. Normal tissue is affected by the tumour cells because of the cancer cell microenvironment interaction. As a result of this interaction, SSEA-1 increase in both normal cells and tumour cells. Furthermore, this research present that the SSEA-1 increasing more significantly in the tumour cells, it might indicate the difficulty of treatment and the tumour relapses in vivo.

Studies have shown that DNA damage induced by ionizing radiation causes arrest in the G1 and G2 phases of the cycle in mammalian cells, and that this observation is related to radiation hypersensitivity [14]. Another finding is that transition from G2 phase to M phase is especially essential for provision of genomic stability and survival after ionizing radiation

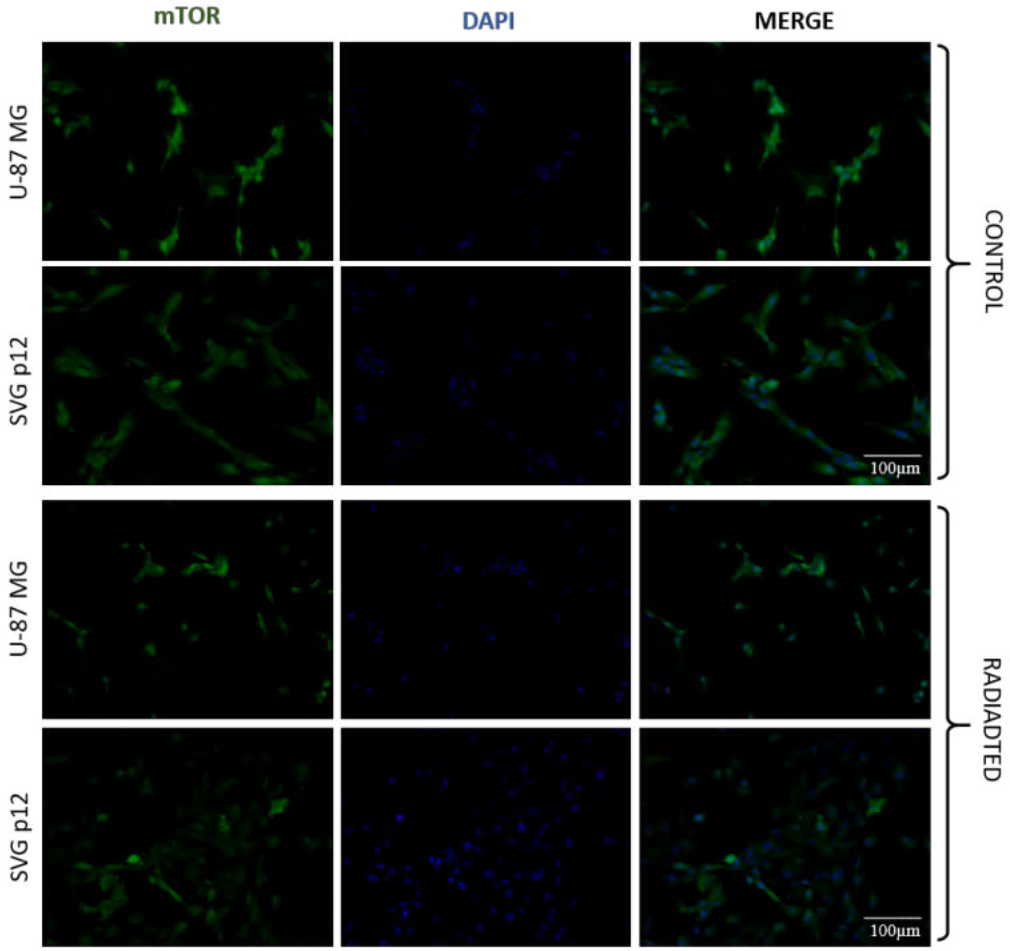

Fig. 5. mTOR expressions of GBMCs and ACs cells after 1 day of culturing (Scale bar 100 µm).

exposure [23].

Both GBMCs and ACs groups were significantly accumulated in S phase. After radiotherapy application for GBMCs in G0/G1 and especially G2/M phase increasing and S phase decreasing observed. These findings suggest that as a result to the radiotherapy effect, GBMCs started to accumulate on check points. On the

Table 4. MIF day 1 IF results

d1	GBMC control < GBMC test
	AC control < AC test
	GBMC control > AC control
	GBMC test > AC test

MIF = macrophage migration inhibiting factor, GBMC = glioblastoma multiforme cell, AC = astrocyte cell, d1 = day 1

Table 5. OPN day 1 and day 12 IF results

d1	GBMC control ~ GBMC test
	AC control > AC test
	GBMC control ~ AC control
	GBMC test > AC test
d12	GBMC control < GBMC test
	AC control ~ AC test
	GBMC control ~ AC control
	GBMC test > AC test
d1 vs d12	GBMC control d12 < GBMC control d1
	AC control 12 < AC control d1
	GBMC test d12 < GBMC test d1
	AC test d12 > AC test d1

OPN = osteopontin, GBMC = glioblastoma multiforme cell, AC = astrocyte cell, d1 = day 1, d12 = days 12

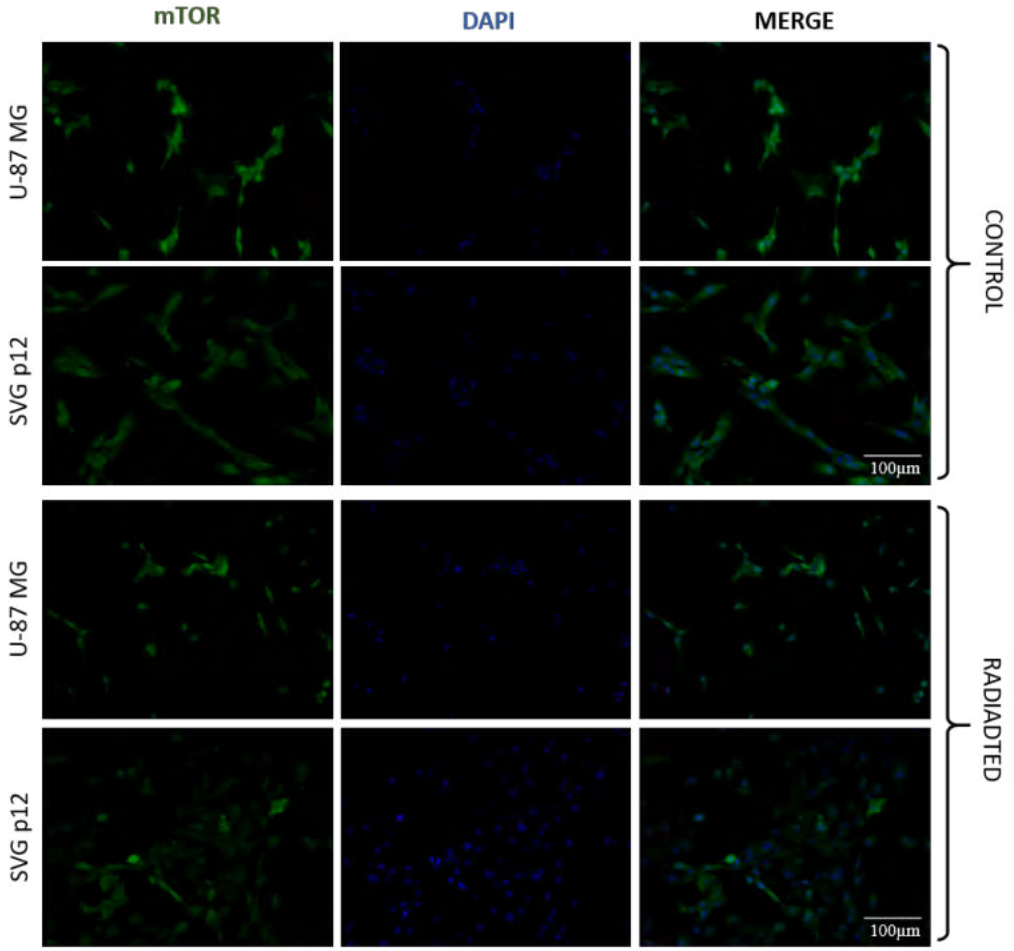

Fig. 6. MIF expressions of GBMCs and ACs cells after 1 day of culturing (Scale bar 100 µm).

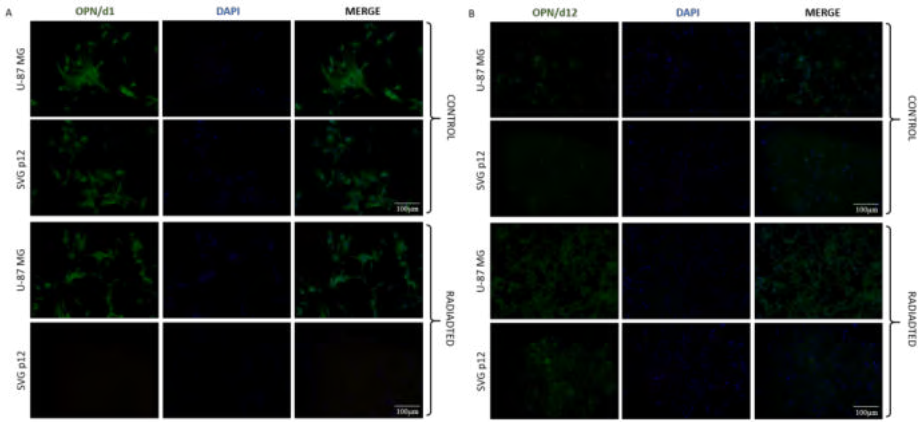

Fig. 7. **OPN expressions of GBMCs and ACs cells after (A) 1 day and (B) 12 day of culturing (Scale bar 100 µm).**

Table 6. **NFATc2 day 1 IF results**

d1	GBMC control < GBMC test
	AC control > AC test
	GBMC control ~ AC control
	GBMC test > AC test

NFATc2 = nuclear factor of activated T-cells cytoplasmic 2, GBMC = glioblastoma multiforme cell, AC = astrocyte cell, d1 = day 1

other hand, ionized radiation affects normal tissue cells differently than tumour cells. For ACs increasing S phase and decreasing in G0/G1 and G2/M phases observed (Fig. 9).

Abnormal signalling in mTOR is may affect tumorigenesis and resistance treatment. p53 mutation, which is frequently seen in tumour formation, increases mTOR activation can be seen in glioblastomas that develops hyper activation of mTOR [24]. In a

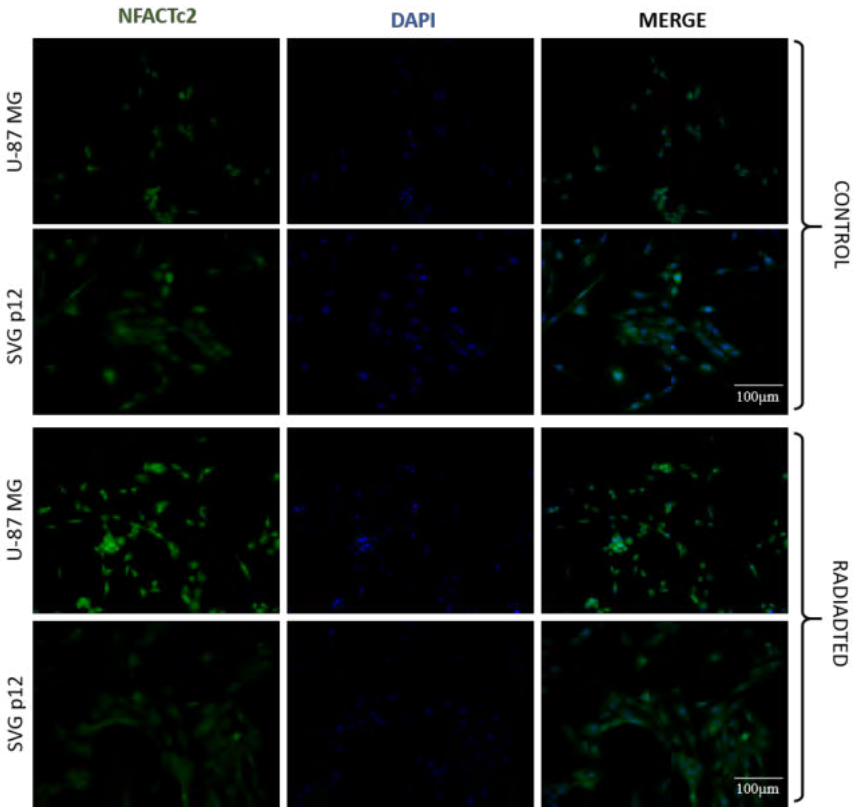

Fig. 8. **NFATc2 expressions of GBMCs and ACs cells after 1 day of culturing (Scale bar 100 µm).**

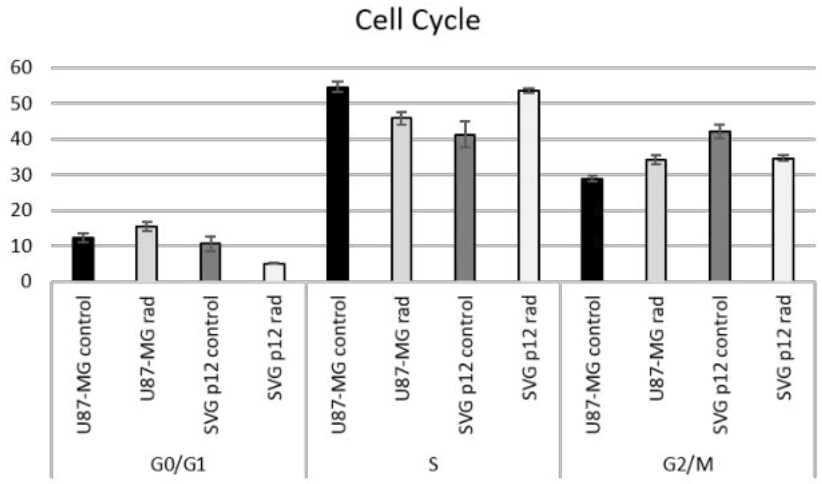

Fig. 9. Cell cycle analysis of GBMCs and ACs cells after 1 day culturing.

study, it was reported that mTOR inhibitors reduce the migration and invasion of GBMCs, also reduce the motility of these cells by the regulation of F-actin and paxillin [25]. In our experiments, mTOR activation was also found to be high in GBMCs with high p53 levels in accordance with the literature. At the same time, as a radiotherapy response, tumour cells and normal tissue cells responded similarly to the ionized radiation with decreasing in mTOR expressions in both GBMCs and ACs (Table 3) (Fig. 5). With treatment, reduced mTOR expressions may be supportive on consistency usage of the radiotherapy and mTOR inhibitors.

MIF expression is strongly associated with the mutational states and activity of p53 in GBMCs. A research study concluded that MIF is strongly expressed in astrocytomas and this increases with higher grades of malignancy [26]. As a cytokine, MIF is the indicator for angiogenesis, cell cycle and p53 which inducing apoptosis and effective in tumorigenesis [16]. Similarly, with the literature, results of this research show that MIF expressions higher in GBMCs more than ACs (Table 4) (Fig. 6). And via radiotherapy stimulation both tumour cell line and astrocytic cell line shows increment in MIF expressions. After radiotherapy tumour and astrocytic cell line increment in MIF expressions might be tone of the reasons for the difficulty of treating relapse tumours.

Researchers found that silencing of OPN expression in GBMCs leads to decrease cell migration and inhibits of tumour growth [27]. In another research stated that high OPN expression was associated with poor survival in GBM patients treated with radiotherapy. Also same researchers indicated that OPN depletion makes GBMCs more susceptible to radiation and DNA damage accumulation after irradiation is higher in these cells than in control cells [19]. In our experiments, we observed OPN expression in GBMCs both in day 1 and day 12. Even though GBMCs test group showed decreasing in OPN expressions on day 1, on day 12 test group OPN expression was higher as similar with ACs. After short time from ionized radiation application loss of OPN expression was found the ACs, however in the long term the OPN expression had increased (Table 5) (Fig. 7). The decrease on day 1 in GBMCs were indicating that loss of OPN expression worsening the effects of radiation the treatment response is compatible with the literature. However, increased OPN expressions, is in concordance with the increased SSEA-1 expressions on day 12. The surviving cells which are resistant and presenting stem cell like markers also displaying increased amount of OPN expressions. And in addition, regular tissue cells OPN pathway affected by the radiation.

According to our study, both GBMCs and ACs control group shows similar expressions of NFATc2 but via radiotherapy increasing in NFATc2 expression in GBMCs compared to GBMCs, ACs control and ACs test groups was found (Table 6) (Fig. 8). Radiotherapy may not affect normal tissue cells as the tumour cells when it comes to NFATc2 but the surviving tumour cells expressing more NFATc2 can contribute the invasiveness of the tumour cells.

CONCLUSION

As a result, radiotherapy is a significant method for treatment of cancer and effects on cell signalling pathways are critical, especially in understanding cancer residues and recurrence. Tumour cells are not only the target of the therapeutics individually, but also cell-cell and cell extracellular matrix interactions act in tumour progression. Detecting cancer cells in the tissue and cells that may have tumour initiating capacities, and learning more about the intracellular and extracellular signal transduction of cells, are the key points that can lead to resolution of treatment failures. It should be kept in mind that when treatment is applied, not only cancerous tissue but also surrounding normal tissue cells will be affected and signal changes in these cells will be effective as tumour niche in both treatment success and tumour recurrences. Given molecular treatments, pathways that target tumour tissue but which will be least effective on the functions of normal tissue cells or cell fates, should be identified. After radiotherapy, it is important to show the change in the characteristics of the cells with time and to determine the tumour initiating properties of the surviving cells and treatment resistance. The determination of the cascade change of signal pathways after radiotherapy is indispensable in the target therapeutic model creation studies.

Authors' Contribution

Study Conception: DÇK, BÖ, YA; Study Design: DÇK, BÖ, YA, SH; Supervision: ESK, GÖ, AU, HA; Funding: ESK, GÖ, AU, HA; Materials: DÇK, BÖ, YA; Data Collection and/or Processing: BÖ, SH, ESK; Statistical Analysis and/or Data Interpretation: DÇK, BÖ, YA, SH, ESK; Literature Review: DÇK, BÖ, YA, HA; Manuscript Preparation: DÇK, BÖ, YA and Critical Review: GÖ, AU, HA.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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The European Research Journal 2023;9(4):630-636 Original Article

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The relationship between epicardial fat volume and myocardial perfusion scintigraphy findings

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ABSTRACT

Objectives: We investigated the epicardial fat volume (EFV) between patients with normal perfusion and reversible perfusion abnormalities in the myocardial perfusion scintigraphy (MPS) in patients with suspected coronary artery disease (CAD). In addition, we aimed to investigate the relationship of automated analysis parameters obtained in the MPS SPECT examination with EFV.

Methods: A total of 295 patients (182 F, 113 M) who underwent MPS in our unit with the suspicion of CAD in the last 1 year and who had a recent thorax CT examination were included. EFV measurement in CT scans was done with Invesalius software. MPS was performed in all patients with a one-day stress and rest imaging protocol. In the stress study, imaging was performed approximately 30-45 minutes after intravenous injection of ~12 mCi Tc99m Sestamibi. Rest study imaging was performed approximately 30-60 minutes after intravenous injection of ~25 mCi Tc99m Sestamibi.

Results: Median EFV was 53.00 ml (interquartile range: 23 ml, range 17-238 ml) in patients with normal MPS, and 62.00 ml in patients with myocardial ischemia on scintigraphy (interquartile range: 53 ml, range: 25-207 ml). The EFV value was statistically significantly higher in patients with reversible ischemia on MPS compared to patients with normal scintigraphy findings ($p < 0.001$). There was a statistically significant, low, and positive correlation between EFV and summed difference score (SDS) values ($p = 0.002$, $r = 0.178$).

Conclusions: The EFV value was significantly higher in patients with reversible ischemia on MPS compared to patients with normal scintigraphy findings. Also there was a statistically low and positive correlation between EFV and SDS values. The automatic calculation of the EFV value during this examination may be a good additional parameter to detect the presence of ischemia.

Keywords: Epicardial fat volume, Tc-99m MIBI, scintigraphy, summed difference score

Coronary artery disease (CAD) can be defined as insufficient blood supply to the myocardium due to narrowing or occlusion of the coronary arteries, usually caused by atherosclerosis. CAD is one of the most important causes of mortality and morbidity all over the World [1]. It is important to determine the risk of cardiovascular disease in these patients. Although coronary angiography is considered the gold standard technique for diagnosing CAD, myocardial perfusion scintigraphy (MPS) using single-photon emission computed tomography (SPECT) with radiopharmaceutical is commonly used in the non-invasive diagnosis of obstructive CAD.

Epicardial fat (EF) is an adipose tissue located be-

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tween the myocardium and the visceral pericardium, which has some metabolic functions. EF has the same embryological origin as visceral adipose tissue and causes to release of proinflammatory cytokines which is involved in the production of atherosclerosis of the coronary arteries [2]. It has been assumed that EF has direct paracrine or vasocrine effects on the coronary arteries due to the presence of anatomical proximity and the absence of a facial barrier between them [3]. In many studies, it has been found that there is a positive correlation between epicardial fat volume (EFV) and atherosclerosis of the coronary arteries [4, 5].

Computed tomography (CT) is considered to be the preferred imaging method for the measurement of EFV due to its high spatial resolution and widespread availability. Traditionally, CT volumetric analysis of EF can be performed in non-contrast thorax CT examination, but it can also be measured with SPECT/CT hybrid cardiac gamma cameras. Low-dose CT for attenuation correction, a standard component of MPS SPECT/CT, can be used to calculate EFV without additional radiation exposure or cost [6].

In clinical practice, MPS are interpreted based on visually by assessment of radiopharmaceutical uptake. Withal, automated analysis data obtained with quantitative softwares may help in visual analysis. Quantification is an important appliance in MPS because it helps determine the severity of ischemia as well as an objective assessment. With MPS SPECT, automated analysis parameters such as summed stress score (SSS), summed rest score (SRS), summed difference score (SDS), stress, and resting mid myocardial perfusion defect percentage rates can be obtained. These parameters can provide important information about the patient's status.

We purposed to compare the EFV between patients with normal perfusion and reversible perfusion abnormalities in the MPS study in patients with suspected CAD. In addition, this study aimed to investigate the relationship of automated analysis parameters obtained in the MPS SPECT examination with EFV.

METHODS

Patients who had previously undergone MPS due to suspected CAD and had recent thorax CT were included in our retrospective study. Only previous imaging of the patients was evaluated and no additional imaging was performed for the study. Informed consent of the patients was obtained before both imaging. All patients approved the use of their medical records for research purposes. This retrospective study was performed in accordance with the Helsinki Declaration.

Study Population

A total of 295 patients (182 F, 113 M) who underwent MPS in our unit with the suspicion of CAD in the last 1 year and who had a recent thorax CT examination were included in the study. There were a total of 196 people in the control group, including 70 males and 126 females. The mean age of the control group was 61.2 ± 10.1 years (range: 26-83 years). There were a total of 99 people in the patient group, including 43 males and 56 females. The mean age of the patient group was 62.9 ± 10.2 years (range: 37-92 years). Patients with a maximum heart rate below 85% of age-predicted maximum heart rate in the exercise stress test, patients with motion artifact or high extra-cardiac activity during MPS, and patients who had previous coronary vascular surgery were excluded from the study.

Computed Tomography Analysis of Epicardial Fat Volume

The non-contrast computed tomography images obtained with a 16 slice CT scanner (Alexion 16 Multi-slice, Toshiba Medical System Corporation) using the following parameters: fixed noise index of 30.9; 0.625-mm collimation; reconstruction slice thickness of 5 mm 120 kVp; variable milliamperage determined by x-, y-, and z-axis dose modulation; gantry rotation time of 0.5 seconds; and 40% ASIR. Thorax CT images of all patients were obtained from the hospital PACS system in DICOMDIR format. EFV measurement in CT scans was performed with free software. (Invesalius 3.1, Centro de Tecnologia da Informação Renato Archer, Brazil). The images obtained in DICOMDIR format were transferred into the software for quantification.

EF is defined as the adipose tissue located between the pericardium and myocardium and also surrounding the coronary arteries. The upper border of the pericardial contours was determined as the pulmonary bifurcation, and the lower border was deter-

mined as the posterior descending artery. EF within pericardium was selected by manual segmentation on sequential trans-axial slices with 5 mm slice thickness and the threshold for EF detection set between −30 HU and −271 HU. During this procedure, other adipose tissues other than epicardial adipose tissue were excluded by paying attention to the pericardial contours (Fig 1). The EFV was then calculated by software that met the threshold parameters for EF in each slice. The measurement result was obtained in ml. The operators did not have patient clinical data, such as the presence or absence of ischemia on MPS.

Myocardial Perfusion Scintigraphy Imaging

MPS was performed in all patients with a one-day stress and rest imaging protocol. In the stress study, imaging was performed approximately 30-45 min after intravenous injection of ~12 mCi Tc99m Sestamibi. In the rest study, imaging was performed approximately 30-60 min after intravenous injection of ~25 mCi Tc99m Sestamibi. MPS imaging was performed using the MEDISO Any Scan S gamma camera system (Mediso Medical Imaging Systems Ltd., Budapest, Hungary). All data collection was carried out by a double-headed SPECT system equipped with a low-energy, high-resolution collimator. The energy photopeak was set to 140 keV. Imaging was done in 64×64 matrix dimensions. Imaging was obtained by taking 64 steps from the right anterior oblique to the left posterior oblique position at 180 degrees, 3-6 degrees in each step. In all patients without contraindications, MPS was performed after a pharmacological stress test with adenosine due to the Covid-19 pandemic. In patients with contraindications for the pharmacological stress test, MPS was performed after the exercise stress test with the Modified Bruce protocol.

Computer-Assisted Quantification of MPS

MPS images were evaluated based on a computer-assisted 17-segment model with 5–point scoring system by an experienced nuclear medicine specialist. Obtained MPS images were grouped as normal or ischemic according to the presence of ischemia signs. MPS quantitative parameters were obtained with com-

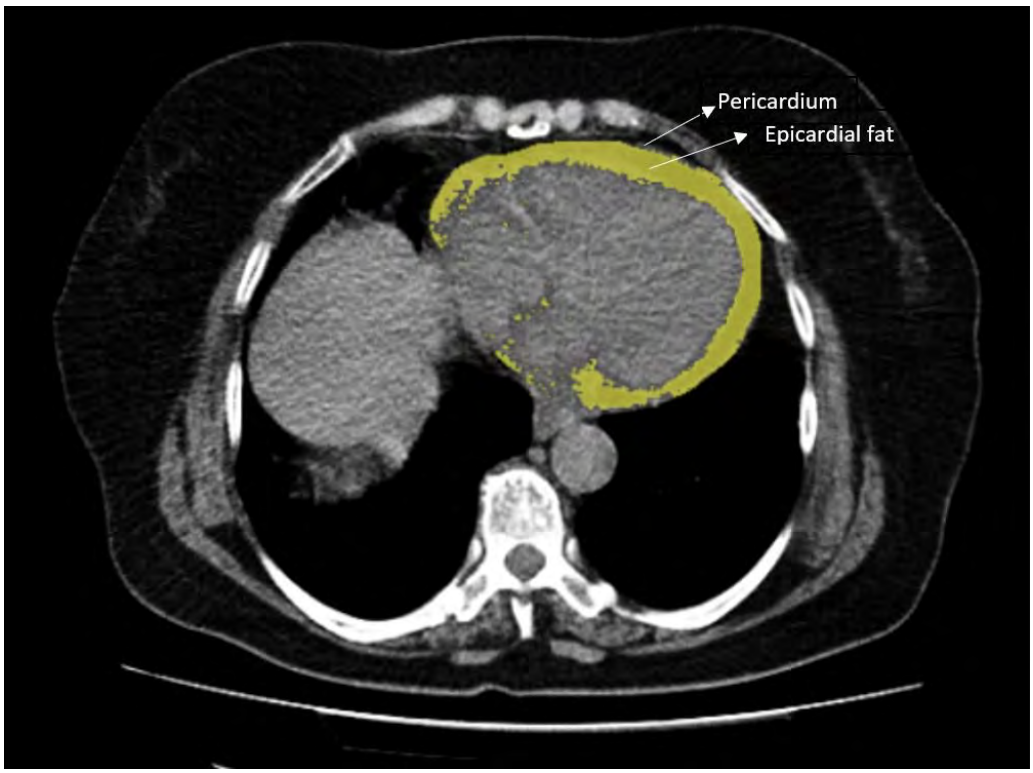

Fig. 1. Manual segmentation of epicardial adipose tissue in the pericardium, which is carried out by paying attention to the contours of the pericardium and distinguishing it from other adipose tissues. This procedure was applied to all consecutive sections. The upper border of the pericardial contours was determined as the pulmonary bifurcation, and the lower border was determined as the posterior descending artery.

mercially available software (Cedars-Sinai Quantitative Perfusion SPECT [QPS] and Quantitative Gated SPECT [QGS]). The total score at stress is called summed stress score (SSS) and reflects the extent and severity of ischemia and infarction. The difference between the SSS and SRS is called summed difference score (SDS), which reflects reversible defect.

Statistical Analysis

We used SPSS statistical software program (SPSS version 26.0, SPSS Inc., Chicago). Kolmogorov-Smirnov/Shapiro-Wilk's test was used to determine whether the data were normally distributed. The Chi-square test was used to examine the differences between categorical variables in the same patient group. While investigating the associations between non-normally distributed and/or ordinal variables, the correlation coefficients and their significance were calculated using the Spearman test. When the numerical data between two independent groups is evaluated, if the normal distribution conditions are met, the Independent Samples t-Test is used, if the normality condition is not met, the Mann-Whitney U test is used. All continuous variables were described as a mean ± SD. A p - value < 0.05 was considered statistically significant.

RESULTS

Median EFV was 53.00 ml (interquartile range: 23 ml, range 17-238 ml) in patients with normal MPS, and 62.00 ml in patients with myocardial ischemia on scintigraphy (interquartile range: 53 ml, range: 25-207 ml). The EFV value was higher in patients with reversible ischemia on MPS compared to patients with

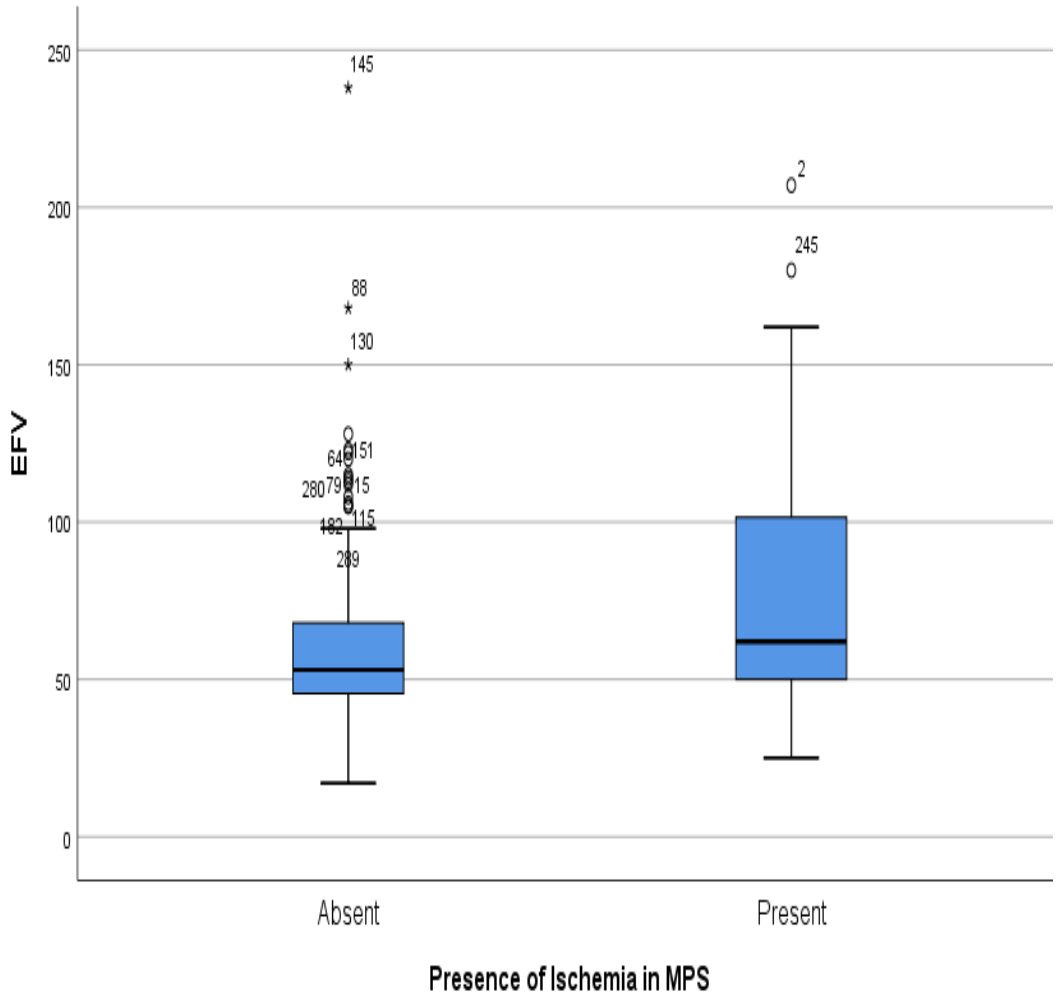

Fig. 2. The relationship between EFV and the presence of ischemia in MPS.

Table 1. Correlation of EFV with SSS, SRS, SDS, age and extent values

		SRS	SDS	SE	RE	Age	SSS
EFV	**Pearson Correlation Coefficient**	**0.240**	**0.178**	**0.262**	**0.236**	**0.227**	**0.254**
	p value	**< 0.001**	**0.002**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**

EFV = epicardial fat volume, SDS = summed difference score, SRS = summed rest score, SSS = summed stress score, SE = stress extent, RE = resting extent

normal scintigraphy findings ($p < 0.001$) (Fig. 2).

The median SDS was found to be 5.00 (range: 0-23) with reversible ischemic patients and 0.00 in patients with normal MPS. The SDS value was statistically significantly higher in patients with reversible ischemia on MPS compared to patients with normal scintigraphy findings ($p < 0.001$). There was a statistically significant, low, and positive correlation between EFV value and SDS values ($p = 0.002$, r = 0.178).

There was a statistically low and positive correlation between EFV and age ($p < 0.001$), r = 0.227). There was no statistically significant correlation between EFV and gender ($p > 0.05$). When ischemic patients were evaluated alone, no statistically significant correlation was found between EFV and SSS, SRS, SDS, and percentage of extent ($p > 0.05$). (Table-1). When the relationship between the presence of myocardial ischemia and age is investigated; While the mean age of patients with myocardial ischemia was 62.9 years, the mean age was 61.3 years in patients without ischemia, and there was no statistically significant relationship between age and presence of myocardial ischemia (p > 0.05) (Table-2).

DISCUSSION

In our study, the EFV value was statistically significantly higher in patients with reversible ischemia on MPS compared to patients with normal scintigraphy findings ($p < 0.001$). There was a statistically significant, low and positive correlation between EFV value and SDS values ($p = 0.002$, r = 0.178).

Kilambi *et al*. [6] compared EFV between patients with normal perfusion and reversible perfusion abnormalities in the MPS. According to the study of Kilambi *et al*. [6], patients with reversible perfusion defects had an increased total EFV compared to patients with normal myocardial perfusion in MPS ($p < 0.001$). In the same study, a statistically significant relationship was found between age and the presence of myocardial ischemia [6].

With the development of technology in recent years, the use of SPECT/CT hybrid gamma cameras has become widespread. At the same time, false-positive findings can be minimized because attenuation correction can be made with cardiac SPECT/CT gamma cameras. In the study of Khawaja *et al*. [7], the relationship between EFV and the presence of is-

Table 2. Age, gender distributions, and EFV in the study groups with regard to the presence of ischemia in MPS

	Normal MPS (n = 196)	Ischemic MPS (n = 99)	*p value*
Age (years), (mean ± SD)	61.2 ± 10.1	62.9 ± 10.2	0.245
Gender, n (%)			0.198
Male	70 (61.9)	43 (38.1)	
Female	126 (69.2)	56 (30.8)	
EFV (mL, median)	53.00	62.00	0.000375
SDS	0	5	< 0.001

MPS = myocardial perfusion scintigraphy, EFV = epicardial fat volume, SDS = summed difference score

chemia in the vascular territories was evaluated in patients who underwent MPS with SPECT/CT cardiac gamma cameras. In this study, patients without perfusion defects had a total EFV of 99.8 ± 82.3 cm3, which was lower than those with ischemia (156.4 ± 121.9 cm3; p = 0.001 versus controls). Nevertheless, EFV is similar to those with fixed perfusion defects that are compatible with myocardial infarction [7].

Although EFV in vascular regions separately not evaluated in this study, we investigated the relationship between the presence of myocardial ischemia and EFV. Our results were similar to these studies. The EFV values of patients with ischemia in MPS examination were significantly higher than those without ischemia ($p < 0.001$). Moreover, positive correlation was found between the SDS values obtained as a result of MPS examination and EFV ($p = 0.002$, r = 0.178).

Tamarappoo *et al*. [8] measured pericardial fat volume from non-contrast thorax CT images taken within the last 6 months of 73 patients with ischemia and 146 patients without ischemia in the SPECT scan. The mean PFV of the ischemic group was significantly higher ($p = 0.0003$). In addition, no significant difference between patients with ischemia and control patients without ischemia in terms of age, gender, and BMI were found. According to the results of this study, myocardial ischemia and pericardial adipose tissue were found to be associated with each other in patients without known coronary artery disease, and it was stated that pericardial fat volume could be used during risk stratification [8]. Janik *et al*. [9] also measured the EFV and coronary artery calcium scores (CAC) of patients who underwent Rb-82 PET/CT with a suspect of myocardial ischemia. Both parameters were significantly higher in patients with ischemia ($p < 0.01$). In addition, EFV values showed a better correlation with ischemia findings than with CAC (r = 0.47 vs r = 0.28, $p < 0.01$) [9]. In a similar study, Otaki *et al*. [10] investigated the relationship between EFV and CAC with impaired myocardial flow reserve (MFR) in Rb-82 PET/CT scans. Both the EFV and CAC values were significantly higher in patients with impaired MFR ($p = 0.01$). According to the results of this study, EFV was determined as the only independent predictor of impaired MFR [10]. Although there were some differences in methods, the results presented in these studies were in parallel with our findings.

In their study in 194 patients with suspected coronary artery disease in 2021, Yu *et al*. [11] showed that those with obstructive disease had higher EFV values ($p < 0.001$). Sun *et al*. [12] demonstrated that indigenous individuals had significantly higher EFV than nonindigenous individuals in Australia and supported the possibility that EAT may result in the greater burden of cardiovascular disease in indigenous populations. Moharram *et al*. [13] concluded that EAT thickness was significantly associated with BMI in European patients from New Zealand, but not in Maori/Pacific patients; this showed that the same BMI level showed different risk in different ethnic groups. These studies also show that EFV is associated with obesity and obviously, cardiovascular risk factors.

When all these studies were evaluated together, it was found that although epicardial fat volume was measured by different methods and myocardial ischemia was revealed by different tests, it was associated with cardiovascular risk factors. Some authors have calculated CAC to EFV in their studies, while others have shown its relevance to clinical situations. In our study, we demonstrated that there is a significant relationship between quantitative values such as SDS and SRS obtained in MPS and EFV.

Limitations

There are several limitations in our study that should be noted. First of all, our study was of a retrospective nature and the cases could not be followed up. The majority of our patient population in terms of gender consisted of female patients, and this leads to a gender imbalance. Coronary artery calcium scoring was not performed in our study. In addition to the patients who underwent MPS, no confirmation was made with coronary angiography. This study was a single-center study and reflects local patient population data only. In addition, SPECT/CT was not used in our study. Instead, SPECT images and recent CT images were used.

CONCLUSION

The EFV was higher in patients with reversible ischemia on MPS compared to normal patients. Also low and positive correlation between EFV and SDS values was found. SPECT/CT gamma cameras are currently used for MPS imaging. Epicardial adipose

tissue volume can be easily calculated from low-dose CT imaging images used for attenuation correction during this hybrid imaging examination. The automatic calculation of the EFV value during this examination may help to detect the presence of ischemia. However, studies with a larger patient population with a prospective study design are needed.

Authors' Contribution

Study Conception: SÖ, SAE; Study Design: SÖ, SAE; Supervision: SÖ, SAE; Funding: SÖ, SAE; Materials: SÖ, SAE; Data Collection and/or Processing: SÖ, SAE; Statistical Analysis and/or Data Interpretation: SÖ, SAE; Literature Review: SÖ, SAE; Manuscript Preparation: AEY and Critical Review: SÖ, SAE.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The European Research Journal 2023;9(3);637-644 Original Article

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Is osteophyte located in the inferior of the intermeniscal ligament an indication for the surgical treatment of degenerative meniscal tear?

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ABSTRACT

Objectives: Our study aimed to determine how often the osteophyte located underneath the anterior intermeniscal ligament is observed in patients who underwent arthroscopic surgery due to degenerative meniscal tears, how often this osteophyte can be diagnosed by magnetic resonance imaging, and whether this osteophyte could be an indication for the surgery to be performed for degenerative meniscopathy.

Methods: Our retrospective study included 47 patients operated for degenerative meniscus tears between 2017 and 2018, with a minimum follow-up of 2 years. Visual analog scale (VAS), Lysholm knee, and Western Ontario Meniscal Evaluation Tool (WOMET) scores were applied to all patients included in the study preoperatively and at the postoperative 3rd, 6th, 12th, and 24th months. The operated patients were grouped into two groups with and without osteophytes beneath the anterior intermeniscal ligament in magnetic resonance imaging (Group A and B). Preoperative and postoperative values of the patients were compared among themselves.

Results: The average age of the patients included in our study was 57 (range: 42 to 72) years. Forty (85%) participants were female. Osteophyte was detected in 36.1% (n = 17) of the patients in preoperative magnetic resonance imagings (Group-A). There was a statistically significant difference between preoperative VAS, Lysholm, and WOMET scores and postoperative 3rd, 6th, 12th, and 24th months ($p < 0.05$). Mean follow-up time was 32 (range: 24 to 60) months.

Conclusions: We believe that arthroscopic control of the inferior intermeniscal ligament for the presence of any osteophytes in patients treated surgically for degenerative meniscal tears is one of the main steps of this surgery.

Keywords: Degenerative meniscus, intermeniscal ligament, osteophyte, meniscectomy

The anterior intermeniscal ligament connects the lateral and medial menisci in the tibial plateau. The length of the anterior intermeniscal ligament (AIL) is approximately 33 mm, and its thickness at the insertion to the meniscus is about 3 mm. Therefore, it can rupture faster [1, 2]. Ex vivo studies have proven

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that the anterior intermeniscal ligament plays a role in the biomechanics of the knee, reducing the femorotibial contact area, spreading the intra-knee pressure force, and distributing the load between the menisci [3, 4].

The etiology of meniscal tears varies depending on age. While tears occur due to major trauma in young people, as age increases, degenerative meniscus tears occur, especially over the age of 45. At the age of 70, its prevalence rises to 50% [5]. Although there is no history of trauma in degenerative tears, they sometimes occur due to minor trauma. Degenerative meniscal tear is also common with knee osteoarthritis. A study conducted with 174 osteoarthritis patients over 45 years of age who presented with unilateral knee pain revealed a symptomatic meniscal tear in 24% on magnetic resonance imaging (MRI) [5]. However, it is also seen in patients without radiological signs of osteoarthritis [2].

The hypothesis that guides our study is that the osteophyte located beneath the anterior intermeniscal ligament is the primary source of pain during walking in patients with a degenerative meniscal tear by causing limitation of extension and debriding this osteophyte will have a positive effect on the postoperative clinical results.

In the study, how often osteophytes located beneath the anterior intermeniscal ligament are observed in patients undergoing arthroscopic surgery due to degenerative meniscal tear, whether it can be diagnosed preoperatively with MRI, and whether it can be used as an indication criterion for surgery for degenerative meniscopathy were investigated.

METHODS

The retrospective study included 57 patients operated due to degenerative meniscal tear in our hospital's orthopedics and traumatology clinic between January 2017 and June 2018, following the approval of the ethics committee (15.05.2020 i4-244-20). Inclusion criteria for the study were patients aged between 40-75 years, clinically and radiologically compatible with degenerative meniscal rupture, conservative treatment was tried for at least three months, but the pain did not decrease, and patients with radiological Kellgren and Lawrence [6] grade 0-I. Exclusion criteria were patients with a traumatic meniscal tear, meniscal root tear, anterior cruciate ligament rupture with knee locking complaint, and radiological Kellgren and Lawrence grade III-IV. Ten patients were excluded from the study, and 47 patients were included. All forty-seven patients were followed up regularly. In 17 (36.17%) of 47 patients who underwent arthroscopic surgery due to degenerative meniscal tear, osteophytes

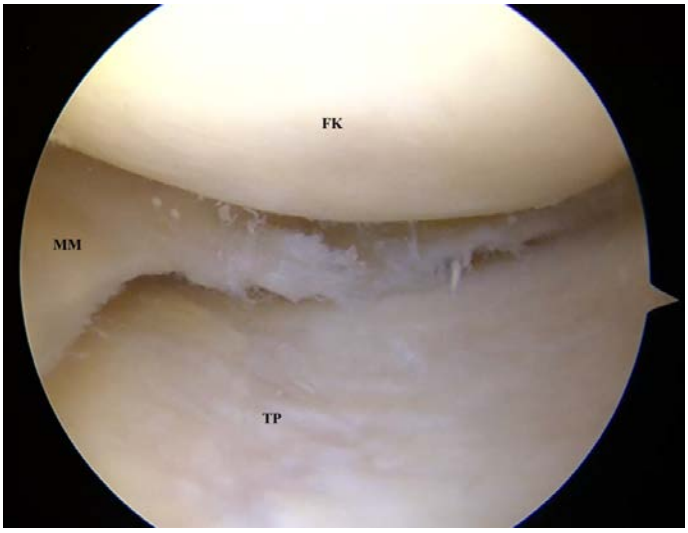

Fig. 1. The left knee is viewed from the anterolateral portal with a 300 arthroscope. Medial femoral condyle, degenerative ruptured medial meniscus, and medial tibial plateau are observed. FK = Femoral condyle, MM = Medial meniscus, TP = Tibia plato.

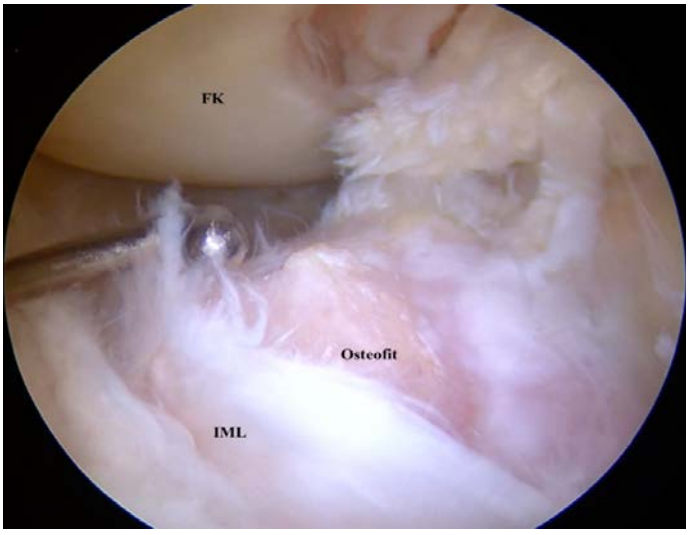

Fig. 2. The left knee is viewed from the anterolateral portal with 300 arthroscope. While the intermeniscal ligament (IML) and its surroundings are examined with the probe's help, the osteophyte borders are seen. FK = Medial femoral condyle.

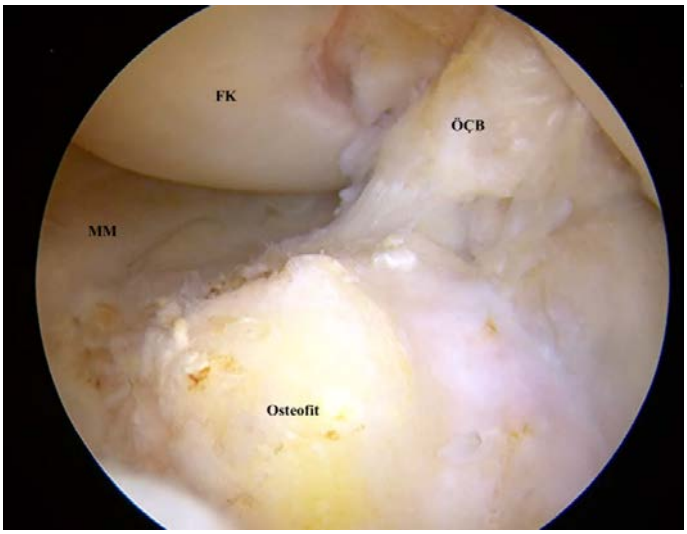

Fig. 3. The left knee is viewed from the anterolateral portal with 300 arthroscope. When the intermeniscal ligament (IML) is retracted anteriorly with a probe, it is seen that the osteophyte is fully revealed. FK = Medial femoral condyle, ÖÇB = Anterior cruciate ligament, MM = Medial meniscus.

located beneath the anterior intermeniscal ligament were observed in MRI (Group-A), whereas it was not seen in 30 (63.83%) patients (Group B). For the clinical evaluation of the patients, visual analogue scale (VAS) [7], Lysholm knee scores [8], Western Ontario Meniscal Evaluation Tool (WOMET) scores [9], joint range of motion were evaluated and recorded at preoperative and postoperative 3rd, 6th, 12th, and 24th months. All patients had weight-bearing and 2-view knee X-rays along with MRI preoperatively.

All patients' surgeries were performed by the same experienced surgeon (RA). After the standard anterolateral and anteromedial knee portals were opened, diagnostic arthroscopy was performed (Fig. 1). Partial meniscectomy was performed on degenerative menisci with 4.5 mm shaver (Dyonics, Smith and Nephew, USA), and arthroscopic hand tools and appropriate contour was given. In all patients, the intermeniscal ligament was examined arthroscopically with the help of an arthroscopic probe and then evaluated for osteophytes at its inferior (Figs. 2 and 3). For patients with osteophytes, osteophytes were debrided and contoured with a 5.5 mm burr (Dyonics, Smith and Nephew, USA) (Figs. 4 and 5). Active isometric quadriceps exercises were started on the 1st postoperative day in all patients. Full weight-bearing was allowed gradually in the first three weeks. Although patients could return to low-impact sports activities three months following surgery, contact sports activities were allowed at the end of the 6th month.

Statistical Analysis

SPSS 2020 package (IBM Corp. Released 2020. IBM SPSS Statistics for Macintosh, Version 27.0. Armonk, NY: IBM Corp) was used to evaluate data collected from patients. Normally distributed data are

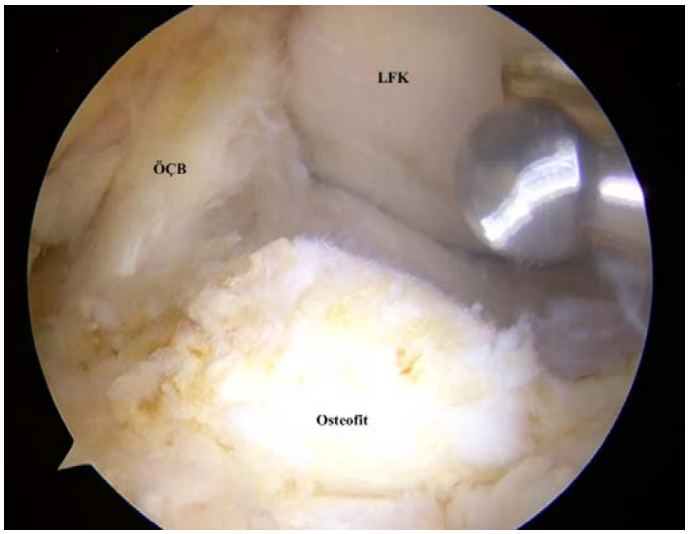

Fig. 4. While the left knee is viewed from the anterolateral portal with 300 arthroscope, 5.5 mm burr in the anteromedial portal. Contouring the osteophyte in the inferior of the intermeniscal ligament with a 5.5 mm burr. LFK = Lateral femoral condyle, ÖÇB = Anterior cruciate ligament..

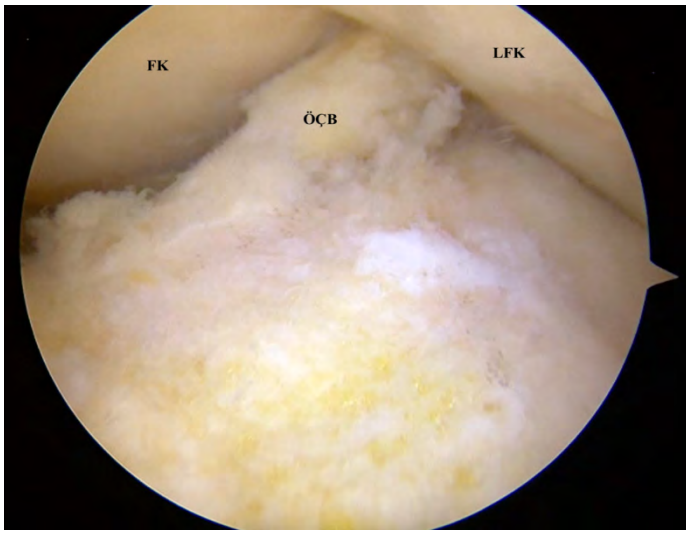

Fig. 5. The left knee is viewed from the anterolateral portal with 300 arthroscope. The osteophyte located beneath the intermeniscal ligament (IML) is seen after the full contouring. FK = Femoral condyle, ÖÇB = Anterior cruciate ligament, LFK = Lateral femoral condyle.

shown as mean and standard deviation, and non-normally distributed data are shown as median and minimum-maximum values. The student's t-test evaluated the patients' pre-and postoperative clinical scores. The Friedman test was used to compare the clinical scores. A $p < 0.05$ was accepted as statistically significant.

RESULTS

Of the 47 patients included in the study, 40 (85.1%) were female, and 7 (14.9%) were male. The mean age was 57.6 ± 6.8 years, and the median value was 57 (min: 42-max: 72). The median value of the body mass index was determined as 28 (min: 24-max: 38) (Table 1). The mean follow-up time was 32 (min: 24-max: 60) months. Patients lost to follow-up were excluded.

A statistically significant difference was found between the preoperative VAS scores of the patients and the postoperative 3rd, 6th, 12th, and 24th months ($p < 0.001$). When the VAS scores between the 3rd month and 6th, 12th, and 24th months postoperatively were compared, a statistically significant difference was observed. Again, the difference between the VAS scores at the 6th and 24th months postoperatively was statistically significant (Tables 2 and 3). When male and female patients were examined separately, there was a statistically significant difference between the preoperative VAS scores and the postoperative VAS scores of the female patients. However, no statistically significant difference was observed between the preoperative VAS scores and the postoperative 3rd and 6th month VAS scores of the male patients ($p = 1.000 – p = 0180$). However, a significant difference was ob-

Table 1. Demographic characteristics of the patients

	Total n = 47	Group A n = 17	Group B n = 30	*p* value
Age (years)	57.57 ± 6.82 (42-72)	55.88 ± 5.66 (46-67)	58.53 ± 7.32 (42-72)	0.2040
Height (m)	1.65 ± 0.64 (1.50-1.84)	1.64 ± 0.71 (1.50-1.82)	1.66 ± 0.67 (1.56-1.84)	0.2343
Weight (kg)	79.70 ± 8.91 (59-106)	79.33 ± 9.02 (60-105)	79.94 ± 8.88 (59-106)	0.6786
BMI (kg/m^2)	29.08 ± 3.2) (23.63-37.50)	28.65 ± 2.52 (23.63-32.87)	29.32 ± 3.65 (23.94-37.50)	0.5100

Data are shown as mean ± standard deviation or (minimum-maximum). BMI =body mass index

Table 2. VAS, Lysholm, and WOMET scores in preoperative and postoperative periods

	VAS	LYSHOLM	VOMET
Preoperative	8.34 ± 1.20 8 (6-10)	26.17 ± 6.27 26 (14-38)	1153.62 ± 129.26 (730-1390)
Postopoperative 3rd month	5.00 ± (2.35) 5.0 (0-10)	68.87 ± (12.04) 71 (41-100)	591.62 ± 1250.19 (40-1080)
Postopoperative 6th month	3.26 ± 2.19 3 (0-10)	81.49 ± 12.71 84 (47-100)	360.0 ± 235.77 (20-1060)
Postopoperative 12th month	2.04 ± 2.33 1 (0-10)	86.89 ± 11.06 90 (42-100)	280.0 ± 223.10 (10-1085)
Postopoperative 24th month	1.26 ± 2.17 0 (0-10)	85.85 ± 11.04 91 (42-100)	200.0 ± 138.95 (0-1095)

Data are shown as mean ± standard deviation or median (minimum-maximum). VAS = Visual Analog Scale, LYSHOLM = Knee Scoring Scale, VOMET = Western Ontario Meniscal Evaluation Tool

Table 3. Statistical analysis of VAS, LYSHOLM, and WOMET scores in preoperative and postoperative periods

	Preoperative	Postoperative 3rd month	Postoperative 6th month	Postoperative 12th month
	p value	*p* value	*p* value	*p* value
Preoperative				
VAS	-			
LYSHOLM				
WOMET				
Postoperative 3rd month				
VAS	**0.004**	-		
LYSHOLM	**0.006**			
WOMET	**0.002**			
Postop 6th month				
VAS	**< 0.001**	**0.037**	-	
LYSHOLM	**< 0.001**	**0.003**		
WOMET	**< 0.001**	0.061		
Postoperative 12th month				
VAS	**< 0.001**	**< 0.001**	0.100	-
LYSHOLM	**< 0.001**	**< 0.001**	0.068	
WOMET	**< 0.001**	**< 0.001**	0.075	
Postoperative 24th month				
VAS	**< 0.001**	**< 0.001**	**0.001**	1.000
LYSHOLM	**< 0.001**	**< 0.001**	0.061	1.000
WOMET	**< 0.001**	**< 0.001**	0.189	0.189

VAS = Visual Analog Scale, LYSHOLM = Knee Scoring Scale, VOMET = Western Ontario Meniscal Evaluation Tool

served between the 3rd month and 12th and 24th month scores ($p < 0.05$) (Table 4).

When the Lysholm scores of the patients were compared, a statistically significant difference was found between the preoperative Lysholm knee scores and the values in the postoperative follow-ups (Tables 2 and 3). A significant difference was found between the preoperative period and the postoperative period in WOMET scores, in which we evaluated both the physical activities and return to sports and the emotional state of our patients. When we evaluated the postoperative follow-ups among themselves, no statistically significant difference was found between the postoperative 3rd and 6th months, 6th and 12th months, and postoperative 12th and 24th months (Tables 2 and 3).

When the correlation between the BMI values of the patients and the preoperative VAS values was examined, it was observed that there was a positive correlation (r: 0.426, $p = 0.003$). Since the correlation between VAS values and BMI values in the postoperative follow-ups was examined, a positive correlation was observed at the 3rd month (r: 0.304, $p = 0.038$), but there was no correlation between the VAS values in the remaining follow-ups. When the BMI values of the patients were compared with the postoperative Lysholm knee scores, a negative correlation was found between the postoperative 6th month Lysholm knee score (r = -0.313, $p = 0.032$). No relationship was observed in the postoperative 3rd, 12th, and 24th month follow-ups. No statistical correlation was found between the BMI values of the patients and the WOMET

Table 4. The mean-median values of VAS scores of male and female patients

Gender	Preoperative VAS	Postoperative 3rd month VAS	Postoperative 6th month VAS	Postoperative 12th month VAS	Postoperative 24th month VAS
Female	8.30-8.00	4.85-5.00	3.27-3.00	2.13-1.50	1.38-1.00
Male	8.57-8.00	5.86-5.00	3.14-3.00	1.57-1.00	0.57-1.00
Total	8.34-8.00	5.00-5.00	3.26-3.00	2.04-1.00	1.26-1.00

scores evaluated in the preoperative or postoperative follow-ups.

The patients who underwent surgery were grouped as those who underwent osteophyte debridement (Group A, n = 17) and those who did not (Group B, n = 30), and comparisons were made in the same way. According to the results of this study sensitivity of the MRI is 100% for the presence of osteophytes underneath the AIL; 17 patients in Group A, has osteophytes seen in MRI and proved by arthroscopy. Also, the 30 patient in Group B no osteophytes in MRI or arthroscopy.

In patients included in Group A, preoperative VAS score was 8.12 (min: 6-max: 10), postoperative 3rd

Table 5. Preoperative and postoperative VAS, LYSHOLM, and WOMET scores and extention angle of patients with osteophytes (+) and osteophytes (-)

	Group A	Group B	*p* value
Preoperative			
VAS	8.12 (6-10)	8.47 (7-10)	0.3425
LYSHOLM	25.41 (14-38)	26.6 (16-38)	0.6256
WOMET	1173.53 (980-1290)	1145.67 (730-1380)	0.5872
Extention angle (^{0})	4.71 ± 5.14	3.50 ± 4.58	0.4296
Postoperative 3rd month			
VAS	4.59 (0-10)	5.23 (0-10)	0.2053
LYSHOLM	67.71 (41-95)	71.37 (41-100)	0.2677
WOMET	623.24 (290-1080)	577.50 (40-1080)	0.7147
Postop 6th month			
VAS	3.29 (0-10)	3.23(0-10)	0.9372
LYSHOLM	79.29 (47-95)	83.50(47-100)	0.4780
WOMET	477.65 (130-1060)	408.65 (20-1050)	0.7987
Postoperative 12th month			
VAS	2.18(0-9)	1.97 (0-10)	0.5941
LYSHOLM	85.18(54-95)	88.23 (42-100)	0.2183
WOMET	380.0 (45-1085)	311.67 (10.1050)	0.6178
Postoperative 24th month			
VAS	1.12 (0-9)	1.33(0-10)	0.7532
LYSHOLM	87.76 (54-99)	88.13(42-100)	0.7985
WOMET	316.47 (0-1095)	261.5(10-1050)	0.9118
Extention angle (^{0})	-9.71 ± 1.21	-0.17 ± 3.07	**< 0.0001**

Data are shown as mean ± standard deviation or median (minimum-maximum). VAS = Visual Analog Scale, LYSHOLM = Knee Scoring Scale, VOMET = Western Ontario Meniscal Evaluation Tool

month of 4.59 (min: 0-max: 10), postoperative 6thmonth of 3.29 (min: 0-max: 10), postoperative 12thmonth of 2.18 (min: 0-max: 9), postoperative 24thmonth of 1.12 (min: 0-max: 9). The mean value of the preoperative VAS score of the patients included in Group B was 8.47 (min: 7-max: 10), the mean value of the postoperative 3rd month was 5.23 (min: 0-max: 10), the postoperative 6th month mean value was 3.32 (min: 0-max: 10), postoperative 12th month mean value was 1.127 (min: 0-max: 10), postoperative 24th month mean value was 1.33 (min: 0- max: 10). When these two groups were compared, no statistically significant difference was found between preoperative, postoperative 3rd month, 6th month, 12th month, and 24th month VAS, Lysholm, and WOMET scores (Table 5).

Mean preoperative extension angle was 3.94 ± 4.77. It was 4.71 ± 5.14 for group A and 3.50 ± 4.58 for group B without significant difference (p = 0.4296). Mean postoperative extension angle was -3.62 ± 5.29. It was -9.71 ± 1.21 for group A and -0.17 ± 3.07 for group B with a significant difference (p < 0.001).

DISCUSSION

A statistically significant difference was found between the patients' preoperative VAS, Lysholm, and WOMET scores and the postoperative 3rd, 6th, 12th, and 24th month results. However, no statistically significant difference was found between the 6th, 12th, and 24th month values. While there was no significant difference in functional scores between the two groups, separated according to the presence of osteophytes beneath the AIL, a significant difference was found when the postoperative range of motion was examined.

In the treatment of degenerative meniscal tears, meniscectomy, which has been frequently applied in previous years, has started to leave its place to conservative treatment methods with the realization of the critical role of menisci in joint stability [10, 11]. Many studies have shown that long-term follow-up results after meniscectomy cause an increase in the development of osteoarthritis, and it does not have a significant advantage over conservative treatment. Li *et al*. [12] compared pain and function results at 12th month and 24th month in patients with degenerative meniscus treated with arthroscopic partial menisectomy (APM) and physical therapy alone. Although patients with APM at 12th month were better, they did not find a significant difference between the two groups in their results at 24th month. This was thought to be because the later onset of osteoarthritic changes suppressed the significant benefits in the short term.

APM, which was frequently applied in previous years, has now started to give way to conservative treatment. In 2016, ESSKA (European Society of Sports Traumatology, Knee Surgery and Arthroscopy) recommended that APM be removed from being the primary treatment for degenerative meniscal lesions, with the "Meniscus Consensus Project" report, in which 84 surgeons and scientists contributed [13]. According to this report, it has been stated that APM can be recommended to patients who have pain and mechanical symptoms after three months of conservative treatment, have normal X-ray findings and have grade 3 meniscus lesions on MRI. It has been reported that early surgery can be recommended in patients with obvious mechanical symptoms or severe osteoarthritis findings on X-rays [13].

Osteophytes are accepted as one of the radiological diagnostic markers of knee osteoarthritis [14]. Osteophytes are abnormal bone spurs around the joint and are frequently seen in middle-aged and older people. Unlike previous studies, we divided the patients who underwent surgery into those with and without osteophytes beneath the AIL in MRI. In 17 of 47 patients who were operated, osteophyte debridement was also performed due to the presence of osteophytes beneath the AIL in MRI. When the postoperative VAS, Lysholm, and WOMET scores of the patients who underwent osteophyte debridement were compared with the other group, no statistically significant difference was observed. When the postoperative joint range of motion was compared, a statistically significant difference was found in favor of those who underwent debridement. Felson *et al*. [14] showed that osteophytes formed in the knee joint restricted the free movement of the femur on the tibia. As a result of our study, although we accept that conservative treatment is the primary treatment method in the general population in patients with degenerative meniscal tears, we think that osteophyte debridement should be performed in patients with osteophytes on MRI. It may provide a higher postoperative clinical outcome and a

better range of motion.

We suggest that patients with degenerative meniscal tears should be evaluated preoperatively for the presence of osteophytes on MRI. The ESSKA report suggests that conservative treatments should be preferred in patients whose osteophytes are not detected in preoperative MRI. Current treatments have begun to focus on preserving these tissues as much as possible, considering the role of menisci in joint stability. Various studies have shown that even partial meniscectomy is insufficient to protect the joint.

Limitations

There are several limitations of our study. Our retrospective study shows the follow-up results of patients with APM who were operated on between 2017-2018. Considering that meniscectomy is no longer the primary treatment, more objective results can be obtained with a prospective study in which patients treated with nonoperative methods and undergoing only osteophyte debridement are examined. Another limitation of our study is that it had a small sample of 47 patients

CONCLUSION

We think that osteophyte debridement, which will be applied without meniscectomy in patients with osteophytes detected in MRI, can preserve meniscus functions as much as possible and contribute positively to the postoperative healing process.

Authors' Contribution

Study Conception: EAÖ; Study Design: EAÖ, RA; Supervision: MOK, RA; Funding: N/A; Materials: EAÖ, MOK; Data Collection and/or Processing: MCG; Statistical Analysis and/or Data Interpretation: MCG, MK; Literature Review: MCG, MK; Manuscript Preparation: MCG, EAÖ, MOK and Critical Review: RA, EAÖ.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The European Research Journal 2023;9(4):645-651 Original Article

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Neutrophil to lymphocyte ratio may be used as a predictor in tendinopathy

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ABSTRACT

Objectives: Tendinopathy is a very common clinical disorder and a complex inflammation and degeneration process. Neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) have been studied and accepted as biomarkers of inflammation, predictors of progression and prognosis in several studies. We aimed to show that NLR and PLR levels can help predict prognosis for tendinopathy by comparing NLR and PLR levels of patients with tendinopathy with healthy control groups.

Methods: Tendinopathy and healthy cases whose complete blood test was requested, were included in the study. Age, gender, white blood cell (WBC), neutrophil, lymphocyte, platelet counts, NLR and PLR of these cases were examined.

Results: There is a statistically significant difference between the tendinopathy (n = 140) and control (n = 51) groups in WBC and NLR values ($p < 0.001$). One unit increase in WBC and NLR levels increases the risk of tendinopathy 1.74 and 10.616 times respectively. According to the results of the ROC analysis, the threshold value of the WBC and NLR values to increase the risk of tendinopathy is 6.47, and 1.64 respectively.

Conclusions: Our study suggested NLR and WBC values are significant indicators of tendinopathy. We consider these results to be a guide for all physicians, especially sports medicine physicians.

Keywords: Tendinopathy, neutrophil to lymphocyte ratio, inflammation, neutrophil

Tendinopathy is a very common clinical problem in the population and athletes [1]. Thirty percent of all running injuries are chronic tendon disorders and a prevalence of 40% in tennis players [2]. Tendinopathy may cause severe morbidity and disability that can last for several months despite appropriate treatment [3]. The words "tendinitis", "tendinosis" and "tendinopathy" are used interchangeably in the literature. According to the consensus prepared to eliminate this terminology confusion, persistent tendon pain and loss of function are called "tendinopathy" [4]. The source of this confusion in terminology is the unclear etiological disagreement about inflammation. The source of this disagreement is the interchangeability of "neutrophil count" and "inflammation" [1]. In the first histopathological studies, unlike other inflammatory processes, it is claimed that "no inflammatory cells" or "few inflammatory cells" were found in sam-

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ples of patients with tendinopathy only because they could not find neutrophils. With this result, it is evaluated that a false perception has been created for years [1]. Neutrophils are seen only in the first two days. The inflammatory process continues with platelets, macrophages, growth factors, matrix metalloproteins (MMPs) and cytokines [2]. Until recent decade, tendinopathy has been defined as tendon degeneration without inflammation due to overuse in the literature [5, 6]. Tendon overload has been shown to be associated with changes in shape of the cell as well as inflammation markers and matrix degradation. It is assumed that tendinopathy develop as a result of the remodeling of the matrix due to inflammation and damage, and is also the source of this condition in cytokines and inflammatory cells. In addition, the number of macrophages in the diseased tendon tendinopathy increasingly used in recent years increased pathogenesis studies showing that support the role of inflammation [1, 7]. The macrophage activation pathways assigned in rotator cuff tendinopathy samples show rised genes and proteins expression stimulated by interferons and NF-kB37 at an early stage and by STAT6 and glucocorticoid receptor activation pathways at an advanced stage [1]. These may be evidence of a complex inflammatory process and persistent inflammation in tendinopathy [1, 7]. Tendinopathy that does not improve in 3-6 weeks in athletes means pain and loss of function, loss of performance, or loss of training/match. The loss of the training/match brings serious losses for both the athlete and the team. Treatment choices should be determined correctly to ending the complaints and returning to sport. Nonsteroidal anti-inflammatory medicines do not help in tendinopathy. Tendinopathy can be diagnosed by examination, the use of imaging method for diagnosis is not the gold standard [4].

Neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) have been studied and accepted as biomarkers of inflammation obtained from complete blood, predictors of progression and prognosis in various cancers, rheumatoid arthritis, cardiovascular and inflammatory and infectious pathologies [8, 9]. However, no similar study on tendinopathy has been found in the literature.

In the light of all this information, we aimed to show that NLR and PLR levels can help predict prognosis for tendinopathy by comparing NLR and PLR levels of patients with tendinopathy with healthy control group.

METHODS

Tendinopathy and healthy cases who applied to the sports medicine outpatient clinic of our hospital between January 2016 and November 2020, and whose complete blood test was requested, and included in the study. Achilles, patellar, biceps, lateral and medial elbow, rotator cuff, and hamstring tendinopathies without inflammatory diseases, infection or any other reason that causes inflammation were included in the study as tendinopathy from records and these cases were defined as tendinopathy group. Individuals who were investigated for a complete blood test to evaluate for iron deficiency and vitamin deficiencies and had a normal result were accepted as healthy cases and were defined as healthy group. Age, gender, white blood cell (WBC), neutrophil, lymphocyte, platelet counts, NLR and PLR of these groups were compared.

Diagnosis of tendinopathy can be done by only physical examination and imaging is not a gold standard [4]. So, we did not compare their diagnosis accuracy retrospectively with imaging results, but the tendinopathies were diagnosed by 3 different experienced sports physician experts in 4 years.

The local ethics committee was approved this retrospective study (Ethics Committee of Health Sciences University Turkey, Date: 25.03.2021 and number: 2021/78).

Statistical Analysis

Statistical analysis was performed using R 3.5.0 (R Core Team, 2018) software. Data were shown as mean ± standard deviation or median (minimum-maximum) values. In univariate comparisons, the Shapiro Wilk test was used to evaluate whether the continuous variables were normally distributed, and the Levene test for the homogeneity of variances. When parametric assumptions were not provided, the Mann-Whitney U test was used to compare two independent groups in terms of a numerical variable, and when parametric assumptions were provided, the significance test of the difference between the two means (student t-test) was used. In categorical variables, the data will be summarized in percentages. In the comparison of two inde-

pendent groups in terms of a categorical variable, the chi-square test or Fisher Exact test was applied by evaluating their assumptions. In multivariate comparisons, a logistic regression model was used for multivariate analysis of prognostic factors. ROC analysis was performed to determine the threshold value for continuous variables that were found to be significant as a result of the logistic regression and threshold val-

Table 1. Paired comparison results in groups with and without tendinopathy

Variable		Control	Tendinopathy	Overall	*p* value
Gender, n (%)	Total	51 (26.70)	140 (73.30)	191	0.623
	Female	31 (60.78)	79 (56.43)	110 (57.59)	
	Male	20 (39.22)	61 (43.57)	81 (42.41)	
Age (years)	Mean ± SD	28.65 ± 9.76	30.41 ± 11.89	29.94 ± 11.36	0.622
	Median (IQR)	27.00 (15.00)	25.50 (22.00)	26.00(21.00)	
	Q1-Q3	21.00-36.00	20.00-42.00	20.00-41.00	
	Min-Max	15.00-50.00	15.00-50.00	15.00-50.00	
WBC	Mean ± SD	5.66 ± 1.12	7.14 ± 1.66	6.75 ± 1.67	**< 0.001**
	Median (IQR)	5.40 (1.65)	7.00 (1.73)	6.60 (2.10)	
	Q1-Q3	4.90-6.55	6.27-8.00	5.45-7.55	
	Min-Max	3.90-8.30	3.40-14.30	3.40-14.30	
Neutrophil	Mean ± SD	3.06 ± 0.73	4.49 ± 1.61	4.11 ± 1.56	**< 0.001**
	Median (IQR)	2.90 (1.15)	4.30 (1.73)	3.90 (1.60)	
	Q1-Q3	2.50-3.65	3.40-5.12	3.10-4.70	
	Min-Max	1.70-4.70	2.10-14.90	1.70-14.90	
Lymphocyte	Mean ± SD	2.02 ± 0.40	2.04 ± 0.54	2.04 ± 0.50	0.774
	Median (IQR)	2.00 (0.45)	2.00 (0.60)	2.00 (0.60)	
	Q1-Q3	1.80-2.25	1.70-2.30	1.70-2.30	
	Min-Max	1.20-3.20	0.70-3.50	0.70-3.50	
Platelet	Mean ± SD	247.57 ± 67.83	266.39 ± 60.61	261.37 ± 62.99	**0.016**
	Median (IQR)	242.00 (67.50)	260.00 (75.75)	255.00 (75.00)	
	Q1-Q3	204.00-271.50	225.75-301.50	221.00-296.00	
	Min-Max	131.00-559.00	133.00-452.00	131.00-559.00	
NLR	Mean ± SD	1.52 ± 0.28	2.37 ± 1.26	2.14 ± 1.15	**< 0.001**
	Median (IQR)	1.50 (0.34)	2.07 (1.02)	1.84 (0.95)	
	Q1-Q3	1.35-1.68	1.65-2.66	1.50-2.45	
	Min-Max	1.04-2.15	1.02-10.43	1.02-10.43	
PLR	Mean ± SD	125.43 ± 34.54	138.83 ± 47.78	135.25 ± 44.94	0.093
	Median (IQR)	115.26 (40.04)	129.98 (55.42)	125.79 (49.53)	
	Q1-Q3	103.50-143.55	106.63-162.05	106.06-155.59	
	Min-Max	63.90-217.51	61.75-320.00	61.75-320.00	

WBC = white blood cell, NLR = neutrophil to lymphocyte ratio, PLR = platelet to lymphocyte ratio, Min = minimum, Max = maximum, SD = standard deviation

ues were determined according to the minimum-maximum rule. In the calculations, the type 1 error rate was accepted as alpha 0.05. Tables were created with Microsoft Excel.

RESULTS

A total of 191 cases were included in the study as 140 tendinopathy group and 51 control group. Comparison of demographics and laboratory features of cases are shown in Table 1. According to paired comparisons of tendinopathy and control groups, there is a statistically significant difference between the groups in terms of WBC and NLR values ($p < 0.001$ for both) (Table 1). There is no significant difference between groups in terms of PLR values ($p = 0.093$). Comparison of NLR and PLR values with box-plot in tendinopathy and control groups are shown in Figs. 1 and 2, respectively.

The results of the logistic regression model made for multivariate analysis are given in Table 2. WBC and NLR were found to be important model variables with the backward selection method. Accordingly, one unit increase in WBC level increases the risk of tendinopathy OR = 1.74 times. Similarly, one unit increase in NLR increases the risk of tendinopathy OR = 10.616 times.

According to the results of the ROC analysis, the threshold value of the WBC value to increase the risk of tendinopathy is 6.47, and the threshold value of the NLR value to increase the risk of tendinopathy is 1.64 (Fig. 3).

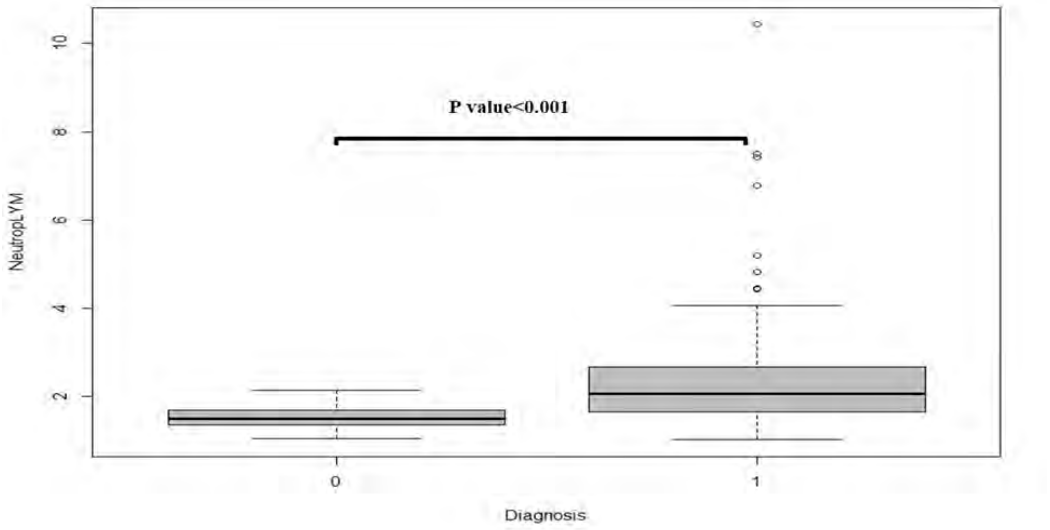


Fig. 1. Comparison of NLR value with box-plot in tendinopathy and control groups.

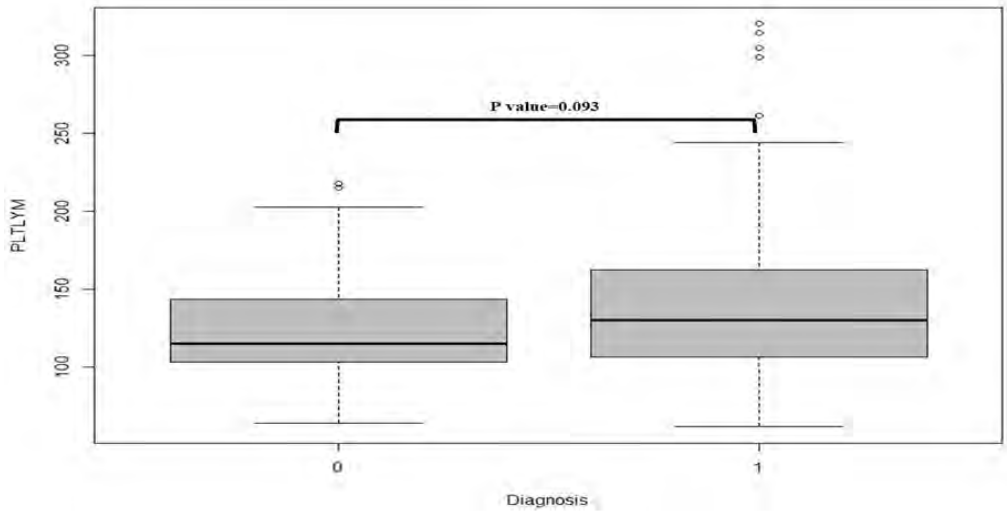


Fig. 2. Comparison of PLR value with box-plot in tendinopathy and control groups.

Table 2. Logistic regression results

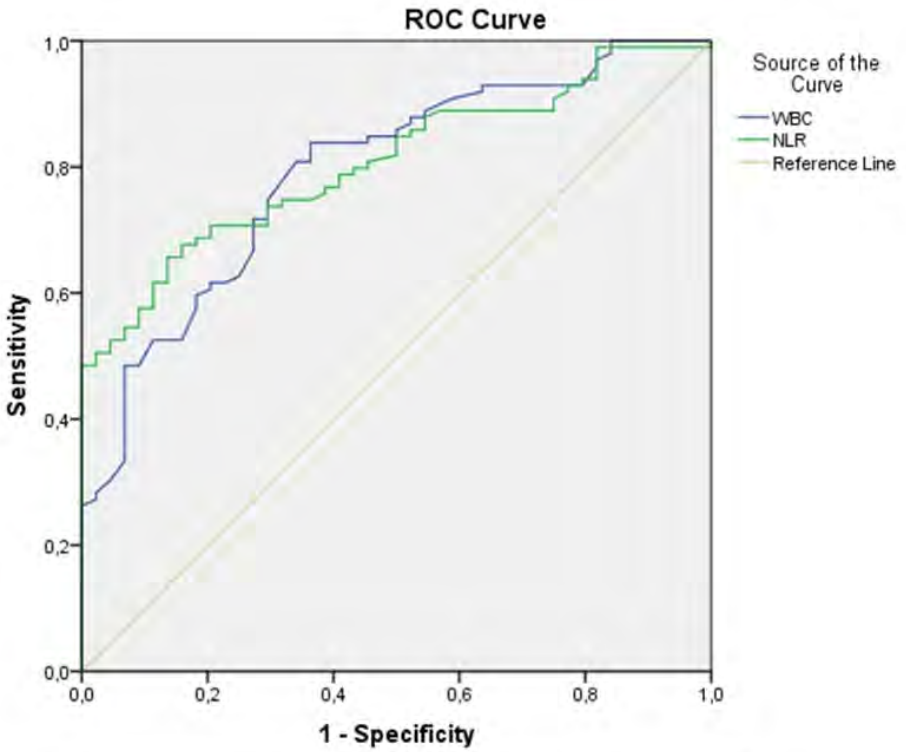
	Dependent variable:
	Diagnosis
WBC	1.746*** (1.261, 2.493)
NLR	10.616*** (3.773, 35.966)
Constant	0.001*** (0.0001, 0.013)
Observations	191
Log Likelihood	-77.892
Akaike Inf. Crit.	161.785

WBC = white blood cell, NLR = neutrophil to lymphocyte ratio

DISCUSSION

This study evaluated NLR and PLR in patients with tendinopathy and in control cases. Our results support the association between NLR and tendinopathy. Moreover, WBC also associated with tendinopathy. NLR and WBC may be a simple and cost-effective method for tendinopathy and also increased NLR and WBC may be predictors of tendinopathy. According to the results of the ROC analysis, the threshold value of 1.64 for NLR and 6.47 for WBC was defined to separate between tendinopathy patients and controls. This is the first study to evaluate the value of NLR and WBC to predict the prognosis of tendinopathy in athletes.

The definition of tendinopathy and the presence of inflammation in tendinopathy has been a discussion issue and opinions change within the framework of histopathological findings over the years. Although it is claimed that it develops with non-inflammatory degeneration due to loading over time [10], some studies showed that inflammatory responses and degeneration are simultaneous with the Iceberg Theory [5, 11]. While the presence of inflammation is being discussed in tendinopathy, our findings supply new evidence for inflammation. A statistically meaningful difference was found in WBC, neutrophil, platelet and NLR values between tendinopathy and healthy group in our



Test Result Variable(s)	AUC	Cut-Off	Asymptotic Sig.[b]	95% CI Lower Bound	95% CI Upper Bound
WBC	,793	6,470	,000	,716	,869
NLR	,806	1,640	,000	,737	,875

Fig. 3. ROC curve analyses of WBC and NLR.

study. Lymphocyte ratios are similar. This shows that the NLR value of tendinopathy group increases due to the rising in the number of neutrophils not from decrease of the lymphocyte counts. In addition, PLR value was found to be high, although it was not statistically significant ($p = 0.093$). Maybe it would have been meaningful if it had been studied with a larger group. High values of WBC, neutrophils and NLR are also indicators of inflammation.

In addition, we can say that the most important result of this study is to show that each unit increase after exceeding the cut-off value in the WBC and NLR values increase the tendinopathy risk 1.7 and 10.6 times respectively. Neutrophils may not be demonstrated or maybe a little demonstrated in tendinopathy in histopathological studies, the fact that the NLR value is such an effective indicator may be evidence of how complex the inflammation cascade is.

WBC and NLR values are obtained from the complete blood test, which is a simple and inexpensive test to apply. NLR value had shown as a valuable inflammatory marker in Ankylosing Spondylitis with a cut off value off 1.91 [12]. Another study had demonstrated the correlation of higher NLR and interleukin-6 in chronic kidney diseases [13]. A study had been found the association between NLR and TNF-α in end stage renal diseases [14] . Fawzy *et al*. [15]'s study proved NLR as a significant inflammation marker in rheumatoid arthritis and showed the correlation between NLR and erythrocyte sedimentation rate, C-reactive protein. In a study comparing inflammation biomarkers of patients with upper extremity overuse musculoskeletal problems for less than 12 weeks and a control group, TNF-α, IL-1β, and IL-6 were moderately correlated, but CRP was strongly associated [16]. Long-term continuous loading increases IL-10 which is a serum biomarker. Serum biomarkers are seen as a useful method considering the relationship between stress, exercise, and injury [17]. TNF-α, IL-1β, IL-6 and IL-10 are beneficial serum biomarkers but more expensive than WBC and NLR.

In a retrospective study by Karakoyun *et al*. [18], there was no correlation between NLR and PLR values and epicondylitis. Different from our study they included patients with medial and lateral epicondylitis and the mean age of patient group was 55.31 ± 2.30 years and the 56.45 ± 4.81 years in healthy group. The mean age of the patient with tendinopathy was 30.41 and control group's was 28.65 in our study. Inflammation in tendon healing and regeneration may be change by age. Excessive inflammation is seen with aging [19], inflammation with tendinopathy may be underestimated due to this excessive inflammation.

Limitations

Our study has some limitations. The main limitation is retrospective design. We did not compare their diagnosis accuracy with imaging results. We did not compare or discuss the duration, grade of tendinopathy, recreational or elite sportsperson or branches of the individuals due to retrospective design. Another limitation is deficiency of comparing with other proinflammatory and inflammatory markers. These findings should be evaluated carefully and repeated with a prospective design for the verification. The strength sides of the study are that it consists of recreational individuals and athletes (absence of sedentary individuals) and an average age of approximately 30.

CONCLUSION

Our study suggested NLR and WBC values are significant indicators of tendinopathy. Also using these values are simple, cheap and cost effective. We consider these results to be a guide for all physicians, especially sports medicine physicians.

Authors' Contribution

Study Conception: AÖ; Study Design: AÖ, İK; Supervision: AÖ, İK; Funding: AÖ, BK, İK, İS; Materials: BK, İK, AÖ; Data Collection and/or Processing: BK, AÖ; Statistical Analysis and/or Data Interpretation: ESY; Literature Review: AÖ, BK, İS, İK; Manuscript Preparation: AÖ, İK and Critical Review: AÖ, İK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Analysis of under-five mortality by diseases in countries with different levels of development: a comparative analysis

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ABSTRACT

Objectives: The right to health is critical for children because they are sensitive beings who are more susceptible to disease and health problems. It would be beneficial to compare child mortality rates in countries with different levels of development and to conduct studies to address them by taking into account their causes. This study aims to analyze the situation of developed, developing and least developed countries in terms of causes under-5 child mortality (U5CM) determined by World Health Organization and to identify the similarities or differences of under-five mortality.

Methods: Child mortality rates per 1,000 live births between 2000 and 2017 years in between different age groups (0-27 days and 1-59 months) by causes (disease-specific) were obtained from World Health Organization for a total 15 countries including developed, developing and least developed countries. Regression analysis was performed to identify which causes have more impact on child mortality. In addition, the relationship between diseases was calculated using Euclidean distance, and diseases were clustered using k-means clustering algorithm for each country.

Results: As a result of mathematical and statistical analysis, it was seen that causes of child mortality have a significant relation with the development level of country where a child was born.

Conclusions: It has been observed that the causes of child mortality in countries with different levels of development vary depending on different factors such as geographical conditions, air quality population and access to medicine.

Keywords: World Health Organization, child mortality, Euclidean distance, linear regression, clustering

The risk of a child dying between birth and the age of 5, expressed per 1.000 live births, is referred to as the U5CM rate. 5.0 million in 2020 [1]. For efforts to improve child survival to be effective, it is necessary to have reasonably accurate information about the causes of child mortality. To determine the effectiveness of disease-specific interventions and assess trends in disease burden in relation to national and international goals, cause-of-death information is required [2].

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The global community agreed on multiple Millennium Development Goals (MDGs) including poverty, hunger, combating disease, environmental degradation and especially two-thirds reduce U5CM rate between 1990 and 2015 (MDG4) [3]. While more than 60 countries managed to achieve MDG4, the target globally was missed by half over declining in the 25 year[4]. It was seen that sustaining progress requires the mobilization and monitoring of resources and collecting appropriate data to assess progress were included in global initiatives. The Sustainable Development Goals (SDG), established in 2015 by United Nation (UN) General Assembly, and including the UN Global Strategy for Women's, Children's and Adolescent's health [5, 6]. The SDG child survival targets aim for all countries to achieve a under-five mortality rate of 2.5 percentage or less by 2030 [6]. However, studies show that the global number of child mortality remain larges and children continue to face widespread inequality in their chance of survival and if countries do not take actions millions children will die in next decade by preventable deaths [7].

According to studies conducted by United Nations Children's Fund (UNICEF) and World Health Organization (WHO) pneumonia, diarrhoea, malaria, preterm birth complications, acute respiratory infections and congenital anomalies continue to be a leading cause U5CM. Furthermore, the following diseases also cause U5CM from past to present: sepsis and other infectious conditions of the newborn (SOICN), birth asphyxia and birth trauma (BABT), HIV/AIDS, injuries, measles, meningitis, communicable diseases, noncommunicable diseases, tetanus.

Congenital anomalies (CAs) are functional or structural abnormalities that occur during intrauterine life. CAs can cause fetal death as well as long-term disability. Each year about 3.2 millions of children are born with a CA and 300,000 newborns with a diagnosis of birth defect die in 28 days [8]. CAs have a significant impact in children born preterm and increase prematurity [9]. Although main reason of CAs is unknown there are several environmental factors such as infection agents, chemical toxicants and individual exposures such as socioeconomic status [8].

Sepsis is a life-threatening medical condition that occurs when the body overreacts to an infection [10]. Every year more than one million neonates are dying because of sepsis which is the second major cause of neonates mortality [11].

Acute lower respiratory infections (ALRI) is one of the main causes of U5CM and human respiratory syncytial virus (RSV) is the most common viral pathogen identified in children with ALRI [12]. Globally in 2015, 1.4 million hospital admission and 27300 in-hospital deaths were due to RSV-ALRI in children younger than 6 months. Socioeconomic status, education, having more than two children at home, nutritional variables are significantly associated with cute lowery respiratory tract infections (ALRTI) [13]. The newborn is susceptible to bacterial and viral infections, The main cause of several disease lye under infection such as malaria, measles, meningitis. Especially ALRI are including pneumonia which is a major cause of morbidity and child mortality disease [14].

Birth asphyxia causes hypoxia and ischemia, resulting in widespread organ system damage. Birth trauma can also result in asphyxia, morbidity, and mortality, depending on the severity and anatomic location of the trauma. Every year, approximately four million neonates die as a result of birth asphyxia [15].

Diarrhoea is defined as three or more loose or liquid stools per day. It is a symptom of an infection in the intestines, which can be caused by a number of bacteria, viruses, and parasites. Diarrhoea, a preventable and treatable disease, is one of the leading causes of infectious in under-5 children [16].

Road traffic accidents, drowning, burns, falls, poisoning, and acts of violence are just few of the causes of injuries. Every year, injuries claim the lives of 4.4 million individuals around the world, accounting for roughly 8% of all deaths [17].

Malaria, a life-threatening parasitic disease besides being preventable and curable, spread by bites from infected female Anopheles mosquitoes. In the African Region, under-5 children accounted for an estimated 80% of all malaria deaths [18].

Measles is a serious virus-borne disease that is typically spread through direct contact and air. In 2018, over 140.000 people died as a result of measles. The measles immunization activities have significantly reduced measles deaths [19].

The infection happens when the membranes cov-

ering the brain and spinal cord are infected. Bacterial meningitis, a major public health problem, in 10% to 20% of survivors, can result in brain damage or hearing loss [20].

Other communicable perinatal and nutritional conditions (OCPNC) are related with several contitions. Communicable diseases are illnesses that can be transmitted by more than one way, such as contact with blood and body fluids or inhaling a virus. Hepatitis A, B, and C, influenza, and salmonella are examples of communicable diseases [21].

Noncommunicable diseases are chronic diseases that are caused by factors that are genetic, physiological, environmental, and behavioral. NCDs claim the lives of 41 million people worldwide each year [22]. Preterm babies are those who are born alive before the 37th week of pregnancy. Preterm birth health problems are the biggest cause U5CM, accounting for about one million deaths in 2015 [23].

Tetanus is a serious infectious disease caused by Clostridium tetani spores. The disease is a major public health problem particularly in low-income nations with low immunization rates and contaminated birth practices [24].

Preventing the deaths of children can be accomplished by increasing access to skilled health professionals during pregnancy and childbirth, increasing access to nutrition and micronutrients, increasing access to water, sanitation, and hygiene, and providing vaccinations. Unfortunately, many of these rescue efforts are out of reach for the world's poorest countries [2].

Due to the scarcity of studies on the relationship between the causes of U5CM and the development of countries, this study has attempted to fill the gap in the literature. U5CM factors were analyzed in 15 countries based on three main categories according to their economic indices: developed, developing and least-developed countries. By comparing these countries rather than other studies, the effect of being economically strong on the U5CM was investigated. In addition, geographically distant countries were tried to be selected to see whether geographical factors contribute to U5CM. Through the findings of this study, countries can take action to prevent future U5CM by contributing to public health education and facilitating health institutes facilities. In addition, the world's poorest countries can access the life-saving interventions mentioned above with the support of international humanitarian organizations for children.

METHODS

In this study, number of deaths due to a specific cause, among under-5 children, per 1.000 live births for 15 countries is collected from World Health Organization (WHO) [2]. These countries are classified into three: (1) Developed: Australia, Canada, Germany, Norway, Singapore. (2) Developing: Colombia, India, Mexico, Tunisia, Turkey. (3) Least-developed: Bangladesh, Chad, Mali, Mozambique, Niger. This classification was made according to the Human Development Index (HDI) for 2019. This value is determined by incorporating a country's scores in a wide range of indicators, such as life expectancy, access to electricity for rural populations, GDP (Gross Domestic Product) per capita, imports and exports, homicide rate, multidimensional poverty index, internet availability, education, income inequality, and plenty more. These indicators are combined into a single value ranging from 0 to 1.0, with 1.0 being the maximum attainable level of human development [25]. Countries tried to be selected from different locations on the world to see the how differences between geographic, cultural and other conditions effect causes of U5CM. Firstly, dataset cleaning was performed in order to extract the appropriate information for our analysis. The new 18-year data contains 14 causes of death and two age group (0-27 days and 1-59-month). Mathematical and statistical analysis then applied to our data to investigate whether underlying cause of U5CM differ between developed, developing and least-developed countries. First, we created heat map and bar graphs to see the differences between the groups (based on the level of development of countries), then we performed linear regression analysis to identify which causes have more impact on U5CM. After that, we have calculated the relationship between diseases using Euclidean distance, and we clustered the diseases using k-means clustering algorithm for each country. In addition, we calculated the correlation between the countries in each group and we have shown the results as a correlation matrix.

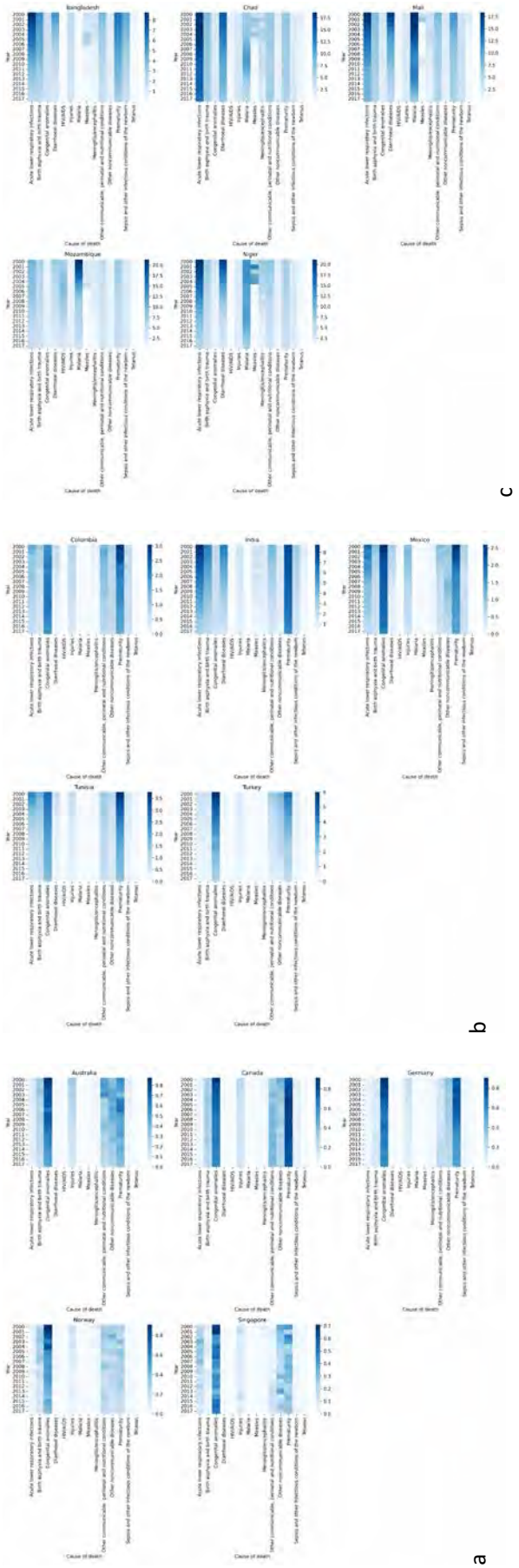

Fig. 1. The heat map graphics shows child mortality rates by cause between 2000 and 2017 years' in (a) developed, (b) developing and (c) least-developed countries.

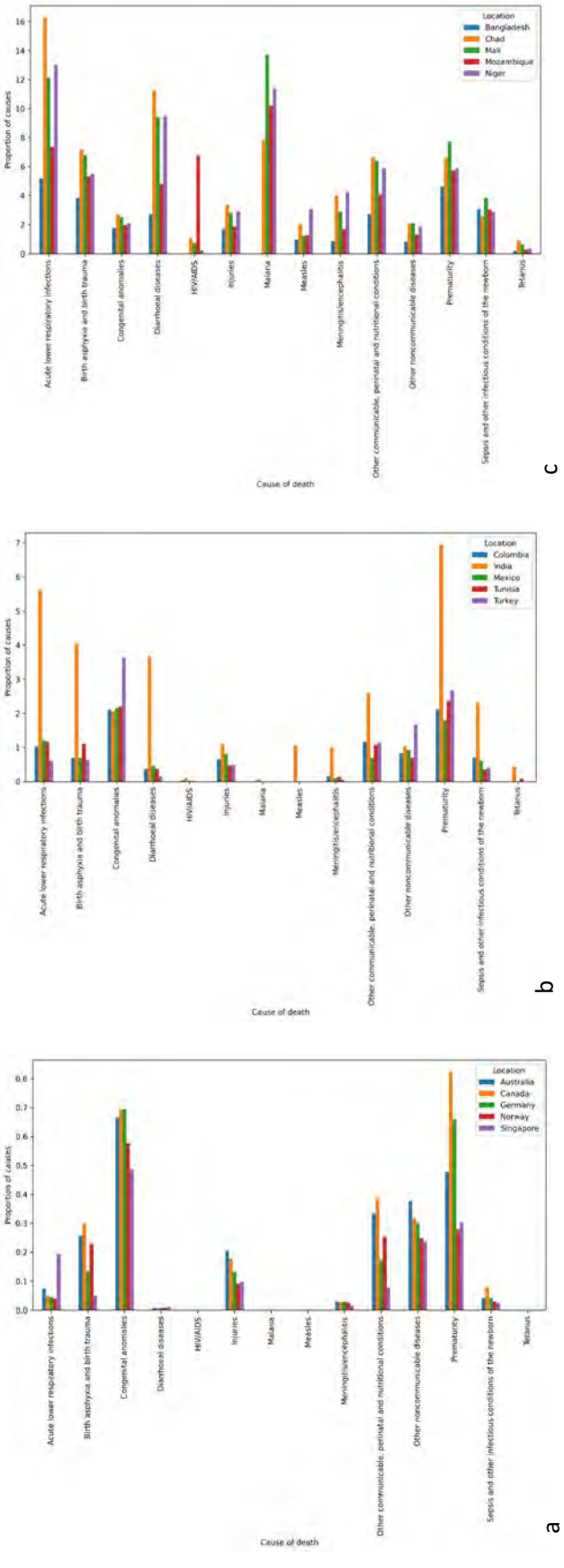

Fig. 2. The graphics show the child mortality rates by cause in (a) developed (b) developing and (c) least-developed countries.

Statistical Analysis

In this section, to obtain consistent outcomes we performed five mathematical and statistical analyses using Microsoft Office Excel and seaborn, scipy, and sklearn packages in Python.

Heat Map and Bar Graphs

According to the development levels of countries, the disparities between the groups (developed, developing and least-developed) are analyzed based on the causes of U5CM and the results are given in Figs. 1 and 2.

Regression Analysis

Linear regression analysis is performed to identify which causes have more impact on U5CM and shows how much diseases are effective in the developing, developed and least-developed countries in Table 1. In the regression analysis the statistical significance level accepted is 0.01 (confidence level is 99%).

Correlation Matrix

The correlation between the countries in each group is calculated to see how much the countries in the same group correlate with each other. The correlation matrix, given in *Supplementary* Table 1 and it shows how countries related with each other's in terms of causes of U5CM.

Euclidean Distance Measurement

In order to show how diseases are related with each other and affect U5CM in a country, we calculated Euclidean distance measures in between two diseases. Euclidean distance results for Germany, Turkey,

Table 1. Regression analysis of developed, developing and least-developed countries based on causes of child mortality

	Developed	Developing	Least-Developed
	coefficient	coefficient	coefficient
Intersection	7.769	103.531	396.477
Period *	-0.003	-0.050	-0.192
Age group *	-0.043	-0.105	3.437
BABT *	0.113	-0.499	-5.069
CA *	0.542	0.504	-8.584
Diarrhoeal disease *	-0.071	-0.929	-3.265
HIV/AIDS *	-0.079	-1.897	-9.036
Injuries *	0.060	-1.222	-8.260
Malaria *	-0.079	-1.913	-2.157
Measles *	-0.079	-1.715	-9.082
Meningitis/encephalitis *	-0.055	-1.639	-8.046
OCPNC *	0.164	-0.597	-5.644
Other noncommunicable diseases *	0.215	-0.901	-9.160
Prematurity *	0.428	1.242	-4.677
SOICN **	-0.037	-1.053	-7.708
Tetanus *	-0.079	-1.826	-10.313

BABT = birth asphyxia and birth trauma, CA = congenital anomalies, OCPNC = other communicable perinatal and nutritional condition, SOICN = sepsis and other infectious conditions of the newborn

*Statistically significant (*p*-value less than 0.01) in all development level groups.

** Statistically significant in two development level groups.

Table 2. Euclidian distance measures of underlying diseases of child mortality in Germany

Causes of child mortality	ALRI	BABT	CA	Diarrhoeal diseases	HIV/AIDS	Injuries	Malaria	Measles	Meningitis/ encephalitis	OCPNC	Other noncommunicable diseases	Prematurity	SOICN	Tetanus
ALRI	0													
BABT	0.8828	0												
CAs	3.9490	3.4218	0											
Diarrhoeal diseases	0.3029	0.9266	4.1672	0										
HIV/AIDS	0.3586	0.9384	4.2059	0.0611	0									
Injuries	0.7104	1.1591	3.4997	1.0035	1.0597	0								
Malaria	0.3638	0.9403	4.2101	0.0659	0.0092	1.0657	0							
Measles	0.3621	0.9398	4.2088	0.0643	0.0081	1.0639	0.0036	0						
Meningitis/encephalitis	0.1446	0.9111	4.0446	0.2078	0.2581	0.8139	0.2646	0.2630	0					
OCPNC	1.1004	0.3309	3.2089	1.1779	1.1944	1.2505	1.1964	1.1958	1.1456	0				
Other noncommunicable diseases	2.1260	2.3916	3.0435	2.4186	2.4765	1.4389	2.4821	2.4803	2.2341	2.3476	0			
Prematurity	4.7889	3.9728	2.6424	4.8667	4.8762	4.7034	4.8776	4.8773	4.8381	3.8033	4.9777	0		
SOICN	0.4802	0.6131	3.9574	0.3609	0.3545	1.0911	0.3548	0.3549	0.4411	0.8910	2.4944	4.5329	0	
Tetanus	0.3638	0.9403	4.2101	0.0659	0.0092	1.0657	0	0.0036	0.2646	1.1964	2.4821	4.8776	0.3548	0

ALRI = acute lower respiratory infection, BABT = birth asphyxia and birth trauma, CA = congenital anomalies, OCPNC = other communicable, perinatal and nutritional conditions, SOICN = sepsis and other infectious conditions of the newborn

Table 3. Euclidian distance measures of underlying diseases of child mortality in Turkey

Causes of child mortality	ALRI	BABT	CA	Diarrhoeal diseases	HIV/AIDS	Injuries	Malaria	Measles	Meningitis/ encephalitis	OCPNC	Other noncommunicable diseases	Prematurity	SOICN	Tetanus
ALRI	0.0000													
BABT	5.5240	0.0000												
CAs	19.0510	19.5407	0.0000											
Diarrhoeal diseases	3.7180	4.6041	22.0752	0.0000										
HIV/AIDS	5.1740	4.6981	23.1375	1.5325	0.0000									
Injuries	1.6743	5.4957	20.0549	2.9120	4.2596	0.0000								
Malaria	5.1848	4.7010	23.1467	1.5420	0.0141	4.2717	0.0000							
Measles	5.1844	4.7008	23.1463	1.5417	0.0143	4.2710	0.0030	0.0000						
Meningitis/encephalitis	4.6789	4.5978	22.7467	1.0773	0.5194	3.7537	0.5310	0.5304	0.0000					
OCPNC	5.5454	3.6387	16.0589	6.8356	7.5729	6.2739	7.5790	7.5788	7.2960	0.0000				
Other noncommunicable diseases	9.8333	14.2657	15.0075	13.3475	14.7465	10.5519	14.7586	14.7581	14.2329	12.1492	0.0000			
Prematurity	19.8015	16.0905	15.3173	20.5762	20.7540	20.4475	20.7559	20.7558	20.6722	14.3033	23.1511	0.0000		
SOICN	5.8771	1.4679	20.9829	3.9617	3.6493	5.5901	3.6493	3.6493	3.6834	5.0356	15.1265	17.1732	0.0000	
Tetanus	5.1848	4.7010	23.1467	1.5420	0.0141	4.2717	0.0000	0.0030	0.5310	7.5790	14.7586	20.7559	3.6493	0.0000

ALRI = acute lower respiratory infection, BABT = birth asphyxia and birth trauma, CA = congenital anomalies, OCPNC = other communicable, perinatal and nutritional conditions, SOICN = sepsis and other infectious conditions of the newborn

Table 4. Euclidian distance measures of underlying diseases of child mortality in Mali

Causes of child mortality	ALRI	BABT	CA	Diarrhoeal diseases	HIV/AIDS	Injuries	Malaria	Measles	Meningitis/ encephalitis	OCPNC	Other noncommunicable diseases	Prematurity	SOICN	Tetanus
ALRI	0													
BABT	95.401	0												
CAs	82.814	41.583	0											
Diarrhoeal diseases	17.735	91.946	73.386	0										
HIV/AIDS	88.989	50.897	11.236	78.063	0									
Injuries	73.966	51.742	13.359	63.808	16.298	0								
Malaria	31.594	123.647	108.370	40.052	113.343	98.031	0							
Measles	84.407	52.869	16.724	72.787	14.432	18.244	109.001	0						
Meningitis/encephalitis	73.948	48.268	12.410	63.732	16.391	10.224	99.125	18.929	0					
OCPNC	48.462	59.096	35.427	40.229	41.603	25.922	73.518	38.820	27.860	0				
Other noncommunicable diseases	77.966	51.871	11.207	67.217	11.481	5.301	102.110	14.815	9.360	30.390	0			
Prematurity	98.744	11.016	49.934	96.414	59.408	59.824	126.986	61.071	56.374	65.431	60.147	0		
SOICN	96.347	20.154	26.039	89.750	33.146	38.220	123.872	37.037	34.893	53.308	36.900	29.778	0	
Tetanus	94.555	45.916	12.842	84.304	9.514	23.037	119.686	18.975	22.007	47.329	18.974	53.804	27.870	0

ALRI = acute lower respiratory infection, BABT = birth asphyxia and birth trauma, CA = congenital anomalies, OCPNC = other communicable, perinatal and nutritional conditions, SOICN = sepsis and other infectious conditions of the newborn

and Mali are given in Table 2-4. Results of other countries is given as *Supplementary* Table 2-13.

K-means Clustering

According to Euclidean distance measurements, diseases are clustered using k means algorithm for each country (Table 5) to see if the same diseases are associated with each other in countries with the same level of development. Fig. 3 represents the clustering results of 3 groups (developed, developing or least-developed countries) based on k means algorithm with k is 3. Clustering results for each country were compared with the clusters of the group to which each country belongs (Fig. 3) and similarity rates are calculated for each country (Table 5).

RESULTS

In this section, firstly, regression analysis results are given, then the findings for each disease are explained in itself, and finally correlation between countries based on the causes of U5CM is expressed.

Coefficient and p-value variables of regression analysis (Table 1) were analyzed based on age group, year and disease. If the p-value of the variable is less than the significance level (0.01), we assume that the variable is statistically significant. According to the Table 1 all variables (age group, year and disease) statistically significant (p-value less than 0.01) in all development level groups except SOINC (It is statistically significant in developed and least-developed countries).

The remainder of this section, the findings for each disease are explained in itself.

While ALRI highly result in U5CM in India as a developing country (Fig. 1b), and Chad, Mali and Niger which are least-developed countries (Fig. 1c) it cause a small number of deaths in developed countries (Fig. 1a). Compared to other developed countries, the highest death rate is seen in Singapore (Fig. 2a). BABT has one of the highest mortality rates in India among developing countries (Fig. 1b) and in Chad among developed countries (Fig. 1c). CAs are the most common problem in the developed countries (Fig. 1a) and developing countries (Fig. 1b). Diarrheal diseases which are caused by infection, have highest percentage as U5CM reason in India (Fig. 1b) when it

Table 5. Disease clusters of fifteen countries (a) developed, (b) developing and (c) least-developed countries based on causes of child mortality.The similarity of the clustering results of each country to the clustering results of the group to which that country belongs.

a)

Causes of child mortality	ALRI	BABT	CAs	Diarrhoeal diseases	HIV/AIDS	Injuries	Malaria	Measles	Meningitis/ encephalitis	OCPNC	Other non-communicable diseases	Prematurity	SOICN	Tetanus	Similarity rate in the group
Norway															0.92
Germany															0.85
Canada															0.85
Singapore															0.78
Australia															0.78

b)

Causes of child mortality	ALRI	BABT	CAs	Diarrhoeal diseases	HIV/AIDS	Injuries	Malaria	Measles	Meningitis/ encephalitis	OCPNC	Other non-communicable diseases	Prematurity	SOICN	Tetanus	Similarity rate in the group
Tunisia															0.78
India															0.78
Colombia															0.65
Turkey															0.57
Mexico															0.57

c)

Causes of child mortality	ALRI	BABT	CAs	Diarrhoeal diseases	HIV/AIDS	Injuries	Malaria	Measles	Meningitis/ encephalitis	OCPNC	Other non-communicable diseases	Prematurity	SOICN	Tetanus	Similarity rate in the group
Mali															1
Chad															1
Bangladesh															0.92
Niger															0.78
Mozambique															0.65

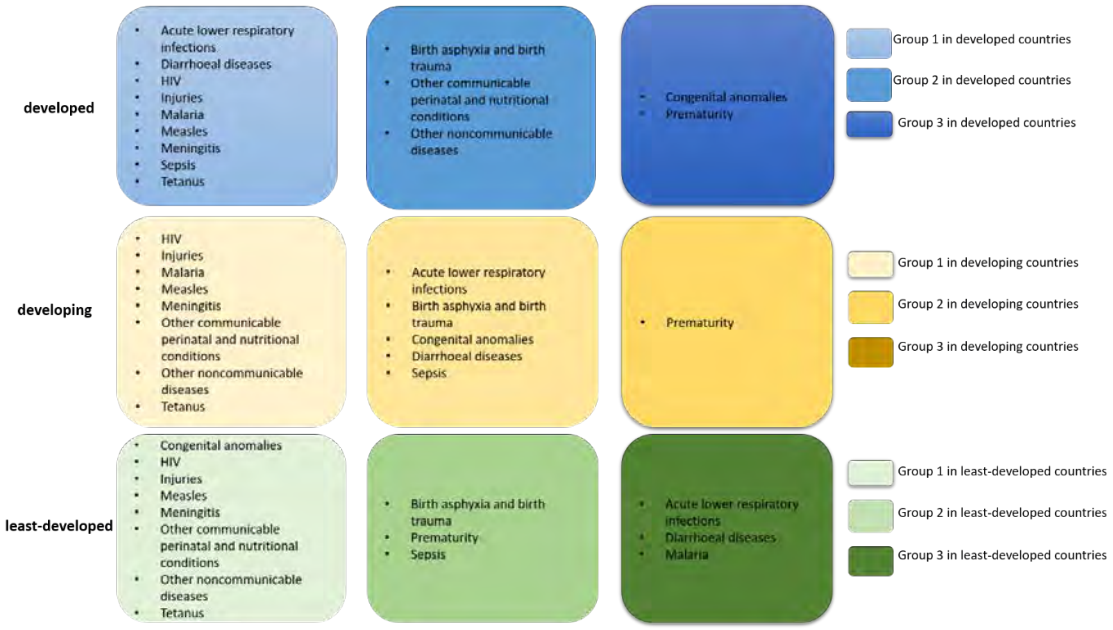

Fig. 3. Disease clusters based on development levels of countries.

compared to other developing countries and commonly seen in Chad, Mali and Niger (Fig. 1c). Unlike these countries diarrheal diseases are hardly ever seen in developed countries (Fig. 1a). While HIV has no effect on U5CM in developed countries, and has a small effect in developing countries, it is effective in least-developed countries, especially Mozambique (Fig. 2). Injuries are related with ALRI in developing countries, and with CAs in least-developed countries. Malaria is commonly seen in least developed countries except Bangladesh as the reason of U5CM (Fig. 1c). In developed and developing countries it almost has no effect on U5CM rate (Figs. 2a and 2b). While Measles can be shown as one of the causes of U5CM in least-developed countries, it has no effect in developed countries and in developing countries except India (Fig. 2). Meningitis/encephalitis cause the most U5CM in least-developed countries among other countries (Fig. 2). Other communicable and noncommunicable diseases cause a number of deaths in all development level groups (Fig. 2). Prematurity is the second highest cause of U5CM in the developed countries especially in Canada and Germany, and the common reason of U5CM in developing countries especially in India (Figs. 1a and 1b). SOICN also cause a number of deaths in all development level groups (Fig. 2). While tetanus has no effect in developed countries, it has a small effect in developing and least-developed countries on U5CM (Fig. 2).

Based on the Euclidean distance results in Table 2 and *Supplementary* Table 2 to 5, we can infer that in developed countries, ALRI, diarrheal diseases, HIV/AIDS, sepsis, measles, meningitis/ encephalitis, and other infectious conditions of the newborn, tetanus are related with each other. ALRI are also generally related with diarrheal diseases in developing and least-developed countries. Prematurity and BABT shows relations in all developed and least-developed countries. Prematurity also shows relationship with SOICN in all developed countries, and Mozambique, Mali, and Chad. Besides, in Australia, Norway, Canada and Germany prematurity also has relationship with CAs. Likewise, prematurity has relevance with CAs in developing countries except India (Table 3, *Supplementary* Table 6 to 9). Prematurity also shows relationship with BABT in India, Tunisia and Mexico. Unlike in developed and developing countries, CAs have a connection with HIV/AIDS, injuries, malaria, measles, meningitis/encephalitis, SOICN, tetanus in least developed countries (Table 4, *Supplementary* Table 10 to13). Clustering results in Table 3 and Fig. 3, it is seen that in developed countries, Norway has the most correlated ratio as 0.92 similarity rate in the group and Australia and Singapore has the lowest similarity rate

with 0.78. It might be because of geographic conditions of the countries (Table 5a). In developing countries, Tunisia and India shows highest rate of similarity in the group as 0.78, Colombia follows them with the rate of similarity as 0.64. However, Turkey and Mexico have the lowest similarity rate in the group as 0.57 (Table 5b). In least-developed countries, Chad and Mali has the highest similarity rate in the group as 1. Mozambique has the lowest similarity in the group with 0.64 similarity rate. Bangladesh and Mozambique differs from the group with the malaria disease than Mozambique (Table 5c).

DISCUSSION

Economic development level has an impact on preventing and treatment disease and improve under-5 children survival chance. However, even if the countries have same economic development level, there are many other reasons associated with U5CM such as geographic conditions, eating habits, culture, exposure to chemicals.

Diarrhoea and ALRI in children 1-50 months of age are almost ten times higher than U5CM from 0-28 days of diarrhea and ALRI. Diarrheal diseases are caused by viral pathogens such as rotavirus, adenovirus and norovirus and bacterial pathogens such as cholera, shigella, campylobacter. The highest risk factors for diarrheal diseases are unsafe water supply, child growth failure, lack of access to handwashing facility, unsafe sanitation in least developed countries [26]. India differs from the other developing countries since it has the highest ALRI, BABT, diarrhoeal diseases, measles, meningitis, tetanus and sepsis and other connectional inflectional diseases U5CM. Cases of infectious diseases are high in India and the causes of epidemics range from lack of sanitation, urbanization change to lack of fresh water [27]. India is the country with the highest number of preterm births all over the world, and there are many causes of preterm birth such as infections, multiple pregnancies, diabetes, high blood pressure [23]. U5CM in India are caused by BABT, sepsis, diarrheal diseases most of which can be avoided by increases in delivery and postnatal care [28].

HIV/AIDS is a major burden in Mozambique which ranks among the 10 countries with the highest HIV burden in the world, with HIV in adults aged 15 to 49 years [29]. HIV can be transmitted from mother to infant during pregnancy, childbirth and breastfeeding [30]. Therefore, cause of U5CM in Mozambique due to HIV/AIDS is highly correlated with the prevalence of HIV/AIDS in the country. A study from Mozambique showing behavioral factors such as men having sex with men or women for money, goods, favor are more associated with HIV prevalence than demographic factors [31]. In the Fig. 2c, malaria hits Mali with highest U5CM rate in the least-developed countries. Because Mali has a subtropical and arid climate, it receives significant rainfall during part of the year and experiences a rainy season for part of the year and extremely dry weather for the rest of the years. Various environmental problems such as deforestation, desertification, soil erosion expose Mali to have many problems related to floods during the rainy season. These conditions create ideal environments for malaria transmission in Mali [32]. Chad has the highest prevalence of ALRIs in the least-developed countries. Globally, 50% of ALRIs deaths occur in sub-Saharan Africa, and Chad is among the 10 countries with the highest prevalence of ALRIs [33]. ALRI is caused by bacterial, fungal, or viral infections of the respiratory tract leading various difficulties [34]. A study was performed to examine the prevalence and predictor of ALRIs among under-5 children in 28 sub-Saharan African countries, including women with under-5 children and the results showed that ALRI was associated with mothers' employment status, child's age, child receiving intestinal parasite and type of toilet facility in the household [35].

Results showed that CAs and prematurity were associated diseases, BABT were associated with OCPNC and non-communicable in U5CM in developed countries. In the literature, it has been shown that there is a correlation between CAs and preterm birth [36]. Prematurity, CAs, BABT and OCPNC are in same group in Australia and Canada. However, in Germany, BABT and OCPNC are in the same group with acute lowery respiratory infections, diarrheal diseases, HIV, malaria, measles, meningitis, sepsis and tetanus. Singapore has the same group with Germany except for acute lower respiratory infection which is in different group in Singapore. Unlike other developed countries, prematurity in Norway is in different group from CAs. Canada has the highest prematurity rate of

U5CM and the highest rate in the group of BABT, SOICN and other communicable conditions. Because preterm birth can cause serious health complications for the baby and increases chronic health conditions that makes the baby susceptible to infections and serious health problem later in their life [37]. Prematurity is associated with CAs in developing countries except India, where prematurity is associated with BABT, and Tunisia, where prematurity is not associated with other diseases (Table 5b). Many different conditions such as low-income, education, susceptibility to infections, population are the reasons of prematurity in India. Bangladesh is geographically far from other least-developed countries however, many disease associations are common in India like CAs are highly related with infectious diseases such as HIV/AIDS, injuries, malaria, measles, meningitis/encephalitis, SOICN, tetanus. ALRI is also associated with diarrheal diseases. Unlike developed countries, in least developed countries water pollution, population, access to the clean food may cause children to be susceptible to infections. In developing countries, Colombia, Turkey and Mexico CAs correlate with prematurity. However, in India, prematurity is associated with BABT.

Even if Mozambique increases investments in malaria control and provide free malaria services, malaria remains a major burden for Mozambique's economy and health system [38]. WHO recorded 229 million cases of malaria in 2019, of which 409,000 deaths in 87 endemic countries and 67 % deaths in under-5 children. Worldwide, 95 % of the malaria cases occurred in 29 countries [39]. Although malaria is preventable, detectable and treatable, it is still more common in poor regions and causes socioeconomics impacts. Especially children and women are at high risk because of their low immunity. In the Chimoio region of Mozambique, under-5 children are three times more prone to malaria than adults [40]. Malaria remains a major public health problem in Africa including Mozambique, Chad, Mali and Niger. Especially in Mozambique, malaria birth defects are particularly prevalent in middle- and low-income countries. Besides poverty, the main reasons for the differences in birth prevalence rates of serious birth defects between high- and low-income countries. Many reasons may be associated with birth prevalence rates, including consanguineous marriage, inequalities in maternal and child health services, advantages of carrying sickle cell for malaria survival [41].

It has been shown that diseases are associated with various effects on countries such as high or low income, education level, health insurance, population, geographic condition, genetic background and culture. Projections for 2030 show that diarrheal diseases and lower respiratory infections will be outranked if countries do not act to prevent diseases. Strategic plans should specify a focus on improving administration of medicine for intestinal worms, more health education to mothers who have under-5 children on ALRIs, reducing poverty and improving the sanitation status of households through the provision of improved toiled facilities. According to current trends, more than 48 million under-5 children will die between 2020 and 2030 however if all countries met the SDG target for U5CM by 2030, around 11 million of these deaths could have been prevented [7]. Countries should evaluate their health plans according to outcomes of these results. It is important to develop national health strategies, and planning health policies and interventions, and track progress towards child survival.

CONCLUSION

Trends in child mortality causes help decision makers assess needs, prioritize interventions, and monitor progress. This study presents information on the proportional distribution of U5CM in countries with different economic levels of development. The findings obtained at the end of the study show that U5CM are caused by different diseases in countries with different levels of development. These differences are due to factors such as the geographical conditions, income levels and populations of the countries. According to our analysis, while diseases that cause mortality in developed and developing countries are mostly caused by genetic factors and birth complications, child mortality in socio-economically unstable least-developed countries are mostly due to lack of adequate sanitation, hygiene and clean water, as well as inability to get vaccine.

Authors' Contribution

Study Conception: MS, NŞE, PG; Study Design: MS, NŞE, PG; Supervision: MS, PG; Funding: MS,

NŞE, PG; Materials: MS, NŞE, PG; Data Collection and/or Processing: NŞE, PG; Statistical Analysis and/or Data Interpretation: MS, NŞE, PG; Literature Review: NŞE, PG; Manuscript Preparation: MS, NŞE, PG and Critical Review: MS, NŞE, PG.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Supplementary Tables 1 to 13

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Musculoskeletal pain, kinesiophobia, and quality of life in obese patients

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ABSTRACT

Objectives: This study aims to examine musculoskeletal pain, kinesiophobia, and quality of life in individuals with normal body mass index and in individuals with obesity, and to determine the relationship between the variables.

Methods: In the study; individuals aged between 20-65 years, with a body mass index of 30 kg/m^2 and above (Group $_{obesity}$) with obesity, and individuals with a range of 18.5-24.9 kg/m^2 (Group $_{normal}$) were included. General Practice Physical Activity Questionnaire, Orebro Musculoskeletal Screening Questionnaire, Short Form-12 Health Survey, and Kinesiophopia Causes Scale were applied to the participants for primary care.

Results: When the descriptive characteristics of the individuals in the sample which were divided into two groups as the Group $_{normal}$ (n = 82; 22.6 ± 1.7 kg/m^2) and Group $_{obesity}$ (n = 92; 33.0 ± 2.8 kg/m^2 were analyzed; while a difference was determined in terms of sex, education level and presence of chronic disease ($p < 0.05$), no difference was found in terms of age, marital status and regular drug use for the treatment of chronic disease ($p > 0.05$). Although the activity level rates of Group $_{obesity}$ were lower according to the physical activity questionnaire classification for primary care, no statistically significant difference was found between the groups ($p = 0.090$). More musculoskeletal pain was found in the individuals of the obesity group ($p = 0.003$), the physical sub-dimension score of the general quality of life questionnaire was negatively affected ($p = 0.014$), and a difference in favor of phobia was determined in all domains of the kinesiophobia causes scale ($p < 0.001$). There was a weak positive correlation between body mass index and musculoskeletal pain, while a moderate positive correlation with kinesiophobia.

Conclusions: Although physical activity level is similar to individuals with normal body mass index, musculoskeletal pain and kinesiophobia are higher in individuals with obesity, and their quality of life is negatively affected.

Keywords: Obesity, kinesiophobia, pain, quality of life

Obesity is a public health problem with personal, social, and financial burdens and increasing its importance globally [1]. Individuals affected by obesity face deep functional and physical limitations. These individuals experience musculoskeletal problems such as joint pain, functional impairment, and walking difficulties [2]. The prevalence of joint pain, especially in load-bearing segments such as the waist,

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increases linearly with high body mass index (BMI) values [3].

Since obesity is associated with clinical conditions related to pain, it is also associated with low physical and emotional well-being. As obesity rates increase, the rates of musculoskeletal disorders and related physical effects also increase [4, 5]. Therefore, obese patients with low back and joint pain experience significant functional limitations and injuries [6]. Here; it is faced with a dual problem, including physical activity limitation in daily life due to movement difficulties and pain caused by excess weight [6]. On the other hand, both conditions affect each other. For example, decreased physical activity due to pain may cause an increase in body weight [6]. On the other hand, individuals with obesity often experience shortness of breath, musculoskeletal disorders, and joint pain during physical activity. These situations which are experienced during exercise can negatively change the perception of individuals with obesity towards the benefits of movement and physical activity. As a result of all these, fear of pain and movement may occur [7]. The pain felt evokes avoidance behaviors against the movement, leading to an ever-increasing spiral of inactivity and more avoidance from exercise [7]. Studies have shown that pain-related fear of movement is strongly associated with perceived and detected injury in individuals with low back pain [7]. Therefore, fear of movement in obese individuals with musculoskeletal complaints may be a clinically important condition called 'kinesiophobia'.

Kinesiophobia is an extreme fear of physical movement and activity, which results from the fear of a painful injury or re-injury and creates a sense of vulnerability [8, 9]. Avoidance behaviors that develop due to kinesiophobia cause the continuation of the cycle of avoiding physical activity. Thus, the mobility and functional limitations of individuals may deteriorate. Patients with a diagnosis of obesity and chronic pain may report higher levels of kinesiophobia and may be exposed to more physical activity restrictions than those with lower BMIs [10]. To clarify the relationship between kinesiophobia, obesity, and pain; it can be predicted that it will facilitate the development of individual and targeted treatment approaches and increase the quality of life of individuals affected by obesity [10].

This study hypothesizes that musculoskeletal pain will be higher in individuals with obesity, although physical activity level is similar to individuals with normal body mass index, obese patients will have high kinesiophobia levels and all these differences will have negative effects on the quality of life of obese patients. In this study, it was aimed to determine the relationship between the variables by examining musculoskeletal pain, kinesiophobia, and quality of life in individuals with normal body mass index and individuals with obesity.

METHODS

The study started after the research protocol was approved by the local ethics committee's decision dated 08/01/2021 and numbered 9.

Individuals aged between aged 20-65 years, with a body mass index of 30 kg / m^2 and above, or between 18.5 -24.9 kg / m^2, who were in our hospital as companions were included to the study. Those who have had joint or back surgery in the last 2 years, those who have had a musculoskeletal injury in the last 6 months, those with cognitive and psychiatric disorders, unstable angina or uncontrolled arterial hypertension, severe pulmonary hypertension, recent cardiac arrhythmia, or myocardial infarction, other clinical conditions (malignancy, etc.) which may be worsened by physical exertion, slightly overweight individuals with BMI less than 18.5 kg / m^2 or between 25.1- 29.9 kg / m^2 were excluded from the study.

To all individuals who voluntarily participated in the study; General Practice Physical Activity Questionnaire, Orebro Musculoskeletal Screening Questionnaire, Short Form-12 Health Survey, Kinesiophopia Causes Scale was applied.

Applied Measurement Tools

General Practice Physical Activity Questionnaire

This questionnaire was developed in England to evaluate the level of physical activity in primary health care institutions and adapted into Turkish by Noğay *et al*. [11]. Questionnaire consists of a total of 7 questions under the headings of activity level at work, the activity level in the last 1 week and walking speed. According to the answers given to the questionnaire, activity

levels are divided into 4 groups as inactive, moderately inactive, moderately active, and active. The Turkish version of the Questionnaire has a Cronbach alpha coefficients value of 0.74 [11].

Orebro Musculoskeletal Screening Questionnaire

This questionnaire, which was developed to determine the risk of musculoskeletal pain by screening method, was adapted into Turkish by Öncü *et al*. [12]. Questionnaire consists of a total of 25 questions, 4 of which contain the descriptive information of the participant and 21 of which will be reflected in the scoring. According to the answers given to the questionnaire, a score can be obtained between 0 and 210. An increase in the score indicates that the risk of pain increases. The Turkish version of the Questionnaire has a Cronbach alpha coefficients value of 0.96 [12].

SF-12 General Quality of Life Questionnaire

The Turkish adaptation of this 12-question questionnaire, which aims to evaluate the general quality of life of individuals with its physical and mental subdimensions, was carried out by Soylu and Kütük [13]. The physical component score of the questionnaire; general health, physical functionality, physical role, and bodily pain sub-dimensions; the mental component score is calculated according to the answers given to the sub-dimensions of social functionality, emotional role, mental health, and energy. A score between 0-100 can be obtained separately from both component scores of the questionnaire. The higher the score obtained, the better the health status. The Turkish version of the Questionnaire has a Cronbach alpha coefficients value of 0.73 for physical component score of SF-12 (PCS-12) and 0.72 for mental component score of SF-12 (MCS-12) [13].

Kinesiophopia Causes Scale

This 20-question scale, which aims to examine the causes of kinesiophobia with its biological and psychological dimensions, was adapted into Turkish by Çayır *et al*. [14] The scale has two sub-dimensions, biological and psychological. The new version of the scale varies between 0-5 points. An increase in the total score of the scale and its sub-dimensions indicates an increase in kinesiophobia. The intra-class correlation coefficient was computed in order to conduct a reliability investigation on The Turkish version of the scale. The scale's overall ICC value was discovered to be 0.863. [14].

Power analysis

The power of the study was examined by post hoc analysis. The α error level was accepted as 0.05 in the G*Power v.3.1 program [15]. The power (1-β err prob) was 0.88 while the effect size was 0.49 for OMSQ, the power (1-β err prob) was 1.00 while the effect size was 1.29 for KCS, the power (1-β err prob) was 0.68 while the effect size was 0.37 for PCS.

Statistical Analysis

The conformity of the continuous variables to the normal distribution was evaluated with the Shapiro-Wilk test. Descriptive statistics were reported as numbers and percentages for categorical variables; mean and standard deviation for continuous variables. Comparisons of continuous variables between two independent groups; independent samples t-test was compared with normal distribution condition and Mann-Whitney U test when normal distribution condition was not met. The Chi-Square test tested the differences between the ratios of categorical variables between groups. Correlation Analysis was used to investigate relationships between continuous variables when the normality assumption was not met, and the Spearman correlation coefficient was also computed; otherwise, the Pearson correlation coefficient was considered. SPSS (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.) was used for statistical analysis. The type I error rate was accepted as 5%. A weak correlation was accepted if r = 0.20-0.39 and a moderate correlation if r = 0.40-0.69 [16].

RESULTS

In the study, the normal body weight (Group $_{normal}$, n = 82) with the mean body mass index 22.6 ± 1.7 kg / m^2 and the obesity class with 33.0 ± 2.8 kg / m^2 (Group $_{obesity}$, n = 92), a total of 174 people's data were analyzed. The mean age of individuals in the Group normal was 42.5 ± 8.8 years, while those in the Group obesity were calculated as 44.8 ± 8.3 years (p = 0.075) (Table 1).

Table 1. Descriptive characteristics of groups

	All	Group $_{normal}$	Group $_{obesity}$	*p* value
Age (years)	43.7 ± 8.6	42.5 ± 8.8	44.8 ± 8.3	0.075
BMI (kg/m^2)	28.1 ± 5.7	22.6 ± 1.7	33.0 ± 2.8	**< 0.001****
Sex, n (%)				**< 0.001****
Female	128 (73.6)	72 (87.8)[a]	56 (60.9)[b]	
Male	46 (26.4)	10 (12.2)[a]	36 (39.1)[b]	
Education Level, n (%)				**< 0.001****
Secondary school	21 (12.1)	3 (3.7)[a]	18 (19.6)[b]	
High school	40 (23)	11 (13.4)[a]	29 (31.5)[b]	
University	113 (64.9)	68 (82.9)[a]	45 (48.9)[b]	
Marital status, n (%)				0.456
Married	146 (83.9)	67 (81.7)	79 (85.9)	
Single	28 (16.1)	15 (18.3)	13 (14.1)	
Chronic disease (Yes), n (%)	71 (40.8)	26 (31.7)[a]	45 (48.9)[b]	**0.021***
Medication use for chronic disease treatment (Yes), n (%)	61 (35.1)	24 (29.3)	37 (40.2)	0.131

Data are shown as mean±standard deviation or n(%). BMI = Body Mass Index. Intependent Samples t-Test was used for age, Mann-Whitney U was used for body mass index, Chi-square test was used for other descriptive characteristics value, **p*-value is significant at the 0.05 level (2-tailed), ***p*-value is significant at the 0.01 level (2-tailed). a-b = There is a difference between them at Chi-square test.

Participants in the Group $_{normal}$ had controlled hypertension (n = 9), diabetes (n = 3), thyroid disease (n = 6), rheumatic disease (n = 6), migraine (n = 2), asthma (n = 2), vitiligo (n = 1). Participants in the Group $_{obesity}$ had controlled hypertension (n = 18), diabetes (n = 13), thyroid disease (n = 4), rheumatic disease (n = 2), osteoporosis (n = 1), migraine (n = 4), asthma (n = 3), angioedema (n = 1), epilepsy (n = 1), polycystic ovary syndrome (n = 1), fibromyalgia (n = 1), psoriasis (n = 1). When the descriptive features of the groups are examined; while a difference was determined in terms of sex, education level, and presence of chronic disease ($p < 0.05$). No difference was found in terms of marital status and regular drug use for the treatment of chronic disease ($p > 0.05$) (Table 1).

When the activity levels of the groups were evaluated with the General Practice Physical Activity Questionnaire, although the activity level rates of Group obesity were lower, no statistically significant difference was found between the groups ($p > 0.05$) (Table 2).

According to the results of the measurement tools

Table 2. Physical activity levels of groups

Activity Level	All n (%)	Group $_{normal}$ n (%)	Group $_{obesity}$ n (%)	*p* value
Inactive	4 (2.3)	0 (0)	4 (4.3)	0.090*
Moderately inactive	33 (19)	13 (15.9)	20 (21.7)	
Moderately active	45 (25.9)	23 (28)	22 (23.9)	
Active	92 (52.9)	46 (56.1)	46 (50)	

*Chi-square test was used.

Table 3. Musculoskeletal pain, kinesiophobia level and quality of life of the groups

	All	Group $_{normal}$	Group $_{obesity}$	*p* value
OMSQ	85.7 ± 29.6	78.5 ± 28.4	92.2 ± 29.2	**0.003 ****
SF-12				
PCS-12	42.7 ± 6.6	44.0 ± 6.4	41.6 ± 6.6	**0.014 ***
MCS-12	42.6 ± 5.4	42.6 ± 5.2	42.5 ± 5.6	0.816
KCS Total Score	2.8 ± 0.8	2.3 ± 0.6	3.2 ± 0.8	**< 0.001****
KCS-Biological Domain	2.8 ± 0.9	2.3 ± 0.6	3.3 ± 0.8	**< 0.001****
KCS-Psychological Domain	2.7 ± 0.9	2.2 ± 0.7	3.1 ± 1.0	**< 0.001****

Data are shown as mean±standard deviation. OMSQ = Orebro Musculoskeletal Screening Questionnaire, SF-12 = Short Form-12 Health Survey, PCS = Physical component score of SF-12, MCS = Mental component score of SF-12, KCS = Kinesiophopia Causes Scale.

Mann-Whitney U was used for OMSQ, Intependent Samples *t*-test was used for other value, **p*-value is significant at the 0.05 level (2-tailed), ***p*-value is significant at the 0.01 level (2-tailed).

Table 4. The relationship of body mass index with pain, kinesiophobia, and quality of life

		All	Group $_{normal}$	Group $_{obesity}$
OMSQ	r_s	**0.222****	0.090	0.036
	***p* value**	**0.003**	0.421	0.736
PCS-12	r	-0.144	0.067	0.064
	***p* value**	0.058	0.547	0.542
MCS-12	r	-0.024	-0.192	0.052
	***p* value**	0.758	0.084	0.625
KCS Total Score	r	**0.534****	0.118	0.186
	***p* value**	**< 0.001**	0.293	0.076
KCS-Biological Domain	r	**0.537****	0.114	0.116
	***p* value**	**< 0.001**	0.310	0.269
KCS-Psychological Domain	r	**0.471****	0.096	**0.223***
	***p* value**	**< 0.001**	0.392	**0.033**

OMSQ = Orebro Musculoskeletal Screening Questionnaire, PCS = Physical component score of SF-12, MCS = Mental component score of SF-12, KCS = Kinesiophopia Causes Scale.

Correlation test was used, "r" was used for indicating Pearson correlation coefficient; and "r_s" was used for the Spearman correlation coefficient, *: *P* - value is significant at the 0.05 level (2-tailed), **: *P* - value is significant at the 0.01 level (2-tailed). A weak correlation was accepted if r = 0.20-0.39 and a moderate correlation if r = 0.40-0.69.

in which musculoskeletal pain, kinesiophobia level, and quality of life were evaluated, more musculoskeletal pain was found in individuals in the Group $_{obesity}$, the physical sub-dimension score of the general quality of life questionnaire was negatively affected, a difference in favor of phobia was determined in all domains of the kinesiophobia causes scale ($p < 0.05$) (Table 3).

In the correlation analysis made considering all the participants, a weak positive relationship was found between body mass index and musculoskeletal pain, and a moderate positive relationship with kinesiophobia. When the analyzes were repeated according to the groups, only in Group $_{obesity}$ was a weak positive correlation was determined between the body mass index and the psychological domain of kinesiophobia ($p < 0.05$) (Table 4).

DISCUSSION

Individuals affected by obesity face physical limitations. These individuals frequently experience musculoskeletal problems such as joint pain, functional impairment, and walking difficulties [10]. Since obesity is associated with clinical conditions related to pain, it is also associated with losses in physical and emotional well-being [1]. Pain that increases with movement can cause individuals with obesity to have difficulties while maintaining physical activity. The perception that will be created by the difficulty felt during physical activity creates fear of movement due to pain and may cause a decrease in the quality of life in individuals with obesity [6]. All these situations are an indication that the fear of movement that develops in obese individuals with musculoskeletal complaints may be clinically important and should be rehabilitated.

In our study, when the descriptive characteristics of the individuals in the sample, which were divided into two groups as normal body mass index and obese patients, were examined; while a difference was determined in terms of sex, education level, and presence of chronic disease, no difference was found in terms of age, marital status and regular drug use for the treatment of chronic disease. Obesity is higher in males and individuals with lower education levels than the university level. In addition, additional chronic diseases of individuals diagnosed with obesity were observed more frequently. In addition, in a study comparing the obese patient and the normal group, the obese patient group was found to be older than the normal group, and the rate of women was found to be higher than men. While more patients were classified as 'working' in the obese patient group, more individuals were identified as 'student' in the normal group. A higher rate of additional chronic disease was found in the obese patient group. Among the normal and obese group, depression (13.8% vs. 12.9%), anxiety (3.1% vs. 2.9%), and arthritis (7.7% vs. 12.9%) prevalence was not found to be different. It has been found that obese patients use more narcotic drugs for pain control compared to the normal group and less of them use nonsteroidal anti-inflammatory drugs for pain control [7]. In a study by Alqahtani *et al*. [17], comparing the obesity patient group with the normal group, most of the participants in both groups were found to be single and their income level was found to be close. Gomes-Neto *et al*. [18], in a study comparing the quality of life of people with knee osteoarthritis (OA) with and without obesity, no statistically significant difference was found between the groups in terms of sociodemographic characteristics and duration of OA.

In our study, although physical activity levels of individuals with obesity were lower according to the General Practice Physical Activity Questionnaire classification, no statistically significant difference was found between the groups ($p > 0.05$). In parallel, in a study conducted with 200 female university students in Saudi Arabia in 2017 using the same questionnaire, although the activity level rates of the obesity group were lower, no statistically significant difference was found between the groups [17]. However, in a study conducted with 4716 adults in Iran, a statistical difference was found in the activity level ratios of the obesity group, reflecting that individuals with obesity were less active [19]. Although there is no statistically significant difference in various studies, results reflecting that individuals with obesity are less active have been determined. This situation, which was also revealed in our study, shows that individuals with obesity are less physically active than individuals with normal body mass index.

In our study, more musculoskeletal complaints were found in individuals with obesity. In the literature, there is a lot of evidence pointing to the coexistence of obesity and pain complaints [20-22]. In the study of Smuck *et al*., less than 3% of people in the normal BMI range reported low back pain in the last 3 months, while 7.7% of obese patients and 11.6% of morbidly obese patients reported low back pain [23]. The results of a population-based study of more than 30000 people over a 10-year period in Norway show that individuals with a diagnosis of obesity, who are not physically active, have a higher risk of developing chronic arm pain [24]. Results of another large population-based study that included prospective follow-up for over ten years showed that people with a diagnosis of obesity developed a higher number of low back pain complaints than those without a diagnosis of obesity [25]. In another study by Maclellan *et al*. [26], multisite musculoskeletal pain was found to be common and severe in obese patients. In another study that studied 6079 Latin American women between the ages of 40-59, it was concluded that obesity is an iden-

tifiable risk factor for musculoskeletal pain in middle-aged women [27]. The results of a survey conducted with more than 1 million people in the United States showed a linear increase in chronic pain cases as BMI increases [28]. Similarly, Hitt *et al*. [29] showed a linearly increasing relationship between obesity and pain. At the end of the study, it was found that the higher the BMI, the more common the complaint of pain, and the probability of suffering from pain in patients with morbid obesity was four times higher than those without a diagnosis of obesity [29].

In the general quality of life questionnaire, we used in our study, the physical sub-dimension scores of the obesity-diagnosed group were negatively affected, while the mental sub-dimension scores were not. It is known that obesity has various negative effects on the functional capacity and quality of life of people. In a study conducted in the USA, a negative correlation was found between BMI and the physical sub-dimension scores of the general quality of life questionnaire, but no significant correlation was found for the mental sub-dimension scores [30]. In another study, it was shown that obesity is associated with low quality of life in patients with low back pain [7]. However, according to the research results reported in the literature, the relationship between mental health and obesity is not consistent. While some studies have shown that obesity is associated with lower mental sub-dimension scores and well-being [31, 32], others have not [33, 34].

In our study, a difference in favor of phobia was determined in all sub-scales of the Kinesiophobia Causes Scale. There are very few studies [1, 6, 7, 10] in the literature examining the relationship between obesity and kinesiophobia. In a study by Varallo *et al*., in patients with chronic low back pain; the relationship between obesity, pain intensity, and kinesiophobia was evaluated. Their findings revealed that kinesiophobia in individuals with low back pain and obesity mediates the relationship between pain intensity and injury [6]. According to the results of another cross-sectional study conducted on 106 participants with obesity and chronic low back pain, kinesiophobia was found to be an important factor in increasing pain-related disability and pain intensity in individuals with chronic low back pain and obesity [1]. Studies supporting this explanation have been previously reported by Vincent *et al*. [7] reported. It was found that adults affected by moderate obesity and chronic low back pain reported higher levels of kinesiophobia compared to those of normal weight, and obese adults with chronic low back pain had a higher fear of movement than adults without a diagnosis of obesity with chronic low back pain [7]. In addition, another study overweight older observed that the degree of kinesiophobia was a significant predictor of low back pain severity and perceived disability in adults with low back pain [10].

Limitations

The first limitation of our study is that it is a cross-sectional study. The second is that the sample consists of individuals from a single center and accompanying individuals in our hospital. On the other hand, it is important that it is a few studied topics in the literature. This study has several strengths, such as the use of validated, reliable measurement tools, the achievement of an adequate sample size, and the fact that it presents data on a population for which there is still little research.

CONCLUSION

Although the physical activity level of an individual with obesity is similar to that of individuals with a normal body mass index, musculoskeletal pain and kinesiophobia are higher, and the quality of life is negatively affected. In the light of the data obtained, it is thought that kinesiophobia should not be ignored when planning an exercise for an individual with obesity, and treatment approaches for kinesiophobia should also be exhibited.

Authors' Contribution

Study Conception: SE; Study Design: SE; Supervision: CÇ; Funding: N/A; Materials: SE, FHK, AÖ; Data Collection and/or Processing: SE, FHK, AÖ; Statistical Analysis and/or Data Interpretation: SE; Literature Review: SE, FHK, AÖ, CÇ; Manuscript Preparation: SE, FHK, AÖ and Critical Review: SE, FHK, AÖ, CÇ.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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C-reactive protein to albumin ratio in atrial fibrillation

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ABSTRACT

Objectives: Atrial fibrillation (AF) may cause thromboembolism and cardiac morbidity and mortality. Patients with cardiovascular diseases are at risk for developing AF. In this study, the relationship between inflammation markers and AF was examined.

Methods: Among 689 people followed up in the cardiology outpatient clinic for reasons such as hypertension, coronary artery disease, and rheumatic valve disease, 88 patients with AF and 601 patients without AF were compared. The blood parameters of the AF group were examined during the period when AF developed in the patients. Hemogram and biochemistry parameters of AF and non-AF groups were compared.

Results: C-reactive protein to albumin ratio (CAR), neutrophil count to albumin ratio, neutrophil count to lymphocyte count ratio, and monocyte count to lymphocyte count ratio were significantly higher in the AF group than in the non-AF group ($p < 0.001$, $p < 0.001$, $p = 0.001$, $p < 0.001$; respectively). According to the Receiver Operating Characteristics analysis, it was found that the CAR value of cut-off: 0.0533 could diagnose AF with 74% sensitivity (AUC: 0.789, CI 95%: 0.726-0.853, $p < 0.001$). Albumin value of cut-off: 3.75 was found to be able to diagnose AF with 82% specificity (AUC: 0.772, CI 95%: 0.707-0.836, $p < 0.001$).

Conclusions: AF is an arrhythmia that should be recognized early due to the complications it causes, and the CAR value can be used in the diagnosis of AF in individuals with cardiac disease.

Keywords: Atrial fibrillation, C-reactive protein, albumin

Atrial fibrillation (AF) is one of the most critical and most frequent arrhythmias, accelerating mortality and morbidity such as hemodynamic instability, thromboembolism, and stroke, increasing hospital readmissions and thus health care costs. In general, AF affects patients' quality of life in negative way [1]. AF alone increases mortality risk by 1.5% to 1.9% in a broad age group in both gender [2].

The pathophysiological mechanism in AF is quite complex and multifactorial. Prothrombotic state, inflammation, and oxidative stress may play essential roles in the formation of supraventricular arrhythmias. Complete blood count (CBC) and biochemical examination are necessary blood tests routinely used in clinical practice to examine cardiovascular diseases. However, the diagnostic performance of blood parameters for AF alone and in combination with other diseases is still unknown [3].

C-reactive protein (CRP)/albumin ratio (CAR) is a recently used marker and is used in the follow-up of infections, malignancies, rheumatic diseases, and serious diseases. It has been emphasized that CAR can also be used to determine the severity of coronary artery disease. It is thought that the CAR value alone gives more significant results than the ratio of CRP or albumin [4].

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Many methods are used to determine the severity of AF, and CBC parameters are the essential tools that can be easily accessed and applied among these. Our information on the use of lymphocyte ratios in AF, which has been the subject of research in many fields recently and can be used as a diagnostic tool and follow-up tool of diseases, is limited.

In this study, we aimed to investigate the relationship between AF and inflammation values, especially CAR, neutrophil to albumin ratio (NAR), monocyte to lymphocyte ratio (MLR), neutrophil to lymphocyte ratio (NLR), and platelet lymphocyte ratio (PLR).

METHODS

Study Design and Population

This research is a cross-sectional study. A total of 689 consecutive patients are followed up in the cardiology outpatient clinic for hypertension, coronary artery disease, and rheumatic valve disease whom are at risk of developing AF were enrolled in this study. All patients were followed for the six-month in term of developing AF. The patients were divided into two groups according to presence of AF at the end of the follow up. Venous blood of the patients who developed AF was taken when AF developed.

Exclusion criteria of this study were follows: 1) patients with hematological malignancies or cancer or thyroid hormone abnormalities. 2) usage of any drug which can affect laboratory parameters. 3) patients with active inflammation 4) Patients with a history of stroke or a recent surgical operation.

Electrocardiography

The diagnosis of AF is made by the absence of P waves on electrocardiography (ECG) and irregular R-R wave distances. 12-lead ECG recordings (25 mm/sec, 10 mm/mV) were obtained in the supine position using the CardioFax S device (Nihon Kohden, Tokyo, Japan).

Laboratory Analysis

Venous blood samples were examined regularly at hospital admission. Total white blood cell count and neutrophil, lymphocyte, monocytes, eosinophil, and basophil counts were measured using a device (CELL-DYN Ruby; Abbott Diagnostics, Abbott Park, IL) and given as x 103 cells/mm^3. Hemoglobin, hematocrit, platelet counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT), mean platelet volume (MCV), platelet distribution width (PDW), red blood cell distribution width (RDW) were also calculated. Albumin and CRP levels were analyzed using biochemistry kits (Abbott Diagnostics) and an Architect c8000 Chemistry System (Abbott Diagnostics) machine.

Statistical Analysis

All analyzes were performed in SPSS26.0 for Mac (SPSS Inc., Chicago, IL). In the findings, categorical data were expressed as numbers and percentages. The conformity of the data to the normal distribution was evaluated using the Kolmogorov-Smirnov test. The mean and standard deviation values of the continuous data were given. Student t-test was used to assess the difference between groups of normally distributed data. The Mann-Whitney U test evaluated the difference between the two groups of non-normally distributed data. The chi-square test was performed to assess the difference in categorical data between groups. Receiver Operating Characteristics (ROC) analysis was performed to evaluate the sensitivity and specificity of data that differed significantly between groups for the diagnosis of AF. A p - value of < 0.05 was considered statistically significant.

RESULTS

The comparison of the laboratory parameters of the AF group and the non-AF group is shown in Table 1. There was no significant difference between groups regarding mean age and gender distribution. The AF group's white blood cell, MCV, RDW, neutrophil count, lymphocyte count, monocyte count, PDW, and CRP values were significantly higher than the non-AF group ($p = 0.049$, $p = 0.006$, $p = 0.002$, $p < 0.001$, $p = 0.011$, $p = 0.003$, $p = 0.03$, and $p = 0.023$; respectively).

The albumin level of the AF group was significantly lower than the non-AF group ($p < 0.001$). In addition, the lymphocyte count was significantly lower in the AF group than in the non-AF group ($p = 0.011$). NLR, MLR, CAR, and NAR values of the AF group

Table 1. Comparison of baseline demographic and laboratory parameters of the study population

	AF Group (n = 88)	Non-AF Group (n = 601)	*p* value
Age	69.60 ± 8.52	68.76 ± 7.61	0.562[1]
Female gender	43 (49%)	264 (44%)	0.478[3]
WBC (1/μL)	7968 ± 628	7524 ± 1530	**0.049[1]**
RBC (10^3/μL)	5001 ± 609	5290 ± 609	**0.002[1]**
HCT (%)	43.61±4.4	44.36 ± 5.42	0.284[1]
HGB (g/dL)	14.13 ± 1.48	14.52 ± 2.04	0.121[1]
MCV (fL)	111.76	89.25	**0.006[2]**
MCH (pg)	107.38	93.63	0.093[2]
MCHC (g/dL)	98.84	102.16	0.685[2]
RDW (%)	112.96	88.04	**0.002[2]**
PLT (1/μL)	97.47	103.54	0.838[2]
Neutrophil (10^3/μL)	113.06	87.95	**< 0.001**
Lymphocyte (1/μL)	2155 ± 832	2488 ± 987	**0.011[1]**
Monocyte (1/μL)	112.63	88.38	**0.003[2]**
Eosinophil (1/μL)	95.29	105.72	0.203[2]
Basophil (1/μL)	100.96	100.04	0.910[2]
PCT (%)	104.98	96.02	0.274[2]
MPV (fL)	108.15	92.86	0.062[2]
PDW (fL)	109.36	91.65	**0.03[2]**
Albumin (g/dL)	3.57 ± 0.32	3.92 ± 0.26	**< 0.001[1]**
CRP (mg/dL)	105.56	95.44	**0.023[2]**
NLR	114.28	86.72	**0.001[2]**
MLR	117.56	83.44	**< 0.001[2]**
PLR	108.51	92.49	0.05[2]
CAR	129.45	71.55	**< 0.001[2]**
NAR	120.11	80.89	**< 0.001[2]**

WBC = White blood cells, RBC = Red blood cells, HCT = Hematocrit, HGB =Hemoglobin, MCV = Mean corpuscular volume, MCH = Mean corpuscular hemoglobin, MCHC = Mean corpuscular hemoglobin concentration, PLT = Platelet, PCT = Plateletcrit, MPV = Mean platelet volume, PDW = Platelet distribution width, RDW = Red blood cell distribution width, CRP = C-reactive protein, NLR = Neutrophil lymphocite ratio, MLR = Monocyte to lymphocite ratio PLR = Platelet to lymphocite ratio, CAR = C-reactive protein to albumin ratio, NAR = Neutrophil to albumin ratio

[1]Student's t test was performed and data were given as mean ± standard deviation.

[2]Mann-Whitney u tests were applied and the data were given as sequence numbers.

[3]Chi-square test was applied and data were given as percentages.

were significantly higher than the non-AF group (p = 0.001, $p < 0.001$, $p < 0.001$, and $p < 0.001$; respectively).

The evaluation of the parameters that differed between the groups by ROC analysis is shown in Table 2. According to the ROC analysis, when the CAR marker was above the cut-off value of 0.0533, its sensitivity was the highest (sensitivity: 74%, specificity: 77%) (AUC: 0.789, CI 95%: 0.726-0.853, $p < 0.001$). According to ROC analysis, albumin of cut-off: 3.75

Table 2. Evaluation of laboratory parameters by ROC analysis

		95% Confidence Interval					
Parameters	**AUC**	**Lower**	**Upper**	**Cut-off**	**Sensitivity**	**Specifity**	***p* value**
CAR	0.789	0.726	0.853	0.0533	74%	77%	**< 0.001****
NAR	0.696	0.624	0.768	1237.18	63%	64%	**< 0.001****
NLR	0.638	0.561	0.714	2.0495	61%	61%	**0.001****
MLR	0.671	0.597	0.745	0.2161	64%	64%	**< 0.001****
WBC (1/µL)	0.579	0.500	0.658	N/A	N/A	N/A	0.053
Neutrophil (10^3/µL)	0.626	0.549	0.703	4631.50	57%	59%	**0.002****
Monocyte (1/µL)	0.621	0.544	0.699	495.50	59%	59%	**0.003****
MCV (fL)	0.613	0.535	0.691	85.19	%60	60%	**0.006****
RDW (%)	0.625	0.542	0.702	11.51	62%	62%	**0.002****
PDW (fL)	0.589	0.510	0.667	19.49	56%	56%	**0.03***
Lymphocite (1/µL)	0.592	0.513	0.670	2277.50	55%	56%	**0.025***
Albumin (g/dL)	0.772	0.707	0.836	3.75	65%	82%	**< 0.001****
CRP (mg/dL)	0.551	0.471	0.630	N/A	N/A	N/A	0.216
RBC (10^3/µL)	0.637	0.560	0.714	5136.50	63%	60%	**0.001****

WBC = White blood cells, RBC = Red blood cells, MCV =Mean corpuscular volüme, PDW = Platelet distribution width, RDW = Red blood cell distribution width, CRP = C-reactive protein, NLR = Neutrophil to lymphocite ratio, MLR = Monocyte to lymphocite ratio, CAR = C-reactive protein to albümine ratio, NAR = Neutrophil to albumine ratio, AUC = Area under curve

ROC analysis was applied. $p < 0.05$ was accepted as statistical significance.

was found to have the highest specificity (sensitivity: 65%, specificity: 82%) (AUC: 0.772, CI 95%: 0.726-0.853, $p < 0.001$).

DISCUSSION

Our study found significant differences between the AF group and the non-AF group. We obtained the most sensitive data for the diagnosis of AF according to the ROC analysis of the CAR value, which has recently come to the fore as a new marker of inflammation.

Since AF is the most common arrhythmia in clinical practice and causes diseases such as stroke and heart failure and significantly increases cardiovascular mortality, early diagnosis and treatment are valuable in disease progression. In addition, AF is known as the most common arrhythmia in hospitalized patients. It has been emphasized in previous studies that AF occurs after an inflammatory process, and the presence of interstitial fibrosis and inflammatory cells in the atrial tissues of AF patients indicates the presence of an inflammatory process. The incidence of AF increases with increasing age [5]. In this sense, our study is compatible with the literature, and the mean age of the AF group was 69.60 ± 8.52 years. But there was no significant age difference between the AF and non-AF groups.

CRP value has been known for a long time as an inflammation marker and has been associated with the risk of atherosclerosis and stroke due to vascular damage in studies. A study found that the incidence of AF increased fourfold in the presence of CRP and microalbuminuria [6]. A study conducted on patients who had an acute myocardial infarction and subsequently developed AF found a higher CRP value in these patients [7]. On the other hand, albumin is decreased in oxidative stress and inflammation as a negative acute-phase reactant. A study conducted in Copenhagen found a relationship between hypoalbuminemia and the development of AF in women, independent of other risk factors [8].

The CAR value has emerged as a marker showing

the inflammatory state and body hunger level and can be elevated in many clinical situations. A study found that the CAR value may predict the risk of new-onset AF in patients with COVID-19 [9]. A survey conducted in 2020 reported that patients who developed AF after bypass surgery had a significantly higher CAR value in the preoperative period [10]. In a study conducted on patients who had a stroke, it was found that the CAR value could predict mortality within 90 days [11]. Çınar *et al*. found that CAR value in ST-elevation myocardial infarction could predict mortality risk better than CRP and albumin alone [4].

In recent studies, NAR value has come to the fore as a new inflammation marker. Yu *et al*. [12] reported that NAR value could be used to predict mortality in patients with cardiogenic shock. In another study, the NAR value was written as a mortality predictor in sepsis and septic shock [13]. In our research, CAR and NAR values were high in the patient group, and it was concluded that they could significantly diagnose the disease according to the ROC analysis.

Chavarria *et al*. [14] found that NLR increased statistically in cases with new-onset AF developing after percutaneous coronary intervention in acute ST-elevation myocardial infarction. Many studies have shown increased inflammation associated with AF. Although CRP is the most widely used indicator of inflammation, NLR has recently been shown to be a marker of inflammation in certain conditions. Karavelioglu *et al*. [15] found that NLR could significantly predict recurrence in AF patients undergoing medical cardioversion with amiodarone.

It has been suggested that neutrophil reflects inflammation, and lymphopenia indicates general health status and physiological stress [16]. Based on this idea, Gibson *et al*. [17] showed that NLR could be a significant predictor in the preoperative and postoperative AF periods that occur after coronary artery grafting.

NLR has been studied for some time in inflammatory diseases, and NLR has been emphasized as an independent predictor of coronary artery disease. In addition, a relationship was found between the frequency of thromboembolic stroke in AF and NLR [18]. Shao *et al*. found that NLR and RDW values were higher in AF patients [19].

MLR, as an inflammation marker, was found to be higher in coronary artery patients [20]. MLR study in AF patients has not been found in the literature. In our research, the MLR value was higher in AF patients, like other inflammation markers.

Limitations

The relatively small study population is the first limitation. As a second limitation, being a single-center study may limit the interpretation of results. The cross-sectional nature of the study may limit the clear relationship between cause and effect. There are needs for more larger and prospective studies to confirm this relationship.

CONCLUSION

Our most striking result is that new inflammation markers such as CAR and NAR values were significantly higher in the AF group, and especially the CAR value had the most heightened diagnostic sensitivity. The CAR value may be used as a suggestive marker in AF that needs to be diagnosed early.

Authors' Contribution

Study Conception: SA; Study Design: SA; Supervision: SA, FE; Funding: SA; Materials: SA; Data Collection and/or Processing: SA; Statistical Analysis and/or Data Interpretation: SA; Literature Review: SA; Manuscript Preparation: SA and Critical Review: SA.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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An examination of the burnout levels of healthcare professionals according to some variables during the COVID-19 pandemic

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ABSTRACT

Objectives: The objective of this study is to examine the effects on burnout levels of socio-demographic characteristics of healthcare professionals in different professions who are on active duty during the COVID-19 pandemic.

Methods: Obtained through socio-demographic information form created by the researchers and Maslach Burnout Scale (MBS) e-data collection method.

Results: It has been found out that gender variable reveals important differences among healthcare professionals and female healthcare professionals are more exhausted than men, the burnout levels of healthcare professionals who have a one-to-one relationship with COVID-19 patients are higher, and healthcare professionals trying to obtain information about the pandemic through social media are more exhausted than others. Variables such as marital status, education level, and being diagnosed with COVID-19 were not found to affect burnout in this study. However, when the data set was divided into two over the gender variable, it was observed that the education level variable affected emotional exhaustion scores among female participants. Although the emotional exhaustion scores of female participants with doctoral and high school education did not reveal a significant difference between the groups in the advanced statistical study, they revealed a significant difference according to the One-Way Analysis of Variance. The group with the highest burnout among healthcare professionals was determined as nurses.

Conclusions: It is important to carry out studies on psychological support and coping with burnout for healthcare professionals who are on active duty during the pandemic.

Keywords: COVID-19, pandemic, healthcare professionals, burnout

As a highly contagious disease affecting the upper respiratory tract, COVID-19 (SARS-CoV-2 virus) has become a global health problem acting upon every aspect of social life since December 2019. In March 2020, the World Health Organization (WHO) declared COVID-19 as a pandemic [1]. As of September 2021, there were 230,418,451 diagnosed COVID-19 patients and 4,724,876 deaths due to COVID-19

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worldwide [2]. Labrague and de Los Santos [3] reported that approximately 6% of diagnosed COVID-19 patients are healthcare professionals.

Burnout syndrome, first described by Freudenberger in 1974 [4] frequently experienced especially in occupational groups that provide face-to-face service [5]. Burnout syndrome occurs as a result of stress reactions that develop due to long-term exposure to disturbing events [6]. The most comprehensive definition of burnout syndrome used today was made by Christina Maslach in 1981 [7]. Maslach defined burnout syndrome as a syndrome consisting of emotional exhaustion, a sense of personal failure, and depersonalization [7].

Due to a virus with such a contagious and high mortality rate, many factors such as increased workload, uncertainty, fear and stress have been considered important for healthcare professionals to develop burnout syndrome. Not only the increased workload and being at the forefront of fighting the virus, but also the risk of contracting the disease are factors that negatively affect healthcare professionals in this process. Hoşgör *et al*. [8] found that there is a significant relationship between fear of COVID-19 and burnout level. Abdelghani *et al*. [9] found that the fear of COVID-19 among physicians working in Egypt during the pandemic increased emotional exhaustion, depersonalization, and feelings of personal failure. Giusti *et al*. [10] found the emotional exhaustion rates of healthcare professionals working during the pandemic to be very high, while they also found that their feelings of depersonalization and personal failure were at average levels. Ozbezek *et al*. [11] found that social support perceived by healthcare professionals reduces the severity of burnout syndrome. Turkili *et al*. [12] have determined that contact with COVID-19 patients, catching COVID-19 disease of healthcare professional, difficulty in accessing personal protective equipment, lack of social support and following social media for information purposes are important risk factors for burnout syndrome.

The aim of this study is to examine the burnout levels of healthcare professionals in different occupational groups working in the field of health according to some socio-demographic variables.

METHODS

Research Design

By using a cross-sectional design, it was conducted via internet. The Maslach Burnout Scale was applied to the participants in order to determine their burnout levels as well as their socio-demographic information. The data of the research, which is a cross-sectional study, was collected in 2021 by publishing the questionnaire form prepared on google forms on social media sharing platforms. The questionnaire was online between 01 June 2021 to 01 September 2021.

Data Collection Tools

Maslach Burnout Scale (MBS)

The inventory developed by Maslach and Jackson in 1981 [7]) was adapted into Turkish by Ergin in 1992 [13]. MBS, a 5-point Likert-type rating scale, consists of 22 items and three sub-dimensions. Three points are obtained from the scale as Emotional Exhaustion (9 items), Depersonalization (5 items) and Feeling of Personal Failure (8 items) [7, 13]. Capri (2006) reported the Cronbach α internal consistency coefficients of the sub-dimensions of the scale as 0.83 for Emotional Exhaustion (EE), 0.72 for Feeling of Personal Failure (PF) and 0.65 for Depersonalization (D) [14].

Ethical Considerations

Ethics committee approval was obtained from European University of Lefke, Ethics Committee 01.11.2021, BAYEK 001.10 approval number. The questionnaire form consists of three parts. The first part is about the participants' consent to participate in the research. When “I give consent” option is selected; the second part of the questionnaire is displayed. This section basically includes questions about the socio-demographic data of the participants, such as age, gender, educational status, and whether they have a physical or psychological disorder. Also, they were asked about their experiences and information on availability of personal protective equipment, whether they or a colleague has contracted the disease, whether they lost patients due to COVID-19, the sources from which they follow information about COVID-19, and whether they were the victim of verbal or physical vi-

olence by relatives of patients during the pandemic. Finally, Maslach Burnout Scale questions were asked to the participants in the survey. The study was conducted in accordance with the Declaration of Helsinki principles.

Participants

The participants of the study consisted of 261 healthcare professionals. The distribution of the participants regarding gender, marital status, education level and occupation is given in Table 1.

The mean age and working years of the participants were calculated as 35.99 ± 10.34 years (range 20-65) for age and 11.91 ± 9.91 years (range 0-40) for working year, respectively. 47.9% (n = 125) of the participants could not go to their homes during the pandemic, 38.7% (n = 101) experienced loss of patients due to COVID-19, and 21.8% (n = 57) were diagnosed with COVID-19. 9.6% (n = 25) stated that their colleague caught COVID-19 and 34.9% (n = 91) stated that they had difficulty in reaching personal protective equipment. 31.8% (n = 83) of the participants stated that they were exposed to verbal and physical abuse by their relatives during the pandemic, 12.6% (n = 33) were physically abused and 8% (n = 21) reported that they started to experience psychological and another 8% (n = 21) reported that they started to experience both physical and psychological problems. Finally, 75.5% (n = 197) of the participants reported that they followed the course of the pandemic and new developments on social media platforms.

Table 1. Distribution of participants by gender, marital status, education level and occupation

	n	%
Sex		
Female	168	64.4
Male	93	35.6
Marital status		
Married	164	62.8
Single	81	31.0
Divorced	13	5.0
Widow	3	1.1
Education level		
High school	35	13.4
Associate degree	61	23.4
Undergraduate	97	37.2
Master's degree	40	15.3
PhD	28	10.7
Occupation		
Healthcare professional	37	14.2
Manager	10	3.8
Pharmacist	9	3.4
Doctor	53	20.3
Nurse	93	35.6
Contact tracing team	59	22.6

Statistical Analysis

The datas were presented as mean ± standard deviation or frequency and percentage values. Normally distributed data were compared with the independent-samples t-test or one-way analysis of variance. One-Way Analysis of Variance was used to determine whether the participants' MBS scores showed a difference between occupational groups. The level of statistical significance was set at $p < 0.05$.

RESULTS

The average values obtained by all of the participants and also from the MBS defined according to their occupational groups are given in Table 2.

According to the results obtained, the personal failures of healthcare professionals working as nurses and pharmacists were found to be higher than those working as healthcare professionals. On the other hand, nurses experience more personal failure than doctors. When the data set is divided into two on the basis of the gender variable, the sense of personal failure of female healthcare professionals and physicians was found to be low enough to make a significant difference compared to female nurses and contact tracing team members. On the other hand, among male healthcare professionals, there was a significant difference between healthcare professionals working as nurses and those working as pharmacists. Accordingly, male healthcare professionals working as nurses experience more emotional exhaustion than those working as pharmacists.

The scores of the participants in the MBS were

Table 2. The mean values obtained by the participants from the Maslach Burnout Scale and the differences between the groups

	Emotional Exhaustion	Depersonalization	Feeling of Personal Failure
All participants	25.45 ± 7.92	8.85 ± 3.17	26.79 ± 4.00
Healthcare professional	26.84 ± 8.51	9.38 ± 3.43	24.38 ± 4.78
Manager	24.00 ± 7.55	7.70 ± 2.66	27.20 ± 2.44
Pharmacist	20.22 ± 6.34	7.22 ± 1.30	27.22 ± 3.07
Doctor	25.79 ± 8.22	9.60 ± 3.27	25.47 ± 3.34
Nurse	25.96 ± 8.18	8.65 ± 3.22	28.31 ± 3.28
Contact tracing team	24.51 ± 6.88	8.63 ± 2.97	26.98 ± 4.38
p value	> 0.05	> 0.05	**< 0.001***

Data are shown as mean ± standard deviation. *$p < 0.05$

compared according to the gender variable. Based on to this calculation, male participants' feelings of personal failure made a significant difference compared to female participants ($p = 0.008$). Personal failure scores of healthcare professionals who were away from their homes during the pandemic were higher than those who did not have to stay away from their homes ($p = 0.034$).

The depersonalization ($p = 0.015$) and personal failure ($p = 0.025$) scores of healthcare professionals who lost patients due to COVID-19 were found to be higher than those who did not, revealing a significant difference. The data set was divided into two based on the gender variable and it was investigated whether the gender variable revealed significant differences between various socio-demographic variables and burnout scores. Accordingly, female participants with loss of patient experience depersonalization ($p = 0.018$) and personal failure (0.003), while male participants ($p > 0.05$) do not.

Emotional exhaustion ($p = 0.012$) and depersonalization ($p = 0.001$) scores of healthcare professionals who were exposed to physical and verbal abuse by the relatives of the patients were calculated higher than those who did not, and this increase created a significant difference. When the data set was divided into two based on the gender variable, it was determined that female participants who were exposed to physical and verbal abuse experienced depersonalization ($p = 0.000$) and emotional exhaustion ($p = 0.039$), whereas male participants did not.

The personal failure scores ($p = 0.007$) of healthcare professionals, whose close colleague had contracted COVID-19 disease, were high enough to make a significant difference. Emotional exhaustion ($p = 0.011$) and depersonalization ($p = 0.023$) scores of the participants who stated that they had difficulty in reaching personal protective equipment created a significant difference. When the data set was divided into two on the gender variable, it was determined that male participants who had difficulty in reaching personal protective equipment experienced emotional exhaustion ($p = 0.001$) and depersonalization ($p = 0.015$), while female participants did not.

Personal failure ($p = 0.044$) scores of healthcare professionals who follow the developments related to COVID-19 on social media were found to be higher. Emotional exhaustion ($p = 0.001$) scores differed between the participants who had both physical and psychological disorders, those who only reported having psychological disorders, and those who did not have any disease. Participants with physical and psychological disorders experience more emotional exhaustion than participants without any disorders. Similarly, a significant difference was found between the participants who reported that they did not have any disorder and those who reported that they had a psychological disorder. Participants with psychological disorders are more depersonalized than those without psychological disorders ($p = 0.030$).

Variables such as marital status, education level, and being diagnosed with COVID-19 were not found to affect burnout in this study. However, when the data set was divided into two over the gender variable, it

was observed that the education level variable affected emotional exhaustion scores among female participants. Although the emotional exhaustion scores of female participants with doctoral and high school education did not reveal a significant difference between the groups in the advanced statistical study, they revealed a significant difference according to the One-Way Analysis of Variance ($p = 0.030$).

The relationship between the years of service and burnout levels of healthcare professionals was examined. Accordingly, as the years of service of female healthcare professionals increase, their depersonalization levels decrease ($r = -0.172$, $p = 0.025$, $p < 0.05$), and similarly, as the years of service of male healthcare professionals increase, their emotional exhaustion ($r = -0.262$, $p = 0.011$, $p < 0.05$) and depersonalization levels ($r= -0.238$, $p = 0.022$, $p < 0.05$) decrease.

DISCUSSION

Among the main findings of the study, it was determined that the gender variable was effective on emotional exhaustion, depersonalization, and the feeling of personal failure. Accordingly, female healthcare professionals experience more personal failure and emotional exhaustion than male healthcare professionals. Especially female healthcare professionals working as contact tracing team members are more exhausted than their male teammates. Barello *et al*. [15] also found that female healthcare professionals have higher emotional exhaustion rates than males. Jalili *et al*. [16] found that being a woman is the variable that reveals the differences between groups in all three dimensions of burnout, and that depersonalization levels are higher especially among young men compared to others. Torrente *et al*. [17] found that female healthcare professionals who are fighting COVID-19 on the front line are more exhausted than others. Hu *et al*. [18] also found the burnout levels of healthcare professionals working on the frontline during the COVID-19 pandemic to be higher than others. Ruiz−Fernández *et al*. [19] found that doctors' burnout levels were higher than nurses, and health personnel working in COVID-19 services were more exhausted than others.

It has been found that the physical, psychological or both physical and psychological disorders of the participants participating in the research or the diagnoses they received during the pandemic also increased the level of burnout. Duarte *et al*. [20] looked at the burnout levels of Portuguese healthcare professionals during the COVID-19 process. According to their studies, they determined that female healthcare professionals are more exhausted than men and that being diagnosed with any health problem is the main factor that increases burnout [20].

Another finding of the study is the significant increase in the burnout levels of healthcare professionals who have difficulties in accessing personal protective equipment. Morgantini *et al*. [21] found that the ease of access to personal protective equipment is a factor that reduces burnout. Martínez-López *et al*. [22] similarly found that the levels of burnout are higher among healthcare professionals who have difficulty in accessing personal protective equipment.

In our study, it was observed that especially nurses were exhausted more than other occupational groups. On the other hand, the burnout levels of the healthcare professionals who were caring for the patients according to the instructions given by the doctor were found to be at least as high as the nurses. This finding is similar to research conducted around the world during the COVID-19 era. For example, Liu et al. [23] found that working in areas with a high prevalence of COVID-19 patients increased emotional exhaustion and depersonalization, while intense working hours increased emotional exhaustion and decreased the sense of personal accomplishment. Matsua et al. [24] found that the burnout levels of healthcare professionals who were working face-to-face during the pandemic were higher than those of healthcare professionals working in the background.

During the COVID-19 pandemic, the burnout levels of healthcare professionals who lost several patients were higher than those who did not. Mong and Noguchi [25], in their study on emergency service workers, similarly found that the level of burnout increased with higher number of patients lost in the emergency room during COVID-19.

The burnout levels of healthcare professionals who reported that they were exposed to physical and verbal abuse during the pandemic were also found to be significantly higher. Elhadi et al. [26] found that the burnout levels of healthcare professionals who worked during the pandemic in Libya and who were exposed

to physical and verbal abuse were similarly higher.

In this research, long working years, in other words, work life and experience in the profession lower the burnout levels. As the years spent in the profession increase, it becomes easier to cope with the burnout syndrome. Alrawashdeh et al. [27], in their study with healthcare professionals, similarly, it was found that more experienced healthcare personnel with longer working years had lower burnout levels than those with fewer working years. In our study, the burnout levels of healthcare professionals who update their information about COVID-19 via social media were calculated to be higher. Shao et al. [28] determined that negative emotions arise and burnout becomes widespread in the case of using social media for updating information about the pandemic and following developments.

CONCLUSION

In this study, in addition to variables such as being a woman, being in a one-to-one relationship with patients with COVID-19 and being obliged to provide care, performing the nursing profession and following the updates about the pandemic on social media were identified as variables that increase burnout. On the other hand, the length of the years spent in the profession has been determined as a factor that reduces burnout depending on the experience gained.

Authors' Contribution

Study Conception: ZO, GBA; Study Design: ZO, GBA; Supervision: ZO, GBA; Funding: ZO, GBA; Materials: ZO, GBA; Data Collection and/or Processing: ZO, GBA; Statistical Analysis and/or Data Interpretation: ZO, GBA; Literature Review: ZO, GBA; Manuscript Preparation: ZO, GBA and Critical Review: ZO, GBA.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Comparison of the performances of non-parametric k-sample test procedures as an alternative to one-way analysis of variance

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ABSTRACT

Objectives: The performances of the Kruskal-Wallis test, the van der Waerden test, the modified version of Kruskal-Wallis test based on permutation test, the Mood's Median test and the Savage test, which are among the non-parametric alternatives of one-way analysis of variance and included in the literature, to protect the Type-I error probability determined at the beginning of the trial at a nominal level, were compared with the F test.

Methods: Performance of the tests to protect Type-I error; in cases where the variances are homogeneous/heterogeneous, the sample sizes are balanced/unbalanced, the distribution of the data is in accordance with the normal distribution/the log-normal distribution, how it is affected by the change in the number of groups to be compared has been examined on simulation scenarios.

Results: The Kruskal-Wallis test, the van der Waerden test, the modified version of the Kruskal-Wallis test based on the permutation test were not affected by the distribution of the data, but by the violation of the homogeneity of the variances. The performance of the Mood's Median test and the Savage test were not found to be sufficient in terms of protection of theType-I error compared to other tests.

Conclusions: It was determined that the Kruskal-Wallis test, the van der Waerden test, the modified version of Kruskal-Wallis test based on permutation test were not affected by the distribution of the data and tended to preserve the Type-I error when the variances were homogeneous.

Keywords: Analysis of variance, conformity of normal distribution, non-parametric k-sample tests

Data analysis methods that will be allied to the data obtained from research with at least interval scale; variance varies according to sample size, distribution of data, and the number of groups to be compared. One of the most critical steps of statistical data analysis is to decide whether the test procedure to be used to analyze the data will be a parametric or non-parametric test. Parametric tests are statistical methods that require data to be measured on an interval or ratio scale, which can be applied due to certain assumptions. Non-parametric test procedures are alternatively preferred when the necessary assumptions are not met for performing parametric tests.

One-way analysis of variance (ANOVA) or F-test,

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which is a parametric test, is used to compare the mean of more than two populations and is one of the most frequently used and most important statistical methods for this purpose [1]. The assumptions for the F test include that the data is normally distributed, the sample variances are equal, and the samples are independent [2]. If the assumptions of conformity to normal distribution or homogeneity of variance are violated, the probability of Type-I error obtained at the end of the trials and the power of the test are adversely affected. This adversely affect becomes even more evident if the sample sizes in the groups compared are not balanced [3]. Non-parametric tests are statistical procedures that are preferred as an alternative to parametric tests when assumptions are not met. Non-parametric tests have less assumptions than parametric tests [4]. The data need not conform to a normal distribution. Non-parametric tests can be applied to data measured with a classifier or ordinal scale.

Pearson [5], Glass *et al*. [6], and Wilcox [7] examined the effect of the normality assumption violation on the Type-I error. Wilcox [7] concluded that samples that do not conform to normal distribution have some impact on the Type-I error rate, but the effect is mini-

Table 1. Sample sizes of the groups

Number of groups	Balanced Sample	Non-balanced Sample		
		Observation combinations where the number of sample sizes are not equal	Observation combinations where the number of sample sizes differs excessively	Observation combinations with inverse matching between variance and number of sample sizes
3	3:3:3 5:5:5 10:10:10 15:15:15 20:20:20 25:25:25 30:30:30 50:50:50 80:80:80 100:100:100	3:5:7 5:10:15 20:25:30 50:60:70 65:75:85 70:90:100	3:25:30 3:80:80 5:20:100	7:5:3 15:10:5 30:25:20 70:60:50 85:75:65 100:90:70
5	3:3:3:3:3 5:5:5:5:5 10:10:10:10:10 15:15:15:15:15 20:20:20:20:20 25:25:25:25:25 30:30:30:30:30 50:50:50:50:50 80:80:80:80:80 100:100:100:100:100	3:5:7:9:11 5:7:9:12:15 20:22:24:28:30 50:55:60:65:70 55:65:75:85:95 60:70:80:90:100	3:20:25:80:100 3:5:30:80:100 5:10:20:25:80 3:5:10:15:100	7:5:3 15:10:5 30:25:20 70:60:50 85:75:65 100:90:70
8	3:3:3:3:3:3:3:3 5:5:5:5:5:5:5:5 10:10:10:10:10:10:10:10 15:15:15:15:15:15:15:15 20:20:20:20:20:20:20:20 25:25:25:25:25:25:25:25 30:30:30:30:30:30:30:30 50:50:50:50:50:50:50:50 80:80:80:80:80:80:80:80 100:100:100:100:100:100:100:100	3:5:7:9:11:12:14:15 20:22:24:25:26:28:29:30 50:55:60:65:70:75:80:85 60:65:75:80:85:90:95:100	3:5:10:20:25:30:80:100 5:10:20:20:25:80:90:100 3:5:10:80:80:90:100:100 20:25:30:80:90:90:100:100	15:14:12:11:9:7:5:3 30:29:28:26:25:24:22:20 85:80:75:70:65:60:55:50 100:95:90:85:80:75:65:60

mal if the variances are homogeneous. Glass *et al*. [6] reported similar results to Wilcox [7] in their studies if the variances were homogeneous. In his study, Bunning [8] examined the performances of the Kruskal-Wallis test, the normal score test and the Welch test, which he included as an alternative to the F test and the F test, in terms of Type-I error and power. He evaluated the performances of the tests under various simulation scenarios in terms of whether the variances are homogeneous or not in equal and unequal sample sizes if the data show normal distribution or not. In his study, Moder [2] stated that the location parameters of the groups should be investigated in detail when there are unbalanced sample sizes.

In our study, we compared the performances of the Kruskal-Wallis test, the Mood's Median test, the van der Waerden test, the modified version of Kruskal-Wallis test based on permutation test and the Savage test, which are among the non-parametric alternatives of the F test, to protect the Type-I error under various simulation scenarios.

METHODS

In our study, the Kruskal-Wallis test, the modified version of Kruskal-Wallis test based on permutation test, the Mood's Median test, the van der Waerden test and the Savage test in terms of maintaining the probability of the Type-I error determined at the beginning of the experiment was compared with the F test. Simulation scenarios were run under the R program [9].

The performance of the tests was evaluated as a result of comparisons between three, five, and eight groups for simulation scenarios involving balanced/non-balanced sample sizes (Table 1), normal distribution or log-normal distribution, homogenous or heterogeneous variances (Table 2). In addition to the specified simulation conditions, observation combinations are also included, where the number of sample size varies excessively among the group with higher variance is assigned a lower number of observations, and the group with a lower variance is assigned a higher number of observations and inverse matching between variance and sample size.

In comparisons made to determine Type-I error, group means were taken equally. The Type- I error probabilities for each of the simulation scenarios were obtained after the numbers of H_0 hypotheses were determined, which were rejected at the end of 50000 repetitions. In our study, the evaluation criterion proposed by Peterson [10] was adopted and it was concluded that the performance of the tests with a probability of the Type-I error between 4.49% and 5.49% was sufficient to maintain Type-I error.

Table 2 shows the variance rates of the groups that are suitable for normal distribution and the scale parameter values of the groups that are suitable for log-normal distribution.

The F Test

One-way analysis of variance (ANOVA) or F test is used to compare the mean of more than two populations. It is one of the most important and frequently used methods of applied statistics [1]. The null hypothesis H_0: $\mu_1=\mu_2=\ldots=\mu_k$ versus alternative H_1: at least one μ_i (i= 1, 2, . . ., k) is different. The F test statistic,

$$F = \frac{\sum_{i=1}^{k} n_i(X_{i.}-\bar{X}_{..})^2/(k-1)}{\sum_{i=1}^{k}\sum_{j=1}^{n_i}(X_{ij}-\bar{x}_{i.})^2/(N-k)} \sim F_{1-\alpha;k-1,N-k} \qquad (1)$$

In Equation, k is the number of groups, N is the total number of observations, Xij is the jth observation (j = 1, 2, . . ., *ni*) in the ith group (i = 1, 2, . . . , *k*), $N=\sum n_i$, $\bar{X}_{..}$ is the overall mean, $\bar{X}_{i.}$ is the sample mean for the *i*th group. The F test is more powerful if the assumptions of normality and variance homogeneity hold. The null hypothesis, H_0: $\mu_1=\mu_2=\ldots=\mu_k$, should then be rejected at the α level of significance when $F \geq F_{1-\alpha;k-1,N-k}$.

The Kruskal-Wallis Test

One of the non-parametric alternatives to the F test is the Kruskal-Wallis (KW) test. The KW test is a non-parametric test procedure used to compare three or more groups independently [11]. It is carried out using ranks given to observation values instead of actual observation values. To calculate the test statistics, the data are sorted from small to large, and each is assigned a rank. $R_i=\sum_{j=1}^{n_i} R_{ij}$ is the sum of ranks assigned to the observations in the ith group. The null hypothesis H_0: $\theta_1=\theta_2=\ldots=\theta_k$ versus alternative H_1: at least one θ_i (i= 1, 2, . . ., k) is different. The test statistic is calculated as,

Table 2. Variance rates of groups

	Normal distribution		Log- normal distribution	
Number of groups	Homogeneous Variance	Heterogeneous Variance	Homogeneous Scale parameter (b)	Heterogeneous scale parameter (b)
3	1:1:1 2:2:2 4:4:4 8:8:8 10:10:10	1:1:2 1:2:2 1:1:4 1:4:4 1:1:8 1:8:8 1:1:10 1:10:10 1:4:8 2:1:1 2:2:1 4:1:1 4:4:1 8:1:1 8:8:1 10:1:1 10:10:1 8:4:1	0.1:0.1:0.1 0.2:0.2:0.2 0.3:0.3:0.3 0.4:0.4:0.4 0.5:0.5:0.5 0.6:0.6:0.6 0.7:0.7:0.7 0.8:0.8:0.8	0.10:0.10:0.20 0.10:0.20:0.20 0.10:0.30:0.50 0.10:0.40:0.50 0.10:0.10:0.50 0.10:0.50:0.60 0.10:0.60:0.80 0.20:0.10:0.10 0.20:0.20:0.10 0.50:0.30:0.10 0.50:0.40:0.10 0.50:0.10:0.10 0.60:0.50:0.10 0.80:0.60:0.10
5	1:1:1:1:1 2:2:2:2:2 4:4:4:4:4 8:8:8:8:8 10:10:10:10:10	1:1:2:2:2 1:1:4:4:4 1:1:8:8:8 1:1:10:10:10 1:2:4:8:10 2:2:2:1:1 4:4:4:1:1 8:8:8:1:1 10:10:10:1:1 10:8:4:2:1	0.1:0.1:0.1:0.1:0.1 0.2:0.2:0.2:0.2:0.2 0.3:0.3:0.3:0.3:0.3 0.4:0.4:0.4:0.4:0.4 0.5:0.5:0.5:0.5:0.5 0.6:0.6:0.6:0.6:0.6 0.7:0.7:0.7:0.7:0.7 0.8:0.8:0.8:0.8:0.8	0.1:0.1:0.2:0.2:0.2 0.1:0.1:0.4:0.4:0.4 0.1:0.1:0.5:0.5:0.5 0.1:0.1:0.6:0.7:0.8 0.1:0.3:0.5:0.7:0.8 0.2:0.2:0.2:0.1:0.1 0.4:0.4:0.4:0.1:0.1 0.5:0.5:0.5:0.1:0.1 0.8:0.7:0.6:0.1:0.1 0.8:0.7:0.5:0.3:0.1
8	1:1:1:1:1:1:1:1 2:2:2:2:2:2:2:2 4:4:4:4:4:4:4:4 8:8:8:8:8:8:8:8 10:10:10:10:10:10:10:10	1:1:1:1:1:1:1:2 1:1:1:1:1:1:1:4 1:1:1:1:1:1:1:8 1:1:1:1:1:1:1:10 1:1:1:2:2:2:4:4 1:1:1:1:4:4:4:4 1:1:1:1:8:8:10:10 2:1:1:1:1:1:1:1 4:1:1:1:1:1:1:1 8:1:1:1:1:1:1:1 10:1:1:1:1:1:1:1 4:4:2:2:2:1:1:1 4:4:4:4:1:1:1:1 10:10:8:8:1:1:1:1 10:10:8:8:4:4:2:1	0.1:0.1:0.1:0.1:0.1:0.1:0.1:0.1 0.2:0.2:0.2:0.2:0.2:0.2:0.2:0.2 0.3:0.3:0.3:0.3:0.3:0.3:0.3:0.3 0.4:0.4:0.4:0.4:0.4:0.4:0.4:0.4 0.5:0.5:0.5:0.5:0.5:0.5:0.5:0.5 0.6:0.6:0.6:0.6:0.6:0.6:0.6:0.6 0.7:0.7:0.7:0.7:0.7:0.7:0.7:0.7 0.8:0.8:0.8:0.8:0.8:0.8:0.8:0.8	0.1:0.1:0.1:0.1:0.1:0.1:0.1:0.1:0.2 0.1:0.1:0.1:0.1:0.1:0.1:0.1:0.1:0.3 0.1:0.1:0.1:0.1:0.1:0.1:0.1:0.1:0.5 0.1:0.1:0.1:0.1:0.1:0.1:0.1:0.1:0.7 0.1:0.1:0.1:0.3:0.3:0.3:0.5:0.5 0.1:0.1:0.1:0.1:0.6:0.6:0.8:0.8 0.2:0.3:0.4:0.5:0.6:0.7:0.7:0.8 0.2:0.2:0.2:0.4:0.4:0.8:0.8:0.8 0.2:0.1:0.1:0.1:0.1:0.1:0.1:0.1 0.3:0.1:0.1:0.1:0.1:0.1:0.1:0.1 0.5:0.1:0.1:0.1:0.1:0.1:0.1:0.1 0.7:0.1:0.1:0.1:0.1:0.1:0.1:0.1 0.5:0.5:0.3:0.3:0.3:0.1:0.1:0.1 0.8:0.8:0.6:0.6:0.1:0.1:0.1:0.1 0.8:0.7:0.7:0.6:0.5:0.4:0.3:0.2 0.8:0.8:0.8:0.4:0.4:0.2:0.2:0.2

$$KW = \frac{1}{s^2}\left(\sum_{i=1}^{K}\frac{R_i^2}{n_i} - \frac{N(N+1)^2}{4}\right) \quad (2)$$

where

$$S^2 = \frac{1}{N-1}\left(\sum_{i=1}^{k}\sum_{j=1}^{n_i} r_{ij}^2 - \frac{N(N+1)^2}{4}\right) \quad (3)$$

Note that, when there are no ties, S^2 simplifies to $N(N+1)/12$.

The null hypothesis H_0: $\theta_1 = \theta_2 = \ldots = \theta_k$,should then be rejected at the α level of significance when $\chi^2_{KW} \geq \chi^2_{k-1;\alpha}$.

The Modified Version of Kruskal-Wallis Test Based on Permutation Test

Permutation test is the test procedure which is presented by Fisher [12] and the probability values obtained are exact probabilities, and it is also stated by Hecke [13] as a simulation method used to determine the strength of the test.

There are two methods for calculating the KW test: permutation and rank transformations. The modified version of the KW test based on the permutation test is obtained by combining the permutation method based on the F statistic with the rank method [11]. The process of obtaining the permutations starts by choosing the test statistic T and the acceptable significance level α. $\pi_1, \pi_2, \ldots, \pi_n$ be a set of all distinct permutations of the ranks of the data set in the experiment. For permutation testing, the data are sorted from small to large, each is given a rank and the KW test statistic is calculated ($H_1=t_0$). Different permutation (π_i) values are obtained for each data sorted from small to large. The KW test statistic is calculated for the obtained permutation (n_i) values ($H_i=H(\pi_i)$) and this process i is repeated (i= 2, 3, ..., M).

The null hypothesis H_0: $\theta_1 = \theta_2 = \ldots = \theta_k$ versus alternative H_1: at least one θ_i (i= 1, 2, …, k) is different. The test statistic is calculated as [13],

$$p_0 = p(H \leq H_i) = \frac{1}{M}\sum_{i=1}^{M}\psi(t_0 - H_i) \quad (4)$$

where

$$\psi(.) = \begin{cases} 1, if\ t_0 \geq H_i \\ 0, if\ t_0 < H_i \end{cases}$$

Under the empirical distribution, if $p_0 \leq \alpha$, reject the null hypothesis.

The Mood's Median Test

The Mood's Median (MM) test is the generalized version of the median test used to test data from two independent groups, used for three and more sample comparisons [15]. The null hypothesis H_0: $\theta_1 = \theta_2 = \ldots = \theta_k$ versus alternative H_1: at least one θ_i (i= 1, 2, …, k) is different.

To obtain the test statistics of the MM test, the common median value of all data is first calculated. As a second step, for each sample, it is determined how many observations are greater than the calculated median value and how many are equal to or less than it. As a result, a 2xk frequency table is obtained. The test statistic is calculated as,

$$\chi^2 = \sum_{i=1}^{k}\frac{(O_{ij}-E_{ij})^2}{E_{ij}} \quad (5)$$

The null hypothesis H_0: $\theta_1 = \theta_2 = \ldots = \theta_k$,should then be rejected at the α level of significance when $\chi^2 \geq \chi^2_{\alpha;(i-1)*(j-1)}$

The van der Waerden Test

The advantage of the van der Waerden test is that it provides the high efficiency of the standard ANOVA analysis when the normality assumptions are in fact satisfied, but it also provides the robustness of the KW test when the normality assumptions are not satisfied [16]. The KW test is based on the ranks of the data. The van der Waerden test converts the ranks to quantiles of the standard normal distribution. These are called normal scores and the test is computed from these normal scores [17]. The null hypothesis H_0: $\theta_1 = \theta_2 = \ldots = \theta_k$ versus alternative H_1: at least one θ_i (i= 1, 2, …, k) is different. The formula for the van der Waerden test is

$$V = \frac{1}{S^2}\sum_{i=1}^{k} n_i \bar{A}_i^2 \quad (6)$$

where

$$A_{ij} = \phi^{-1}\left(\frac{R_{ij}}{N+1}\right), \bar{A}_i = \frac{1}{n_i}\sum_{j=1}^{n_i} A_{ij}, S^2 = \frac{1}{N-1}\sum_{i=1}^{k}\sum_{j=1}^{n_i} A_{ij}^2, \phi^{-1}$$

is the normal quantile of x. The null hypothesis should then be rejected at the α level of significance when $V \geq \chi^2_{\alpha;k-1}$

The Savage Test

The Savage test is among the non-parametric alternatives to the F test used to test the differences between location parameters. The Savage test is powerful to compare scale differences or position differences in the extreme value distribution, which are compatible with exponential distribution [18].

The Savage test statistic is calculated by Savage scores. The null hypothesis H_0: $\theta_1 = \theta_2 = \ldots = \theta_k$ versus alternative H_1: at least one θ_i (i= 1, 2, …, k) is different. The formula for the Savage test is

$$T_E = \frac{1}{S_E^2} \sum_{i=1}^{k} n_i \bar{S}_i^2 \qquad (7)$$

where

$$S_{ij} = \sum_{i=1}^{k} \frac{1}{N-i+1} - 1 \; ; \; \bar{S}_i = \frac{1}{n_i} \sum_{j=1}^{n_i} S_{ij} \; ; \; S_E^2 = \frac{1}{(N-1)} \sum_{i=1}^{k} \sum_{j=1}^{n_i} S_{ij}^2$$

The null hypothesis should then be rejected at the α level of significance when $T_E \geq \chi^2_{\alpha;k-1}$.

RESULTS

In this study, the tests were compared with the help of simulation scenarios in terms of the Type-I error protection. Simulation scenarios were performed under the R program [9]. The obtained Type-I errors are given in tables.

Comparisons in which sample size is balanced, the group variances are homogeneous, and the data follow to the normal distribution (Table 3, Supplementary Table 1, 2)

The F test is the test that shows the most successful performance when the non-parametric alternatives are taken into consideration and the predetermined Type-I error level was determined.

In addition to the F test, the KW test also tends to maintain the Type-I error level in terms of observation combinations, and the increase in the number of groups to be compared (especially in the case of eight groups) has a positive effect on its performance.

Comparisons in which the sample size is not balanced, the group variances are homogeneous, and the data follow to the normal distribution (Supplementary Table 3-8)

The F test and the KW test based on permutation test are the most successful tests for estimating the Type-I error level initially determined. The F test and the modified version of KW test based on permutation test are followed by the KW test with deflection estimates shown only in a single simulation scenario.

The other tests included in the study were found to be adversely affected by the imbalance of the number of sample sizes in the groups, and their performance to protect the Type-I error determined at the beginning was not sufficient.

When simulation scenarios involving observation combinations in which the number of sample sizes in groups differ excessively, it was observed that the F test and modified version of KW test based on permutation test were not affected by the extreme differences in the number of sample sizes in groups and tended to maintain the Type-I error level initially determined in all simulation scenarios according to the Peterson criterion.

On the other hand, in cases where the number of sample size in the groups varies in a balanced manner, the KW test, which performs at a level that can accompany these two tests, was observed to have affected its performance and gave deviated results if the difference in the number of sample size was excessive.

Comparisons in which the sample size is balanced, group variances are heterogeneous, but the data follow to the normal distribution (Supplementary Table 9-11)

It has been seen that the tests included in the study generally give deviated results in terms of protecting the Type-I error and their performance was not found sufficient.

Comparisons in which the sample size is not balanced, group variances are heterogeneous, but the data follow to the normal distribution (Supplementary Table 12-20)

It has been seen that the tests included in the study generally give deviated results in terms of protecting

Table 3. Type-I error rates (%) for k=3 groups where $\sigma_1^2:\sigma_2^2:\sigma_3^2$= 1:…:1~10:…:10, $\mu_1=\mu_2=\mu_3=0$, sample size is balanced ($n_1=n_2=n_3$)

σ^2	n	F	KW	permKW	MM	VW	Savage
1	3	4.70%	8.51%	5.03%	0%	1.11%	0.71%
	5	4.98%	5.49%	4.90%	6.67%	4.85%	2.74%
	10	5.03%	5.11%	4.42%	3.48%	4.47%	3.97%
	15	5.09%	5.21%	4.74%	4.69%	4.78%	4.29%
	20	5.07%	5.15%	4.78%	4.74%	4.87%	4.43%
	25	5.08%	5.14%	4.83%	4.77%	4.78%	4.48%
	30	4.93%	4.92%	4.75%	4.15%	4.68%	4.63%
	50	5.11%	5.13%	4.99%	4.88%	4.99%	4.68%
	80	5.07%	5.08%	4.98%	5.54%	5.04%	4.89%
	100	5.04%	5.05%	5.00%	5.12%	5.00%	4.84%
2	3	4.82%	8.31%	4.60%	0%	1.02%	0.71%
	5	4.93%	5.51%	4.82%	6.80%	3.86%	2.64%
	10	4.88%	5.15%	4.29%	3.37%	4.42%	3.71%
	15	5.03%	5.20%	4.64%	4.67%	4.77%	4.19%
	20	4.80%	4.99%	4.51%	4.61%	4.62%	4.24%
	25	5.15%	5.26%	4.95%	4.88%	5.01%	4.67%
	30	5.03%	5.09%	4.86%	4.40%	4.96%	4.62%
	50	4.93%	5.01%	4.84%	4.79%	4.93%	4.79%
	80	4.99%	5.06%	4.92%	5.48%	4.95%	4.78%
	100	5.00%	4.97%	4.95%	5.10%	4.98%	4.76%
4	3	5.08%	8.57%	4.70%	0%	1.03%	0.67%
	5	4.96%	5.46%	4.79%	6.59%	3.83%	2.64%
	10	5.06%	5.29%	4.46%	3.31%	4.55%	3.82%
	15	4.77%	4.90%	4.60%	4.49%	4.73%	4.19%
	20	5.10%	5.10%	4.78%	4.74%	4.87%	4.43%
	25	4.95%	4.94%	4.84%	4.81%	4.85%	4.61%
	30	5.21%	5.13%	4.60%	4.14%	4.62%	4.52%
	50	5.10%	5.01%	4.80%	4.84%	4.78%	4.87%
	80	4.89%	4.85%	4.95%	5.42%	4.97%	4.83%
	100	4.86%	4.92%	4.85%	5.09%	4.88%	4.80%
8	3	4.97%	8.59%	4.84%	0%	1.13%	0.72%
	5	4.78%	5.29%	4.67%	6.66%	4.84%	2.73%
	10	5.07%	5.20%	4.46%	3.43%	4.55%	3.85%
	15	5.15%	5.35%	4.76%	4.72%	4.84%	4.42%
	20	4.90%	4.93%	4.62%	4.79%	4.56%	4.37%
	25	5.01%	5.12%	4.79%	4.70%	4.85%	4.55%
	30	5.00%	5.04%	4.82%	4.36%	4.76%	4.53%
	50	5.08%	5.03%	4.97%	4.82%	4.95%	4.84%
	80	5.21%	5.22%	5.13%	5.51%	5.12%	4.99%
	100	5.09%	4.93%	5.05%	5.14%	5.07%	5.01%
10	3	5.10%	8.66%	4.90%	0%	1.16%	0.72%
	5	4.99%	5.47%	4.91%	6.74%	3.90%	2.71%
	10	4.88%	5.08%	4.29%	3.46%	4.40%	3.76%
	15	5.01%	5.05%	4.61%	4.47%	4.61%	4.23%
	20	5.02%	5.09%	4.73%	4.85%	4.77%	4.28%
	25	5.08%	5.08%	4.85%	4.82%	4.83%	4.55%
	30	4.90%	4.99%	4.73%	4.38%	4.82%	4.52%
	50	5.06%	4.99%	4.84%	4.85%	4.86%	4.60%
	80	5.08%	5.10%	4.95%	4.88%	4.92%	4.68%
	100	5.09%	5.06%	5.04%	4.95%	5.00%	4.80%

F: F test; KW: Kruskal-Wallis test; permKW: the modified version of Kruskal-Wallis test based on permutation test; MM: Mood's Median test; VW: van der Waerden test

the Type-I error and their performance is not sufficient.

Comparisons in which the sample size is balanced, group variances are homogeneous, and the data follow to log-normal distribution (Supplementary Table 21-23)

As expected, the F test is the test that shows the most successful performance in order to estimate the level of Type-I error determined at the beginning when considering the non-parametric alternatives available.

In addition to the F test, the KW test also tends to maintain the Type-I error level in terms of observation combinations, and the increase in the number of groups to be compared (especially in the case of eight groups) has a positive effect on its performance.

The performance of the MM test was also positively affected by the increase in the number of groups. Although its performance in protecting the Type-I error is lower than that of the KW test, its performance in the case of eight groups has increased significantly compared to the number of groups to be compared with three and five.

Comparisons in which the sample size is not balanced, group variances are homogeneous, and the data follow to log-normal distribution (Supplementary Table 24-29)

When simulation scenarios involving observation combinations in which the number of sample size in the groups are not equal are examined, the F test and the KW test are the tests that show the most successful performance in order to estimate the Type-I error level determined at the beginning. These tests are followed by modified version of KW test based on permutation test.

When the simulation scenarios involving observation combinations in which the number of sample size in the groups differ excessively, it was observed that the permutation version of the F test and the KW test was not affected by the extreme differences in the number of sample size in the groups.

T he other tests included in the study were found to be adversely affected by the imbalance of the number of sample size in the groups, and their performance in maintaining the Type-I error level determined at the beginning was not sufficient.

Comparisons in which the sample size is balanced, group variances are heterogeneous, and the data follow to log-normal distribution Supplementary (Table 30-32)

It has been seen that the tests included in the study generally give deviated results in terms of protecting the Type-I error and their performance was not found sufficient.

Comparisons in which the sample size is not balanced, group variances are heterogeneous, and the data follow to log-normal distribution (Supplementary Table 33-41)

It has been seen that the tests included in the study generally give deviated results in terms of protecting the Type-I error and their performance was not found sufficient.

DISCUSSION

The F test is the test that shows the most successful performance as expected in cases where the conformity to the normal distribution and the homogeneity of the variances are provided. When the simulation scenarios where the assumption of homogeneity of variances are not met, as expected, the F test was highly affected by the deterioration in group variances and failed to maintain the Type-I error at the nominal level ($\alpha = 0.05$). The results of our study reach similar results to the studies conducted by Buning [8] and Moder [2]. It is the test that shows the most successful performance compared to other alternative tests in cases where the data conform to the log-normal distribution, and the variances are homogeneous. Blanca *et al*. [19] Clinch and Keselman [20], Gamage and Weerahandi [21], Lantz [22] and Schmider *et al*. [23] reported that the F test tends to protect the Type-I error in cases where the assumption of conformity to the normal distribution is violated. It was observed that the effect of violation of the homogeneity of variances on the performance of the F test was more than the violation of the assumption of conformity to normal distribution. Bishop and Dudewicz [3], Blanca *et al*. [19], Brown and Forsythe [24], Buning [8], Debeuckelaer [25], Lee and Ahn [26], Li *et al*. [27], Lu and Mathew [28], Markowski [29], Keselman *et al*. [30], Tomarken and Serlin [31] concluded that the F test is highly affected by the deterioration in group variances.

In this study, the KW test was not affected by the distribution of the data. It was concluded that the violation of the homogeneity of variances and the number of sample sizes (equal and unequal) in the groups were effective on the performance of the KW test to protect the Type-I error. In their studies, Hoeffding [32] ve Terry [33] concluded that the performance of the KW test was not sufficient in terms of protecting Type-I error in cases where the variance was not homogeneous. Lantz [22], Luh and Guo [34], Jett and Speer [35] found in their studies that the KW test was not affected by the distribution of the data, and in cases where the variances were homogeneous, they tend to protect the Type-I error.

The modified version of the KW test is not affected by the distribution of the data; It is highly affected by the homogeneity violation of variances such as the KW test. It can be suggested as an alternative for the F test for observation combinations where the number of sample sizes in the groups are not equal and excessively different. Odiase and Ogbonmwan [14] reported in their study that the permutation test does not require assumptions for the distribution of the data, and that it performs well on data that are normally distributed and not normally distributed.

The van der Wearden test was not affected by the distribution of the data and showed successful performance in protecting the Type-I error in observation combinations where the number of sample sizes in the groups where the group variances were homogeneous differed significantly. The van der Wearden test was greatly affected by the breakdown in group variance. Luepsen [1] stated that the van der Wearden test was the most successful test after the F test in estimating the Type-I error level in cases where there is no relationship between group variances and the number of observations belonging to the groups.

Although the MM test performed well as the number of groups compared increased, it did not show a successful performance in protecting the Type-I error in general. Jett and Speer [35] stated in their simulation studies that the performance of the MM test was not sufficient to protect the Type-I error and reported our study with supporting findings.

The Savage test could not perform adequately to protect the Type-I error at nominal level and gave biased results. There is no study in the literature regarding the Savage test. Our study aims to contribute to the literature by reporting that the Savage test's performance in protecting the Type-I error compared to other tests gives very poor and biased results.

CONCLUSION

In conclusion as stated in the literature, it was determined that the F test tends to maintain its robustness in case of violation of the normal distribution, however, it is more affected by the violation of the homogeneity assumption of variances. It was concluded that the distribution of the data was not effective on the KW test's performance in protecting Type-I error, the violation of homogeneity of variances and the sample size in the groups were effective. The modified version of KW test based on permutation test is not affected by the distribution of the data; like the KW test, it is highly affected by the violation of homogeneity of variances. It can be suggested as an alternative to the F test for combinations of observations where the sample sizes in the groups are not equal and vary excessively. The van der Wearden test was not affected by the distribution of the data and showed successful performance in protecting the Type-I error in observation combinations where the number of sample sizes in the groups where the group variances were homogeneous differed significantly. In general, the MM test did not show a successful performance in protecting the Type-I error. It has been found that the Savage test's performance in protecting the Type-I error compared to other tests gives very poor and biased results.

Authors' Contribution

Study Conception: GO; Study Design: GO; Supervision: GO; Funding: N/A; Materials: N/A ; Data Collection and/or Processing: ACM; Analysis and/or Data Interpretation: ACM, GO; Literature Review: ACM; Manuscript Preparation: ACM, GO and Critical Review: ACM, GO.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Supplementary Tables 1 to 41

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The relationship between pathological internet use and psychiatric difficulties in adolescents during COVID-19 pandemic

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ABSTRACT

Objectives: Internet addiction has been attracting widespread interest due to the increase in screen time related to lectures, decrease in social activities and spending more time at home due to the education restriction all over the world. It was aimed to determine the psychiatric difficulties for before pandemic period and distance education period, separately and to evaluate the relationship between adolescents' pathological internet usage and difference in psychiatric difficulties.

Methods: Four hundred thirty-eight adolescents aged 11-17 years, secondary and high school students were included in the study. An online survey was created consists of "Young Internet Addiction Test-brief form" (IAT), "Strengths and Difficulties Questionnaire" (SDQ) and researcher form using Google forms application. Youths completed the SDQ retrospectively for two separate time points: during the school restriction period and before the pandemic.

Results: According to the results of our study, the number of changed areas (sleep, eating habits, leisure/social/physical activities) (B = 3.071, $p < 0.001$) and the change in SDQ-Peer relationship subscale score (B =1.193, $p = 0.019$) were among the factors significantly predict pathological internet use levels, respectively.

Conclusions: Studies enable understanding the relationship between pathological internet usage and psychiatric difficulties would be useful for the organization of preventive interventions in case similar outbreaks occurred in the future.

Keywords: COVID-19, pandemic, internet addiction, adolescent, psychiatric difficulties

Due to the pandemic that has been experienced all over the world for about 1.5 years, social life has been greatly restricted, and works, meetings, shopping and even education have begun to be made over the internet. In addition to the numerous benefits of technological devices and the internet, which is increasingly set to become an essential part of our daily lives, it has commonly been discussed that excessive use of internet is responsible for academic/family domain issues and medical/physical or psychological problems [1, 2]. Although the internet provides many opportunities for disadvantaged children (poverty, ethnicity,

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gender), it has also hazardous content that might be inappropriate for the child's development or leading to risky behavior [3, 4]. Pathological internet use has become a critical issue regarding public health, considering that 1/3 of internet users all over the world are under the age of 18.

It is well-known that the internet could be used by individuals to cope with stress, to relax or to communicate more easily due to the anxiety in social situations. It is also possible that children and adolescents with psychiatric disorders such as Depressive Disorder, Anxiety Disorder or attention deficit hyperactivity disorder, who use internet for short-term relaxation [5], easier communication or a relatively better focusing [6, 7], might lose control over the internet. Various explanations have been given for the frequent co-morbidity of pathological internet use with psychiatric disorders. The explanations were suggested such as psychiatric disorders have a role on either onset or maintain internet use, that excessive use of the internet deteriorates the course of psychiatric disorders, or that both occur with common etiological mechanisms. Studies, evaluating the relationship between internet addiction and co-morbid psychiatric disorders are mostly cross-sectional, which is insufficient to explain the causal relationship between them.

In recent years, including before pandemic, a growing body of literature has examined excessive internet use /internet addiction and related psychological factors. One of the factors, that was commonly emphasized associated with the internet/technological device overuse in the studies regarding this field, has been the time spent on the internet [8]. During the restrictions due to the pandemic, the length of time that adolescents are required to be online, including education, and therefore the risk of internet addiction has increased [9]. Studies on the long-term negative effects of the COVID-19 pandemic crisis we are experiencing as the whole world on adolescent mental health are required as such a crisis could be prolonged or repeated [10]. Most studies evaluating related factors with problematic internet use have only focused on, changes in sleep and eating habits, and quality of life in adults and adolescents during the pandemic, but not much is known about differences regarding psychiatric symptom levels between before and during the pandemic.

The aim of this study was to evaluate the difference in psychiatric difficulties before and after the pandemic and to assess the relationship of this difference with internet excessive use.

METHODS

Participants and Procedure

Adolescents aged 11-17, living in Bursa, a big city in the west of Turkey and studying 5th, 6th, 7th, 9th, 10th and 11th levels were recruited for our study. 8th and 12th grade students were excluded because they continued face-to-face education during the pandemic restriction period. Students from a secondary school and a high school in the Nilüfer district of Bursa were invited to participate to the study. The necessary permissions for the study were obtained from Bursa State Hospital Clinical Research Ethics Committee (date 02.06.2021 and number 2021-10/11) and T.C. Bursa Provincial Directorate of National Education (date 07.10.2021 and number 34082522). This study has been carried out in accordance with Declaration of Helsinki.

Adolescents were asked to fill out a detailed form prepared by the researcher, which evaluates the sociodemographic characteristics, as well as the technical devices/internet usage areas and durations, changes in eating, sleeping habits, physical activity levels, hobbies and socializing activities. (for two separate times, before the pandemic and during the school restriction period). In addition to this form, participants were asked to fill out two scales. An on-line survey was created consists of "Young Internet Addiction Test-short form" (IAT), "Strengths and Difficulties Questionnaire" (SDQ) and researcher form using Google forms application. The questionnaires were distributed to the students 15-31 of October in 2021 by their school counselor via WhatsApp. All the scales took approximately 10 minutes in total. Responses of the participants were automatically stored on the Google drive storage.

Measures

Strengths and Difficulties Questionnaire (SDQ)

SDQ is a short scale used in order to screen emotional and behavioral problems with a self-report form for ages 11-18. The scale consists of 5 subscales: Conduct Problems, Emotional Problems, Attention Deficit

and Hyperactivity, Peer Relationships and Prosocial Behaviors. A mean score can be obtained for each subscale, as well as the "Total Difficulty Score" can be calculated with the sum of the first four. Turkish validity and reliability study of the scale was made by Güvenir *et al*. [11]. Authors have suggested that SDQ had a high internal consistency except the peer problem scale. In addition to being short and practical, it is useful among the tools developed for similar purposes, as it contains items that question positive areas and contributes to the evaluation of the current strength of the youth. The SDQ scales were filled for two time points, before the pandemic and during the face-to-face education restriction, in our study.

Young Internet Addiction Test (IAT)-Brief Form

It is used to assess the youth's internet usage. Young *et al*. [12] created the Internet Addiction Test (IAT) by adapting features common to all addictions such as excessive use by ignoring basic needs, needing to use more and more (tolerance), negative emotions (anger, nervousness) in lack of internet, continuing to use the internet even though it makes life difficult. The study, in which the brief form of the scale developed by Pawlikowski *et al*., was used in university students and adolescents in our country, has showed that the Young Internet Addiction Test is a valid and reliable tool for our culture as well [13]. Explanatory factor analysis indicated that the scale explained 39.52% of the total variance in university students and 48.9% of that in adolescents. The authors have claimed that Young IAT-brief form Cronbach's alpha coefficient was found as .91 in university students and .86 in adolescents. Correlation coefficient for test-retest reliability was found as .93 in university students and .86 in adolescents. Although the scale has no cut-off value, high scores indicate a high level of internet addiction.

Statistical Analysis

The Statistical Package for the Social Sciences (version 20) program was used to analyze the data. Descriptive statistics are shown as mean-standard deviation or frequency (%). The data obtained for two time points, pre-pandemic and distance education period, were compared using the Wilcoxon Signed-Rank test. Mann Whitney U test was performed to explore differences in the scores of psychiatric difficulty areas according to the changes in sleep, eating habits, the time allocated to daily physical activity/leisure activities/social activities during school restriction (two separate groups: change is or not in the eat and sleep habits, leisure activities, social activities; decrease is or not in the physical activities). The comparison of the restriction period-SDQ subscale scores and IAT total scores between two groups set on the basis of their education preferences was also made using the student t test. The relationship among the variables: age, monthly income and change in the SDQ subscale scores between before the pandemic and school restriction time, and the IAT total score were evaluated by Spearman correlation analysis. Subtracting the score for pre-pandemic from the school restriction time score, it was obtained the score of "the change in SDQ subscales". Whether there is an alteration in the sleep and eating habits, leisure activities, social activities and a decrease in physical activities which are actually nominal variables were accepted as continuous variables in our study. The sum of the scores was expressed as "number of changed areas". Correlations between SDQ change scores and this new variable, "number of changed areas" were also examined by Spearman Correlation analysis. Finally, the variables that might influence IAT total scores were evaluated by using hierarchical linear regression analysis. The independent variables were gender and number of changed areas in the first step and the changes in the SDQ subscales (conduct, emotional and peer relationship) were in the second step. Significance was set at $p < .05$.

RESULTS

A total of 462 adolescents agreed to participate to the study and completed the survey. Since 24 of them significantly missed completing the questionnaire, the sample was composed of 438 adolescents with a mean age of (14.15 ± 2.23 years). Two-hundred and twenty two of 438 participants (50.7%) were girls; 216 (49.3%) were boys.

The answers given to the questionnaire prepared by the researcher were evaluated in order to detect the sociodemographic variables that might be associated with excessive use of internet. One of the questions, asked in the researcher survey was how many hours per day the participants spent with the internet/tech-

nological devices apart from online education. According to answers given for the pre-pandemic, 35.8% of the participants (142 of 397) stated that they spent less than 2 hours, 37.5% (n = 149) 2-4 hours, 16.4% (n = 65) 4-6 hours, 10.1% (n = 40) spent more than 6 hours with internet/technological devices. In the period when face-to-face education was restricted, it was stated that 23.2% of them (92 of 397) used the internet for less than 2 hours, 30.7% of them (n = 122) for 2-4 hours, 21.4% of them (85) for 4-6 hours, and 24.4% of them (n = 97) for more than 6 hours.

Regarding their sleep duration in a day, 4% (16 of 397) stated that they slept less than 5 hours in a day, 25.7% (n = 102) slept 5-7 hours, 51.6% (n = 205) slept 7-8 hours, 18.4% (n = 73) slept more than 8 hours. When asked how much time they spent on physical exercise before the pandemic, 33.3 percent (132 of 396) indicated less than 30 minutes, 31.8 percent (n = 126) stated 30 minutes to an hour, 20.2 percent (n = 80) replied 1-2 hours, and 14.6 percent (n = 58) said more than 2 hours. According to the answers, 93.4%of the participants (370 of 396) had pre-pandemic leisure activities and 89.1% of them (353 of 396) were spending time with their friends before the pandemic.

Changes in sleep, eating habits, physical activity, leisure time and social activity levels compared to before pandemic were evaluated at the time of education restriction. In response to the question of sleep habit change, the majority of those surveyed (305 of 395) (77.2%) indicated that there was a change in their sleep habits during the face-to-face education restriction period. Eighty-five of 300 respondents (28.3%) stated that the amount of sleep decreased, while 73 of them (24.3%) that the amount of sleep increased. The positive response rate was 66.9% (265 of 396) for change in the adolescents' eating habits. While 42.3% of the youth (113 of 267) stated that they ate unhealthier during the restriction period; 19.5% (52 of 267) stated that they ate healthier. Additionally, 262 of 396 teenagers (66.2 %) reported reducing or discontinuing physical activities in comparison to the pre-pandemic era; 181 of 382 (48.6%) reported reducing or stopping leisure activities; and 297 of 355 (83.6%) reported reducing or discontinuing social activities.

In terms of education preference, 67.2% of the participants (266 of 396) answered that they preferred face-to-face education and 32.8% (n = 130) preferred distance.

Young Internet Addiction Test-brief form (IAT) was used to determine the pathological internet use levels of the youths participated in our study. The average score from the IAT was (28.45 ± 11.7). When the IAT mean scores of the participants (n = 438) were evaluated according to varied ranges; the rates were as follows: 12-23 (178, 40.6%), 24-35 (146, 33.3%), 36-47 (77, 17.6%), 48-60 (37, 8.4%).

Secondary aim of our study was to evaluate the psychiatric difficulty areas of adolescents according to their answers for pre-pandemic and post-pandemic and to investigate the relationship between pathological internet use and psychiatric difficulties. Preliminary analyses showed that scores from all SDQ subscales were higher during the school restriction period than before the pandemic, except for the social

Table 1. Descriptive statistics related to psychiatric difficulty areas (n = 356)

	Before pandemic (Mean ± SD)	During restriction (Mean ± SD)	95% confidence interval of the difference		Z value	*p* value
			lower	upper		
Conduct problems	1.82 ± 1.5	2.29 ± 1.6	- 0.59	- 0.35	- 7.489	**< 0.001**
Emotional problems	2.75 ± 2.1	3.69 ± 2.6	- 1.13	- 0.75	-9.202	**< 0.001**
Hyperactivity-inattention	4.28 ± 1.9	4.63 ± 2.0	- 0.50	- 0.18	- 4.191	**< 0.001**
Peer relationship problems	2.55 ± 1.8	2.94 ± 1.9	-0.52	- 0.25	- 5.238	**< 0.001**
Prosocial behavior	8.03 ± 1.9	7.39 ± 2.0	0.49	0.78	8.388	**< 0.001**
Total difficulties score	11.39 ± 4.7	13.55 ± 5.7	- 2.55	- 1.75	- 10.457	**< 0.001**

Table 2. Comparison of psychiatric difficulty areas scores (during pandemic) according to the changes in the sleep, eating habits, the time allocated to daily physical activity, leisure activities and social activities during school restriction

	Decreased PA		Sleep change		Eating change		Leisure activity change		Social activity change	
	- (122)	+ (235)	- (79)	+ (277)	- (119)	+ (238)	- (83)	+ (254)	- (26)	+ (294)
Conduct problems	2.03 ± 1.5	2.44 ± 1.6*	1.83 ± 1.3	2.44 ± 1.6*	2.10 ± 1.8	2.40 ± 1.5	1.85 ± 1.3	2.40 ± 1.6*	2.15 ± 1.6	2.29 ± 1.5
Emotional problems	3.17 ± 2.5	3.97 ± 2.6*	3.02 ± 2.1	3.90 ± 2.7*	3.03 ± 2.3	4.02 ± 2.6*	2.85 ± 2.2	3.97 ± 2.6*	3.34 ± 2.7	3.74 ± 2.6
Hyperactivity-inattention	4.21 ± 1.9	4.85 ± 2.1*	4.15 ± 2.1	4.78 ± 2.0*	4.39 ± 1.9	4.75 ± 2.1	4.00 ± 1.9	4.82 ± 2.0*	4.03 ± 2.0	4.67 ± 2.0
Peer relationship problems	2.69 ± 1.7	3.07 ± 1.9	2.82 ± 1.9	2.98 ± 1.9	2.62 ± 1.8	3.10 ± 1.9*	2.73 ± 2.0	3.01 ± 1.8	2.34 ± 1.3	2.90 ± 1.8
Prosocial behavior	7.65 ± 1.8	7.26 ± 2.1	7.21 ± 1.8	7.43 ± 2.1	7.62 ± 2.0	7.28 ± 2.1	7.67 ± 2.0	7.42 ± 2.0	7.38 ± 2.8	7.56 ± 1.8
Total difficulties score	12.11 ± 4.8	14.30 ± 5.9*	11.83 ± 5.1	14.09 ± 5.7*	12.07 ± 5.5	14.28 ± 5.6*	11.44 ± 5.4	14.18 ± 5.7*	11.88 ± 4.5	13.59 ± 5.7
IAT total score	25.64 ± 10.2	30.75 ± 12.0*	24.98 ± 10.1	30.16 ± 11.8*	25.71 ± 10.8	30.74 ± 11.8*	25.66 ± 10.9	30.28 ± 11.8*	26.07 ± 8.6	29.30 ± 11.9

Note: (-/+) two categories according to whether there was a change in eating, sleep habits, leisure activity and social activity patterns, and whether there was a decrease in physical activity levels.

skills sub-domain, where high scores indicate positive functioning. Descriptive statistics related to psychiatric difficulty areas are seen in table 1. When the scores of psychiatric difficulties and IAT total score were compared according to whether there was a change in eating, sleep habits, leisure activity and social activity patterns, and whether there was a decrease in physical activity levels, the results obtained are shown in Table 2.

Further analyses indicated that there was no significant relationship between IAT scores and age, r (432) = 0.082, and monthly income, r (323) = -0.011. There were positive correlations between IAT and SDQ change- conduct, r (312) = 0.180, $p = 0.001$), SDQ change- emotional, r (312) = 0.243, $p < 0.001$), SDQ change- peer relationship, r (312) = 0.221, $p < 0.001$), and SDQ change-total difficulties, r (311) = 0.290, $p < 0.001$) variables. Significant mild correlations also between the number of changed areas and SDQ change- emotional r (303) = 0.203, $p < 0.001$, SDQ change- hyperactivity r (302) = 0.117, $p = 0.042$), SDQ change- peer relationship r (303) = 0.116, $p = 0.043$ and SDQ change-total subscale r (302) = 0.212, $p < 0.001$ scores (see Table 3).

The variables that might influence IAT total scores were assessed using hierarchical linear regression analysis. Gender and "number of changed areas" variables were entered as the first block when examining predictors of IAT total scores, and the results indicated that the model was significant, and 13.2 % of the variance was explained by the model (F = 18.206, $p < 0.001$). In the model 1; gender did not significantly predict IAT total scores and "number of changed areas" was statistically significant. After entry of the change in the SDQ-conduct, change in the SDQ-emotional and change in the SDQ-peer relationship variables at the second block, the model was still significant (F = 11.611, $p < 0.001$) and total variance explained by the model as a whole was 18.4 % (R squared change = 0.052). In the final model, the number of changed areas (B= 3.071, $p < 0.001$) and the change in the SDQ- Peer relationship subscale score variables were statistically significant (B = 1.193, $p = 0.019$) (4).

The restriction period-SDQ scores of the participants were evaluated after the sample was subdivided on the basis of education preference (n = 357); scores from the "Emotional problems" (t = 2.714, $p = 0.007$,

Table 3. Correlations among the variables: age, monthly income, change in the SDQ subscale scores between before the pandemic and school restriction time, number of changed areas and the IAT total score

	IAT total score		Number of changed areas	
	r	*p* value	r	*p* value
Age	0.082	0.087		
Monthly income	-0.011	0.840		
SDQ Change- Conduct problems	0.157	**0.005**	0.045	0.440
SDQ Change -Emotional problems	0.169	**0.003**	0.174	**0.002**
SDQ Change- Hyperactivity-inattention	0.079	0.167	0.152	**0.008**
SDQ Change- Peer relationship problems	0.113	**0.046**	0.056	0.334
SDQ Change- Prosocial behavior	-0.177	0.107	-0.060	0.300
SDQ Change- Total score	.179	**0.002**	0.179	**0.002**
IAT total score			0.335	**< 0.001**

*Spearman correlation analyses. Note: Number of changed areas: change in the sleep and eating habits, leisure activities, social activities and decrease in physical activities which are actually nominal variables were accepted as dummy variables, treated as a continuous variable, and summed.

95% Confidence Interval (CI): 0.21-1.35), "Peer relationship problems" (t = 4.090, $p < 0.001$, 95% Confidence Interval (CI): 0.44-1.26) and "Total difficulties" (t = 3.304, p = 0.001, 95% Confidence Interval (CI): 0.84-3.31) subscales were statistically significantly higher in the group prefer distance education compared to the group prefer face to face education. And the scores obtained from the "Pro-social behaviors" sub-scale, which indicates positive functionality, were significantly higher (t = -2.471, $p = 0.014$, 95% Confidence Interval (CI): -1.01--.11) in the group prefer face to face education. The IAT total score of the participants who preferred online education was statistically significantly higher than those who preferred face-to-face education (t = 3.81, $p = 0.002$, 95% Confidence Interval (CI): 1.63-6.92).

Table 4. Hierarchical linear regression analysis for variables predicting IAT total score (n = 267)

	Unstandardized Coefficients		Standardized Coefficients Beta	*p* value
	B	Std. Error		
Model 1				
Gender	-0.351	1.321	-0.015	0.791
Number of changed areas	3.532	0.593	0.337	**< 0.001**
Model 2				
Gender	0.222	1.294	0.010	0.864
Number of changed areas	3.071	0.588	0.293	**< 0.001**
SDQ change-conduct	0.969	0.599	0.094	0.107
SDQ change- emotional	0.728	0.396	0.112	0.067
SDQ change- peer relationship	1.193	0.505	0.136	**0.019***

DISCUSSION

In the present study, it was determined that the rate of those who used daily internet/technological devices less than 2 hours a day as recommended by the guidelines was 35.8% for the pre-pandemic period and 23.2% for the restriction period. Moreover, it was detected that the rate of those using the internet for more than 6 hours a day, excluding the time allocated for education, increased during the school restriction period (10.1%-24.4%) compared to the pre-pandemic period. In studies evaluated the effects of social isolation on adolescents due to the pandemic, it was a common finding that adolescents spent more time on the internet during lockdown than before the pandemic [14, 15]. It's noteworthy that the daily internet/technological device usage time in our study has apparently increased during the restrictions. Despite that, the average IAT score was 28.45, that was lower than the value we anticipated. While there is no cut-off value for IAT-brief form to diagnose internet addiction, it was found that approximately 8% of respondents appear to have scores in highest range. Our findings are in line with previous studies. According to the results of two studies from Turkey, which used the same scale "Parent Child Internet Addiction Test" to measure the level of internet addiction during the pandemic period, the rates of those with limited symptoms, although not at the level of internet addiction, were found as 9.7% [16] and 4.8% [17]. The fact that the time, adolescents spend with the internet/technological device is much higher than the recommended duration by the guides may mislead to perceive as addiction. That's because the age-appropriate activities youths used to do offline such as shopping, doing homework, socializing, watching movies or listening to music have recently been done online.

Our study results have revealed that significant changes have also been found in the daily routine of youths who spent a significant part of their time at home during distance education. According to our data, less than 20% of adolescents reported that they slept more than 8 hours a day before pandemic. Although there is no definite opinion about how much children and adolescents should sleep, it has been argued that modern life, technology/internet use may be related to the decrease in the total sleep time of youths in recent years [18]. When the answers given about whether there was a change in their sleeping habits during the period they were educated from home, it has been found that sleep duration decreased in 28.3% of the youths and increased in 24.3% of them. According to the literature, there are several studies in which it has been reported that sleep duration increased [19-21] or decreased [22] in adolescents during the social isolation period [23] have found that overall poor sleep quality was associated with poor oral hygiene during social distancing. Although, sleep habits may vary depending on cultural characteristics or parental attitudes, it has been suggested that adolescents with quality sleep spend less time on the internet/social media [24, 25].

Regarding changes in the other daily habits; 66.2% of the participants that their physical activities, 48.6% of them that their leisure activities; 83.6% of them that their social activities reported that they have reduced or completely quit compared to the pre-pandemic period. A recent systematic review has revealed that daily physical activity levels of adolescents declined, and sedentary life has increased during the period of staying at home due to the pandemic [26]. Positive effects of physical activities on mental health have been known. In a study conducted during COVID-19 pandemic with physiotherapy students, high physical activity levels were found to be associated with lower depressive symptom scores [27]. Relationship between physical activity levels and screen addiction was also examined; A 2015 study found that smart phone overuse might reduce physical activity levels such as walking [28]. In another study investigating the relationship between friendship network and the physical activity levels and indirectly screen time, it was found that being with active friends, especially for boys, had a positive effect on physical activity levels [29]. Due to physical distance and not being able to go to school, adolescent's activity levels have decreased considerably during the restrictions. Physical activity levels have probably not been among the priorities of parents who both had to work at home and were interested in their children's education [30].

There were positive correlations between the number of changes in daily habits such as sleep, eating, physical activity, leisure time/social activities and changes in the psychiatric diffilties particularly in emotional area in the current study. It's well known that mental problems can cause changes in sleep, ap-

petite and activity levels, as well as changes in daily habits can lead to psychiatric symptoms. Ingram *et al* suggested that the changes in diet, sleep quality, and physical activity related to differences in negative mood during COVID-19 lockdown [31]. In a study conducted with university students in China, adverse changes in the living rhythms of students who stayed at home during the pandemic were associated with higher depressive symptom levels; despite, it was determined that favorable living rhythms led to better perceived self-efficacy [32].

Our study results have demonstrated that the adolescents obtained higher scores from all psychiatric difficulty areas during the face-to-face education restriction compared to the pre-pandemic period. In a review evaluating the effects of the COVID-19 pandemic on the mental health of children and adolescents, including studies most of which determined the diagnoses with symptom checklists; Depression and anxiety were most frequent. It was emphasized that the rates of depression were similar to the studies before the pandemic, but it could be said that the anxiety increased compared to the pre-pandemic period [33]. Ozturk *et al*. [34] found that conduct problems and anxious temperament are more frequent in adolescents with internet addiction than in those without in their study investigated the relationship among the internet addiction, affective temperament and psychiatric difficulties in high school students in 2013.

According to our study results, positive significant relationships were also found between the SDQ subscales change scores (between two time points) except for the hyperactivity and the IAT total score, which indicates the level of internet excessive use. In a meta-analysis study, conducted before the pandemic, a positive correlation was found between ADHD and internet addiction and ADHD symptom severity was detected to be higher in those with internet addiction. Thus, it has been suggested that just as ADHD may initiate internet addiction, internet overuse may also negatively affect the prognosis of ADHD [35]. It has been probable that the reason for our findings related to the hyperactivity subscale were not significant might be the relative decrease in school-related responsibilities, the looser rules and the relatively better sustain of attention in front of the screen during the distance education period. The fact that psychiatric difficulty areas were assessed only by adolescents' self-report and the absence of parental reports might be responsible for the outcome.

Regarding regression analyses, "the number of changed areas" was significantly explained 11.5% of the variance and adding the changes in psychiatric difficulty subscale scores significantly increased 5.8 percent of the variance explained ($R2 = 0.173$, $p < 0.001$). The regression coefficients indicated that the number of changed areas ($p < 0.001$, beta = 0.293) have the most effect among the factors that predict pathological internet use levels. This factor was followed by the change in the peer relationship problems subscale scores ($p = 0.019$, beta = 0.136). Our study demonstrated that during the period of school restrictions, adolescents whose daily routines changed and kept away from social life increased significantly various psychiatric difficulties, and especially increase in the problems related to peer relationships significantly predicted their pathological internet use levels. Studies reporting that the idea of loneliness is a predictor of internet addiction [36-38] and on the contrary that social support plays a role in preventing internet abuse [39, 40] emphasizes the importance of strengthening personal relationships and increasing offline social/leisure activities instead of banning the internet [41]. In a recent study, role of peer relationships in the effect of internet addiction on internalizing and externalizing symptoms has been investigated. The authors have suggested that internet addicted youths, who had positive friendships and less delinquent peers, exhibited less aggressive behavior [42]. In addition to strategies to improve peer relationships, emotion regulation techniques (relaxing, meditation, cognitive-behavioral therapies) and integrative treatments including yoga and mindfulness have been demonstrated as effective treatments in the prevention of addiction, including internet addiction [43, 44].

Youths' psychiatric difficulties were compared also according to their educational preferences in our study. Considering that the emotional and peer relationship problems are significantly higher in the group that prefers distance education, it is understandable that the anxiety levels of the adolescents who are away from their peers due to the restriction and lost the opportunity to improve their social skills have increased. Thus, the importance of being with peers, supporting social development and taking psychological protective measures becomes evident. Both, the ability of

adolescents to seek help when they feel that they have lost their control over the internet, or being identified of pathological usage would be easier with face-to-face education.

Limitations

Our study has a number of restrictions. First, in the duration when the study data were collected, face-to-face education had just started after a long time. Because social isolation was ended mainly, and social activities started except school in this period, the findings need to be interpreted with attention. Secondly, since the questionnaire was not administered in person to the adolescents, it should be taken into account that its reliability was low. Gathering information about a time point in the past, is also a limitation as there is a risk of forgetting. Final limitation is that the socioeconomic levels of the participants are not homogeneous. However, no correlation was found between the primary outcome of our research: the levels of internet overuse, and the monthly income of the families.

CONCLUSION

Despite these, the use of standardized self-report scales to determine psychiatric difficulty areas and pathological internet use levels and the fact that the design of the study is based on the comparison of evaluations made for two separate time points provides an advantage over the other studies. It has been well known that psychiatric difficulties experienced in adolescence negatively affect school success, social functionality and family relationships, and therefore reduce the quality of life in adulthood. So, understanding negative effects of social distancing on mental health and internet addiction and determining the risk or resilience factors would be beneficial for similar situations that may be encountered in the future [45].

Authors' Contribution

Study Conception: DA; Study Design: DA, FG; Supervision: DA, FG; Funding: N/A; Materials: N/A; Data Collection and/or Processing: DA; Statistical Analysis and/or Data Interpretation: DA, FG; Literature Review: DA; Manuscript Preparation: DA, FG and Critical Review: FG.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Should encountering atypia of undetermined significance / follicular lesion of undetermined significance after thyroid biopsy lead to the operation?

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ABSTRACT

Objectives: Nodular goiter is the most common disease of the thyroid gland. Thyroid nodules are malignant in 3-5% cases. To determine the incidence of malignancy in patients defined as atypia of undetermined significance (AUS) or follicular lesion of undetermined significance (FLUS) as a result of fine needle aspiration biopsy (FNAB) and evaluate the clinical, biochemical and sonographic features as possible predictors of malignancy.

Methods: Patients who had undergone at least one FNAB and diagnosed as AUS/FLUS from January 2011 to December 2015 were included in the study. Age, gender, benign disease, thyroid stimulating hormone (TSH) level, size, localization, number, time of FNAB, ultrasonography (USG) characteristics, follow-up data on repeated FNAB results and, if surgical excision was performed, final pathological results were analyzed.

Results: A total of 5181 thyroid nodules were biopsied in 4089 patients, and the biopsy specimen taken from 611 nodules was diagnosed histopathologically as AUS/FLUS (11.79%). After FNAB, 167 of 611 patients diagnosed with AUS/FLUS were operated. While 65.9% (n = 110) of 167 patients who underwent surgery were made a benign diagnosis; malign diagnosis was made to 34.1% (n = 57).

Conclusions: The rate of malignancy in surgically confirmed nodules was 34.1% in this study, which is higher than the Bethesda classification. Due to the high malignancy rate which determined in cytologically diagnosed AUS/FLUS, we think that the repeat decision of the FNAB should be reconsidered and the surgical plan after the first FNAB should be considered more seriously.

Keywords: Thyroid biopsy, thyroid cancer, thyroidectomy

Nodular goiter is the most common disease of the thyroid gland, especially in areas with iodine deficiency [1]. Thyroid nodules are found in 4-8% by palpation in adults, 41% by ultrasonography (USG), and 50% in autopsy series [2]. Thyroid nodules are malignant in 3-5% cases although thyroid cancers are more rare, constituting less than 1% of all malignant neoplasms [3]. Important information is obtained by thyroid function tests (TFT), scintigraphy and USG in clinical and diagnostic approach in thyroid nodules but

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the distinction of benign and malignant lesions cannot be made with these tests [4].

Today, the most commonly used, fast, simple, least invasive, inexpensive and reliable method for the malignant-benign differentiation of thyroid nodules is cytological examination of fine needle aspiration materials [5]. Diagnostic accuracy of thyroid fine needle aspiration biopsy (FNAB) increases up to 80-95% in experienced clinics [6].

The Bethesda Classification Reporting Thyroid Cytopathology was defined by the National Cancer Institute (NCI) to improve communication between cytopathologists and clinicians and standardize the terminology used for FNAB. This system is divided into six diagnostic categories that provide an assessment of the risk of malignancy and management guidelines for each category [7]. Atypia of undetermined significance (AUS) or follicular lesion of undetermined significance (FLUS) is a Bethesda system category that includes various nuclear abnormalities that do not fit into another category after FNAB [8]. According to the Bethesda system, the AUS/FLUS category covers approximately 7% or less of all thyroid FNABs and the expected risk of malignancy in the AUS/FLUS category according to NCI is between 5% and 15% [9]. However, several recent studies have reported various malignancy rates ranging from 6% to 76% for surgically confirmed cases [10].

In this study, by analyzing the data of our hospital, we aimed to determine the incidence of malignancy in patients defined as AUS and FLUS as a result of FNAB in our own patient group and evaluate the clinical, biochemical and sonographic features as possible predictors of malignancy. In addition, this study aims to contribute to the follow-up and treatment processes of patients.

METHODS

This study was conducted in an education and research hospital in the Eastern Black Sea Region, which is considered an endemic region for goiter. The patient consent forms required for the FNAB and surgical procedures to be performed within the framework of ethical recommendations were obtained. The study was approved by the hospital ethics committee (No: 2016/44). FNAB cytologic evaluation of the cases was performed according to the widely accepted and applied Bethesda classification.

A retrospective review of institutional electronic medical records was performed and cases diagnosed as AUS/FLUS from January 2011 to December 2015 were identified. Patients who had undergone at least one FNAB and diagnosed as AUS/FLUS were included in the study. Patients with malignant, malignant suspicious, follicular neoplasia or follicular neoplasia suspected as a result of biopsy performed in different thyroid nodules and patients with synchronous malignancy in another organ and contralateral thyroid lobe were excluded from the study. Age, gender, benign disease, thyroid stimulating hormone (TSH) level, size, localization, number, time of FNAB, ultrasonography (USG) characteristics, follow-up data on repeated FNAB results and, if surgical excision was performed, final pathological results were analyzed. Only patients who underwent surgical excision were included in the final analysis after adequate follow-up data and exclusion of cases without a descriptive pathological diagnosis. All FNABs were performed by an experienced endocrinologist or interventional radiologist with USG guidance from a suspected thyroid nodule. USG features of thyroid nodules were recorded using electronic database or by scanning patients' files. Among these features, hypoechogenicity, microcalcification, presence of halo and presence of solid component were defined as suspicious criteria for malignancy [11].

National Cancer Institute recommendations are followed in our hospital; for all patients diagnosed with AUS / FLUS in the first FNAB, a repeated FNAB is recommended [12]. After repeated FNAB, surgical excision is recommended for patients having AUS/FLUS, follicular neoplasia suspicious, follicular neoplasia, malignant suspicion and malignancy. If the diagnosis cannot be made after repeated FNAB, it is recommended to repeat the FNAB or perform surgery. If the results of repeated FNAB are benign, follow-up is recommended. Despite these recommendations, several factors (eg, clinical features, USG features, cosmetic problems, or patient preferences) may influence treatment decision.

The electronic medical records were reviewed using the database of our hospital and in case of insufficient data in the medical records, the current status of the patients was asked by telephone interview.

Table 1. Distribution of patients by gender

Gender	n	%
Female	504	82.5
Male	107	17.5

When the follow-up data were insufficient to confirm the prognosis of the nodule, the patient refused FNAB recurrence and / or surgical excision, or the patient died for other reasons, the patient was considered a missing case (Data Loss = DL).

Statistical Analysis

Continuous variables were presented as mean ± standard deviation in the parametric distribution and median in the nonparametric distribution. For statistical analysis, Chi-square test, Fisher's exact test, Mann-Whitney U test and Student's t-test were used for intergroup comparisons based on categorical or continuous properties of variables. Univariate analysis of the variables was performed using a logistic regression test to identify possible markers for malignancy. For logistic regression testing, categorical variables were converted to binomial data using known risk factors for a more appropriate assessment. In order to determine the lower and upper threshold values of malignancy rates, all nodules followed without surgery were assumed to be benign or malignant. The low threshold value was calculated by dividing the number of patients with malignancy detected after surgery by the total number of AUS/FLUS nodules after assuming benign patients. The upper threshold value was calculated by dividing the total number of patients who had malignancy with surgery and the patients under follow-up after the assumption of malignant patients, by the total number of AUS/FLUS nodules. Statistical analysis package (SPSS 20.0, IBM Corp, Armonk, NY, USA) was used for all statistical analysis. All reported p values are bilateral and $p < 0.05$ is considered significant. Also, confidence intervals (CI) were calculated at the level of 95%.

RESULTS

Clinical Findings in Nodules Diagnosed as AUS/FLUS with FNAB

A total of 5181 thyroid nodules were biopsied in 4089 patients, and the biopsy specimen taken from 611 nodules was diagnosed histopathologically as AUS/FLUS (11.79%). Patients whose histopathological diagnoses were reported as AUS/FLUS were included in the study group. Five hundred and four (82.5%) of these patients are females; 107 (17.5%) of them were males (Table 1). The female / male ratio was determined as 4.72. The mean age of the patients included in the study group was 52.97 ± 13.2 years, the average TSH level was 1.88 ± 5.8 mIU/L, and the average diameter of the biopsied nodules was 20.8 ± 9.86 mm (Table 2). Twenty-six of the patients had undergone a surgical operation due to benign disease. Diagnosis was made by performing biopsy from 495 (81%) patients from a single nodule, 113 (18.5%) patients from two nodules, three (3.5%) patients from three or more nodules (Table 3).

Histopathological Results in Operated Cases

After FNAB, 167 of 611 patients diagnosed with AUS/FLUS were operated and clear information about histopathological diagnosis was reached. While 65.9% (n = 110) of 167 patients who underwent surgery were made a benign diagnosis; malign diagnosis was made to 34.1% (n = 57) (Table 4).

Clinical Features

Twenty-seven (16.2%) of 167 patients who had undergone surgery were male and 83.8% (n = 140) were female. In the examination among female patients, 67.1% (n = 94) and 32.9% (n = 46) were diag-

Table 2. Age-TSH value-nodule diameter values of the patients

	n	Minimum	Maximum	Mean	Standart deviation
Age (years)	611	18	93	52.97	13.296
TSH (mIU/L)	547	0.01	127.5	1.887	5.82794
Nodule diameter (mm)	581	4	61	20.82	9.86

Table 3. Number of nodules taken by biopsy according to the number of patients

Number of biopsied nodules	n	%
From one nodule	495	81
From two nodules	113	18.5
From three or more nodules	3	0.5
Total	611	100

nosed as malignant. The average age of 110 patients in the benign disease group was 48.22 ± 11.39 years, and the average age of the patients in the malignant group was 52.3 ± 12.30 years (p = 0.034). Among male patients, this rate was 59.3% (n = 16) benign and 40.7% (n = 11) malignant (p = 0.429). Only 1.2% (n = 2) of 167 patients had undergone a previous surgical operation for thyroid due to a benign disease (p = 0.306) (Table 5). Sixty-four (38.3%) of the patients were operated after the first diagnosis of AUS/FLUS, and 47.9% (n = 80) were second and 13.8% (n = 23) were third. Of these, 31.3% (n = 20) of those who were operated after the first biopsy, 38.8% (n = 31) of those operated after the second biopsy, and 26.1% of those who were operated after the third biopsy (n = 6) a malignant diagnosis was made (p = 0.437) (see Table 5). Data related to TSH were reached in 159 of 167 patients whose histopathological diagnosis was confirmed surgically, while 8 patients could not. The average TSH value of 104 patients in the benign group was measured as 1.205 (0.01-127.49) mIU/L; the average TSH value of 55 patients in the malignant group. was measured as 1.08 (0.01-18.09) mIU/L (p = 0.621) There was no significant difference between malignant and benign groups in terms of gender, thyroid surgery, median TSH level, number of FNAB. It was observed that the average age was higher only in the malignant group than in the benign group. (p = 0.034) (Table 5).

Table 4. Histopathological distribution of the AUS/FLUS diagnosed nodules after surgical confirmation (n = 167)

Pathological diagnoses	n	%
Benign pathological diagnoses		
Adenomatous nodule	8	7.3
Follicular adenoma	4	3.6
Hashimoto's thyroiditis	9	8.2
Nodular goiter	10	9.1
Multinodular goiter	79	71.8
Total	110	100
Malign pathological diagnoses		
Papillary carcinoma (Classic variant)	28	49.1
Papillary carcinoma (Follicular variant)	23	40.4
Papillary carcinoma (Oncocytic variant	4	7
Follicular carcinoma	2	3.5
Total	57	100
General total	167	100

USG Features

Echogenicity, wall irregularity, halo presence, nodule component, calcification and nodule size were evaluated, and the data availability was 86.82%, 86.22%, 88.62%, 89.82%, 88.62% and 98%, respectively. Data on echogenicity could not be obtained in 22 of 167 patients whose histopathological diagnosis was clarified after surgery. 28.3% (n = 41) of the patients whose data were available had hypoechogenicity, 66.2% (n = 96) isoechogenicity and 5.5% (n = 8) hyperechogenicity. 36% (n = 15) of patients with nodules with hypoechogenicity, 34.4% (n = 33) of patients with nodules with isoechogenicity and 37.5% (n = 3) of patients with nodules with hyperechogenicity were diagnosed as malignant (p = 0.96) (Table 6). While 144 patients' USG information was obtained about wall irregularity, 23 patients did not have any data. While 3.5% (n = 5) of 144 patients had wall irregularities; no wall irregularity was found in 96.5% (n = 139). To 20% (n = 1) of patients with wall irregularities in thyroid nodules taken biopsy; 35.3% (n = 49) of patients without wall irregularities were given a malignant diagnosis. One hundred forty-eigth patients had information about the presence of halo, while 19 patients could not. While halo was found in 19% (n = 29) of the patients whose data were reached, 80.4% (n = 119) did not. While 37.1% (n = 11) of patients with halo in the nodule undergoing biopsy had a malignant diagnosis after surgery; This rate was 34.5% in patients with nodules without halos (n = 41) (p = 0.725) after surgery (p = 0.482). In the data analysis made for the components of the nodules, the data could be accessed in 150 patients. Data were not available in 17 patients. The patients whose data were available had 54.7% (n

Table 5. Clinical findings in surgically diagnosed nodules

		Cases		
		Benign (n = 110)	**Malign (n = 57)**	***p* value**
Age (years)		48.22 ± 11.39	52.3 ± 12.30)	**0.034**
Gender				
	Female	94 (85.5%)	46 (80.7%)	0.429
	Male	16 (14.5%)	11 (19.3%)	
Previous surgery				
	Yes	2 (1.8%)	0 (0%)	0.306
	No	108 (98.2%)	57 (100%)	
Time of surgery				
	After first FNAB	44 (40%)	20 (35.1%)	0.437
	After second FNAB	49 (44.5%)	31 (54.4%)	
	After third FNAB	17 (15.5%)	6 (10.5%)	
TSH value (mIU/L)		1.205 (0.01-127.49)	1.08 (0.01-18.09)	0.621

= 82) pure solid, 0.7% (n = 1) pure cystic and 44.7% (n = 82) mixed nodules. Of these, 35.4% (n = 29) of patients with pure solid nodules, 100% of pure cystic (n = 1) and 3.8% of mixed type (n = 22) were diagnosed as malignant after surgery ($p = 0.368$) (see Table 6). USG information about the calcification properties of 148 nodules with FNAB was reached. While 18.2% (n = 27) of them have micro, 7.4% (n = 11) macro and 6.1% (n = 9) mix type calcification; there was no calcification in 68.2% (n = 101). 33.3% of patients with nodules with microcalcification (n = 9), 54.5% of patients with nodules with macrocalcification (n = 6) and 33.3% of patients with nodules with mixed type calcification (n = 3) a malignant diagnosis was made. This rate was 32.7% (n = 33) in patients without calcification (p = 0.546). Data on the size of 165 nodules were available. While the average size of nodules in the benign group is 20 ± 11.00 mm; In the malignant group, this ratio was 21 ± 11.70 mm ($p = 0.937$) (Table 6).

In the univariate analysis of clinical and USG data, only age (> 55 years) was found as a sign of malignancy. (odds ratio 0.321; 95% CI: 0.161-0.633; p : 0.001). Univariate analyzes for malignancy are shown in Table 7.

DISCUSSION

In this study, FNAB was performed on 5181 thyroid nodules, and 611 nodes (11.79%) were diagnosed with AUS/FLUS. This rate is slightly higher than the Bethesda forecast, but within acceptable limits. The reason why the rate is higher than the Bethesda classification may be that the hospital where we conduct the study is in an endemic region for goiter and the differences in interpretation among pathologists. According to the prediction of the Bethesda classification, the expected malignancy rate in the AUS/FLUS category is between 5-15% (9). However, the frequency of true malignancy in this group is not fully known, since not all nodules are evaluated histopathologically. In studies involving only cytological follow-up, the rate of malignancy was low, whereas in a large-series study, the rate was reported to be 27.5% [13]. In cases where patients are selected for the operation, this rate varies between 6-48% [14, 15].

In this study, 167 of 611 thyroid nodules diagnosed with AUS/FLUS were surgically diagnosed and 57 (34.1%) of these 167 patients received a malignant diagnosis. This rate is higher than Bethesda's estimate. Possible reasons for the high may include the nodules

Table 6. **USG findings in surgically diagnosed nodules**

		Cases		
		Benign (n = 110)	**Malign (n = 57)**	***p* value**
Echogenity				
	DL	16	6	
	hypoechoic	26 (27.7%)	15 (29.4%)	0.96
	Isoechoic	63 (67%)	33 (64.7%)	
	Hyperechoic	5 (5.3%)	5 (5.9%)	
Calcification				
	DL	13	6	
	Micro	18 (18.6%)	9 (17.6%)	0.546
	Macro	5 (5.2%)	6 (11.8%)	
	Mix	6 (6.2%)	3 (5.9%)	
	Absent	68 (70.1%)	33 (64.7%)	
Halo				
	DL	14	5	
	Yes	18 (18.8%)	11 (21.2%)	0.725
	No	78 (81.3%)	41 (78.8%)	
Component				
	DL	12	5	
	Pure solid	53 (54.1%)	29 (55.8%)	0.368
	Pure cystic	0 (0%)	1 (1.9%)	
	Mix	45 (45.9%)	22 (42.3%)	
Size (mm)		20 (7-53)	21 (9-52)	0.937

DL = Data loss

that are considered to be at risk of high malignancy radiologically and clinically, and heterogeneity in the content of the AUS/FLUS. Assuming that all patients who were under follow-up and not surgically diagnosed with pathology were malignant or benign, the lower limit was 16.3% for malignancy and the upper limit was 68.3%. According to this wide range, the rate of malignancy in our study was higher than the predictions of Bethesda. However, some recent studies have indicated the malignancy rate in a range of 6% to 76% for cases confirmed surgically [10, 16]. This high range suggests that the malignancy rate predicted by Bethesda possibly for AUS/FLUS must be reconsidered. Although the actual incidence is currently unknown, this is likely due to the fact that only a small proportion of patients diagnosed with AUS/FLUS have been surgically confirmed and while determining these rates in the researches, the patients who underwent surgery should not be included in this rate and the patients who are under clinical follow-up should not be included. Recent studies have focused on increasing the validity and reliability of FNAB [17, 18]. In this study, 55 (97%) of 57 patients in the malignant group were detected as papillary carcinoma. In various studies, it was observed that the most common subtype among thyroid cancers diagnosed in patients diagnosed with AUS/FLUS was papillary carcinoma [19-22].

Table 7. Univariate data analysis according to clinical and USG data

		Odds ratio	95% CI	*p* value
Age	< 55y	reference	0.161-0.633	**0.001**
	≥ 55y	0.321		
Gender	Female	reference	0.604-3.27	0.43
	No	1.405		
Previous surgery	Yes	reference	0.542-2.063	0.871
	No	1.057		
TSH value	Less / Normal	reference	0.754-3.078	0.241
	High	1.523		
Size of nodule	≤ 20mm	reference	0.632-2.284	0.576
	> 20mm	1.201		
Time of surgery	After first FNAB	reference	0.635-2.392	0.536
	After second FNAB	1.233		
Hypoechogenity	No	reference	0.432-1.949	0.823
	Yes	0.918		
Microcalcification presence	No	reference	0.483-2.365	0.87
	Yes	1.068		
Halo presence	No	reference	0.502-2.693	0.725
	Yes	1.163		
Pure solid	No	reference	0.544-2.105	0.843
	Yes	1.071		

USG features of patients who were diagnosed with AUS/FLUS and who underwent surgical confirmation were evaluated with univariate analysis method for each feature and no significant relationship was found between USG features and malignancy. The accuracy of these results, which were obtained due to the retrospective nature of the study, the loss of data and this evaluation only in patients undergoing surgery, is uncertain and open to discussion. In addition to the fact that the data are different in various studies after the literature review, some researchers have suggested that the diameter of the nodule may be helpful in assessing the risk of malignancy, while some researchers have suggested that the diameter of the nodule is not associated with malignancy [23].

The relationship between the TSH values of the patients and the gender difference with malignancy were also evaluated statistically, but no statistical difference was found similar to previous studies [24].

There are differences between studies for the relationship between age and malignancy rate. In one of these studies, the rate of malignancy was analyzed higher in patients under 40 years of age [25], while in a similar study [26] patients with thyroid malignancy were younger than patients in the benign group. In current study, after the evaluation for malignant and benign groups without determining a reference value, the mean age was higher in the malignant group (p = 0.034).

According to the Bethesda classification, the biopsy should be repeated 3-6 months after the initial diagnosis of AUS/FLUS, and surgery should be performed in the case of a suspected malignant or malignant diagnosis [14].

In this study, no significant difference was found between the nodules repeating biopsy and the nodules not performed in terms of malignancy frequency. Considering the whole study, we think that surgical decision can be made earlier rather than repeat biopsy due to our higher rate of malignancy than expected.

CONCLUSION

Since the Bethesda classification predicts malignancy rate low, it recommends repeat biopsy for patients diagnosed with AUS/FLUS [27]. In this study, the rate of malignancy in surgically confirmed nodules was 34.1%, which is higher than the Bethesda classification. When the USG features of the malignant nodules were examined, it was seen that none of the features supported malignancy. As for clinical features, data were obtained that only over 55 years of age increased the risk of malignancy. According to our study, the risk of malignancy was three times higher in patients over 55 years old compared to those less than 55 years old. Due to the high malignancy rate which determined in cytologically diagnosed AUS/FLUS, we think that the repeat decision of the FNAB should be reconsidered and the surgical plan after the first FNAB should be considered more seriously. Based on data, it was not possible to create a successful algorithm using USG features or clinical features. Alternatively, new imaging techniques and molecular analyzes are promising methods to increase predictive validity of AUS/FLUS [28]. However, valuable analyzes are needed for all these newly defined methods.

Authors' Contribution

Study Conception: BÇ; Study Design: HAT; Supervision: HAT; Funding: EK, HAT; Materials: EK, HAT; Data Collection and/or Processing: BÇ, HAT; Statistical Analysis and/or Data Interpretation: EK; Literature Review: BÇ; Manuscript Preparation: BÇ, HAT and Critical Review: EK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Associating craniofacial morphometry determined by photo analysis with somatotype in healthy young individuals

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ABSTRACT

Objectives: Evaluation of the relationship between craniofacial parameters and somatotype provides important contributions to specialist physicians and anatomists in determining diseases and obtaining objective results of anthropometric measurements. The study was designed in line with this hypothesis and the aim was to find out how this relationship changed in healthy individuals.

Methods: The study was conducted by examining 191 healthy individuals between the ages of 18 and 30. The individuals' faces were photographed from a distance of 1 meter and craniofacial parameters were measured in Image J program. Somatotype analysis was conducted by using Heath-Carter somatotype method.

Results: As a result of our study, the individuals were found to be grouped in four classes according to Heath-Carter somatotype method: (1) mesomorph endomorph, (2) endomorph ectomorph, (3) endomorph mesomorph and (4) central. Significant correlation was found between the second and first somatotype groups in terms of total nasal length, while significant correlation was found between second and first/second and third/fourth and third/first and third somatotype groups in terms of body mass index (BMI) parameter ($p < 0.05$). Craniofacial parameters were also evaluated and a very high correlation was found between total facial height and mandibular height, while there was a high correlation between total facial height and the other 16 parameters.

Conclusions: As a result of our study, a relationship was found between somatotype groups and craniofacial parameters, within craniofacial parameters, and between somatotype and BMI. We believe that this relationship will guide morphological studies in basic medical sciences and surgical interventions in clinical sciences.

Keywords: Heath-Carter somatotype analysis, craniofacial parameters, photo analysis

Anthropometry is a universal, inexpensive and reliable method evaluating morphological characteristics of human beings. Body structure, age, gender, body proportion and body mass index (BMI) are critically important in correct evaluation of anthropometric parameters. Knowing the aforementioned information about the human body is important in terms of both the reliability of anthropometric parameters and also fully showing factors such as individuals' health and nutritional habits [1-4].

Anatomic measurements vary according to age, gender, height, weight, race, physiological and patho-

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logical factors. Therefore, determination of somatotype with advanced anthropometric methods is critically important in the objective evaluation and interpretation of anatomical measurements [5]. For this reason, knowing about craniofacial anthropometry is important for both basic medical sciences and clinical sciences. Craniofacial anthropometry is clinically important in Down syndrome, nasal septum deviation, facial asymmetry, dentofacial disorders, facial and orthofacial surgery [5-10]. Different face types have been formed with the data obtained as a result of craniofacial anthropometry. Some of these are Bruges facial index, Vitruvian facial index and the ratio of lower facial height to total facial height [8].

Somatotype is a method based on anthropometric measurements that allow to determine body composition [11, 12]. Somatotype method was introduced by Sheldon in 1940. In this method, classification was made from 1 to 7 based on shape of the body. According to Sheldon, these classes occur due to genetic differences and they do not change throughout life. However, many researchers rejected this hypothesis in the following years. In 1967, Heath and Carter modified the Sheldon somatotype method and introduced the Heath-Carter somatotype method [12-14]. This somatotype method is a method in which body types are determined with anthropometric measurements, which are widely used in health and sport sciences [15-18]. Skin thickness from four points, two circumference, two width, weight and height measurements are required to calculate this method. Body types are grouped in three main categories as endomorphy, mesomorphy and ectomorphy with the measurements obtained [17, 19]. Roughly, endomorphy is defined as obesity, mesomorphy is defined as muscularity and ectomorphy is defined as being thin [20]. Endomorphy occurs with the increase in physical fat mass of the body due to the increase in the energy stores of the individual. Mesomorphy occurs due to the development of the body's musculoskeletal system. Ectomorphy occurs with the decrease in height-weight ratio [13].

This study was conducted to reveal the relationship between somatotype and craniofacial parameters and to guide this relationship to morphological (anatomical, radioanatomical), surgical (fascial, dentofascial, orthofascial intervention) studies in the field of health.

METHODS

The study was initiated with the 2022/798 decision of non-interventional local ethics committee and the study was funded by Karabük University Scientific Research Projects Unit (Project Number: KBÜBAP-22-DS-008). This study was carried out within the framework of ethical rules in accordance with the Declaration of Helsinki. 191 healthy individuals between the ages of 18 and 30 who had no pathologies (septum deviation, nose, face and chin anomaly, strabismus) or no surgical intervention in craniofacial parameters were included in the study population.

Study Protocol

A fixed board was placed next to the individuals and a guiding paper with a space of 1 mm was placed on the board. A professional camera was placed at a

Fig. 1. Craniofacial parameters. (a) Total nasal length, (b) Nose width, (c) Pupillary distance, (d) Lower facial height, (e) Total face height, (f) Mandibular height and (g) Face width.

distance of 100 cm from the individuals with a tripod. Facial photos of each individual were taken and saved to computer in jpeg format. These saved photos were transferred to Image J (Version 1.53e) program and craniofacial measurements were made.

The following craniofacial measurements were made (Fig. 1):

- (a) Total nasal length: Nasion-subnasal
- (b) Nose width: Distance between alae ancillary
- (c) Pupillary distance: Pupillary distance
- (d) Lower facial height: Subnasal-gnathion
- (e) Total face height: Supraorbitale-gnathion
- (f) Mandibular height: Stomion-gnathion
- (g) Face width: Distance between zygomatic bones

Facial indices suitable for these craniofacial parameters were included in the study. Face indices were: Bruges face index, The ratio of lower facial height to total face height and Vitruvian index.
The following formulas were used:

Bruges face index:

$$\frac{\textit{Total face height}}{\textit{Pupillary distance}}$$

The ratio of lower facial height to total face height:

$$\frac{\textit{Lower facial height}}{\textit{Total face height}}$$

Vitruvian index:

$$\frac{\textit{Mandibular height}}{\textit{Lower facial height}}$$

Following the photo shooting, the individuals' heights were measured with a tape measure, while their weights were measured with a digital calliper and saved in excel format with their BMI. Next, in order to determine the somatotypes of individuals, their triceps, subscapular, suprailiac and calf skinfold thicknesses were measured and recorded with a skinfold calliper. In addition, contracted arm and calf circumference were measured and recorded by using tape measure and elbow and knee widths were measured and recorded with digital calliper.

Statistical Analysis

Heath-Carter somatotype method and Somatotype (Version 1.2.5) were used in somatotype analysis of the individuals. Height, weight, triceps, subscapular, suprailiac, calf skin thickness and contracted arm and calf circumference widths were used in somatotype analysis. Median, minimum and maximum values were included in descriptive statistics. Normality distribution of the data was tested with Kruskal Wallis H test. The correlation between somatotype groups and the parameters was analysed with Kruskal Wallis H test. Significant correlation was determined between which groups by Pairwise Comparisons test. In addition, the relationship between parameters was tested

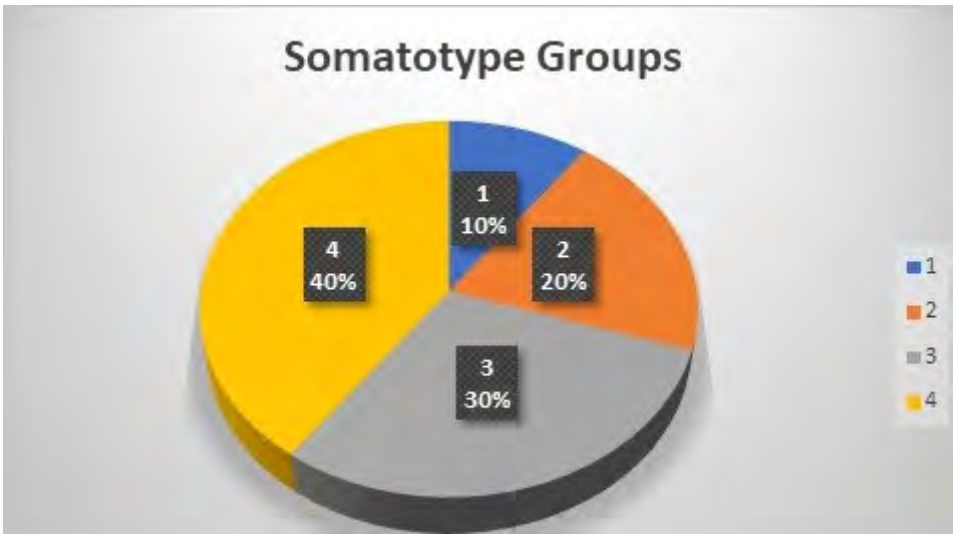

Fig. 2. Somatotype groups. (1) Mesomorph endomorph, (2) Endomorph ectomorph, (3) Endomorph mesomorph and (4) Central.

with Spearman rho test in our study. $P < 0.05$ value was considered as statistically significant in analyses. Minitab 17 and SPSS (Version 21) programs were used for statistical analysis in the study.

RESULTS

It was found that the individuals in the study were distributed in four different groups in terms of somatotype. It was found that there were 111 individuals in the first group (mesomorph endomorph), 33 individuals in the second group (endomorph ectomorph), 32 individuals in the third group (endomorph mesomorph) and 15 individuals in the fourth group (central) (Fig. 2). As a result of the study, it was found that 40% of the individuals had mesomorph endomorph body type, 30% had endomorph ectomorph body type, 20% had endomorph mesomorph body type and 10% had central body type.

Table 1 shows descriptive statistics of age, BMI and craniofacial parameters used in the study in terms of somatotype groups. Table 2 shows the descriptive statistics of face index parameters. Table 3 shows the descriptive statistics of Heath-Carter somatotype method.

With Kruskal Wallis H test, a significant correlation was found between total nose length and BMI according to somatotype groups ($p < 0.05$). Pairwise Comparisons test were found between 1 vs 3 groups in total nasal length and between 2 vs 1/2 vs 3/4 vs 3/1 vs 3 groups in terms of BMI ($p < 0.05$) (Tables 4 and 5).

The correlation between Spearman rho test and craniofacial parameters were examined and a very high correlation was found between total face height and mandibular height. A high correlation was also found between 16 parameters. High correlation was found between total nasal length and nose width, pupillary distance, total face height and face width; between nose width and pupillary distance, lower facial height, total face height, mandibular height and face width; between pupillary distance and total face height and face width; between lower facial height and

Table 1. Descriptive statistics of age, BMI and craniofacial parameters in terms of somatotype

Parameters	Mesomorph endomorph	Endomorph ectomorph	Endomorph mesomorph	Central
Age (year)	20.00 (18.00-27.00)	20.00 (18.00-28.00)	20.50 (19.00-30.00)	20.00 (18.00-23.00)
BMI (kg/m^2)	23.18 (17.91-36.01)	19.05 (17.26-23.05)	25.62 (18.98-32.39)	20.98 (19.03-23.24)
Total nasal length (cm)	5.25 (3.39-7.24)	5.69 (4.09-8.02)	5.88 (3.90-6.99)	5.25 (4.10-6.33)
Nose width (cm)	3.41 (2.19-5.99)	3.47 (2.57-5.18)	3.65 (2.19-4.65)	3.37 (2.59-4.55)
Pupillary distance (cm)	5.42 (4.04-7.43)	5.49 (4.45-7.86)	5.97 (4.23-7.00)	5.33 (4.55-6.66)
Lower facial height (cm)	5.95 (4.53-8.69)	6.20 (5.02-9.32)	6.17 (4.89-9.55)	6.20 (5.53-8.22)
Total face height (cm)	12.50 (9.51-17.51)	12.20 (10.13-17.41)	13.08 (9.92-17.569	12.32 (10.75-14.93)
Mandibular height (cm)	4.36 (3.07-6.15)	4.36 (3.66-6.48)	4.68 (3.45-7.45)	4.67 (3.44-5.91)
Face width (cm)	10.72 (7.25-14.40)	10.47 (8.66-14.81)	11.02 (7.25-14.41)	10.97 (9.18-14.03)

Table 2. Descriptive statistics of face index parameters in terms of somatotype

Parameters	Mesomorph endomorph	Endomorph ectomorph	Endomorph mesomorph	Central
Bruges face index	2.27 (1.83-2.70)	2.32 (1.76-3.13)	2.27 (1.76-2.70)	2.36 (1.75-3.14)
The ratio of lower facial height to total face height	0.50 (0.40-0.65)	0.49 (0.41-0.77)	0.50 0.42-0.58)	0.52 (0.41-0.73)
Vitruvian index	0.72 (0.59-0.85)	0.70 (0.62-0.79)	0.73 (0.56-0.92)	0.71 (0.42-0.96)

Table 3. Descriptive statistics of Heath-Carter somatotype method in terms of somatotype

Parameters	Mesomorph endomorph	Endomorph ectomorph	Endomorph mesomorph	Central
Triceps skin thickness (mm)	13.00 (5.00-27.00)	11.00 (7.00-27.00)	13.00 (7.00-25.00)	13.00 (5.00-23.00)
Subscapular skin thickness (mm)	15.00 (5.00-29.00)	15.00 (7.00-25.00)	13.50 (7.00-27.00)	15.00 (8.00-27.00)
Suprailiac skin thickness (mm)	12.00 (4.00-29.00)	11.00 (3.00-21.00)	11.00 (3.00-22.00)	11.00 (5.00-19.00)
Calf skin thickness (mm)	17.00 (5.00-33.00)	17.00 (9.00-27.00)	15.00 (6.00-29.00)	17.00 (7.00-37.00)
Contracted arm circumference (cm)	28.00 (21.00-35.00)	28.00 (21.00-38.00)	28.25 (21.00-35.00)	29.00 (24.00-42.00)
Calf circumference (cm)	34.00 (29.00-41.00)	35.00 (29.00-43.00)	34.00 (27.00-41.50)	34.00 (29.00-40.70)
Elbow width (mm)	61.19 (49.62-83.09)	60.66 (51.68-76.10)	62.52 (50.51-71.58)	61.48 (54.78-87.08)
Knee width (mm)	91.79 (77.53-108.26)	91.99 (80.18-129.40)	91.75 (82.34-103.34)	91.91 (85.66-115.73)

total face height and face width; between total face height and mandibular height and face width and between mandibular height and face width (Table 6).

DISCUSSION

The present study was conducted to find out the relationship between somatotype and craniofacial parameters. As a result of the study, it was found that 40% of the individuals had mesomorph endomorph body type, 30% had endomorph ectomorph body type, 20% had endomorph mesomorph body type and 10% had central body type. Significant correlation was found between total nasal length and BMI parameters in different somatotypes ($p < 0.05$). In addition, very high correlation was found between total face height and mandibular height ($p < 0.05$).

The relationship between somatotype and BMI has been examined in many studies in which different diseases and parameters have been used and significant correlations have been found between these two

Table 4. Pairwise comparisons test results for total nasal length parameter

Parameters	Somatotype Groups	*p* value
Total nasal length	1 vs 2	0.21
	1 vs 3	**0.08**
	1 vs 4	0.85
	2 vs 3	0.77
	2 vs 4	0.19
	3 vs 4	0.13

Table 5. Pairwise comparisons test results for BMI

Parameters	Somatotype Groups	*p* value
BMI	1 vs 2	**0.00**
	1 vs 3	**0.00**
	1 vs 4	**0.01**
	2 vs 3	**0.00**
	2 vs 4	0.06
	3 vs 4	**0.00**

parameters. In a study in which they examined the correlation between Q angle and Stork balance stand and somatotype, Şenol *et al*. [3] found a significant correlation between somatotypes and BMI. As a result of the cohort study they conducted on somatotype and pituitary adenocarcinoma, Cote *et al*. [21] found a significant correlation between somatotype and BMI. In our study, significant correlation was found between BMI and 2 vs 1/2 vs 3/4 vs 3/1 vs 3 somatotype groups ($p < 0.05$). These results support the literature.

The relationship between somatotype and diseases has been the subject of many articles and a correlation has been found between many diseases and somatotype. In a study in which they examined the relationship between COPD phenotypes and somatotype, Divo *et al*. [22] found correlations between smoking-related COPD and somatotype. In a study they conducted on the relationship between sarcopenia and somatotype, Yasuda *et al*. [23] found correlation between presarcopenia and somatotypes of young female individuals. Mantarkov *et al*. [24] found significant correlations among schizophrenia and somatotype. The literature shows that somatotyping, which is a detailed body composition analysis, has started to gain

Table 6. Correlation analysis of craniofacial parameters

Parameters		Total nasal length	Nose width	Pupillary distance	Lower facial height	Total face height	Mandibular height
Nose width	r	0.623[c]					
	p	**0.000**					
Pupillary distance	r	0.711[c]	0.794[c]				
	p	**0.000**	**0.000**				
Lower facial height	r	0.306[a]	0.613[c]	0.566[b]			
	p	**0.000**	**0.000**	**0.000**			
Total face height	r	0.654[c]	0.776[c]	0.751[c]	0.757[c]		
	p	**0.000**	**0.000**	**0.000**	**0.000**		
Mandibular height	r	0.391[a]	0.624[c]	0.513[b]	0.800[d]	0.769[c]	
	p	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	
Face width	r	0.642[c]	0.770[c]	0.766[c]	0.640[c]	0.748[c]	0.660[c]
	p	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**

[a] weak correlation, [b] moderate correlation, [c] high correlation, [d] very high correlation

popularity in studies conducted in the field of health. However, it is noteworthy that no studies were found in literature examining the effects of somatotype on craniofacial measurements of healthy young individuals.

There are complex relationships between craniofacial parameters and the relationships are sometimes not fully recognized by visual examination. These complex relationships can be the indicator of many diseases in clinic [5, 6]. In a study they examined the craniofacial parameters of schizophrenic individuals, Demir *et al*. [25] reported relationship between schizophrenia and craniofacial parameters. In a study they examined craniofacial parameters and nasal septum deviation, Arpacı *et al*. [4] reported correlation between these two. In a study they conducted on 180 individuals, Lee *et al*. [26] found a correlation between obstructive sleep apnoea and craniofacial parameters. Our study was based on the hypothesis that somatotype could affect craniofacial parameters in healthy individuals and a very high correlation was found between total face height and mandibular height, while high correlation was found between 16 parameters. We believe that knowing about these characteristics of healthy individuals will guide clinical studies.

In a study conducted on 76 females and 76 males individuals between the ages of 17 and 25, Kosif [27] found lower facial height as 77.54 ± 8.16 mm in women and as 86.70 ± 9.87 mm in men; total nasal length as 52.34 ± 8.05 mm in women and as 56.49 ± 5.86 mm in men; nose width as 41.65 ± 2.24 in women and as 44.62 ± 4.59 mm in men. In this study, we found lower facial height media value as 5.95 cm in the first group, as 6.20 cm in the second group, as 6.17 cm in the third group and as 6.20 cm in the fourth group; total nasal length median value as 5.25 cm in the first group, as 5.69 cm in the second group, as 5.88 cm in the third group and as 5.25 cm in the fourth group; nose width median value as 3.41 cm in the first group, as 3.47 cm in the second group, as 3.65 cm in the third group and as 3.37 cm in the fourth group. In a study they conducted on face types of Anatolian men, Özdemir *et al*. [8] examined 300 individuals between the ages of 20 and 40 and found Bruges face index as 193.85 ± 16.48 mm, the ratio of lower facial height to total face height as 30.92 ± 2.37 mm. In the present study, we found Bruges face index median value as 2.27 cm in the first group, as 2.32 cm in the second group, as 2.27 cm in the third group and as 2.36 cm in the fourth group; we found the median value of the ratio of lower facial height to total face height as 0.50 cm in the first group, as 0.49 cm in the second group, as 0.50 cm in the third group and as 0.52 cm in the fourth group. These results support the results we found. We believe that the partial highness in the ratio of lower facial height to total face height is due to regional differences.

Limitations

Limitations of the study;
- Low number of individuals,
- It is the narrowness of the age range.

CONCLUSION

We believe that the results of this study, which examines the relationship between craniofacial parameters and somatotype, will contribute greatly to clinical (fascial surgery, maxillofacial surgery, nasal septum deviation) and morphological (anthropological, radioanatomical, anatomical) studies.

Authors' Contribution

Study Conception: ŞT, YS; Study Design: ŞT, YS; Supervision: ŞT; Funding: N/A; Materials: ŞT, YS, ZÖ, DŞ; Data Collection and/or Processing: YS; Statistical Analysis and/or Data Interpretation: YS; Literature Review: ŞT; Manuscript Preparation: ŞT, YS and Critical Review: ŞT.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Y chromosome polymorphism in Turkish patients with reproductive problems: a genetic centre experience

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ABSTRACT

Objectives: Male infertility is a large and unexplored global health problem in terms of prevalence. Chromosomal polymorphisms may be associated with infertility and recurrent spontaneous abortions. Non-protein coding and frequently repetitive satellite DNA sequences are found in these regions.

Methods: This study aims to present a genetic laboratory experience in the evaluation of frequency, type and significance of Y chromosome polymorphism of Turkish patients with reproductive system problems. The study included 435 patients aged 18-60 years with a documented clinical diagnosis of infertility.

Results: In our study, 435 individuals were analyzed cytogenetically and 75 of them (17.24%) were found to carry chromosomally polymorphic variants in Y chromosome. We detected increased heterochromatin structure in the long arm of chromosome Y (Yqh+) as a common variant in our patient group. The frequency of chromosomal polymorphism Yqh- is % 11.26. The rate of chromosomal polymorphism we detected is close to those reported in the literature (10-15%) and statistically significant ($p < 0.001$), twice that found in the normal population (2-5%).

Conclusions: Findings support that Y chromosome polymorphisms may be associated with infertility risk and may play an important role in the development of infertility. More research combining genome studies and other fields is needed to clarify the relationship of Y chromosome polymorphisms with and to infertility.

Keywords: Male infertility, chromosomal polymorphisms, Y chromosome

By definition, infertility is the clinical absence of pregnancy despite regular and unprotected sexual intercourse for 1 year. It is a disease characterized by the deterioration of the reproductive capacity of the person or his partner [1]. Male infertility is a health problem that has not been studied or studied to fully understand its magnitude and prevalence. Globally, accurate information on male infertility rates is lacking and not properly reported [2]. Nowadays, assisted reproduction centers allow couples, with a previous diagnosis of male infertility linked to chromosomal aberrations, to procreate using the assisted reproductive technique: Intracytoplasmic sperm injection [3, 4]. Therefore, it is important to perform genomic screening in male patients for the detection of genetic etiologies linked to male infertility.

There are chromosomal polymorphisms, inherited variation that spans heterochromatic regions within the genome. They are most frequently seen in the pericentric heterochromatin region of the long arms of chromosomes 1, 9 and 16, heterochromatin on the long arm of the Y chromosome, and heterochromatin in the

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satellites or bodies of chromosomes 13, 14, 15, 21, 22. Chromosomal polymorphisms may be associated with infertility and recurrent pregnancies resulting in miscarriage (RSA). These regions are composed of frequently repetitive satellite DNA sequences that do not code for proteins. When located on the same chromosome, repeat sequences may predispose to homologous unequal recombination leading to chromosomal micro rearrangements, deletions, duplications and inversions that can affect a clinical condition such as infertility and recurrent miscarriage. This study aims to present an experience from a different geography in frequency and type evaluation and significance of Y chromosome polymorphism of Turkish patients with reproductive system problems. First, we screened the frequencies of heteromorphisms in our patient cohort retrospectively. In continuation, we examined whether there are significant manifestations of heteromorphisms, as the complex nature of heterochromatin structure may indicate common occurrences that may have clinical implications.

METHODS

The study was conducted in the Laboratory of Medical genetics, University hospital Trakya, Edirne, Turkey, within 4 year period (2017-2021). The design was the retrospective type of observation.

Inclusion criteria are the following: (1) Men with no previously known history of infertility, (2) Men of legal age, and (3) Men who signed informed consent and agreed to participate in the study. Exclusion criteria: 1. Men older than 65 years, 2. Men with previous surgical treatments in the reproductive area, 3. Foreign patients.

The study included 435 patients aged 18-60 years with a documented clinical diagnosis of infertility. Karyotyping was performed using peripheral blood lymphocytes cultured for 72 hours in RPMI-1640 medium supplemented with fetal bovine serum and phytohemagglutinin. The chromosomes were stained by GTG banding technique. At least twenty GTG banded metaphases (450-500 band levels) were analyzed for each patient. Karyotyping of metaphases was performed and defined according to the International System for Human Cytogenetic Nomenclature 2016 guideline. Karyotypes were independently examined under the light microscope by three laboratory technicians at different time intervals for consistency of analysisPolymorphisms of Y chromosome were determined as Yqh + or Yqh-. Additionally, Y pericentric inversion is a structural anomaly of the human Y chromosome, with a prevalence of 1 in 1000 for males with this structural abnormality. The inverted Y chromosome has often been associated with specific phenotypic abnormalities. Its association with fertility problems has not been reported. It is considered to be one of the heteromorphisms.

The karyotype analyzes of all patients included in the study were normal. Patients with numerical and structural chromosomal abnormalities, including mosaicism, and AZF deletion by Y microdeletion analysis were not included in the study.

Ethical Approval

The study was carried out with the approval of the Trakya University Hospital Ethics Committee (TÜTF-BAEK 2021/210).

Statistical Analysis

SPSS 25.0 (IBM Corporation, Armonk, New York, United States) was used for statistics. Monte Carlo stimulation technique, Pearson Chi-square and Fisher Exact tests were used for categorical variable analysis. Column ratios were compared among themselves. Expressed as p value with Benjamini-Hochberg correction. Categorical variables were expressed as n (%). A $p < 0.05$ were considered statistically significant.

RESULTS

Four hundred and thirty-five individuals were evaluated cytogenetically within the project. It was determined that 75 individuals (17.24%) of the patient group carried polymorphic variations in the Y chromosome. The most common polymorphism detected in this population under analysis was an increased heterochromatin domain on the long arm of chromosome Y (Yqh+) (Fig. 1). The frequency of chromosomal polymorphism Yqh- is % 11.26 (Fig. 2). The rate of chromosomal polymorphisms is in line with those re-

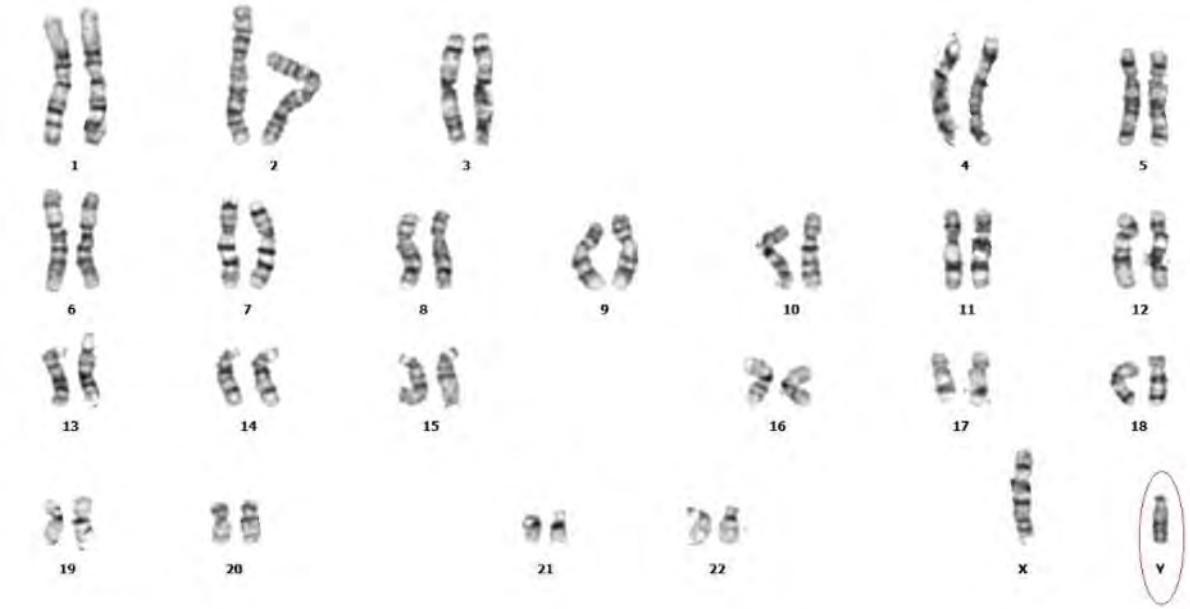

Fig. 1. **Methaphase image of a 34-year-old male patient. An example of the Yqh+ polymorphism.**

Fig. 2. **Methaphase image of a 30-year-old male patient. An example of the Yqh-polymorphism.**

ported in the literature (10-15%) and statistically significant ($p < 0.001$), twice that found in the normal population (2-5%) [5, 6]. It varies greatly between individuals and different populations.

DISCUSSION

The result obtained is a unidirectional trend with data from Turan, which is explained by variants of the Y chromosome, which are thought to have an adverse effect on spermatogenesis and may have an adverse effect on the outcome of ART (Assisted reproductive technology) procedures. The Y chromosome shows variations not only among individuals, but also between different population groups. Information on the importance of Y chromosome polymorphisms in infertility is still conflicting [7]. Whether or not Y chromosome heteromorphisms are of clinical importance, there are reports suggesting that epigenetic modifica-

tions in the heterochromatic region of the Y chromosome may play a factor in male factor infertility. Madon *et al*. [8], Minocherhomji *et al*. [9], Şahin *et al*. [10], Mierla and Stoian [11] and Yakin *et al*. [12] are some of the researchers who found significant associations between heteromorphisms and infertility.

Y chromosome polymorphism in 29.2% of infertile men [13]; 30.7% [14]; It was determined as 65.1% [15]. These data are consistent with our study results. The increased heterochromatin region for Yqh+ was thought to play an important role in the reproductive process. It has been reported to be associated with suppression of gene expression, especially for genes related to fertility in spermatogenesis. On the other hand, altered heterochromatin and euchromatin regions by induction of epigenetic changes in the male-specific region may be the reason for heterochromatin polymorphisms of the Y chromosome and its association with infertility. There is increasing evidence that heterochromatin regions contain significant amounts of repetitive DNA in heterogeneous content and that Chromosomal polymorphisms are an expression of morphological variability associated with increases or decreases in heterochromatin area. Chromosomal variations are thought to regulate gene expression with an opposite shift between heterochromatin formation, heterochromatin (non-coding DNA sequences) and euchromatin. (expressed DNA sequences) [16].

The number of studies examining the association of heteromorphisms with IVF failure apart from infertility is relatively less. Liang *et al*. [17] and Xiao Z *et al*. [18] observed that the effect of male polymorphisms on fertilization rates was negative. According to the Yqh- variation, the results of IVF treatment were found to be significantly more unsuccessful in couples carrying Yqh+ [17, 18].

Recent studies have reported that Y chromosome polymorphisms can affect homologous chromosome pairing and chromosome segregation. In addition, polymorphisms of the Y chromosome can cause errors in homologous chromosome pairing during cell division, resulting in disorders in cell division, developmental disorders in the embryo, teratogenicity disorders, stillbirth, and abortion.

A study in the field of cytogenetics shows that an increase (Yqh+) or decrease (Yqh-) in heterochromatin in the long arm of the Y chromosome can cause mitotic errors and thus cause stillbirth or miscarriage [19].

During meiosis, the pairing and synapse of the X and Y chromosomes are altered due to DNA repeats in certain regions of the Y chromosome, which can reduce reproductive capacity [20-21]. Similarly, we believe that molecular studies will help identify more precise roles for heterochromatin and chromosomal polymorphic variations, which have yet to be realized. Variations in the Y chromosome may occur due to inhibition of gene transcription, possibly due to the silencing effect on genes/gene promoters.

In the study of Ghahfarrokhi *et al*. [22] Y chromosome polymorphisms were also observed in people with Azoospermia and severe oligospermia. In their study, 6% of men with azoospermia and 4% of all men had qh+ chromosome. Also, infertile people have long and short Y chromosomes. Y chromosome variants are frequently seen as Yqh + or Yqh- with a frequency of 3.4% and 27.3%, respectively [22].

CONCLUSION

Y is fighting a losing battle: Loss of genes on Y may make fewer loci develop or suppress driving, while accumulation of repeats and heterochromatinization may make Y an easier target for drivers on X. This means that the silencing effect of heterochromatin increased on the chromosome can inhibit expression on Y within the genome. As a summary, our data show that Y chromosome polymorphisms may be associated with infertility risk and may play an important role in infertility. In conclusion, it is clear that more research, including molecular genetics, genomics, and other fields, is urgently needed to elucidate the mechanisms by which Y chromosome polymorphisms are particularly associated with and lead to infertility.

Authors' Contribution

Study Conception: EİA, HG; Study Design: EA; Supervision: ÇM, SD; Funding: N/A; Materials: HG, SY; Data Collection and/or Processing: EA; Statistical Analysis and/or Data Interpretation: EİA; Literature Review: EİA; Manuscript Preparation: EİA and Critical Review: HG, SD.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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The European Research Journal 2023;9(4):730-736 Original Article

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Third generation dual-source computed tomography coronary angiography with high-pitch spiral mode versus prospectively-gated sequential mode: comparison of radiation exposure and image quality

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ABSTRACT

Objectives: To compare high-pitch spiral (HPS) and prospectively-gated step-and-shoot (SAS) coronary CT angiography (CCTA) using third generation dual-source CT regarding objective and subjective image quality parameters and radiation exposure.

Methods: Eighty pairs of patients matched for gender, age, heart rate and BMI were enrolled in this retrospective study. High-pitch spiral and prospectively ECG-gated sequential CCTA were performed using third generation dual-source CT. The signal-to-noise ratio (SNR) and contrast-to-noise ratio (CNR) in the left ventricle were calculated for each group. Image quality were also scored using four-point scale. Student t-test was used to compare SNR, CNR and mean effective dose values (ED) and Wilcoxon test was used to compare image quality scores. Interrater aggreement were evaluated using Cohen's kappa statistics.

Results: Between-group differences in terms of age, gender, BMI, heart rate, and Agatston score were statistically not significant. Mean SNR and CNR was higher in prospective SAS protocol (16.5 ± 6.2 vs. 14.7 ± 4.9, $p = 0.047$ and 13.0 ± 5.2 vs. 11.2 ± 4.3, $p = 0.02$). Image quality scores showed no significant difference between two scan protocols ($p > 0.05$). Regarding radiation exposure, CT dose index (CTDIvol), dose length product (DLP) and ED was significantly lower for high-pitch group ($p < 0.0001$).

Conclusions: HPS CCTA using DSCT enables > 70% dose reduction while maintaining the image quality compared to prospectively ECG-gated SAS protocol. Therefore, HPS CCTA protocol can be preferred in patients appropriate for prospective ECG-triggered protocol.

Keywords: Coronary CT angiography, high-pitch, image quality, radiation dose

Coronary CT angiography (CCTA) is frequently used in routine clinical practice and partially replaced invasive coronary angiography to detect coronary artery disease (CAD) with its high sensitivity and negative predictive value [1]. CCTA has also been included in guidelines for the exclusion of CAD in intermediate-risk patients with symptoms [2, 3].

As radiation exposure was the major concern in CCTA, various innovations have been generated to reduce radiation exposure without comprising image

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quality [4]. Among the dose reduction tecniques developed, the prospective electrocardiographic (ECG)-gated CCTA caused the highest reduction in the effective dose (ED) [5]. In contrast to performing data acquisition at the entire cardiac cycle in retrospective ECG-gated CTCA or low pitch spiral (LPS) mode, the diastolic stage of the cardiac cycle is used for imaging in prospective electrocardiographic (ECG)-gated step-and-shoot (SAS) mode which avoids X-ray exposure in other phases [6]. Besides, second and third-generation dual-source CT (DSCT) systems have introduced prospectively ECG-gated high-pitch spiral scan mode (HPS), which enables entire data acquisition within a single heartbeat allowing even more radiation dose reduction [7].

Despite the fact that prospective ECG-triggered protocols provide an opportunity to scan with less radiation exposure, in clinical practice, they are not appropriate in patients with tachycardia, arrhythmia, and obesity due to higher motion artifacts and image noise. Retrospective ECG-gated CTCA protocols are still needed in patients with rapid unstable heart rates or to perform functional analysis [8, 9]. Although HPS technique provides further dose reduction, image quality should also be considered to select the optimal prospective ECG-triggered protocol in this selected patient group. Neefjes *et al*. [10] revealed that the image quality obtained with HPS mode was lower compared with SAS mode in patients with heart rate of ≥ 55 beats per minute (bpm). In addition, Seppelt *et al*. [11] compared HPS and SAS mode in a matched study population and higher signal-to-noise (SNR) ratio and image quality was found in SAS protocol. On the other hand, Smettei *et al*. [12] reported no significant difference in subjective image quality between SAS and HPS protocols.

In this study, we aimed to compare objective and subjective image quality parameters and radiation exposure between prospective ECG-triggered SAS and HPS protocols in age, gender, body-mass index (BMI) and heart rate matched patient cohorts.

METHODS

Study Population

This retrospective study was approved by Institutional Ethical Review Board and written informed consent was waived (IRB Approval number:377, Date: June 8th, 2020). From July 2017 to August 2021 a total of 80 consecutive patients (Group A) who underwent CCTA with clinical suspicion of CAD using 192-slice DSCT scanner with ultra-fast, low dose high-pitch mode (FLASH) were enrolled. An age, gender, BMI and heart rate- matched control group (Group B) were also selected retrospectively from a cohort that had CCTA with prospective SAS scanning protocol from July 2017 to August 2021. Patients with stent, coronary artery bypass grafts, cardiac devices, and patients younger than 18 were excluded from the study.

Patient Preparation and CTA protocol

A 192-slice DSCT scanner (Somatom Force, Siemens Healthineers) was used to perform both scanning protocols. Propranolol hydrochloride (10 mg, up to 40 mg if needed) was administered orally prior to examination to achieve a heart rate below 70 beats/min. Two different CCTA acqusition protocols were used. In group A, prospectively ECG-triggered HPS protocol were used. Group B received a CCTA with prospectively ECG-triggered SAS mode. Scan parameters of both protocols were summarized in Table 1. Intravenous contrast agent was injected with a flow rate of 5-6 ml/sn followed by 50 ml isotonic saline chaser. The scan was initiated using the real-time bolus tracking method with a region of interest placed at left atrium lumen and attenuation treshold set to 75 Hounsfield units. Automatic tube potential selection and tube current modulation (CareDose4D, Siemens Healthineers) was used for all examinations. A model-based iterative algorithm (ADMIRE, strength level 3; Siemens Healthineers) and a soft reconstruciton kernel (Bv40) were used to reconstruct all images. For the radiation exposure evaluation, CT-dose index (CTDI) and dose-length product (DLP) provided by the scanner were recorded. The effective dose (ED) was calculated by multiplying DLP with a conversion factor (0.014 mSv/mGycm) [13].

Image Quality Assessment

All images were evaluated by a radiologist with 8 years experience in cardiovascular imaging and a radiology resident who completed cardiac imaging training independently. Both readers were blinded for the scanning protocols and clinical information of the patients. Image quality was scored on a 4-point scale as

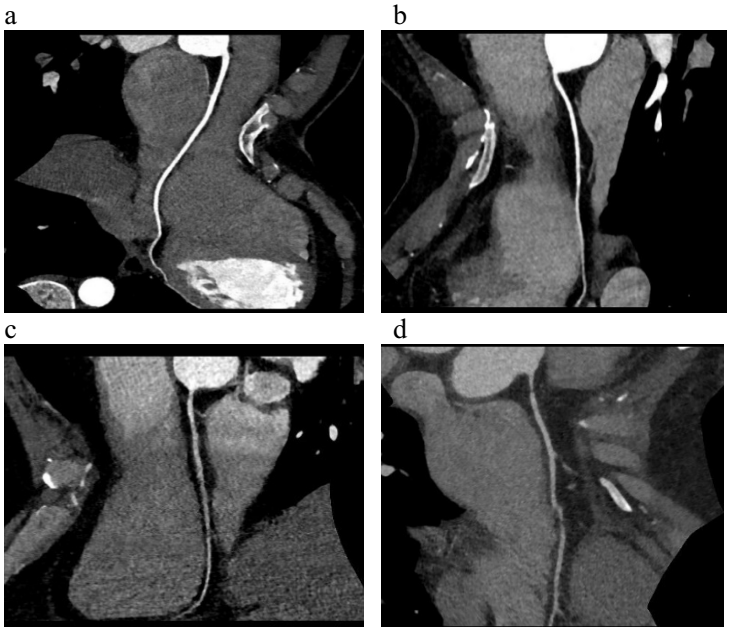

Fig. 1. Images of right coronary artery showing examples of 4-point subjective image quality scale for coronary CT angiography with high-pitch spiral mode (A-D). A Very good image quality, without any artifacts, image quality (IQ) score = 4 B Good image quality, presence of minor artifacts, IQ = 3 C Adequate image quality to exclude severe stenosis, IQ = 2 D Non-diagnostic image quality, presence of severe artifacts, IQ = 1.

follows: 4 = very good, complete delineation of vessel walls without motion artifacts; 3 = good, presence of minor artifacts but maintained ability to evaluate luminal stenosis; 2 = adequate, presence of major artifacts but sufficient to rule out severe stenosis, 1 = non-diagnostic, presence of severe artifacts (Fig. 1). Both readers assessed the images independently and discrepancies between readers were resolved by consensus in another reading session.

Quantitative assessment was performed by measuring attenuation values of the left ventricle with a region-of-interest (ROI, size 80 mm^2). The mean attenuation value was determined as signal and the standard deviation value as noise. The signal-to-noise ratio was defined as the quotient of signal value and the noise value. The contrast-to-noise ratio was calculated by dividing the difference between left ventricle lumen attenuation and left ventricle wall attenuation by the image noise.

Statistical Analysis

The data were analyzed by using IBM SPSS Statistics Software (version 22; IBM, USA), and a p value of 0.05 or less indicate statistically significance. The continuous data were expressed as mean ± standard deviation (SD), and categorical data were expressed as counts and percentages. Chi-square and Student t-test were used to compare categorical variables and continuous variables, respectively. The Wilcoxon test was used to compare subjective image quality scores between two patient groups. Interrater agreement was evaluated using Cohen κ coefficient. Agreements were considered as: poor, $\kappa < 0.21$; fair, $\kappa = 0.21$-0.40; moderate, $\kappa = 0.41$-0.60; substantial, 0.61-0.80; and excellent, $\kappa > 0.80$.

RESULTS

A total of 160 patients (n = 80 in each group) were enrolled in this study. Both groups were consisted of 40 men and 40 women. As the patient groups were matched in regard to gender, age, heart rate and BMI, there was no statistically significant difference between groups. The mean age of the patients was 51.90 years in high-pitch protocol and 53.34 years in prospective step-and-shoot protocol. BMI was 27.55 ± 3.68 kg/m^2 versus 28.16 ± 4.05 kg/m^2 in high-pitch protocol and prospective step-and-shoot protocol respectively. Mean heart rate was 72.21 bpm in high-

Table 1. Baseline characteristics of study population

Characteristics	High-pitch spiral CCTA	Step-and-shoot CCTA	*p* value
Age	51.90 ± 12.280	53.34 ± 12.793	0.469
Male sex, n (%)	40 (50)	40 (50)	1
Body mass index	27.55 ± 3.683	28.16 ± 4.055	0.328
Heart rate (beats/min)	72.21 ± 9.612	74.41 ± 9.567	0.149
Agatston score	58.37 ± 159.73	71.75 ± 207.65	0.654

Table 2. Image noise and image quality parameters in both groups

	High-pitch spiral CCTA	Step-and-shoot CCTA	*p* value
Noise	33.684 ± 8.859	32.649 ± 10.113	0.502
Signal-to-noise ratio (SNR)	3.640 ± 1.275	3.452 ± 1.300	0.371
Contrast-to-noise ratio (CNR)	12.149 ± 5.279	13.171 ± 5.294	0.235

pitch protocol and 74.41 bpm in prospective step-and-shoot protocol. No significant difference were found in terms of Agatston score between groups ($p = 0.65$). Baseline characteristics of the study population are summarized in Table 1.

There were no significant difference between groups regarding noise levels (33.6 ± 8.8 HU vs. 32.6 ± 10.1 HU, $p = 0.50$). No significant difference in mean SNR and CNR was observed between two scan protocols (3.6 ± 1.2 vs. 3.4 ± 1.3, $p = 0.37$ and 12.1 ± 5.2 vs. 13.1 ± 5.2, $p = 0.23$) (Table 2). Image noise and objective image quality parameters for each acquisition mode are shown in Table 2.

Regarding subjective image quality scores, no significant differences were found between two scan protocols ($p > 0.05$). Interrater agreement for image quality assessment on per-vessel level was substantial (LMCA κ: 0.69; LAD κ: 0.62; Cx κ: 0.61; RCA κ: 0.61).

With regard to radiation dose, CTDIvol, DLP and ED was significantly lower for high-pitch group ($p < 0.0001$). The mean ED for the patients was 1.29 ± 1.01 mSv (range: 0.12-3.93) in low dose high-pitch group and 6.72 ± 4.43 mSv (range: 1.30-29.61) in prospective SAS protocol. Radiation doses of two acquisition modes are summarized in Table 3.

DISCUSSION

In this study we compared prospective ECG-gated SAS and HPS protocol regarding objective and subjective image quality parameters and radiation exposure in a age, gender, heart rate and BMI matched patient population using third-generation DSCT system. There was no significant difference in objective and subjective image quality between two scan protocols. Radiation dose with HPS mode was significantly lower than SAS mode.

The main point of selecting an optimal CCTA scan protocol is to achieve high-quality images with the lowest possible radiation exposure. Among the improvements in the CT technology, the use of prospective ECG-triggered protocols provides greatest dose

Table 3. Radiation doses in both groups

	High-pitch spiral CCTA	Step-and-shoot CCTA	*p* value
CTDIvol (mGy)	9.424 ± 10.419	34.310 ± 23.226	**< 0.001**
DLP (mGy×cm)	92.720 ± 72.377	480.216 ± 316.884	**< 0.001**
ED (mSv)	1.298 ± 1.013	6.723 ± 4.436	**< 0.001**

CTDIvol = volume CT dose index, DLP = dose length product, ED = effective dose

reduction while maintaining image quality [14, 15]. With the help of DSCT systems, the high-pitch protocol has become available, which leads reduction in acquisition time hence decreasing radiation exposure. Third-generation DSCT systems with their high performance x-ray tube, wider longitudinal coverage, faster rotation time and iterative reconstruction algorithms contribute to obtain better image quality with a lesser radiation exposure. Lin *et al*. [16] reported that third-generation DSCT systems provide a 20% incerase in image quality of HPS scan compared to second-generation CT systems. Linsen *et al*. [17] also showed that HPS CCTA with third-generation DSCT offers better subjective and objective image quality despite a more rapid heart rate compared to second-generation DSCT.

Despite the ongoing improvements in CT technology, prospective ECG-triggered protocol can not totally replace retrospective ECG-triggered protocols. Traditionally, prospective ECG-triggered protocols require a low (< 70 bpm) and stable heart rate as these protocols are more susceptible to motion artifacts compared to retrospective ECG-triggered protocol. It has been shown that at least one coronary segment is nondiagnostic in patients who couldn't achieve the target heart rate despite premedication [18]. Ochs *et al*. [19] stated that HPS protocol is most applicable to non-obese patients with stable heart rate ≤ 65 bpm and a calcium score ≤ 600 since image quality worsen above these limits.

Among the factors affecting image quality, it has been revealed that calcium score calculated by Agatston method is the factor that most affect image quality of HPS protocol. Obesity and heart rate ≥ 65 bpm were also reported as other factors that reduce image quality [19]. Besides, the fact HPS protocol is more susceptible to motion artifacts compared to SAS and LPS protocols, may also result in impaired image quality.

Comparing the image quality of different CCTA acquisition methods is difficult as repeating each protocol in the same patient would not be appropriate regarding radiation exposure. Matching both patient groups in terms of factors affecting image quality is necessary to create comparable patient cohorts. Previous studies comparing HPS CCTA and prospective SAS CCTA protocols have generally made evaluation without patient selection. Nevertheless, in studies that selected patient groups and built comparable cohorts reported variable results. Jia *et al*. compared turbo high-pitch CCTA and prospective SAS mode regarding diagnostic accuracy and image quality and revealed that turbo high-pitch CCTA scan mode offers highly accurate images for significant stenosis, especially in patients with heart rate < 71.5 bpm and coronary calcium score < 444.1 [20]. On the other hand, Seppelt *et al*. [11] found significantly lower SNR and subjective image quality scores for HPS compared to SAS protocol. In the present study, no significant difference in SNR and CNR values were observed. Moreover, there was no significant difference in subjective image quality between two scan mode.

With regard to radiation exposure, the mean ED in the HPS mode was 1.29 ± 1.01 mSv which is in line with previous studies. Seppelt *et al*. [11] found that the mean ED in the SAS mode was more than three times that of the HPS mode. Smettei *et al*. [12] also revealed that FLASH mode provide 62% radiation dose reduction than SAS mode while maintaining image quality. In our study, HPS mode provides > 80% reduction in the radiation exposure compared to SAS protocol without comprising image quality.

Limitations

This work has several limitations that need to be mentioned. First, this retrospective study analyzed limited study population with relatively low heart rate at a single-center. Multi-center studies with larger sample size should be conducted to validate our results. Second, the diagnostic accuracy of detecting coronary artery stenosis for both groups were not evaluated since it was not the aim of this study. Lastly, the potential effect of heart rate variability on image quality was not included in this study.

CONCLUSION

In summary, the current study results revealed that HPS CCTA provides significant dose reduction compared to prospective SAS CCTA without comprising image quality. In this context, HPS CCTA protocol can be preferred in patients appropriate for prospective ECG-triggered protocol.

Authors' Contribution

Study Conception: AAA, GE, LS, KK, MA; Study

Design: AAA, GE, LS, KK, MA; Supervision: GE; Funding: N/A; Materials: N/A; Data Collection and/or Processing: AAA, LS; Statistical Analysis and/or Data Interpretation: AAA; Literature Review: AAA; Manuscript Preparation: AAA, and Critical Review: GE.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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The European Research Journal 2023;9(4):737-742 Original Article

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The effect of early surgical treatment on functional outcomes in terrible triad of elbow: a comparative study

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ABSTRACT

Objectives: In our study, we aimed to evaluate the effect of early surgical treatment on functional results in the terrible triad of the elbow.

Methods: Fifty-four patients operated for the terrible triad of elbow (TTE) injuries were included in the study. The study groups were determined as Group A (operated within 24 hours) and Group B (operated after one week). The same surgical procedure and rehabilitation program were applied to all patients. The follow-up period was 16.4 ± 8.4, with a minimum of 6 months. In the last follow-up, the flexion-extension range and supination-pronation interval were evaluated. Mayo Elbow Performance Score (MEPS) and Oxford Elbow Score were used for functional scoring.

Results: There was no significant difference between the two groups in terms of age, gender, injury type, and fracture classification. Elbow motion in group A was higher than in group B ($p < 0.01$). Moreover, group A had better higher MEPS and Oxford Elbow Score, an excellent and good rate than group B at the final clinical visit ($p < 0.01$). No postoperative pain or complication rate differences were found between the two groups.

Conclusions: Early operation for TTE patients resulted in better functional recovery than delayed operation.

Keywords: Terrible triad, elbow dislocation, early surgical treatment

Dislocation of the elbow accompanied by radial head and coronoid fracture has been named by Hotchkiss as a terrible triad of elbow [1]. Typically occurs with axial and valgus trauma to the elbow while the forearm is in supination [2, 3]. Surgical treatment is required because it causes instability in the elbow joint. Elbow fracture-dislocations are difficult to treat and have poor outcomes [4]. The primary goal of treatment is to provide stable fixation that will allow early elbow movements. Radial head fixation or arthroplasty, coronoid fixation, and lateral collateral ligament complex (LCLC) repair constitute the standard treatment [5, 6]. Despite all treatment protocols, pain, joint stiffness, instability, osteoarthritis are common complications [7, 8]. The optimal time for surgical treatment is controversial publications are reporting that the frequency of joint stiffness is high in patients operated after two weeks [9, 5]. In our study, we aimed to show the effect of early surgical treatment on functional results.

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METHODS

This study was started retrospectively with the approval of Istanbul Training and Research Hospital's Ethics Committee dated 06.12.2019 and numbered 2102. Fifty-four patients who were operated on with a terrible triad of elbow between 2011 and 2019 were included. Inclusion criteria are the following: (1) Being over 18 years of age, (2) Previously normal elbow functions, (3) No additional diseases affecting joint movements, (4) Applying the same surgical protocol (combined medial-lateral approach), and (5) At least 6 months of the follow-up period. The patients were divided into two study groups those operated within 24 hours (Group A) and those operated on after one week (Group B). Group A consists of twenty-eight patients and Group B consists of twenty-six patients. Ten patients were operated on late due to delayed admission to the hospital and nine patients were operated on late due to preoperative anesthesia preparation. In addition, seven patients were operated on late due to open fractures. Coronoid fractures were classified according to Regan-Morrey classification, radial head fractures according to Mason-Johnston classification.

Surgical Technique

Closed reduction and long arm splint were applied to all patients after vascular and neurological examination in the Emergency Service. Fracture components were determined by CT (computed tomography). In standard therapy, coronoid fracture fixation, radial head fracture fixation or arthroplasty, lateral collateral ligament complex (LCLC) repair was performed using a combined medial and lateral approach. Medial collateral ligament (MCL) repair was also performed in patients with persistent instability (Fig. 1).

Postoperative Care and Follow-up

A long arm splint was applied to all patients postoperatively. Antibiotic prophylaxis was started for 72 hours. 25 mg indomethacin was continued for six months, three times a day. In the follow-up in the second week, the long arm splint was removed, surgical sutures were removed, and an angle-adjusted elbow brace was applied. Passive movements were initiated in the 30°-90° flexion-extension range until the 1st-month follow-up. Active flexion-extension and supination-pronation exercises were started in the 1st month. Bone union was followed by direct radiography at every control. Flexion-extension, supination-pronation range were recorded during his follow-up for at least six months. Mayo Elbow Performance Score (MEPS) and Oxford Elbow Score were used for functional scoring. Complications and visual analog scale (VAS) pain scale were recorded (Fig. 2).

Statistical Analysis

SPSS 22.00 program was used for data analysis. Mean, standard deviation and ratio were used in the descriptive statistics of the data. Kolmogorov-Simirnov test was performed for the distribution of variables. The Mann-Whitney U test was used for the

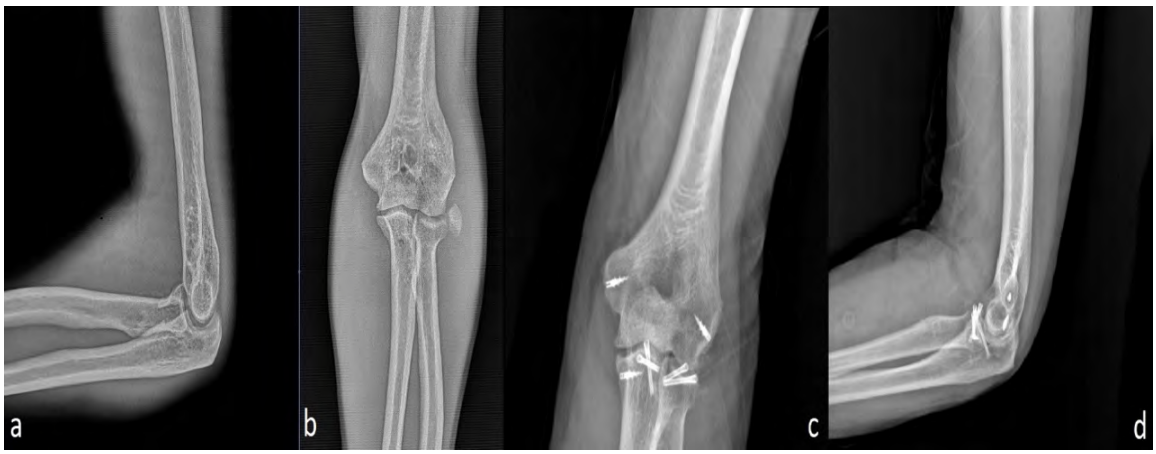

Fig. 1. (a, b) preoperative X-ray scans show that terrible triad of elbow, (c, d) postoperative X-ray scans show that anatomical repair of bony and soft structures.

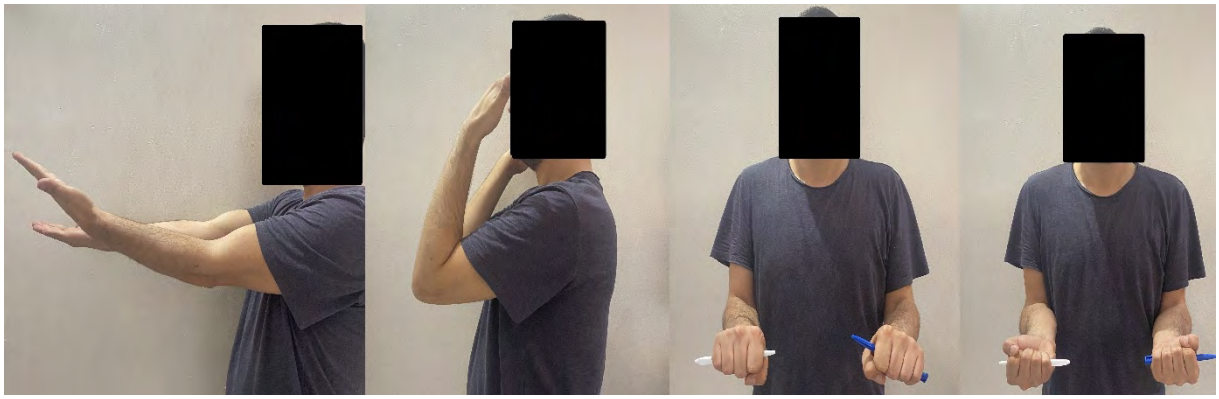

Fig. 2. Clinical photos at 12 months after surgery reveal excellent clinical outcomes.

analysis of quantitative independent data, and the Chi-square test or Fisher test was used for the analysis of qualitative independent data. The statistical significance level was determined as $p < 0.05$.

RESULTS

Fifty-four patients, including 40 men and 14 women, were included in the study. The patients who operated within 24 hours were determined as Group A (n = 28), and the patients who operated after one week as Group B (n = 26). Group A had twenty male and eight female patients, and Group B had twenty male and six female patients. The mean age was 40.14 ± 11.6 years in Group A, and 38.5 ± 14.8 years in Group B. According to the cause of trauma, 18 (33.3%) patients were admitted to the emergency service after a traffic accident, 24 (44.4%) patients with a fall from a height, and 12 (22.2%) patients with a simple fall. Thirty-two

Table 1. Preoperative data among two groups

	Group A	Group B	*p* value
Gender (Male/Female)	20/8	20/6	0.645
Age (Years)	40.14 ± 11.6	38.5 ± 14.8	0.861
Injury			0.196
Traffic accident	12	6	
Fall from height	12	12	
Simple fall	4	8	
Left/Right	16/12	16/10	0.743
Regan-Morrey			0.037
I	16	6	
II	8	12	
III	4	8	
Mason-Johnston			0.355
I	14	8	
II	8	10	
III	6	8	
Follow-up (Months)	18.4 ± 8.6	16.3 ± 8.5	0.445

(59.2%) patients were operated on the left elbow and 22 (40.9%) on the right elbow. According to the Regan-Morrey coronoid fracture classification, 22 (40.7%) patients were typed 1, 20 (37%) patients were typed 2, and 12 (22.2%) patients were type 3. According to the Mason-Johnston Radius head fracture classification, 22 (40.7%) patients typed 1, 18 (33.3%) patients typed 2, and 14 (25.9%) typed 3. Our average follow-up period was 16.4 ± 8.4 months, at least 6 months (Table 1).

Elbow Functional Outcomes

Patients in group A showed better elbow flexion-extension and forearm rotation recovery than those in group B (range of elbow flexion-extension 135 ± 18.7 to 117.7 ± 10.1, p < 0.01; forearm rotation 160 ± 28.5 to 132.3 ± 19.6, p < 0.01) (Table 2).

In addition, at the final clinical visit, the total MEPS (94.2 ± 9.3 to 86.1 ± 7.4, $p < 0.01$) and Oxford Elbow scores (44.2 ± 5.5 to 38.9 ± 4.4, $p < 0.01$) in group A was higher than group B which indicates that op¬erations performed earlier were associated with regaining better elbow function (Table 3).

In Group A, two patients had osteoarthritis, one patient had heterotopic ossification, and five patients had joint stiffness. In group B, three patients had osteoarthritis, one patient had ulnar nerve deficit, two patients had heterotopic ossification and six patients had joint stiffness. The VAS score was 2.71 ± 1.38 in group A and 3.38 ± 1.12 in group B (Table 4).

Table 2. Comparison of joint range motion among two groups

	Flexion-Extension Range (Mean ± SD)	Supination-Pronation Range (Mean ± SD)
Group A	135 ± 18.7	160 ± 28.5
Group B	117.7 ± 10.1	132.3 ± 19.6
***p* value**	**< 0.01**	**< 0.01**

SD = standard deviation

Table 3. Comparison of MEPS and Oxford elbow score

	MEPS (Mean ± SD)	Oxford Elbow Score (Mean ± SD)
Group A	94.2 ± 9.3	44.2 ± 5.5
Group B	86.1 ± 7.4	38.9 ± 4.4
***p* value**	**< 0.01**	**< 0.01**

MEPS = Mayo elbow performance score, SD = standard deviation

DISCUSSION

Terrible triad of elbow are injuries that damage bone and ligament structures, leading to instability [7, 10, 11, 2]. Therefore, almost every patient requires surgical treatment [5, 12, 13]. The treatment protocol is generally radial head fixation or arthroplasty, coronoid fracture fixation, LCLC repair, and MCL repair in patients with persistent instability [14-17]. Despite all treatment protocols and ideal bone and soft tissue reconstructions, they are injuries with high complication rates [4, 18, 19].

Complex elbow dislocations are complicated injuries that are challenging to treat; however, not all of these injuries are equivalent. Understanding elbow biomechanics and the mechanism of injury provides valuable insight into the variations of pathology that may be observed. Identification of the particular fracture pattern encountered helps guide appropriate treatment. Systematic protocols to address these injuries have resulted in improved functional outcomes through the optimization of elbow stability and articular congruency. Although results are often acceptable, complex elbow dislocations still frequently result in residual elbow stiffness and arthrosis. Further investigation into methods of fixation following complex

Table 4. Complications and VAS score among two groups

	Group A	Group B	*p* value
Osteoarthritis	2	3	0.663
Nerve deficit	0	1	0.481
Heterotopic ossification	1	2	0.604
Elbow stiffness	5	6	0.634
VAS	2.71 ± 1.38	3.38 ± 1.12	0.33

VAS = visual analog scale

elbow dislocations should continue the trend of improved patient outcomes [8].

When the literature is reviewed, there is a consensus about better results in early surgical treatment. McKee *et al*. [5] reported in their study that the incidence of joint stiffness was low in the early surgical treatment group. Lindenhovius et al. [9] in their study with 36 patients, two study groups were determined as those who were operated on within two weeks (acute) and those who operated on for more than three weeks (subacute). The same surgical protocol was applied to all patients. A better flexion-extension arc was found in the study group compared to the control group [9]. Zhou et al. [20] evaluated fifty-eight TTE patients by dividing them into three study groups. Group A was determined to be operated on within 24 hours (emergency), Group B operated between 24 hours and two weeks (early) and Group C operated after two weeks (delayed). The same surgical protocol was applied to all patients. Range of motion was compared between the groups at the first, third and sixth-month follow-up. MEPS was used for functional scoring at the sixth-month controls. While there was no significant difference between the emergency and early operated groups in the long-term controls, a better range of motion was observed in both groups compared to the delayed operated groups [20]. The clinical difference between early and late surgical treatment may be due to the alteration of soft tissues. Fracture hematoma occurs early in the injury, soft tissues become congestive. Inflammatory mediators are released between 24-48 hours and the process of necrosis begins with cell degeneration. This chain can be broken with early surgical treatment. In addition, as time passes, capsule contracture and muscle atrophy begin to appear. This makes it difficult to gain range of motion. In addition, soft tissues and bone fragments can be better identified in early surgical treatment, which benefits anatomical reduction and fixation. After the good repair of bone and soft tissues, patients can start their functional exercises early.

The general approach in open fractures is to wait for wound healing after wound debridement. This can prolong the time to surgery and lead to poor results. However, studies have shown that Gustilo type 1 and 2A are more common in open elbow dislocations [20, 21]. In our study, four patients in Group A had Gustilo tip 1 open elbow injury. We performed urgent debridement and repair operation within 24h but wound infection did not develop in any patient. Therefore, open terrible triad of elbow can be treated early after complete debridement.

CONCLUSION

In summary, terrible triad of elbow has many complications and poor prognosis. There are many factors that effect the results. The common opinion for good results is to achieve stable joint and early rehabilitation. When the literature is reviewed, there are no comprehensive studies examining studies the effect of time to surgery on outcomes in terrible triad of elbow. In our study, we showed that early operation for TTE patients resulted in better functional recovery than delayed operation. Although it varies according to the wound condition in open TTE patients, closed TTE patients should have an early operation.

Authors' Contribution

Study Conception: BA, EÇ, SS; Study Design: BA, EÇ, SS, AD; Supervision: BA, EÇ, SS; Funding: EG; Materials: BA, SS; Data Collection and/or Processing: BA, SS; Statistical Analysis and/or Data Interpretation: BA, SS; Literature Review: BA, EÇ; Manuscript Preparation: BA, EÇ and Critical Review: SS.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The European Research Journal 2023;9(4):743-752 Original Article

DOI: 10.18621/eurj.1022099 *Neurology and Pain Management*

Investigation of the relationship between lumbar spine MRI findings and pain in patients who received and did not receive Parkinson's treatment

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ABSTRACT

Objectives: Parkinson's disease is a chronic, progressive neurodegenerative disorder. Pain is a common symptom in Parkinson's disease, but the prevalence, characteristics, and documentation of its relationship with Parkinson's disease are insufficient. In this study, it was aimed to evaluate the relationship between lumbar spine magnetic resonance imaging (MRI) findings in patients who received and did not receive Parkinson's treatment.

Methods: The demographic characteristics of patients with diagnosed Parkinson's disease were retrospectively obtained from the records. Their pain was grouped and the Hoehn Yahr stage at the first examination, and the interventional treatments performed were recorded. MRI measurements were made in the axial plane and sagittal plane of the spinal canal, whereas Ligamentum flavum measurements were made on both the right and left sides.

Results: Twenty-six patients were included in the study. The average age was 73.5. Notably, 57.7% of patients were not diagnosed with Parkinson's disease prior to admission, while the Hoehn Yahr stage mostly comprised Stage 2 with 53.8%. Additionally, low back, waist, and hip pain was observed in 84.6% (n = 22), whereas 61.5% (n = 16) of patients experienced radicular pain. Epidural injections accounted for 33.2%. On lumbar MRI, the most narrow spinal segment on axial measurement was shown to be L4-L5. The axial spinal canal measurement of the Hoehn Yahr 1 group was observed to be significantly lower than the Hoehn Yahr 2 group.

Conclusions: Optimal management for lumbar pain that increases with age is currently inadequate. There is a need to conduct larger studies on pain complaints, which is one of the frequently experienced non-motor symptoms in Parkinson's disease, as well as the interventional methods applied.

Keywords: Pain, Parkinsonism, low back pain, epidural, spinal stenosis

Parkinson's disease (PD) is a chronic, progressive, and neurodegenerative disease. As the disease progresses, motor impairments and non-motor symptoms create significant disease burdens. Therefore, the goal of treatment is to relieve symptoms. Pain is common in patients with Parkinson's disease, but the

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prevalence, characteristics, and documentation of its relationship with Parkinson's disease are insufficient [1].

To the best of our knowledge, different studies have elucidated all kinds of pain with a high frequency of up to 65% or 85% at different rates. In a study evaluating that the pain perceived by patients is directly related to PD, the pain experienced by 46% of patients was attributed to PD. Some studies have investigated specific types of pain or specifically localized pain areas in PD patients. For example, the prevalence of low back pain has been reported to be 60-74% [2-7]. The need for large population-based studies on the review of non-motor symptoms and the prevalence of pain and other non-motor symptoms in PD patients has also been emphasized [2-4].

Pain management may indicate the magnitude of pain as a clinical problem in PD patients. As the disease progresses, musculoskeletal, radicular, and dystonic pain become more frequent. This is probably attributed to pathologically increased muscle tone and the long-term worsening of postural reflexes. Moreover, as these postural disturbances (anteflexion, kyphoscoliosis) progress, the non-radicular back pain gradually turns into radicular pain. Furthermore, central pain is likely to worsen with neurodegeneration spreading to the central sensory pathways [3, 5, 6].

In addition to the deterioration of muscular imbalance limb deformities, degeneration of the neck, trunk, or lumbar region, and axial sagittal or frontal deformities can also cause pain due to the movement disorder itself. The lumbar region has been shown to cause more musculoskeletal pain in PD compared to the control group. Back pain appears to be an early and common symptom in PD. Patients with PD deal with chronic low back pain more than those belonging to the same age group [4, 7-9]. Lumbar spinal canal stenosis (LSCS) is known to be the most common spinal disorder among elderly patients. The narrowing of the canal is partly caused by ligamentum flavum (LF) hypertrophy, which mechanically compresses the nerve root or cauda equina [10].

Joints between vertebrae are strengthened and supported by many ligaments. One of them is LF, which adheres to the front of the upper lamina and to the back of the lower lamina. Due to the connective tissue, it affects the instability of the spine, controls the intervertebral movement, and creates a protective surface for the posterior dural sac. LF hypertrophy is considered an important cause of radiculopathy in lumbar degenerative disease [11-13].

It is argued that altered posture and abnormal muscle tone in Parkinson's patients can increase stress on both lumbar discs, soft tissue, as well as bone structures of the lumbar spine. Truncal dystonia may additionally contribute to focal distress and other related problems. Whether the relationship between PD and low back pain is specific remains unclear as it is common in both cases [7, 14].

This study aims to describe the clinical and demographic predictors of pain, including the types of pain, painful areas, and the diagnosis that cause pain in patients admitted to the pain outpatient clinic with primary pain complaints or before the diagnosis of PD, as well as the use of different interventional pain treatments. This is done to evaluate the relationship between lumbar spinal canal measurement, LF measurements, and PD staging in patients with low back pain.

METHODS

The study was initiated in accordance with the Helsinki declaration and after obtaining the approval of the local ethics committee (Sakarya University Faculty of Medicine ethical committee, number E-71522473-0.50.01.04-15084). Patients who applied to Sakarya Training and Research Hospital Neurology and Pain outpatient clinic between 2019-2021 were diagnosed with Parkinson's or had Parkinson's disease, but were not diagnosed in another center. Patients who were diagnosed by pain specialists and neurologists according to UK PD Brain Bank Criteria [15] were retrospectively removed from the hospital records. A pain physician and neurologist examined the patients.

Inpatient and outpatient records were reviewed, focusing on the presence of pain complaints in the personal history of patients with PD or those who were newly diagnosed. Demographic characteristics, pain that prompted patients to seek medical help, and first-time complaints were noted. In pain classification, especially those with back pain, waist, hip pain is in the Back/Waist/ Hip Pain group; in the radicular pain group, those with radicular pain that spread to the extremity towards the arm or leg; in the joint pain group,

with or without movement disorders, the knee or shoulder area is specified; in the peripheral neuropathic pain group, patients with symptoms of mononeuropathy such as tingling and dysesthesia in the extremities, bilateral distal symmetrical neuropathic pain or entrapment neuropathy and clinical laboratory evidence and also who met diagnostic criteria for neuropathic pain; orofacial pain or neuralgiform pain was collected in the neuralgia/orofacial pain group. Lumbar magnetic resonance imaging (MRI) records were checked, since the treatment of patients and the most common low back pain was observed. Anteroposterior (AP) spinal canal measurement in the sagittal plane in lumbar MRI was measured at three different levels in T2-weighted imaging (T2WI) and the narrowest AP spinal canal measurement in T2WI in the axial section. Multiple measurements made in the sagittal plane were taken at the lumbar vertebra levels comparatively, and at the levels closest to the AP measurement in the axial section, in order to minimize the error caused by postural posture disorders during imaging. LF measurements were done with the help of hospital imaging software. The maximum thickness of the LF was measured on both the right and left sides at the most narrow lumbar levels. The measurement was made in T2WI perpendicular to the lamina, right and left-sided, mid-width distance (Fig. 1).

The types of treatment received by the patients and the Hoehn Yahr (HY) stages at the first examination were also noted. The two most important conditions in the HY staging are whether the disease is bilateral or postural disorder. Therefore, increased parkinsonian motor impairment can encompass unilateral (Stage 1) to bilateral disease (Stage 2), the presence of postural imbalance (Stage 3), the loss of physical independence (Stage 4), and being tied to a wheelchair or bed (Stage 5) [16].

The pain types of patients were classified as acute, duration less than three months, whereas those with a long duration were classified as chronic. For the pain that caused the patient to come to the pain clinic, the diagnosed pain syndrome or its etiological cause, and the interventional procedure for pain were recorded.

Statistical Analysis

The research data were evaluated by being uploaded to the computer environment via ―SPSS for Windows 21.0 (SPSS Inc, Chicago, IL). Descriptive statistics were presented as median (minimum-maximum), frequency distribution, and percentage. The variables' suitability to normal distribution was examined using visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk Tests). Kruskal-Wallis and Mann-Whitney U Tests were used for groups that did not conform to normal distribution. Statistical significance level was accepted as $p < 0.05$.

RESULTS

Twenty-six patients were included in the study and their data were analyzed. It was observed that the av-

Table 1. Sociodemographic features (n = 26)

Parameter		Data
Age (years)		Median 73.5 (Q1:69-Q3:78)
Gender, n (%)	Male	13 (50)
	Female	13 (50)
Previously diagnosed with PD, n (%)	Yes	15 (57.7)
	No	11 (42.3)
HY stage, n (%)	1	4 (15.4)
	2	14 (53.8)
	3	8 (30.8)

PD = Parkinson disease, HY = Hoehn Yahr

Table 2. Diagnosed pain syndromes

Parameter	n	%
Lumbar radiculopathy + Lumbar spinal stenosis	13	32.5
Lumbar radiculopathy	4	10
Failed back surgery	4	10
Knee osteoarthritis	4	10
Cervical radiculopathy	3	7.5
Lumbar vertebra fracture	2	5
Shoulder impingement syndrome	2	5
Postzoster neuralgia	2	5
Polyneuropathy	2	5
Carpal tunnel syndrome	1	2.5
Trigeminal neuralgia	1	2.5
Sciatica axonal neuropathy	1	2.5
Plexopathy	1	2.5

Table 3. Interventional treatments applied

Interventional treatments	n	%
Genicular block	1	3.3
MILD procedure	1	3.3
Intercostal block	1	3.3
Caudal epidural steroid injection	5	16.6
Lumbar epidural steroid injection	4	13.3
Sacroiliac injection	1	3.3
Mandibular block	1	3.3
Lumbar faset median nerve block	5	16.6
Epiduroscopy	1	3.3
Transforaminal epidural steroid injection	1	3.3
Facet median branch radiofrequency coagulation	2	6.6
Piriformis injection	1	3.3
Spinal cord stimulator implantation	1	3.3
Paravertebral trigger point injection	1	3.3
Vertebroplasty/kyphoplasty	2	6.6
Intraartricular knee injection	1	3.3
Erector spina plane block	1	3.3

MILD = Minimal Invasive Lumbar Decompression

erage age of these patients was 73.5 and that the number of males and females was equal. Notably, 57.7% of patients were not diagnosed with PD before the application, and the HY stage mostly comprised Stage 2 with 53.8%. Moreover, 80.8% were found to have an additional disease. Table 1 illustrates the sociodemographic characteristics of the patients.

The prevalence of comorbid diseases in patients was as follows: 50% hypertension, 38.5% coronary artery disease, 26.9% diabetes mellitus, 11.5% thyroid disease, and 11.5% cancer. Previous joint or lumbar surgery was observed in 42.3% (n = 11) of patients. Similarly, lumbar surgery was seen in 38.5% (n = 10) of patients. The presence of rotoscoliosis was observed in 23.1% (n = 6) of patients.

In terms of pain classification, the following observations were made: 84.6% (n = 22) of patients had low back, waist, and hip pain, 61.5% (n = 16) experienced radicular pain, and 15 of these patients had pain radiating to the radicular lower extremity. Similarly, 38.5% (n = 10) of patients with knee or shoulder joint pain, 23.1% (n = 6) of patients experiencing peripheral neuropathic pain, including distal symmetrical, or mononeuropathy/plexopathy, and 15.4% (n = 4) of patients with neuralgia/orofacial pain were determined. The etiological diagnoses based on these complaints and imaging methods are shown in Table 2. In some patients, more than one diagnosis was made for the painful condition.

It was observed that 57.7% (n = 15) of patients were using drugs for PD. The most common drugs used by the patients were dopamine agonist or MAO-B inhibitor and L-dopa combination therapy. Most of them were already on medical treatment for pain. Interventional pain treatments applied to patients are depicted in Table 3. The frequency percentage within the procedures was given due to the interventional treatments performed on a patient undergoing more than one procedure.

The minimum and maximum values of the patients' lumbar MRI measurements are shown in Table 4, whereas the relationship between the HY phase and Canal measurements is shown in Table 5. In the study, the narrowest lumbar range was found to be 42.3% (n = 11), most frequently at the L4-5 level. We know that the disability of the disease and posture disorders is

Table 4. **Measurements**

Parameter		Median	Q1-Q3
Lumbar MRI sagittal spinal canal measure 1		13.3	(10.4-14.4)
Lumbar MRI sagittal spinal canal measure 2		12.8	(9.9-14.05)
Lumbar MRI sagittal spinal canal measure 3		12.8	(10.0-13.5)
Lumbar MRI sagittal spinal canal measure mean		12.96	(10.53-14.74)
Lumbar MRI axial spinal canal AP measure		12.0	(9.9-15.1)
Lumbar axial lig. flavum measure 1		6	(5.3-6.7)
Lumbar axial lig. flavum measure 2		5.7	(4.8-6.6)
Lumbar axial lig. flavum measure mean		6.05	(5.05-6.6)
Parameter		**n =26**	**%**
Lumbar MRI	L1-L2	2	7.7
The most narrow spinal segment on axial	L2-L3	2	7.7
	L3-L4	7	26.9
	L4-L5	11	42.3
	L5-S1	1	3.8

Measurements are given as mm. MRI = Magnetic Resonance Imaging

Table 5. **HY and measurements**

Parameter	HY1 (n = 4)	HY2 (n = 14)	HY3 (n = 8)	KW	*p* value
Lumbar MRI sagittal spinal canal measure 1	7.55 (4.7-9.45)	13.8 (12.25-14.3)	13.35 (9.72-15.6)	3.481	0.175
Lumbar MRI sagittal spinal canal measure 2	8.15 (6.4-10.23)	13.8 (11.4-14.7)	12.3 (8.65-13.55)	4.927	0.084
Lumbar MRI sagittal spinal canal measure 3	8.95 (7.9-9.2)	12.8 (11.65-13.7)	13.0 (9.05-13.7)	2.537	0.281
Lumbar MRI sagittal spinal canal measure mean	8.21 (6.5-9.34)	13.53 (11.41-14.86)	12.81 (9.39-14.36)	4.134	0.127
Lumbar MRI axial spinal canal AP measure	7.2 (5.1-8.7)	14.0 (11.81-15.6)	10.7 (8.77-12.27)	9.043	**0.011***
Lumbar axial lig. flavum measure 1	6.4 (6.1-6.73)	5.9 (5.1-6.5)	6.05 (5.35-6.57)	1.170	0.557
Lumbar axial lig. flavum measure 2	5.3 (4.0-6.1)	5.6 (4.85-6.55)	6.25 (4.65-6.62)	0.195	0.907
Lumbar axial lig. flavum measure mean	5.85 (5.05-6.32)	6.0 (4.77-6.72)	6.35 (5.37-6.58)	0.656	0.720

Analyzed with the Krukal-Wallis (KW) Test. In the post-hoc Kruskal-Wallis one-way ANOVA test, it was observed that the "Lumbar MRI Axial Spinal Canal AP Measure" measurement of the HY1 group was significantly lower than the HY2 group.

Measurements are given as mm. HY = Hoehn Yahr

positively correlated with the progression of the HY stage. When we looked at the relationship between the HY phase and the canal measurements, it was evident that the Lumbar MRI Axial Spinal Canal Ap measurement was significantly lower in the HY1 group than in the HY2 group ($p = 0.011$).

When looking at the relationship between the group that received and did not receive Parkinson's treatment and the lumbar MRI measurements, no significant difference was found (Table 6).

DISCUSSION

Pain in PD can occur in both treated and untreated PD patients due to the objective pain perception impairment, related to the disease itself, and secondary diseases such as musculoskeletal or visceral pain. The most commonly used clinical classification of pain in PD is the classification proposed by Ford, which divides pain into musculoskeletal, radicular/neuropathic, dystonia-related, akathisic discomfort/pain, and central pain. The prevalence of pain in PD patients ranges from 40-85%. [6, 17-19].

Musculoskeletal problems are the most common cause of physical disability in the general population. Although musculoskeletal problems are common, a few reports describe the prevalence or clinical features of musculoskeletal problems in PD. In a survey on the effect of comorbidities on health-related quality of life (HRQoL) in patients with PD, Andreadou *et al*. reported that arthritis, low back pain, and osteoporosis, musculoskeletal problems (45%) were the most common comorbid disorders in patients with PD [9, 20].

When age increases, degenerative spine problems are also known to increase. The prevalence of disc or posterior facet degeneration increases exponentially after age 50. Bijkerk *et al*. [21] showed that 68.5% of men and 66.2% of women have degenerative disc disease. In addition, Lomber Spinal Stenosis (LSS) is a

Table 6. Measurements in the group that received and did not receive Parkinson's treatment

Parameter	Parkinson's treatment + (n = 15)	Parkinson's treatment – (n = 11)	Z	*p* value
Lumbar MRI sagittal spinal canal measure 1	13.4 (12.2-14.4)	11.6 (9.1-14.35)	0.872	0.392
Lumbar MRI sagittal spinal canal measure 2	12.8 (11.1-14.5)	11.7 (8.4-13.8	0.873	0.392
Lumbar MRI sagittal spinal canal measure 3	12.9 (11.2-13.8)	12.2 (8.57-13.25)	0.743	0.392
Lumbar MRI sagittal spinal canal measure mean	12.96 (12.16-14.83)	11.68 (8.77-11.43)	0.743	0.466
Lumbar MRI axial spinal canal AP measure	11.6 (10.6-15.4)	12.1 (8.55-13.85)	0.775	0.466
Lumbar axial lig. flavum measure 1	6.0 (5.5-6.6)	5.7 (4.82-6.56)	0.775	0.466
Lumbar axial lig. flavum measure 2	6.2 (5.10-5.72)	4.6 (4.02-6.25)	1.774	0.087
Lumbar axial lig. flavum measure mean	6.1 (5.15-6.65)	5.57 (4.75-6.55)	0.840	0.428

Analyzed by Mann-Whitney U test. Measurements are given as mm.

clinical problem that becomes increasingly common as the population ages [4, 21, 22]. The deterioration in the proprioceptive mechanism, which increases with age, causes deterioration in the agonist-antagonist muscles in the joint structure that normally absorb shocks. Moreover, with the concomitant changes in postural control, PD may exacerbate the back problems associated with pre-existing degenerative diseases [4, 23, 24].

In our patients, the most common type of pain was back, hip, and radicular pain. Although 38.5% of patients had lumbar spine surgery, it was remarkable that the most common pain was waist, back, hip, and radicular pain. The most common diagnosis is lumbar radiculopathy with lumbar spinal stenosis, followed by lumbar radiculopathy with failed back surgery. In fact, diagnoses made for pain in the lumbar region have a total share of 57.5% among all diagnoses.

Buhrmann *et al*. [17] stated in a study conducted on PD that 71.4% of patients had back pain, followed by joint pain with 52.4% and that only 15.3% of the pain had a neuropathic character. They showed that pain was independent of the HY stage and that anti-parkinson drugs had a positive effect on their pain in only one-third of patients. Based on this, they stated that it is unlikely that the pain in PD can be solely explained by changes in dopaminergic pathways. Compared to pain frequency, only a quarter of patients were found to be diagnosed for their pain, and pain specialists were involved in only 10.9% of pain treatments, and neurologists - although one of the main treating doctors in PD - were only involved in pain management in 3.3% of cases [17, 25]. This study is similar to our study in that waist and back pain are the most common symptoms. Since our study was retrospective and was performed only on patients who applied to the neurology-pain outpatient clinic, there was no scale given in the follow-up phase regarding the effectiveness of dopaminergic treatment on pain or pain. This is one of the limitations of our study.

In our study, interventional pain therapies were applied to the patients as treatment. These patients were either currently receiving medical therapy or did not benefit from medical therapy. Some patients underwent more than one interventional procedure for pain palliation. In Buhmann *et al*.'s study [17], peripheral nerve blockade (22.6%) was found to be the most common interventional treatment option for pain, followed by epidural injection (8.9%) and infusion (7.3%). They noted that despite the high prevalence of chronic pain, only a quarter of their patients had a formal diagnosis of pain, thus confirming that pain in PD is an undergraded and inadequately treated symptom [17].

If we look at the interventional treatments (Table 3) in our study, epidural injections are the most common 33.2%, followed by facet median bundle branch block and median branch radiofrequency procedures with 23.3%. Vertebroplasty/kyphoplasty was performed on two patients before. In this age group, which also has osteoporosis, we should be careful against pain due to vertebral fracture.

In the geriatric age group with rotoscoliosis, lumbar surgery, posture disorders, it is very difficult to optimally measure the sagittal cross-section from a single level in MRI. For this reason, measurements were made at the lumbar levels most suitable for MRI. This is the reason for the measurements made at different levels in the sagittal section, which is one of the limitations of our study. Foraminal measurements could be more reliable than AP measurement in this patient group, where foraminal stenosis is also common, but MRI sections were not optimal for foraminal measurements. The narrowest level of axial spinal canal measurement was found to be L4-L5.

LSS can be defined both clinically and radiologically. MRI is often used to evaluate the radiological signs of LSS. However, there are no detailed classification criteria for defining LSS using MRI. Indeed, significant variability has been identified in both quantitative, semi-quantitative, and qualitative descriptions. However, as assessed by MRI and clinical symptoms, there appears to be only a weak correlation between spinal morphology. Cautious interpretation of LSS and results has been recommended, as there is considerable variation in defining diagnostic criteria between studies for both clinical symptoms and radiological signs [26-29].

Measurements in our study are shown in Table 4. The differences with the HY stage in Table 5 and the relationship between the groups that received and did not receive Parkinson's treatment are depicted in Table

6. Although our patients comprised both clinically symptomatic and asymptomatic groups with no complaints in the lumbar region, they were predominantly clinically symptomatic. Advanced age, low number of patients, and additional comorbid diseases such as osteoporosis can be considered confounder. Between the HY stage and axial spinal AP measurement, those in the HY1 stage were observed to be significantly lower than those in the HY2 stage. In fact, as the HY stage progresses, the postural disorders are expected to become evident, and axial involvement increases. However, there is a major difference between HY1 and other stages, such as unilateral disease and bilateral disease.

In this case, the question arises whether the mechanical stress increases more in the presence of unilateral disease to ensure stability and the secondary canal diameter is measured smaller with axial measurement. We believe that the staging and measurements of PD patients with low back and leg pain should be investigated in future studies.

There was no significant difference between the groups that received and did not receive PD treatment. Since there is no pain secondary to dystonia, the difference may not be observed. However, the effects of PD treatment, which is also effective on rigidity and axial degeneration, assumes significance. Additionally, back pain is another issue that needs to be investigated in larger numbers. Although studies suggest that LF thickens with age, there are also studies stating that LF thickening is independent of age and gender and that mechanical stress and degeneration are the most important ones [11-13, 30, 31].

It has been stated that LF has different thicknesses at different levels. Kolte *et al*. [11], Altinkaya *et al*. [30], and Abbas *et al*. [31] stated that the thickest level in their study was L4-L5. They demonstrated the increased thickness at the level of L4-L5 instead of L5-S1, the stabilization of the L5-S1 segment with iliolumbar ligaments, and the greater transverse process of the L5 vertebra, in addition to the greater coronal alignment of the S1 facets and the ability to reduce shearing stress. In the study of Kolte *et al*. [11], in the 61-80 age group, right and left LF measurements averaged 4.35 and 4.43 mm, respectively in the thickest L4-L5. In the study of Sakamaki *et al*. [32], the thickest LF in the 60-69 age group was 3.8 mm, and it was 3.9 mm in the 70-79 age group. In the study conducted by Altinkaya *et al*. [30], it was measured as 5.1 mm above the age of 80 and in the thickest segment. In our study, the age group was homogeneous and the mean LF measurement was found to be higher than other studies with 6.05 mm.

Limitations

One of our limitations is ; we could not evaluate the all patients while they are in the ON period. But we enrolled only the chronic pain patients, so ON period can be negligible. Dystonia that painful situation in the ON period were not seen in our patients while examining. Although the absence of a control group in our study is another limitation and also we couldn't get the records of electromyography (EMG), it is noteworthy that the measurements of LF are significantly higher than in other studies. In conclusıon, in our study, LF measurements were found to be thicker in a similar geriatric age group compared to other observed studies. The most common clinical pain syndrome was lumbar stenosis and lumbar radiculopathy. These Parkinson's patients are mostly observed in the geriatric age group. However, are these measurements related to age or axial involvement due to the disease itself? We opine that our study will inspire further studies on this subject. We would also like to draw attention to pain complaints which are non-motor symptoms but are often overlooked.

CONCLUSION

We found that lumbar stenosis and lumbar radiculopathy was the most common clinical pain syndrome in our Parkinson patients. LF measurements in PD were found to be thicker in a similar geriatric age group compared to other observed studies. Pain is an important and often overlooked symptom in PD.

Authors' Contribution

Study Conception: ZTI, FAE; Study Design: ZTI, GGÇ; Supervision: ZTI, SGB; Funding: N/A; Materials: ZTI; Data Collection and/or Processing: ZTI, FAE, GGÇ; Statistical Analysis and/or Data Interpretation: ZTI, SGB; Literature Review: ZTI, FAE; Manuscript Preparation: ZTI, SGB and Critical Review: ZTI, SGB.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Role of laboratory markers in identifying the severity of acute appendicitis in geriatric patients

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ABSTRACT

Objectives: Objectives: The main aim of our study is to investigate the effectiveness of laboratory markers in geriatric patients diagnosed with acute appendicitis in the emergency department.

Methods: A total of 73 cases aged over 65 who underwent appendectomy were reviewed retrospectively. Age, sex, laboratory values of leukocyte count, neutrophil and lymphocyte values, platelets, serum sodium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), C-reactive protein (CRP) and serum total bilirubin levels and pathological findings were investigated. The patients were divided into two different groups according to the pathology reports of appendicitis: complicated appendicitis (gangrenous/perforated appendicitis) (Group 1) and simple (uncomplicated) appendicitis (catarrhal or phlegmonous appendicitis) (Group 2).

Results: A significant difference was found between Groups 1 and 2 concerning age, WBC, CRP, neutrophil, leukocyte, total bilirubin levels and neutrophil/lymphocyte ratio (NPR), and platelet/lymphocyte ratio.(PLR) No statistically significant differences were found for platelets, Na, AST and ALT levels.

Conclusions: Elderly patients with complicated appendicitis often presented with higher WBC, CRP, total bilirubin, neutrophil, NLR and PLR levels, and lower lymphocyte levels. Moreover, these parameters are adjunctive data with a lower cost, easily available, and rapid markers which can be obtained in the emergency department.

Keywords: Appendicitis, complicated, geriatric, laboratory markers

Acute appendicitis (AA) is the most common abdominal emergencies that require emergency surgery (appendectomy) and are encountered in emergency departments. While the incidence of AA in all age groups in the population is 7%, the incidence of perforation in patients with AA varies between 17-20%. The mortality rate in the general population is below 1%, but this rate approaches 50% in the geriatric age group (> 60 years) [1, 2].

Several scoring systems have been proposed for the diagnosis of AA, such as the Alvarado score. These scoring systems were created based on the patient's physical examination findings, clinical characteristics, and laboratory data. In some cases, the classic signs and symptoms of appendicitis may not be seen. This makes it difficult to make a definitive diagnosis of AA. Predictors of acute appendicitis severity are urgently needed, as delayed diagnosis and treatment of severe

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appendicitis is associated with perforation, abscess formation, postoperative morbidity, mortality, and prolonged hospital stay [3, 4].

For this reason, it is important to identify geriatric patients at risk of complicated appendicitis as early as possible, as it will determine the type of further examination and treatment. Recently, some authors have stated the predictive value of laboratory markers for inflammation such as White blood cell count (WBC), neutrophil/lymphocyte ratio (NLR), platelet count (PLT), platelet/lymphocyte ratio (PLR), serum total bilirubin level, C reactive protein (CRP) level, serum sodium (Na), aspartate aminotransferase (AST), alanine aminotransferase (ALT), which can be practical as a diagnostic-parameters in the peri-operative predictor of diagnosing AA. By the reason of the advantageous cost-effectiveness of these tests [5-8], we wanted to investigate the effectiveness of laboratory markers in diagnosis in geriatric patients diagnosed with AA in the emergency department.

METHODS

The medical records of 73 cases aged over 65 at a single institution who underwent an open or laparoscopic appendectomy in our clinic and had histo-pathological results reported as AA were analyzed retrospectively. For each patient; the age; sex, laboratory values of WBC, neutrophil and lymphocyte values, PLT, Na, AST, ALT, CRP, serum total bilirubin levels, NLR, PLR, and pathological findings were investigated.
The 73 patients were separated into 2 groups according to the pathological grade of AA: complicated AA (gangrenous/perforated) (Group 1) and simple (uncomplicated) AA (catarrhal/phlegmonous) (Group 2). We evaluated the pre-operative factors we identified between the groups and analyzed the risk parameters for complicated AA by multivariate analysis. Exclusion criteria were the presence of mortality, malignancy, the current course of oncological treatments, intra-operative diagnosis of other abdominal pathologies and presence of known liver or kidney diseases.

This study was approved by the Clinical Research Ethics Committee, informed consent was obtained from all patients and all procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Statistical Analysis

The conformity of continuous variables to the normal distribution was evaluated using the Shapiro-Wilk test. Continuous variables were expressed as mean ± standard deviation if the data followed the normal distribution and median (25^{th} percentage-75^{th} percentage) values if the data did not follow the normal distribution. The Mann-Whitney-U test and independent samples t-test were used for continuous variables to compare groups. The chi-square test was used to compare the gender distribution between the groups. In order to estimate the sensitivity and specificity of the NLR ratio for predicting the presence of complicated appendicitis, receiver operator characteristic (ROC) curve analysis was performed. The SPSS program (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) was used for statistical analysis, and the type I error rate was set at 5%.

RESULTS

A total of 73 patients who underwent appendectomies between January 2017-August 2021 were included. The median age was 74 years (25^{th} percentage: 70 years – 75^{th} percentage: 80 years) in Group 1 and 69.50 years (25^{th} percentage: 66 years - 75th percentage: 73.25 years) in Group 2 (Table 1). The median age was found to be higher in Group 1 ($p = 0.020$). In Group 1; 48.70% (n = 19) of the participants were female and 51.30 (n = 20) were male. In Group 2; 32.40% (n = 11) were female and 67.60% (n = 23) were male. When compared in terms of gender, it was revealed that there was no significant difference between the groups. ($p = 0.156$) (Table 1).

Group 1 had a significantly higher median value in total bilirubin level compared to Group 2 ($p = 0.003$). Mean WBC and neutrophil levels were also found to be higher in Group 1 ($p = 0.003$ and p = 0.006), whereas the mean lymphocyte level was found to be higher in Group 2 ($p < 0.001$). The mean N/L level was significantly higher in Group 1 ($p < 0.001$). There was no difference between the groups according

Table 1. Comparison of the two groups

	Group 1 (n = 39)	Group 2 (n = 34)	*p* value
Age (years), median (IQR)	74 (70- 80)	69.5 (66-73.25)	**0.020**
Female, n (%)	19 (48.7%)	11 (32.4%)	**0.156**
Total Bilirubin (mg/dL)	1.22 (0.77-1.79)	0.81 (0.53-0.97)	**0.003**[a]
WBC ($\times10^3/mm^3$)	14.49 ± 3.99	11.06 ± 3.92	**0.003**[b]
Neutrophil count ($\times10^3/mm^3$)	11.73 ± 4.03	9.05 ± 4.06	**0.006**[b]
Lymphocyte count ($\times10^3/mm^3$)	1.20 ± 0.41	1.84 ± 0.66	**< 0.001**[b]
Neutrophil / Lymphocyte ratio	10.16 (6.50-15.14)	5.17 (3.12-8.24)	**< 0.001**[a]
PLT ($\times10^9$/L)	241 (183-296)	238 (176-276)	0.682[a]
Platelet/ Lymphocyte ratio	200 (153.96-266.25)	134.58 (95.10-181.25)	**< 0.001**[a]
CRP (mg/dL)	101 (83-169)	35.40 (17.68-77.50)	**< 0.001**[a]
Na (mEq/L)	137.39 ± 4.14	137.79 ± 5.04	0.704[b]
AST (U/L)	22 (16-31)	20 (18-28.25)	0.553[a]
ALT (U/L)	13 (11-21)	16.50 (12-21.25)	0.482[a]

Data were presented as median (25th percentile – 75th percentile) and mean ± standard deviation. WBC = White Blood Cell, PLT = platelet, CRP = C-reactive protein, Na = Sodium, AST = aspartate aminotransferase, ALT = alanine aminotransferase
[a]Mann-Whitney U Test, [b]Independent Samples t-Test

to PLT level ($p = 0.682$), but PLR and CRP levels were higher in Group 1 ($p < 0.001$ and $p < 0.001$). There was no significant difference between the two groups in the median of Na, AST, and ALT levels ($p > 0.05$) (Table 1).

ROC curve analysis was performed to forecast the sensitivity and specificity of NLR for predicting the presence of complicated appendicitis, and the cut-off point for NLR was determined as >5.85. The area under the curve for NLR was 0.81 (sensitivity 84.60%, specificity 64.70%, $p < 0.001$), showing that a NLR > 5.85 was significantly related to an increased risk of the presence of complicated appendicitis (Fig. 1).

To determine the risk factors that may affect the

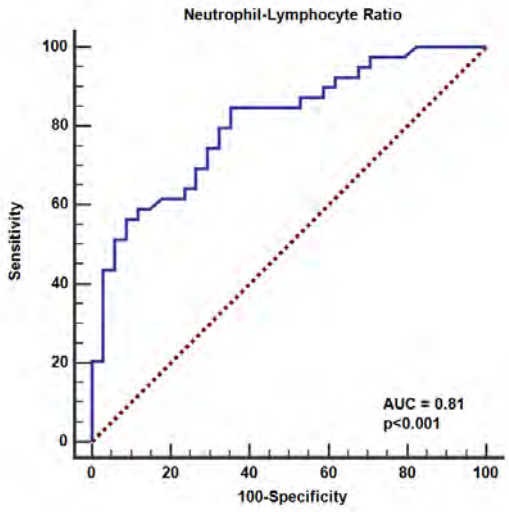

Fig. 1. Receiver-operator characteristic (ROC) curves for determining the presence of complicated appendicitis. The area under the curve (AUC) for Neutrophil to Lymphocyte Ratio (NLR) is 0.81 with $p < 0.001$

Table 2. Risk factors affecting of complicated appendicitis

	Wald	*p*-value	OR	95% CI	
				Lower	Upper
Total bilirubin	4.74	**0.029**	4.41	1.16	16.75
Lymphocyte	7.16	**0.007**	0.02	0.01	0.34
CRP	12.44	**< 0.001**	1.06	1.03	1.10

OR = Odds ratio, CI = Confidence Interval, CRP = C-reactive protein
Model Significance: $\chi^2 = 66.86$; $p < 0.001$, Hosmer & Lemeshow Test: $p = 0.809$

patients with complicated appendicitis, age, gender, and the variables given in Table 1 were first examined with univariate logistic regression analysis, and the variables were analyzed with multivariable logistic regression analysis. The forward selection approach was selected as the variable selection method in the analysis, and the final step's findings are given in Table 2.

When the results of the analysis were examined, it was found that the logistic regression model obtained in the final step was compatible with the data ($p = 0.809$) and the logistic regression model was also statistically significant ($p < 0.001$). As a result of the analysis, it is predicted that a one-unit increase in the total bilirubin level will increase the risk of complicated appendicitis 4.41 times. An increase of 1 unit in the lymphocyte level is predicted to reduce the risk of complicated appendicitis by 98%. On the other hand, CRP level was also determined as a risk factor that may affect the formation of complicated appendicitis, and it was determined that an increase of 1 unit in the CRP level would increase the risk of complicated appendicitis 1.03 times.

DISCUSSION

AA is one of the most prevalent causes of abdominal pain and emergency surgery. The mechanism of AA formation is in the form of inflammation of the vermiform appendix's lumen resulting from an obstruction. This obstruction can be caused by lymphoid hyperplasia (mostly in young patients), a fecalith, parasitic infection, or a tumor (carcinoid, adenocarcinoma or metastatic). Depending on its clinical forms, AA is divided into two groups: uncomplicated and complicated. Uncomplicated (simple) appendicitis is characterized by the absence of perforation or abscess; complicated AA is the result of rupture of the appendix with or without abscess formation. Appendicitis can develop in patients of any age, but it is most commonly seen in patients between the ages of 10-30 [9]. Simple AA is most common in younger patients, but complicated AA is more common in very old and very young patients. Babies and young children often have a significant delay in evaluation, diagnosis, and treatment. The main reason is that they cannot specify pain symptoms like an adolescent/adult. Similar latency may occur for elderly patients because of cognitive deficits (such as dementia) or the reduced ability to sense and localize pain due to age-related changes in pain receptors [10]. Objective criteria, such as laboratory parameters, are needed to determine diagnostic parameters in geriatric AA patients. The main reason for this is that appendiceal perforation, formation of abscess, and pan-peritonitis are still common in elderly patients with AA. In this study, we investigated the value of several laboratory markers that are frequently used in the clinic and easily accessible in terms of their usability in the prediction of complicated AA in the elderly.

In acute appendicitis, the usual presentation involves leucocyte counts of between 10-18000 /mm3. Rarely, do they have moderate polymorphonuclear-Leucocytosis dominancy (left shift). WBC in uncomplicated appendicitis cases is rarely greater than 18000. If they do reach this level, then appendicitis should be considered perforated or complicated [7]. The high mortality rate in the elderly due to AA is usually attributed to the delayed diagnosis and treatment of the condition and concomitant diseases. This is one

of the reasons why the mortality rate in the elderly is higher than in younger patients. Classical symptoms in geriatric patients may not be very clear. Physical examinations are generally not very obvious. Abdominal distension is usually seen. In about 30% of cases, appendiceal perforation can only be detected intraoperatively. Therefore, early surgical treatment is recommended in suspected cases of AA [11].

Previous studies have reported that levels of CRP were correlated with the severity of appendiceal inflammation and may be precise in predicting complicated appendicitis. On the other hand, various studies revealed that CRP levels and WBC counts are not sensitive and specific to descry between simple / complicated appendicitis [12, 13]. We evaluated in our study that both WBC counts and CRP levels were significantly higher in patients with complicated appendicitis compared with simple appendicitis.

The systemic inflammatory response may lead to lymphocytopenia or neutrophilia. This may lead to an increase in NLR and PLR, which are markers of inflammation in appendicitis. In addition, changes in platelet count are thought to be comprised of the inflammatory process. PLR, and NLR are inflammatory markers. Their advantageous features are that they can be easily calculated from the blood count, and they are simple, cost-effective, and non-invasive.

Ishizuka *et al*. [14] established a cut-off value of 8.0 for the NLR to distinguish gangrenous AA from catarrhal AA. Kahramanca *et al*. [15] reported a cut-off value of 5.74 to differentiate complicated appendicitis from simple appendicitis. In the present study, NLR cut-off value was 5.85 for differing complicated appendicitis from simple appendicitis. In these studies, patients in all age groups were calculated. Yavuz *et al*. [7] stated that NLR can be used to diagnose acute appendicitis in the geriatric patient group. In our study, we think that NLR is also effective in demonstrating complicated appendicitis in the geriatric age group. Despite conflicting recommendations regarding cut-off values, we believe that NLR is an important parameter in the diagnosis of acute appendicitis and in differentiating complicated cases. There was no significant difference in platelet count between patients with complicated and simple appendicitis, but higher PLR was reported in patients with complicated appendicitis.

Hyperbilirubinemia and cholestasis occur in systemic infections caused by various diseases, diseases such as generalized peritonitis and sepsis. It has been previously established as a predictive factor for AA [16, 17]. Sevinc *et al*. [6] reported that hyper-bilirubinemia (> 1.0 mg/dL) was significantly associated with perforated appendicitis. Eren *et al*. [18] found that hyper-bilirubinemia (>1.2mg/dL) was related to complicated AA. In our study, hyperbilirubinemia is significantly associated with complicated appendicitis.

We also analyzed and compared Na, ALT - AST levels between simple and complex groups. The results showed that these parameters did not differ significantly between the two groups.

Limitations

There were some limitations of our study. The most important of these are that our study was retrospective, the number of patients was limited and it was a single-center study.

CONCLUSION

Our study suggested that elderly patients with complicated appendicitis often presented with higher WBC, CRP, total bilirubin, neutrophil and PLR, NLR levels, and lower lymphocyte levels. It has been shown that there is currently no simple but perfect test for diagnosing and recognizing complicated appendicitis. However, an increase in NLR can be considered a highly reliable indicator for the diagnosis of complicated appendicitis in geriatric patients. Moreover, these parameters are adjunctive data with a lower cost, easily available, and rapid markers which can be obtained in the emergency department. We recommend that clinicians use these values with the results of physical examination and imaging studies to define high-risk geriatric appendicitis patients earlier.

Authors' Contribution

Study Conception: SA, MA; Study Design: SA, MFE; Supervision: MFE, NDT; Funding: SA, MA, SŞ; Materials: SŞ, MHB; Data Collection and/or Processing: MA, SŞ, MHB; Statistical Analysis and/or Data Interpretation: SA, MFE, MHB; Literature Review: SA, MA, NDT; Manuscript Preparation: SA, MA and Critical Review: MFE, NDT.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The European Research Journal 2023;9(4):759-769 Original Article

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The impact of liraglutide treatment on erectile function of the diabetic rats

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ABSTRACT

Objectives: Glucagon like peptide-1 (GLP-1) is a hormone released from intestinal L-cells following nutrient consumption. It potentiates secretion of insulin from pancreatic beta-cells thus GLP-1 analogues are used for the treatment of type-2 diabetes mellitus (T2DM). This study aims to evaluate impact of GLP-1 receptor agonist liraglutide on erectile function of diabetic rats.

Methods: Male Sprague-Dawley rats (n = 30, 13-weeks old, 240-335 gr) were fed with fatty diet for 2-weeks and divided into 3 groups (n = 10 each). The rats in the first group served as controls (Group C) whereas the rats in the remaining two groups were injected with streptozocin and became T2DM for forming diabetic group (Group D) and treatment group (Group DT). Rats in group D received citrate buffer injections whereas rats in the group DT received liraglutide injections (0.3 mg/kg/12h) subcutaneously. Erectile functions of all rats were evaluated with intracavernosal pressure (ICP)/mean arterial pressure (MAP) measurements. Moreover, plasma sex hormone levels (Testosterone, FSH, LH) were measured and histological assessment of midpenile tissue were performed (Collagen-Type-IV, rat epithelial antigen-1, nNOS).

Results: Maximum ICP/MAP ratios were 0.790 ± 0.164, 0.263 ± 0.139 and 0.652 ± 0.131 in Group C, Group D and Group DT. Although mean ICP/MAP ratios were similar in Group C and Group DT ($p = 0.076$), mean ICP/MAP ratio was significantly lower in Group D ($p < 0.001$). Testosterone and FSH results were significantly lower in the Group D as well ($p = 0.001$). Histological analyses revealed that nNOS ($p < 0.001$), rat epithelial antigen-1 ($p = 0.016$) and muscle/collagen ratio ($p = 0.015$) were also lower in Group D, compared with the other groups.

Conclusions: GLP-1 receptor agonist liraglutide demonstrated protective effects on the erectile tissues of the diabetic rats. Clinical trials are required to confirm if liraglutide treatment has similar beneficial effects on men who have T2DM.

Keywords: Erectile dysfunction, GLP-1 agonist, intracavernosal pressure, liraglutide, nNOS, type 2 diabetic rat

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Erectile dysfunction (ED) is a permanent impairment in initiating and/or sustaining sufficient erection for a satisfactory sexual performance [1]. It is estimated that there are 18 million ED patients in United States of America [2]. The most important risk factors for ED are ageing, atherosclerosis, diabetes mellitus (DM), hypertension, smoking and dyslipidemia [3, 4]. ED frequency in DM patients ranges between 35-90% [5, 6]. Vascular, endothelial, neuronal, endocrine and metabolic pathways play role in the pathogenesis of ED in DM patients [7-12]. In diabetic patients, ED occurs earlier than in the normal population and these cases respond less to phosphodiesterase type 5 inhibitors, which is considered as the first line treatment of ED [13].

Liraglutide is a long acting glucagon-like peptide-1 (GLP-1) receptor agonist used in the treatment of DM [14]. This molecule enhances insulin secretion by stimulating beta cells of the pancreas. Liraglutide controls blood glucose level, while at the same time delays gastric discharge and suppresses postprandial glucagon secretion [15]. Several studies have proven that GLP-1 receptors are also present in extrapancreatic tissues [16, 17]. Although the effects of other new generation DM drugs on erectile function have been examined [18-20], the effect of GLP-1 receptor agonists on diabetic ED has not been analyzed. Although Galli *et al*. [21] have received a patent on that the GLP-1 receptor agonists can be beneficial for the ED treatment by activating GLP-1 receptors in the penile tissue, there are not any clinical or translational data to confirm this hypothesis. Yue *et al*. [22] and Yuan *et al*. [23] have suggested that GLP-1 receptor agonists may protect the endothelial function, regulation of smooth muscle dysfunction, oxidative stress and autophagy, independently of a glucose-lowering effect and regulating oxidative stress, by acting through the Akt/eNOS signal pathway and the RhoA/ROCK pathway but there is not yet enough scientific data on this topic.

Therefore, we aimed to reveal the hormonal, functional and morphological effects of liraglutide treatment on erectile function of rats with type 2 DM (T2DM).

METHODS

This study was carried out at Istanbul Bagcilar Training and Research Hospital Experimental Research and Skill Development Training Centre (BADABEM) with the approval of project numbered 2016-30 from Bagcilar Training and Research Hospital Local Ethics Committee of Animal Tests (SBU B.E.H HADYEK). All expenses of the study were met from the research grant provided by Istanbul Bagcilar Training and Research Hospital Training Planning Committee.

A total of 30 healthy male Sprague-Dawley rats weighing between 240-335 g were included in the study. During the study, rats were fed with tap water (ad libitum) with 2% cholesterol and 10% fat containing rat feed (Ziegler Bros. Gardners, PA). The animals were monitored at a temperature of 22°C for 12 hours in the light and 12 hours in the dark. After feeding them in physiological cages with a cycle of 12-hour night/day for 2 weeks, 10 rats were randomly assigned to form the Control Group (Group C). The remaining 20 rats received intraperitoneal streptozocin (STZ) injection (30 mg/kg) for 2 times within a period of 3 days [24]. Blood glucose levels were measured daily and then weekly throughout the period of the study from the rats' tails with the glucosemeter (Accu-Chek, Roche, Mannheim, Germany) until it is confirmed that the rats are hyperglycemic (blood glucose level > 300 mg/dL). For measurement of the sensitivity to a challenge of insulin, 1 IU/kg bovine insulin in phosphate-buffered solution (PBS) (1 IU/mL) was administered by intraperitoneal injection and tail snip capillary blood samples were collected at 0, 15, 30, 45, 60, 90, and 120 minutes after injection for obtaining a blood glucose response curve.

After these 20 rats became hyperglycemic, they were randomly divided into two groups. Rats assigned to the Diabetic Treatment Group (Group DT) were injected subcutaneously with liraglutide (Victoza®, Novo Nordisk, Denmark) for 12 weeks (0.3 mg/kg/12h) whereas rats in the Diabetic Group (Group D) were given citrate buffer (0.25 ml/kg/12h) solution for 12 weeks.

Liraglutide dosage has been used with reference

to previous studies [17, 25]. After 12 weeks of treatment, rats were anaesthetized with ketamine (60 mg/kg) and xylazine (7.5 mg/kg) intraperitoneally for the physiological testings (24 hours after the last injection to Group D and Group DT). A single dose of ketamine (50 mg/kg) was repeated as needed to continue spontaneous breathing of the rats during the measurements.

Intracavernosal Pressure / Mean Arterial Pressure Measurements

After the anesthesia, the rats were placed in supine position and the incision site was cleared. With transverse neck incision, the left internal carotid artery was identified next to the trachea. The artery was ligated distally and cannulated with PE50 tube after a small incision. PE50 tube was connected to booster unit with pressure transducer (Commat Pharmacology & Physiology Instruments, Ankara, Turkey). This booster unit was connected to the information conversion module (MP35 data acquisition system, Ankara, Turkey) so that the systemic Mean Arterial Pressure (MAP) could be recorded and measured from the computer (Biopac Systems Inc, CA, USA).

Afterwards, a circular incision was performed onto the penis and a 24 G needle tip was placed into the right crurale of the penis for the measurement of Intra Cavernosal Pressure (ICP) (mmHg). Subsequently, a midline laparotomy incision was performed and the main pelvic ganglion was identified with 3.5 × operation magnifying glass in the dorsal section of the prostate. The cavernosal nerve was detected from the satellite ganglia in the lateral of the main pelvic ganglion. The nerve was captured with bipolar electrode just at the distal part of the main ganglion. The electrode line was connected to the STPT02 stimulator (COMMAT Pharmacology & Physiology Instruments, Ankara, Turkey) and the stimulation parameters were adjusted as; 1.5 milliamps, 20 Hz, 5 milliseconds pulse interval, 35 milliseconds delayed, 7.5 volts for 60 seconds. During the nerve stimulation, the maximum ICP/MAP was calculated and the percent of these values in the confidence interval of 95% was reported. High-dose single stimulation was used due to the surgeon and technical equipment performing the procedure, and either single stimulation or low dose to high dose stimulation is not standard. In this study, ICP measurement was made in accordance with the measurement made by Mullerad *et al*. [26].

Serum Analyses

The intracardiac blood samples were placed in biochemical tubes (BD Vaccutainer SST II, Beliver Industrial Estate, UK) and centrifuged at 20,000 rpm for 90 seconds for the decomposition of the serum. The FSH, LH and total testosterone levels were measured in a biochemical analyzer (Roche Cobas 6000 Immunoassay, Roche Diagnostics, Basel Switzerland) according to the manufacturer's instructions by using the sandwich enzyme immunoassay method.

Histopathological Examinations

All penile tissue specimens were first fixated in a 10% Neutral Buffer formaldehyde solution for light and immunofluorescence microscopic examination. After the fixation process, the tissue samples were placed in trays and washed under running water for 2 hours. For draining the water, the tissues were passed from the alcohol series (70%, 80%, 90%, 100%) at increasing degrees. Subsequently, the tissues were passed from xylol solution and then buried into the molten paraffin. Both Hematoxylon-Eosin Staining and Immunohistochemical Staining for rat epithelial antigen-1 (RECA-1 Antibody, ab9774, Abcam, Cambridge, UK), Collagen IV (Collagen IV Antibody, ab6586, Abcam, Cambridge, UK) and neuronal nitric oxide synthase (nNOS Antibody, ab5586, Abcam, Cambridge, UK) were performed. The sections were evaluated in the Leica DC-4000 (Germany) computer-supported imaging system in the Leica Q Vin 3 program. During polarized light microscopy and immunofluorescence microscopic examinations nNOS and RECA-1 expressions could be evaluated whereas we could not obtain sufficient staining for Collagen Type IV. Therefore, the evaluation of collagen accumulation was carried out by polarized light microscopy with Masson's Trichrome staining (Table 1).

Statistical Analysis

The statistical analyses were performed by using the NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) package program. Besides the descriptive statistical methods (mean,

Table 1. Histopathologic scoring table for nNOS, RECA-1, collagen type IV expressions 0: No staining; 1: Minimal level of positivity (< 10%); 2: Medium level of positivity (< 10%-50%); 3: High level of positivity (> 50%)

Histopathological scoring table			
N-NOS	**Group C**	**Group D**	**Group DT**
1	3	1	2
2	2	1	2
3	2	2	2
4	2	1	2
5	2	1	2
6	3	2	3
7	2	1	2
8	3	1	3
9	2	1	
10	3	1	
RECA-1	**Group C**	**Group D**	**Group DT**
1	1	0	0
2	1	1	2
3	2	1	0
4	2	1	1
5	2	1	1
6	1	0	1
7	1	1	0
8	2	0	1
9	2	0	
10	2	1	
Collagen type IV	**Group C**	**Group D**	**Group DT**
1	No staining	No staining	No staining
2	No staining	No staining	2
3	1	2	2
4	1	No staining	2
5	No staining	1	No staining
6	No staining	No staining	No staining
7	No staining	No staining	No staining
8	No staining	No staining	No staining
9	1	2	
10	No staining	2	
Collagen review with trichrome	**Group C**	**Group D**	**Group DT**
1	1	3	1
2	1	2	1
3	2	3	1
4	1	1	2
5	1	3	1
6	1	3	1
7	1	3	2
8	1	3	1
9	1	2	
10	2	3	

standard deviation, median and interquartile range), Kruskal Wallis test was used for comparing the variables with non-normal distribution between the groups, Dunn's multiple comparison test was used for subgroup comparisons, and Chi-square test was used for qualitative data comparisons. The results were evaluated at $p < 0.05$ significance level

RESULTS

Twenty-four hours after the initiation of the treatment, 2 rats died in Group DT and the study was completed with 28 rats, 12 weeks after the injection of STZ (Group DT: 8, Group D: 10, Group C: 10 rats). There was no statistically significant difference among the weight averages ($p = 0.448$) and mean blood glucose levels ($p = 0.546$) of the rats in different groups before the treatment. However, rats in Group D were significantly lighter ($p = 0.001$) and their blood glucose levels were significantly higher ($p = 0.001$) compared with the rats in Group C and Group DT (Fig. 1).

Intracavernosal Pressure / Mean Arterial Pressure Measurements

No statistically significant difference was observed between the MAP values of Group C, Group D and Group DT ($p = 0.363$). Maximum ICP/MAP rates were 0.790 ± 0.164, 0.263 ± 0.139 and 0.652 ± 0.131 in Group C, Group D and Group DT respectively. Although there was no statistically significant difference between Group C and Group DT ($p = 0.076$), the maximum ICP/MAP values of Group D were significantly lower than Group C ($p = 0.001$) and Group DT ($p = 0.001$) (Table 2 and Fig. 2).

Serum Analyses

Average total testosterone values are shown in Table 3. Mean total testosterone level was significantly lower in Group D compared with Group C ($p = 0.001$) and Group DT ($p = 0.001$). Mean total testosterone level of Group DT was also lower than that of Group C but this difference did not reach to a statistically significant level ($p = 0.076$) (Table-3). FSH values were also significantly lower in Group D compared with Group C ($p = 0.001$). Although mean FSH in Group C was lower than Group DT, this was not statistically significant ($p = 0.076$). LH values of Group C were lower than Group D ($p = 0.001$) and Group DT ($p = 0.001$). Although the mean LH levels in Group DT were greater than Group D, this was not statistically significant ($p = 0.086$) (Table 3).

Histopathological Examinations

Statistically significant difference was observed between the nNOS values of Group C, Group D and Group DT ($p = 0.0001$). High level of positivity in Group D was found lower than the Group C and Group DT (Table 4, Fig. 3). Statistically significant difference was also observed between the RECA-1 values of Group C, Group D and Group DT ($p = 0.016$). Medium level of positivity in Group D was

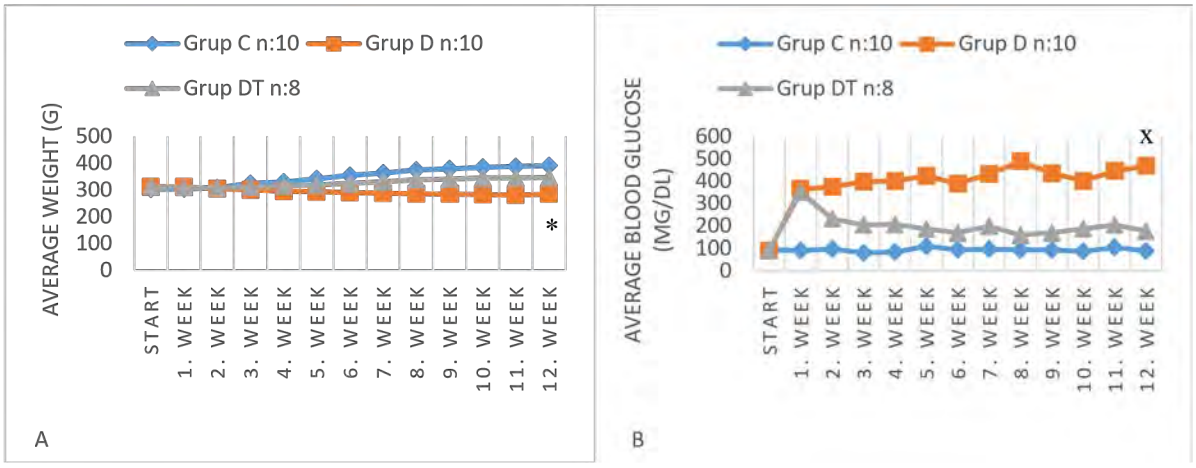

Fig. 1. (A) Weekly weight measurement average results (*$p = 0.001$ for Group D), (B) Weekly blood glucose measurement average result s(xp = 0.001 for Group D)

Table 2. Intracavernosal pressure (ICP) and mean arterial pressure (MAP) measurements, ICP/MAP ratios and intergroup evaluation results

Kruskal Wallis Test		Group C	Group D	Group DT	*p* value
ICP max	Mean ± SD	32.06 ± 22.29	9.33 ± 7.23	33.35 ± 7.06	**0.001**
	Median (IQR)	31.9 (12.51-44.15)	7.1 (5.06-10,43)	34.7 (29.05-38.8)	
MAP ave	Mean ± SD	38.75 ± 23.18	37.41 ± 17.36	51.57 ± 9.52	**0.363**
	Median (IQR)	38.42 (16.41-58.34)	37.56 (22.68-53.5)	47.5 (45.38-62.78)	
ICP/MAP	Mean ± SD	0.790 ± 0.164	0.263 ± 0.139	0.652 ± 0.131	**0.0001**
	Median (IQR)	0.755 (0.665-0.9)	0.205 (0.16-0.378)	0.606 (0.551-0.782)	
ICP/MAP (%)	Mean ± SD	79.13 ± 16.26	26.3 ± 13.91	65.18 ± 13.14	**0.0001**
	Median (IQR)	75.5 (66.88-90)	20.45 (16.03-37.93)	60.6 (55.08-78.2)	
Dunn's multiple comparison test		**ICP/MAP (*p* value)**		**ICP/MAP(%) (*p* value)**	
Group C / Group D		**0.0001**		**0.0001**	
Group C / Group DT		0.076		0.076	
Group D / Group DT		**0.001**		**0.001**	

found lower than the Group C and Group DT (Table 4 and Fig. 4). The collagen distributions of Group C, Group D and Group DT were also significantly different ($p = 0.015$). High level of positivity in Group D was found higher than the Group C and Group DT (Table 4 and Fig. 3).

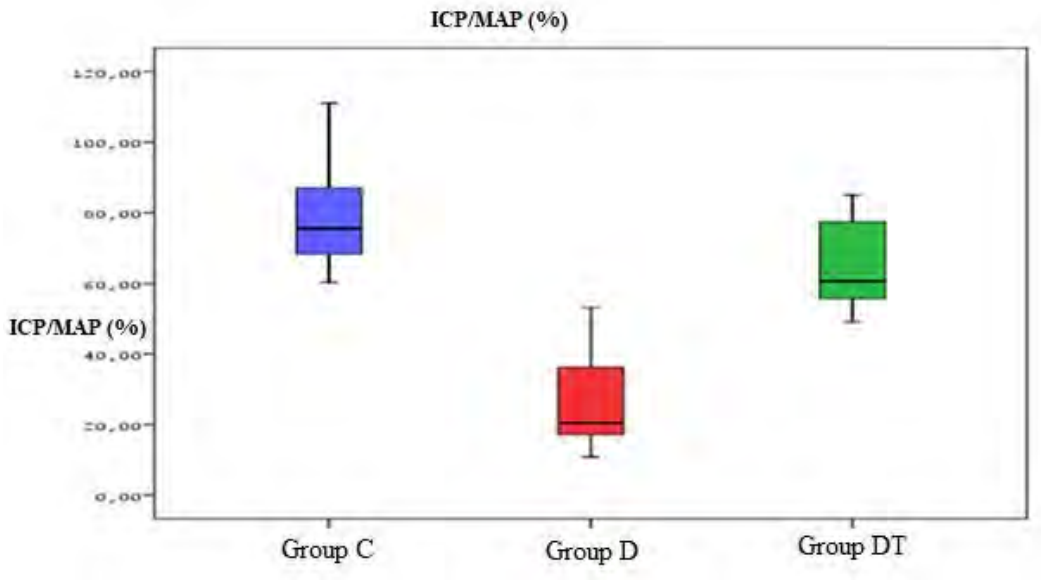

Fig. 2. Intracavernosal pressure (ICP) / mean arterial pressure (MAP) ratios, Group C: 0.79 ± 0.16; Group D: 0.26 ± 0.13, Group DT: 0.65 ± 0.13.

Table 3. Serum mean total testosterone(ng/ml), FSH(IU/l), LH(IU/l) values and intragroup evaluation results

Kruskal Wallis Test		Group C	Group D	Group DT	*p* value
Total testosteron (ng/mL)	Mean ± SD	2.57 ± 0.63	0.58 ± 0.21	1.61 ± 0.23	**0.0001**
	Median (IQR)	2.58 (1.97-3.0)	0.6 (0.525-0.671)	1.63 (1.43-1.79)	
FSH (IU/L)	Mean ± SD	0.107 ± 0.006	0.054 ± 0.007	0.132 ± 0.01	**0.0001**
	Median (IQR)	0.107 (0.102-0.11)	0.052 (0.048-0.06)	0.130 (0.128-0.139)	
LH (IU/L)	Mean ± SD	0.335 ± 0.009	0.398 ± 0.028	0.399 ± 0.027	**0.0001**
	Median (IQR)	0.335 (0.33-0.339)	0.397 (0.378-0.416)	0.402 (0.368-0.421)	

Dunn's Multiple Comparison Test	**Total Testosteron**	**FSH**	**LH**
Group C / Group D	**0.0001**	**0.0001**	**0.0001**
Group C / Group DT	0.076	0.076	**0.0001**
Group D / Group DT	**0.001**	**0.001**	0.859

DISCUSSION

The pathophysiology of diabetes related ED has not been fully enlightened, however increased hyperglycemia-induced glycosylation and reduced NOS phosphorylation are thought to be the key mechanisms [27]. Albersen *et al*. demonstrated decreases in nNOS expression, impairment in endothelial integrity, and decreases in smooth muscle/collagen ratio in cavernosal tissues of the diabetic rats, supporting these theories [24].

Better glycemic control is associated with improved diabetes-related complications. Among the new generation DM treatments, pioglitazone has been found to be associated with improved erectile functions after radical prostatectomy [18, 20], which has

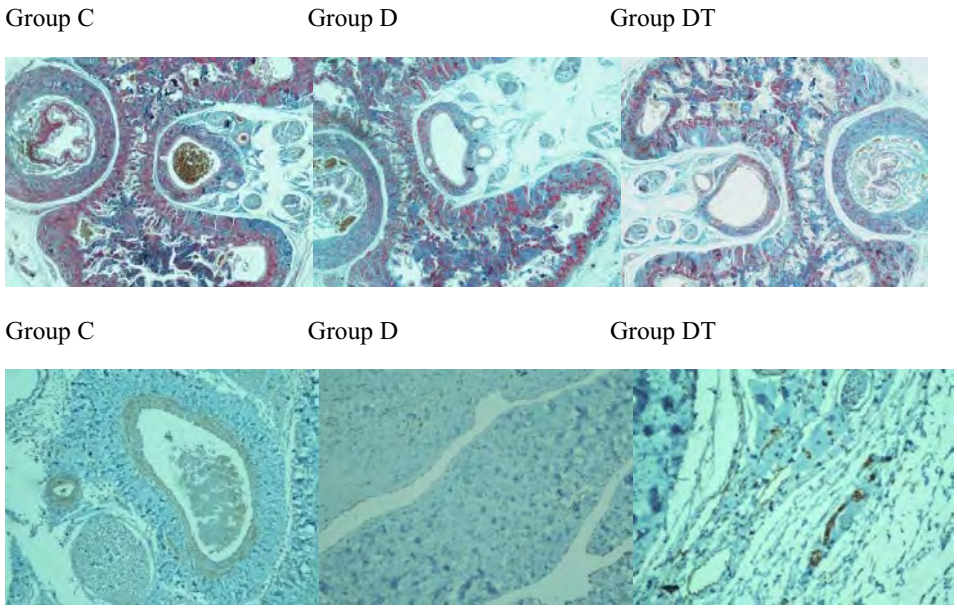

Fig. 3. First row: Collagen tissue in stroma and vein wall in Grup C, increase in Group D, minimal increase in Group DT. Second row: Positivity in vein wall and nerve tissue in Group C with nNOS (× 10), No positivity in Group D with nNOS (× 20), Positivity in vein wall and nerve tissue in Group DT with nNOS (× 10).

Table 4. Histopathologic scoring table and intragroup evaluation results for nNOS, RECA-1, collagen type IV expressions

Chi-Square Test		Group C		Group D		Group DT		*p* value
		n	%	n	%	n	%	
N-NOS	**Positive at minimal level**	0	0.00	8	80.00	0	0.00	**0.0001**
	Positive at medium level	6	60.00	2	20.00	6	75.00	
	Positive at high level	4	40.00	0	0.00	2	25.00	
RECA-1	**Negative**	0	0.00	4	40.00	3	37.50	**0.016**
	Positive at minimal level	4	40.00	6	60.00	4	50.00	
	Positive at medium level	6	60.00	0	0.00	1	12.50	
Trichrome-Collagen	**Positive at minimal level**	8	80.00	1	10.00	6	75.00	**0.015**
	Positive at medium level	2	20.00	2	20.00	2	25.0	
	Positive at high level	0	0.00	7	80.00	0	0.00	

been explained with the presence of its extrapancreatic receptors. Similarly, GLP-1 receptors are also detected in extrapancreatic tissues such as the myocardial and pulmonary arterial systems, which exert their effects via NO dependent mechanisms (possibly NOS2 activation over Giα and MAP kinase dependent p38) [16, 17]. Zhou*et al*. Have shown thatGLP-1 agonist liraglutide treatment protects eNOS activity with inhibition of NF-κB pathway, in addition to the maintaining glycemic control in diabetic rats and has direct beneficial effects against diabetic nephropathy [17]. GLP-1 receptors also exist in the penile tissues and GLP-1 receptor agonists are hypothesized to be beneficial in the ED treatment [21]. In a Chinese study Yue *et al*.

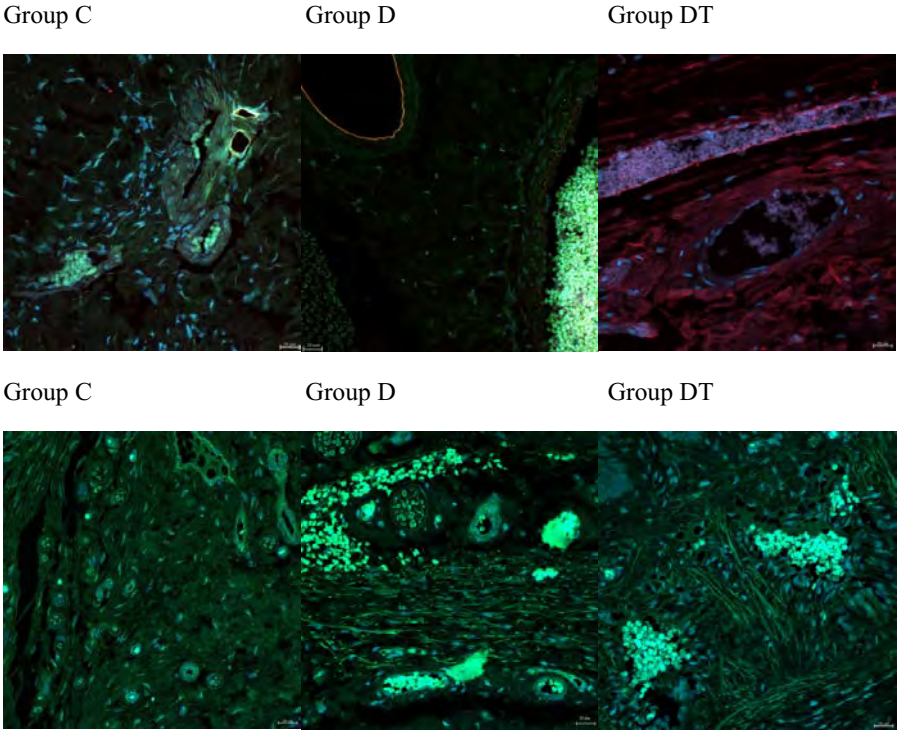

Fig. 4. First row: Fluorescence microscopic image of nNOS positivity in vein wall and nerve tissue. Second row: Fluorescence microscopic image of endothelial RECA-1 expressions.

[22] demonstrated that GLP-1 receptor agonist liraglutide can protect the endothelial functions of diabetic rats by acting through the Akt/eNOS signal pathway. However, the authors did not conduct any physiological testings for the assessment of erectile function of these rats. Yuan *et al*. [23] conducted physiological testings for the assessment of erectile function but they conducted experiments in type 1 diabetes model, which impeded generalization to the general diabetic population. In a retrospective study conducted in 2015 shown that adding GLP-1 agonist, life style change, metformin and testosterone replacement therapy improved erectile dysfunction due to hypogonadism in patients with T2DM [28].

In order to elucidate the actual impact of GLP-1 receptor agonists on erectile function of T2DM rats, we have conducted hormonal, functional and morphological analyses on rats with T2DM. Our results revealed more favorable ICP/MAP values in diabetic rats treated with liraglutide compared to untreated diabetic rats. Moreover, we evaluated the endothelial function of diabetic rats after liraglutide treatment by comparing the morphologic changes in cavernous endothelium along with the RECA-1 expressions in corpus cavernosum. We found that the endothelial integrity was impaired in the diabetic rats and RECA-1 expressions decreased significantly, which could be protected with liraglutide treatment. Moreover, liraglutide treatment maintained the nNOS expressions, which was decreased in the diabetic rats.

Poor glycemic control is associated with decrease in cavernosal smooth muscle content, increase in the collagen amount and decrease in the contractile capacity of the penile tissues [19, 29, 30]. Although we aimed to evaluate the collagen type IV accumulation, which is known to play a significant role in the contractile capacity of cavernous tissue [31, 32], we could not achieve comparable staining. However, trichrome staining method revealed that smooth muscle/collagen ratio decreased in the diabetic rats, but it was maintained in the liraglutide treatment receiving rats. Future studies must elucidate the actual mechanisms explaining how GLP1 receptors ameliorate the detrimental effects of diabetes on the penile tissues.

DM also impairs erectile functions through their effects on the hypothalomohypophyseal axis. DM causes secondary testicular failure by decreasing gonadotropin hormone levels [33]. We also detected decreased total testosterone and FSH levels in the diabetic rat group whereas the levels of these hormones were preserved in the treatment group. There is a need for additional studies to understand the effects of diabetes and GLP-1 agonists on the hypothalamohypophyseal axs and testes.

To our knowledge, our study is the first study that evaluated the effects of GLP-1 agonists on erectile function in diabetic rats, both functionally and histologically. Our results revealed that liraglutide treatment has the potential of preserving the penile tissues and maintaining erectile functions in patients with DM. This study was done in rats, and it is much too early to extrapolate it to humans. Future clinical trials are required to confirm this hypothesis.

Limited histopathological examination and limited quantitative evaluation are among the limitations of the study.

CONCLUSION

GLP-1 agonist liraglutide treatment has protective effects on erectile function of rats with T2DM. There is a need for clinical trials to verify whether liraglutide treatment has similar beneficial effects in patients with DM.

Authors' Contribution

Study Conception: SG, MT, MGÇ, SS, YB; Study Design: SG, MT, MGÇ, SS, YB; Supervision: SG, MT, MGÇ, SS, YB; Funding: SG, MT, MGÇ, SS, YB; Materials: SG, MT, MGÇ, SS, HHT, YB, ECS, AS; Data Collection and/or Processing: SG, MT, MGÇ, SS, HHT, YB, ECS, AS; Statistical Analysis and/or Data Interpretation: SG, MT, MGÇ, SS, HHT, YB, ECS, AS; Literature Review: SG, MT, MGÇ, SS, HHT, YB, ECS, AS; Manuscript Preparation: SG, MT, MGÇ, SS, HHT, YB, ECS, AS and Critical Review: SG, MT, MGÇ, SS, HHT, YB, ECS, AS.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2023;9(4):770-778 Original Article

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The relationship between body mass index and clinical properties/survival in patients with breast cancer

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ABSTRACT

Objectives: The aim of this study was to investigate relationship between body mass index (BMI) and recurrence/survival and to determine whether body mass index is a risk factor.

Methods: A retrospective study of 125 patients with breast cancer was performed. Age, sex, menopausal situation, body mass index, tumor size, axillary lymph node involvement and number, tumor degree, hormone receptor situation, cerbB-2 overexpression, tumor grade, chemotherapy dose and treatments, radiotherapy, metastasis, comorbidity, total survival and time of death were evaluated. Patients were compared as BMI < 30 kg/m^2 and BMI ≥ 30 kg/m^2. Kaplan-Meier method and Log Rank test was utilized for the overall survival of patients.

Results: In these 72 patients, 57.6% of patient's body mass index BMI < 30 kg/m^2 (non-obese) while 53 of the group (42.4%) BMI ≥ 30 kg/m^2 (obese) were found in the group. The median follow-up was 158.32 months (95% CI: 130.45 to 186.19). The mean follow-up was 146 ± 46 months (95% Cl: 55.36-236.63) and 144 ± 28 months (95% CI: 88.19-199.81) for non-obese patients and obese patients respectively. Kaplan Meier plots were drawn for determination of the effect of body mass index in total survival. No significant difference was found statistically between two groups.

Conclusions: No significant difference was found statistically between BMI and recurrence/death. The future studies with long term follow up are needed to investigate the effect of body mass index on recurrence and survival in patients with breast cancer.

Keywords: Breast cancer, body mass index, survival, relapse, obesity

Breast cancer has surpassed lung cancer as the most commonly diagnosed cancer, with an estimated 2.3 million new cases followed by lung, colorectal, prostate and stomach cancers İN 2020 according to GLOBOCAN for Research on Cancer Reports [1]. Since 1990, breast cancer incidence rates have been increasing approximately 1.5% yearly [2]. Although advances in diagnosis and treatment, breast cancer rank first resulting in death among women worldwide.

According to reports of Ministry of Health in 2015, breast cancer is the most common cancer type among women in Turkey with a rate of 25% [3]. Surgical techniques, chemotherapy and radiotherapy have been used for the treatment of breast cancer as well as

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other cancer types. In addition, hormon therapy has been applied particularly in the treatment of breast cancer in recent years. However, between 5 and 20 years after primary diagnosis, the risk of distant recurrence ranged from 13% to 41% depending on the tumor and nodal status of the primary tumor [4].

Breast cancer is a multi-step disorder which factors like genetic, endocrine, nutriment play a crucial role in the development and spread. Several studies showed that obesity or increased body mass index as two significant side effects is associated with breast cancer. The first one is the connection between increased body mass index in pre- and the post-menopausal women and advanced breast cancer. The second one is that increased body mass index corelated with a poor prognosis and accordingly an increase of metastasis to distant sites and high mortality [5-7]. On the other hand, few studies have observed no association between body mass and breast cancer prognosis [8-12]. Up to now, it is uncertain if obesity is related to the risk of occuring particular breast cancer subtypes (luminal A, luminal B, basal-like and human epidermal growth factor receptor (HER-2)) in before and after menopause.

The purpose of this study are to evaluate relationship between BMI and recurrence/survival and to determine whether BMI is a risk factor in breast cancer patients.

METHODS

Patients

The Ankara University Ibni Sina Hospital Database was searched and 125 patients with breast cancer from 2005 to 2014 were identified. The institutional review board approved the retrospective evaluation of medical records for the aim of this study.

Age, sex, menopausal situation, body mass index, co-morbidity, total survival and time of death were recorded. Tumor properties, comprising size, grade, number of involved axillary nodes, and estrogen and progesterone receptor status, cerbB-2 overexpression were summarized from related diagnostic pathology papers. Other knowledge taken from medical reports contain adjuvant therapy components (chemotherapy, hormonal therapy, radiotherapy) and details on first distant recurrence and death.

Obesity was determined according to body mass index (BMI), identified by weight in kilograms (kg) divided by the square of height in meters (m^2). According to National Centre for Disease Control and Prevention guidelines [13], body mass index were categorized as underweight, < 18.5 kg/m^2; normal weight, 18.5-24.9 kg/m^2; overweight, 25.0-29.9 kg/m^2; obese, ≥ 30 kg/m^2. Self-reported height and weight 1 year, as reported in diagnosis were used.

Inclusion and Exclusion Criteria

Breast cancer patients at least 18 years old were included in Ankara University Faculty of Medicine, Oncology Department. Studies were eligible for exclusion in retrospective analysis if they met the following criteria: (i) had a metastasis to distant organs, (ii) not applied in clinics for the last 2 years and (iii) had a synchronic or metachoronous malignite.

Pathology

Breast pathologists examined all pathologic samples. The grade and immunohistochemical analysis of estrogen receptor (ER), progesterone receptor (PgR), and HER-2 status were decided. In brief, invasive breast cancer was diagnosed by needle biopsy of breast. Clinical stage was specified by the 6th edition of Cancer Staging Manual of the American Joint Committee on Cancer [14]. The histological special type of cancer was determined consistent with the classification system of WHO [15]. The grade was specified on the authority of modified Black's nuclear grading system. Immunohistochemical evaluation to find ER and PGR status was carried out using standard immunohistochemistry methods with monoclonal antibodies. Nuclear staining 10% was regarded a positive outcome. HER-2 status was determined using immunohistochemistry or by fluorescence in situ hybridization. Polymerase chain reaction (pCR) was specified as no basis of invasive breast carcinoma and axillary lymph nodes at surgery.

Treatment

Generally, all patients were given 3 to 6 courses of anthracycline or taxane-based chemotherapy regimens. All patients had axillary staging with axillary lymph node dissection or sentinel node biopsy. Radiation therapy was applied in case of breast conservation surgery, locally advanced disease, primary tumor

size before chemotherapy of 5 cm and ≥ 4 involved axillary nodes. Hormonal therapy was given on the authority of standard practice.

Statistical Analysis

Statistical analysis was performed using SPSS (Statistical Package for Social Sciences) for Windows version 15.0 (standard version) programme (SPSS Inc., Chicago, IL). Numeric data are given as mean ± standard deviation (SD).

Chi-square test incorporating Yates correction and Fisher's exact test were used for 2×2 probability tables in nonnumeric data. Spearman rho correlation test was done for correlation analyses between quantitative parameters. To compare groups, one-way or multi-way analysis of variance (ANOVA) were used. Kruskal Wallis-H variance analyses was used for more than two groups in case of non-normal distribution. For survival analysis, Kaplan-Meier curves were plotted, and differences between the curves were analyzed with the log-rank test. Results of $p < 0.05$ were considered statistically significant at 95% confidence interval.

Disease-free survival (DFS) is defined as the time between diagnosis of disease and recurrence or distant metastasis. Overall survival is defined as the time from diagnosis of disease to death of patients of breast cancer.

RESULTS

Total of 125 operated breast cancer patients were studied retrospectively. Among these women, the mean age was 55 ± 12 years. 38.4% of patients were under the age of 50, while 61.6% were up to 50 years. Of the participants who reported menopausal status at diagnosis, 52% were pre-menopausal and 48% were post-menopausal. Blood group A is the most frequent blood group (65.6%) among patients with breast cancer. The mean height of the study participants was 158 ± 5.6 cm, and mean weight was calculated as 73 ± 18.7 kg. The mean BMI was of 28.6 ± 5.43 kg/m². BMI of 72 patients (57.6%) was non-obese group (BMI < 30 kg/m2), while BMI of 53 patients (42.4%) were found obese (BMI ≥ 30 kg/m²).

As can be seen in Table 1, the relationship between BMI and other prognostic factors of breast cancer were evaluated. Significant correlation was found between BMI and age, axillary dissection, stage, comorbidity. No relationship was found between BMI and histology, grade, ER, PGR, HER2 positivity, tumor diameter, positive lymph nodes, lymphovascular invasion, surgical procedure type, tamoxifen use, smoking, blood groups, hormone therapy, radiation therapy, chemotherapy, a number of localization in recurrence statistically. The most common diseases were diabetes (29.87%) and hypertension (28.57%) was observed.

No relationship was found between BMI and and CA-15-3/CEA values. No significant difference was found between obese and non-obese cases from the point of BMI and CA-15-3/CEA values ($p = 0.08$ and $p = 0.509$). The average of CA-15-3 and CEA values to BMI were given in Table 2.

To determine the relationship between BMI and death in univariate analysis, no significant difference was found between average BMI of death group and average BMI of survival group using Mann Whitney U test ($p = 0.6$). To group all patients according BMI, no significant difference was found BMI and death using Pearson chi square test (BMI > 30 kg/m² vs BMI 18.5-24.9 kg/m² $p = 0.81$). A total of 125 patients, 82 patients (65.6%) is still alive, 43 patients (34.4%) were dead.

Kaplan Meier plots were withdrawn to establish the impact of BMI in cerbB2 and disease-free survival (DFS) period. As can be seen in Fig. 1, there were no significant differences between 2 groups statistically ($p = 0.1566$). The mean DFS follow-up was 34 ± 5.4 months (95% Cl: 23.34-44.66) and 25 ± 3.12 months (95% CI: 18.87-31.12) for non-obese patients and obese cases, respectively.

To establish BMI in CEA value, the mean DFS follow-up was 31 ± 3.80 months (95% Cl: 23.54-38.46) and 28 ± 5.61 months (95% CI: 16.98-39.013) for non-obese patients and obese patients, respectively. The mean overall survival follow-up was 70 ± 7 months (95% Cl 57-83) and 70 months ± 10 (95% CI 50-90) for non-obese patients and obese patients, respectively.

To establish BMI in CA-15-3 value, the median DFS follow-up was 28 ± 2.32 months (95% Cl: 23.45-32.55) and 35 ± 9.31 months (95% CI: 16.74-53.25) for non-obese patients and obese patients, respectively. The mean overall survival follow-up was 70 ± 4 months (95% Cl: 62-78) and 97 ± 26 months (95% CI: 45-149) for non-obese patients and obese patients, re-

Table 1. Relationship between BMI and patient properties in non-obese and obese patients

	Body Mass Index (BMI)				*p value*
	< 30		≥ 30		
	n	%	n	%	
Age					0.536
< 50	15	44.1	33	36.3	
≥ 50	19	55.9	58	63.7	
Menopausal Situation					0.689
Pre	19	55.9	46	50.5	
Post	15	44.1	45	49.5	
Grade					0.954
1	1	3.1	2	2.3	
2	15	46.9	43	48.9	
3	16	50	43	48.9	
Stage					**0.02**
1	4	12.5	5	5.7	
2	18	56.3	35	44.2	
3	10	31.3	48	48.3	
ER					0.528
Negative	13	38.2	29	31.9	
Positive	21	61.8	62	68.1	
PGR					1
Negative	9	26.5	24	26.7	
Positive	25	73.5	66	73.3	
HER-2					0.544
Negative	21	61.8	49	53.8	
Positive	13	38.2	42	46.2	
Positive Lenf Node					1
No	15	44.1	39	43.8	
Yes	19	55.9	50	56.2	
Axillary Dissection					**0.024**
No	12	35.3	14	15.4	
Yes	22	64.7	77	84.6	
Lenfovascular Invasion					1
Negative	11	33.3	31	35.2	
Positive	22	66.7	57	64.8	
Surgery Type					0.734
Mastectomy (MRM)	30	88.2	83	91.2	
Prophylactic (BCS)	4	11.8	8	8.8	
Tamoxifen Usage					0.193
No	14	41.2	25	27.5	
Yes	20	58.8	66	72.5	
Cigarette Usage					1
No	28	82.4	73	80.2	
Yes	6	17.6	18	19.8	
Hormone Therapy					1
No	13	38.2	35	39.3	
Yes	21	61.8	54	60.7	
Radiation Therapy					0.081
No	14	41.2	22	24.7	
Yes	20	58.8	67	75.3	
Comorbidity					**0.026**
No	25	73.5	46	50.5	
Yes	9	26.5	45	49.5	

ER = Estrogen receptor; HER-2 = Human epidermal growth factor receptor, PGR = Progesterone receptor, MRM = Modified Radical Mastectomy, BCS = Breast-conserving Surgery

Table 2. Relationship between BMI and cerbB2, CA-15-3, CEA values

Body Mass Index (kg/m^2)	c-erbB-2 (%)	CA 15-3	CEA
< 18.5	50	494.7	25
18,5-24.9	357	47.31	5.86
25-29.9	47.5	55.43	9.45
≥ 30	45.5	43.,26	13.68

BMI = Body Mass Index, CA 15-3 = Carcinoid antigen, CEA = Carcinoembriyonic antigen

spectively.

Kaplan Meier plots were withdrawn to establish the impact of BMI in DFS period. As can be seen in Fig. 2, there were no significant differences between 2 groups statistically (p = 0.761). The mean DFS follow-up was 28 ± 4.49 months (95% Cl: 19.18-36.81) and 31 ± 3.35 months (95% CI: 24.44-37.56) for non-obese patients and obese patients, respectively.

Kaplan Meier plots were drawn to establish the effect of BMI in overall survival. As can be seen in Fig. 3, there were no significant differences between 2 groups statistically (p = 0.659). The mean overall survival follow-up was 75 ± 4.49 months (95% Cl: 56-94) and 73 ± 3.35 months (95% CI: 46-100) for non-obese patients and obese patients, respectively.

DISCUSSION

In this study, there was no significant difference between BMI and recurrence/death. Overall survival of non-obese patients (< 30 kg/m^2) was found to be longer compared to obese patients (> 30 kg/m^2) in survival analysis.

To date, relationship between obesity and risk of developing different breast cancer subtypes have not been clarified. In our study, a statistically significant association of obesity with HER2 status was not identified in terms of disease-free survival as can be seen in Fig. 1. Evaluation of HER2 status in the large groups would be of particular interest because that would allow investigation of obesity associations in women with HER2(+) as well as HER2(-). Biglia *et al*. [16] studied BMI effect in disease-free survival in 2150 women that experienced breast tumor surgery and follow up. In conclusion, a larger tumor size has been found to be associated high BMI both pre and postmenopausal women. Obese pre-menopausal women had large number of metastatic axillary node

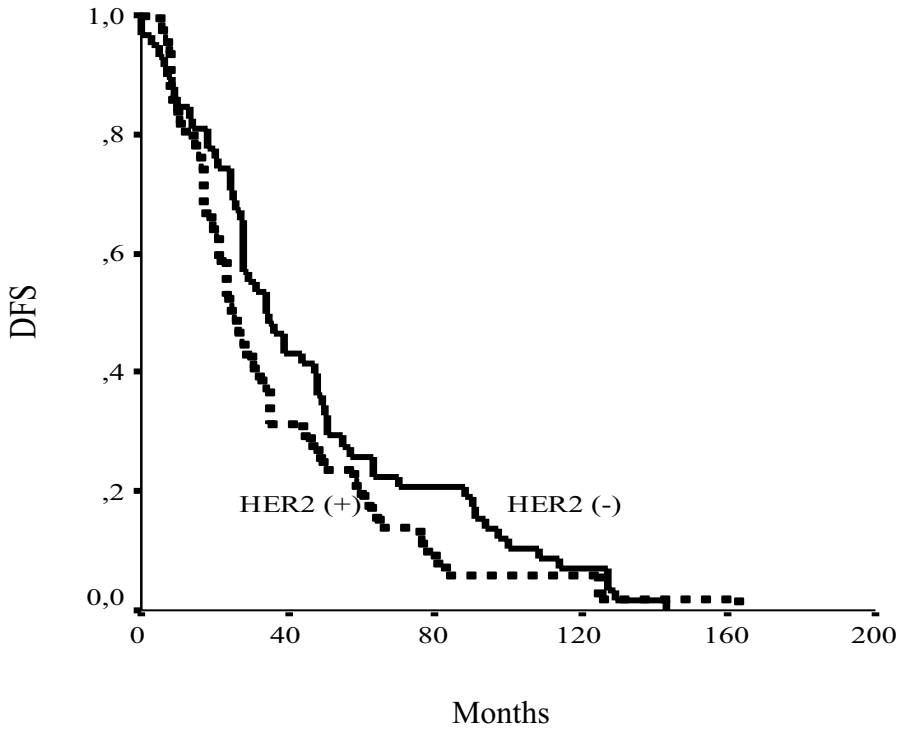

Fig. 1. Disease-free survival graphics between BMI and cerbB2.

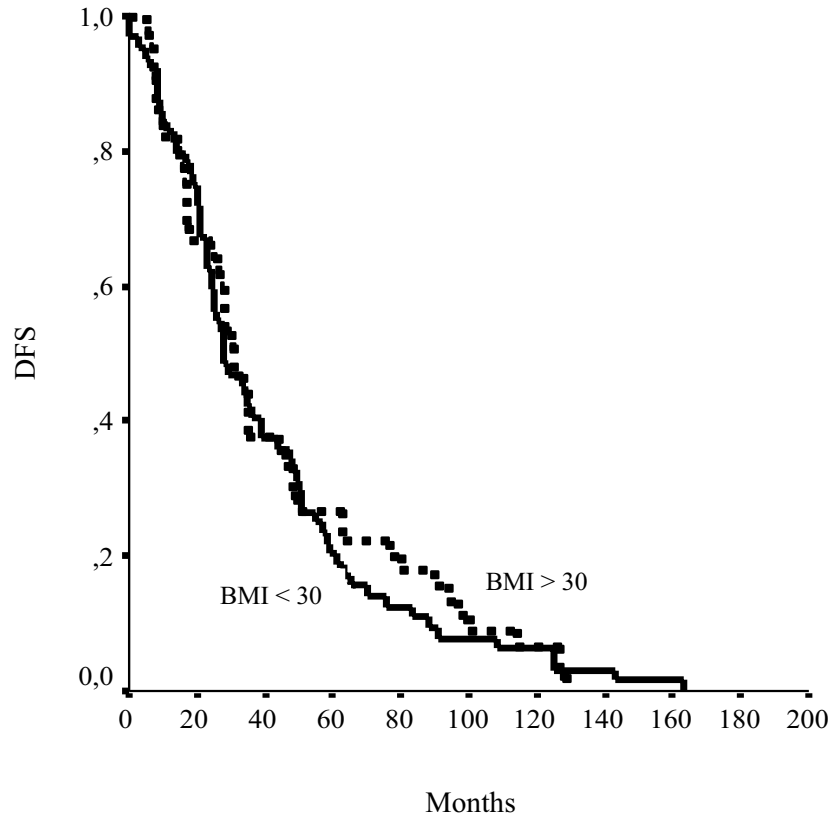

Fig. 2. Relationship between BMI and disease-free survival.

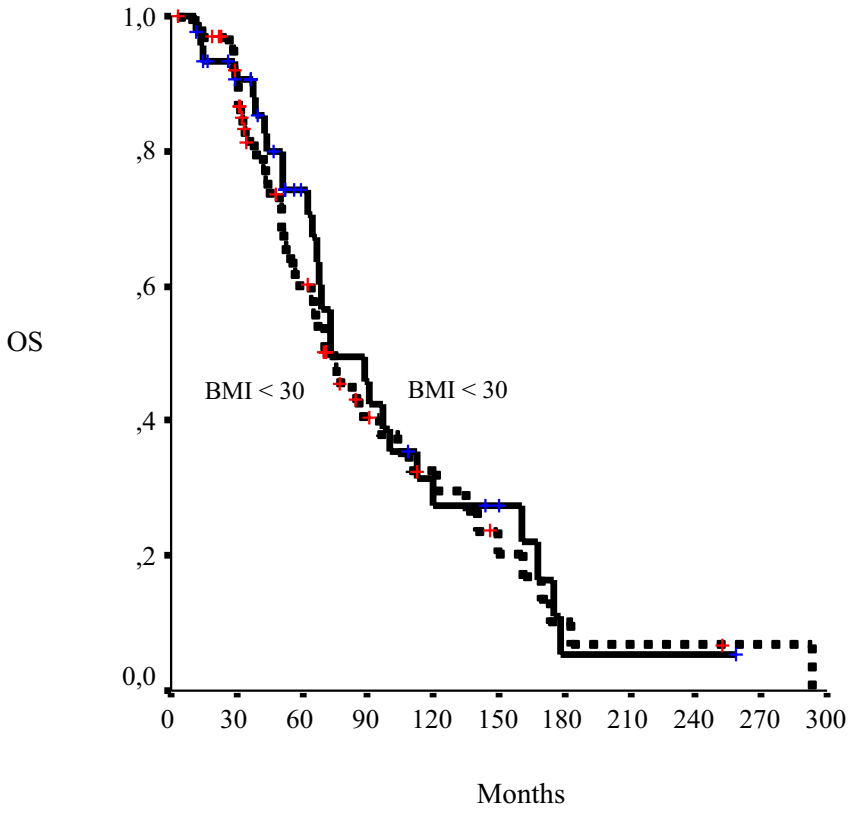

Fig. 2. Relationship between BMI and overall survival.

and a more commonly tumor vascular invasion than non-obese women [16]. Turan *et al*. [17] searched the relationship of breast cancer and breast cancer subtypes with menopausal status and body mass index. It was found that the number of overweight and obese patients was statistically significantly higher in the postmenopausal group. However, no statistically significant correlation was found in the analyses performed between BMI and breast cancer immunohistochemical subtypes in either group. The findings of this study support that obesity causes an increased risk of breast cancer in the postmenopausal period, independent of breast cancer immunohistochemical subtypes. The relationship between obesity and breast cancer should be evaluated together with risk factors [17].

Beypınar *et al*. [18] studied BMI effect to prognosis in young breast cancer patients. Computed tomography (CT) was applied to determine body composition precisely. Although the median overall survival cannot be reached at the end of the follow-up period for both groups (sarcopenic and non-sarcopenic), the difference between groups statistically insignificant. In this study, sarcopenia, may be seen in patients with breast cancer under 40 years old, was not found to be have a prognostic effect [18].

De Azambuja *et al*. [19] studied the impact of BMI on OS and DFS in node-positive breast cancer cases who doxorubicine and docetaxel-based adjuvant chemotherapy. Estimated five years overall survival was 87.5% and 82.9% for non-obese and obese cases, respectively ($p = 0.013$). Estimated five years DSF survival was 75.9% and 70.0% for non-obese and obese cases, respectively ($p = 0.041$). In a multivariate model, obesity continued an independent prognostic determinant for overall and disease-free survival [19].

Chan *et al*. [20] searched for 82 follow-up meta analysis of breast cancer patients with BMI before and after diagnosis, and total and cause-specific mortality until 2013. For BMI previous to diagnosis, comparison with non-obese women, the relative risks of total mortality were 1.41 for obese (BMI > 30.0), 1.07 for overweight (BMI 25.0- < 30.0) and 1.10 for underweight (BMI < 18.5) women, respectively. Obesity is related with poor overall survival and breast cancer survival in pre-menopausal and post-menopausal breast cancer. Being overweight is also associated with a higher risk of mortality [209.

Dawood *et al*. [21] examined the prognostic effect of obesity amongst women with early stage triple receptor-negative breast cancer (TN), a subtype of breast cancer known to be related poor prognosis. Five years DFS was 61%, 62%, and 62% amongst patients with BMI of < 25, 25 to 29.9, and ≥ 30, respectively. There was no significant increase among all groups in risk of distant metastases. Patients with TN disease were shown a poor prognostic result without considering BMI category. The outcome of this study suggest that obesity did not have a function as a prognostic indicator amongst patients with TN disease [21]. In another study, no significant relation was found between obesity and recurrence-free survival (RFS) or overall survival emerged in patients with TN disease after controlling for clinically significant factors [22].

Ewertz *et al*. [23] searched BMI effect in recurrence and survival for 18,967 women treated for early-stage breast cancer. Both chemotherapy and hormone therapy appear less efficient after ≥ 10 years for cases with BMI greater than 30 kg/m^2. In addition, obesity is an independent predictive component for occuring distant metastases and for death as a consequence of breast cancer [23].

Absence of significant molecular biomarkers such as cerbB2 in previous studies and breast cancer which is a heterogenous group in current studies can be interpreted as main reasons of differences between studies. Although no significant differences were observed between cerbB2, CEA, CA 15-3 and DFS, overall survival as a result of analysis between BMI and tumor markers in our study, significant difference is expected to be seen by increasing the patient data.

In this study, it was found to be significant difference between BMI and comorbidity. Diabetes (29.8%) and hypertension (28.6%) are the most common type of disease, body mass index 30 kg/m^2 is greater (obese) patients are seen by 59.3%. Several studies confirmed that hypertension and diabetes in women with breast cancer is related to decreased survival. However, there is no explanation about comorbidity cause whether increase of breast cancer recurrence and breast cancer-specific mortality risk. At the same time, which comorbidities that affect the mechanism of recurrence risk has not been announced yet. Comorbidities are associated with particularly poor quality of life, especially physical health [24]. Lower scores in physical health scale are related to low physical activ-

ity and obesity is also linked to high concentrations of circulating estradiol [25].

Tobias *et al*. [26] studied the relationship between BMI and mortality among patients with type 2 diabetes. No confirmation was obtained lower mortality amongst patients with diabetes who were overweight or obese at diagnosis, in comparison with non-obese patients, or of an obesity paradox. Patterson *et al*. [27] studied whether comorbid medical conditions predict additional breast cancer events and all-cause mortality in women with a history of early-stage breast cancer. Patients with diabetes has two times more risk in terms of incidence and mortality. The presence of multiple comorbidities was found to be significantly lost on additional breast cancer events.

In this study, it was found to be significant difference between BMI and stage. Obese patients have advanced cancer compared with non-obese patients. Although, obesity is known risk factor in postmenopausal breast cancer, relationship between obesity and stage can not be explained. Many hypothesis declared that increased breast size cause to delay diagnosis of breast cancer.

Cui *et al*. [28] examined whether there is an association between BMI and stage in breast cancer. At the end of this study, higher BMI was found to be related advanced breast cancer. Due to difficulties of palpation and access to mass which is the most common symptom, diagnosis delays so women with higher BMI are be diagnosed at a later stage of breast cancer [28]. In addition, several studies showed that women with higher BMI has larger tumors and advanced breast cancer compared to women with low BMI [29].

Limitations

Our work that is a retrospective kohort study is a limiting factor. Due to limitation of patient data, access to patients by phone, follow up in other medical centers, treatment schemes and death time, a number of patients leads to limited. The prognostic effect of obesity in breast cancer and new treatment approaches should be considered with long follow up studies. In the future, a large number of randomized, prospective studies will provide more appropriate treatment approaches. It can be described that obesity has any negative effects before and after diagnosis of breast cancer. Therefore, we think that ideal weight is be considered in follow up for breast cancer patients.

CONCLUSION

In conclusion, no significant difference was observed between BMI and recurrence/death. The coming studies with long-term follow up are required to search the effect of body mass index on recurrence and survival in breast cancer patients.

Ethical Approval

The study protocol was approved by the Institutional Ethics Committee of Ankara University School of Medicine.

Authors' Contribution

Study Conception: BE, GU; Study Design: BE, GU, AD; Supervision: GU; Funding: GU; Materials: GU; Data Collection and/or Processing: BE, GU; Statistical Analysis and/or Data Interpretation: GU; Literature Review: BE, GU; Manuscript Preparation: BE, GU and Critical Review: GU.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Knowledge levels of mothers with children aged 0-6 about autism spectrum disorder

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ABSTRACT

Objectives: In this study, it was aimed to measure the level of knowledge about autism spectrum disorder (ASD) in mothers of children aged 0-6 and to determine the necessity of informative studies on this subject and the characteristics of the group that should be focused on in future studies.

Methods: The population of the study consisted of mothers with children aged 0-6 years who applied to the pediatric outpatient clinic. In order to measure ASD knowledge, 24 questions were created by scanning the literature.

Results: 388 mothers were included in the study. There was a significant difference in educational status regarding ASD knowledge score ($p = 0.042$). There was a significant difference between economic status in terms of ASD knowledge score ($p = 0.026$). Those who had heard of ASD had a significantly higher ASD knowledge score than those who had not heard of ASD ($p < 0.001$). The ASD knowledge score of those who had acquaintances with ASD was significantly higher than those who had not ($p = 0.001$). There was a significant negative correlation between ASD total score, age, and first gestational age.

Conclusions: Increasing the level of knowledge about ASD will enable early diagnosis and early intervention in the course of the disease and the rapid integration of individuals with ASD into society.

Keywords: Autism spectrum disorder, awareness, early diagnosis

Autism spectrum disorder (ASD) is a chronic neurodevelopmental disorder that has the potential to emerge in the first years of life. While ASD patients do not look physically different from others, their understanding, communication, learning processes, and behaviors differ. This difference has a broad spectrum [1]. While some patients can lead a near-everyday life with minimal support, others may need care.

ASD was defined separately from other childhood diseases by the American Psychiatric Association in 1980 and was classified as five diseases in 1994: Autism, Asperger's Syndrome, Atypical Autism, Childhood Disintegrative Disorder, and Rett Syndrome. However, in 2013, Rett Syndrome was excluded from this category, and all were defined as ASD based on social communication and isolated-repetitive sensory activities [2]. While one out of 59 children is diagnosed with ASD in the United States, this rate is estimated to be around 1% globally [3, 4]. It has been observed that the prevalence of ASD has

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been rising in recent decades, and it has been determined that boys are at 4.2 times more risk than girls [2]. It is thought that the increasing prevalence, developing technology and facilitating access to information, and the fact that families seek to diagnose their children are compelling.

Although ASD can be identified after 1.5 years of age, the average age of diagnosis is estimated to be 3.3 [5]. It has been found that the diagnosis of children with an older brother/sister can be made earlier than those without [6]. Although there is no specific indicator or diagnostic test to be a reference, behavioral disorders such as avoiding eye contact at an early age, not spending time with peers, limited vocabulary, and the desire to comply with daily routines are valuable findings for diagnosing ASD [1]. Despite its pathogenesis is not known for sure, it is believed that factors such as lifestyle, environmental factors, genetic components, and maternal age over 40 play a role in the etiology [7].

It is crucial that awareness of ASD is kept high throughout society. Because when families have sufficient information about ASD, their awareness level will increase in order to diagnose children earlier, and they will be able to provide the necessary social conditions for the child to have a chance to lead an everyday life in addition to the professional help that the child should receive [8]. In addition, it is essential to keep the knowledge level of society high so that children with ASD and their families are accepted by society and receive the necessary social support.

This study intended to measure the level of knowledge about ASD in mothers of children aged 0-6 and to determine the necessity of informative studies on this subject and the characteristics of the group that should be focused on in future studies.

METHODS

Study Design

The research was undertaken as a cross-sectional and descriptive study. This study was approved by Local Ethics Committee (Decision Number: 2021/09-13, Decision Date: 16/11/2021). The study was conducted through face-to-face interviews with parents with children aged 0-6 years who were referred to the pediatric outpatient clinic. Data collection was carried out between December 2021 and March 2022. The study population consisted of mothers with children aged 0-6 years who applied to the pediatric outpatient clinic. Three hundred eighty-eight mothers were included in the study with a 95% confidence level and a margin of error of 0.05. A questionnaire form was used as a method of obtaining data, and a consent form was obtained from the participants. A questionnaire was administered to the mothers in the form of in-person interviews.

Questionnaire Form

In order to measure ASD knowledge, 24 questions

Table 1. Sociodemographic characteristics of the participants

	Data
Age (years), Mean ± SD	27.7 ± 5.8
Educational status, n (%)	
Middle school and below	182 (49.6)
High school	135 (34.8)
University	71 (18.3)
Spouse's educational status, n (%)	
Middle school and below	126 (32.5)
High school	189 (48.7)
University	73 (18.8)
Economical situation, n (%)	
Good	179 (46.1)
Moderate	127 (32.7)
Bad	82 (21.1)
First gestational age (months), Mean ± SD	22.4 ± 3.1
Number of children, Mean ± SD	1.8 ± 1.1
Number of children aged 0-6, Mean ± SD	1.2 ± 0.4
The state of hearing about autism spectrum disorder, n (%)	
Yes	234 (60.3)
No	154 (39.7)
Do you know anyone with autism spectrum disorder?, n (%)	
Yes	150 (38.7)
No	238 (61.3)

were created by scanning the literature. Those who answered the questions correctly were given 1 point, and those who gave wrong answers and said they had no idea were given 0 points. The ASD knowledge level of those who answered more than half of the right questions (13 or more) was accepted as high.

Statistical Analysis

Analyzes were conducted using the SPSS (Statistical Package for Social Sciences; SPSS Inc., Chicago, IL) 22.0 software package. In the study, descriptive statistics are displayed as n and % values in categorical data and mean±standard deviation (Mean±SD) values in continuous data. Chi-square analysis (Pearson Chi-square) was carried out to compare categorical variables between groups. The Kolmogorov-Smirnov test evaluated the conformity of continuous variables to normal distribution. Student t-test was used for the comparison of paired groups. One Way ANOVA analysis was performed to compare more than two variables. The Pearson correlation test was used to examine the relationship between continuous variables. The statistical significance level in the analysis was assumed to be $p < 0.05$.

RESULTS

Three hundred eighty-eight women were enrolled in the study. The mean age of the women was 27.7 ± 5.8 years (min = 20-max = 55). The education level of 182 (46.9%) women was secondary school or below, 135 (34.8%) were high school graduates, and 71 (18.3%) were university graduates. Education level of 126 (32.5%) spouses in secondary school or below, 189 (48.7%) are high school graduates, and 73 (18.8%) are university graduates. The economic status of 179 (46.1%) of the women is favorable, 127 (32.7%) of

Table 2. Comparison of ASD knowledge score according to educational status, economic status, hearing about ASD, and acquaintance with ASD

		Total score Mean ± SD	*p* value
Educational status			
	Middle school and below	8.7 ± 3.5[a]	**0.042***
	High school	9.3 ± 4.0[a,b]	
	University	10.0 ± 4.5[b]	
Spouse's educational status			
	Middle school and below	9.2 ± 4.2	0.968*
	High school	9.2 ± 3.7	
	University	9.1 ± 3.9	
Economical situation			
	Good	9.7 ± 4.5[a]	**0.026***
	Moderate	8.9 ± 3.3[a,b]	
	Bad	8.4 ± 2.7[b]	
The state of hearing about autism spectrum disorder			
	Yes	9.7 ± 4.1	**< 0.001****
	No	8.3 ± 3.4	
Do you know anyone with autism spectrum disorder?			
	Yes	10.0 ± 3.5	**0.001****
	No	8.7 ± 4.0	

*One Way ANOVA analysis, **Student t test was applied. [a,b]Group from which the difference originates

them are moderate, and 82 (21.1%) of them are bad. The mean first gestational age of the mothers was 22.4 ± 3.1 months (min = 18-max = 36). The mean number of children of the mothers was 1.8 ± 1.1 (min = 1-max = 8), and the mean number of children aged 0-6 years was 1.2 ± 0.4 (min = 1-max = 3). Of the mothers, 234 (60.3%) had heard of ASD, and 150 (38.7%) had acquaintances with ASD (Table 1). The mean ASD total knowledge score of the mothers was 8.2 ± 3.6, and the median score was 18 (min = 0-max = 22).

There was a significant difference in educational status regarding ASD knowledge scores (p = 0.042). This difference was due to the difference between those who graduated from secondary school and below and those who were university graduates. It was determined that the scores of those who graduated from university were higher. There was a significant difference between economic status in terms of ASD knowledge score (p = 0.026). This difference was due to the difference between those with good economic status and those with bad economic status, and it was determined that the scores of those with good economic status were higher.

Those who had heard of ASD had a significantly higher ASD knowledge score than those who had not heard of ASD (p < 0.001). The ASD knowledge score of those who had acquaintances with ASD was significantly higher than those who had not (p = 0.001). (Table 2). There was a significant negative correlation between ASD total score, age, and first gestational age (Table 3).

ASD knowledge level was high in 70 (18%) mothers and low in 318 (82%) mothers. It was observed that the level of knowledge was high in 12.4% of those with secondary education and below, 20% with high school graduates, and 28% with a university degree. This difference was found to be statistically significant (p = 0.01). ASD knowledge level was high in 23.7% of those with good economic status, 18.4% with moderate, and 3.9% with poor economic status. There was a significant difference between economic status and ASD knowledge level (p = 0.001).

Twenty-two point six percent of those who had heard of ASD and 11% who had not heard of ASD were found to have a high level of ASD knowledge, and a significant difference was found between them (p = 0.004). ASD knowledge level was high in 24% of those who had acquaintances with ASD and 14.3% of those who had not, and a significant difference was found between them (p = 0.015).

Table 3. Correlation of ASD knowledge score with age, gestational age and number of children

	Total score	
	r	p value*
Age	**-0.127**	**0.012**
First gestational age	**-0.117**	**0.021**
Number of children	0.017	0.743
Number of children aged 0-6	0.040	0.438

*Pearson correlation analysis was applied.

DISCUSSION

Determining the level of knowledge about ASD is essential not only for the individual with ASD and his/her immediate environment but also for society in general. Early diagnosis of the disease will provide early support for the development of the individual with ASD. It will enable the individual to adapt to family life and the social order more quickly. ASD does not only affect individuals. The implementation of the individual's daily routines and conditions, such as sleep disorders, can affect the whole family.

For this reason, preparations for this disease group should target the ASD-affected community, which includes individuals with ASD, as well as their families and the immediate affected environment. In this study, 24 questions were asked to determine the knowledge level of the participants, but the participants were able to answer 8.2 of these questions correctly on average. The level of knowledge is considered relatively low. In a study conducted with 339 parents in Pakistan in 2018, it was found that the knowledge level of the participants about ASD was low in parallel with our study [8]. On the other hand, the public's knowledge level was high in studies conducted in the United States of America, Australia, and Saudi Arabia [2, 9, 10]. In order to increase the level of knowledge, it is recommended to provide parents with short-term training in pairs. In a study conducted in India, informative training on ASD was organized for parents, and a significant increase was observed in the knowledge levels of

the participants after the training [8].

In this study, the ASD knowledge levels of those who heard about ASD and had contact with individuals with ASD were significantly higher than those who did not hear about ASD and did not know anyone with ASD. In a study conducted in Australia, the level of knowledge of those who had contact with individuals with ASD was significantly higher than those who did not [9]. In a study in Pakistan in which families with children with ASD and health professionals were not evaluated, the level of knowledge of participants who heard about ASD was significantly higher than those who did not [8]. Previous research supports our study. Again, in a study conducted with 1054 participants in Australia, it was stated that the factor affecting knowledge level the most was spending time with individuals with ASD [11]. It is thought that individuals who have heard of ASD and know people with ASD have researched this area and obtained information about ASD from mass media, such as social media or television, in their daily lives [2].

In this study, when the level of knowledge was measured according to education level, it was determined that university graduates had a significantly higher level of knowledge than other education levels. In a study conducted in Australia with 478 participants, a correlation was found between educational status and the level of knowledge [9]. In a study conducted in Saudi Arabia with 500 participants, the knowledge level of master's/doctorate graduates was significantly higher than other education groups [2]. Previous studies show that there is a correlation between the level of education and the level of knowledge about ASD in general.

When the income status of the family and the level of knowledge about ASD were examined, it was observed in our study that participants with a good income had a higher level of knowledge than participants with poor income. In a study comparing the knowledge levels of ASD in America and China, a correlation was found between the economic situation and knowledge levels in China [12]. Likewise, the level of knowledge of those with good economic status in Pakistan was significantly higher than those of the middle and lower classes [8].

Although no significant correlation was detected between the level of knowledge and age in the literature, it was observed in this study that the level of knowledge declined with increasing age. This situation makes accessing informative channels (social media, television, etc.) challenging as age increases and adaptation to technology decreases.

CONCLUSION

Based on previous studies and the results of this study suggests that the ASD knowledge level of those who have not encountered individuals with ASD may be insufficient. For this reason, in order to increase the ASD knowledge level of the public, it is necessary to use social media actively, to include informative programs about ASD through channels such as television/radio, and to organize training that is easy to reach, especially for parents and prospective parents. Increasing the level of knowledge in ASD will enable early diagnosis and early intervention in the course of the disease. This will enable the rapid integration of individuals with ASD into society.

Authors' Contribution

Study Conception: MEP, EÖ, MYÖ; Study Design: OK, YK; Supervision: MEP, YK, MYÖ; Funding: N/A; Materials: N/A; Data Collection and/or Processing: EÖ, OK, MYÖ; Statistical Analysis and/or Data Interpretation: MEP, YK; Literature Review: MEP, EÖ, YK; Manuscript Preparation: MEP, OK, YK, MYÖ and Critical Review: EÖ, YK, MYÖ.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The European Research Journal 2023;9(4):785-791 Original Article

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The relationship of university students' childhood mental trauma with depression, anxiety and stress

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ABSTRACT

Objectives: The aim of this study is to examine the relationships between childhood mental trauma experiences, depression, anxiety and stress concepts.

Methods: The research is a descriptive and relationship-seeking type of study. The study sample consisted of 192 students. Data were collected with the "Personal Information Form, Childhood Mental Trauma Scale and Depression Anxiety Stress Scale Short Form".

Results: The rate of students who stated that they were exposed to any trauma in their childhood is 74%. The majority of students reported physical (28.2%) and emotional abuse (33.1%). Those who were exposed to childhood traumas had high Depression Anxiety Stress Scale anxiety and depression subscale scores, and the difference between them was statistically significant. When the correlations between the scales are examined; There was a positive correlation between Depression Anxiety Stress Scale depression sub-dimension and Childhood Mental Trauma Scale total and physical abuse sub-dimensions, and a positive significant relationship between anxiety sub-dimensions and Childhood Mental Trauma Scale total, physical abuse, emotional abuse, and emotional neglect sub-dimensions.

Conclusions: Childhood traumas are one of the important risk factors especially for depression and anxiety disorders. It can be recommended to take mental health measures to prevent the negative consequences of childhood traumas observed in adulthood, to provide psychosocial support to students and to include practices that will increase psychological resilience.

Keywords: Childhood, mental trauma, depression, anxiety, stress, university students

Childhood traumas are the most difficult to detect and treat; are traumas that usually have long-term effects. Child abuse and neglect, which is examined under childhood traumas, negatively affects the health of the child and leaves permanent traces for life [1]. Childhood psychological traumas in childhood; cause cognitive, emotional, and physical problems. In adulthood, it causes psychopathological conditions such as stress, anxiety and depression. This situation negatively affects the well-being of individuals [2]. In addition, childhood traumas increase the risk of suicide and sleep problems [3, 4].

With this study, it is thought to reach information about the psychological problems experienced by university students in their young adulthood. When the literature is examined, it has been determined that the

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studies in which all concepts are examined together are limited. In this study, it is aimed to examine the relationships between the concepts of childhood mental trauma, depression, anxiety and stress, which have been the subject of various studies.

METHODS

The research is a descriptive and relationship-seeking type of study. The universe of this research consists of university students who actively continue their education at the associate degree level in the spring semester of the 2021-2022 academic years in the Vocational School of Health Services of a university. While determining the sample of the study, primarily departments (Elderly Care, Physiotherapy, Operating Room Services, Medical Documentation and Secretariat) were determined by random disproportionate cluster sampling. The sample size of the study was calculated as n = 178 with 90% confidence interval and ±5% sampling error. The study was completed with 192 students.

Criteria for Inclusion in the Research are the flolowing: (1) Be 18 years or older, (2) Be in a position to understand the study and give informed consent. Also, exclusion criteria include having a hearing or speech impairment that cannot communicate meaningfully, and receiving any psychiatric diagnosis.

Data Collection Tools

Personal Information Form, Childhood Mental Trauma Scale and Depression-Anxiety-Stress Scale Short Form were applied by the researcher.

Personal Information Form

This form consists of 9 questions containing sociodemographic information.

Childhood Trauma Scale (CTQ)

The scale developed by Bernstein *et al*. (1994) to evaluate pre-adulthood trauma experiences with the name "Childhood Traumas Inventory" was adapted into Turkish by Şar *et al*. [12] in 2012, Cronbach alpha value was found to be 0.93. The scale consists of 5 sub-dimensions and 28 items in order to determine experiences in three types of abuse (physical, emotional and sexual) and two types of neglect (physical and emotional). High scores obtained from the 5- Likert-type scale based on self-evaluation indicate the high level of childhood experiences based on trauma. Subscores are between 5-25, total score is between 25-125. In this study, the Cronbach alpha coefficient was calculated as .86.

Depression-Anxiety-Stress Scale (DASS21)

The Depression-Anxiety-Stress Inventory developed by Lovibond and Lovibond (1995) consists of a 42-item long form. The Turkish adaptation of the scale (short form, 21 items) was made by Yılmaz *et al*. [5] in 2017. Cronbach Alpha internal consistency coefficients of the three sub-dimensions of the scale; .84 for anxiety sub-dimension; It was tested as .91 for depression sub-dimension and .90 for stress sub-dimension. High scores on each of the depression, anxiety and stress dimensions indicate that the individual has a related problem. In this study, the Cronbach alpha coefficient for the whole scale was found to be .80.

Research Ethics

Approval for the research was obtained from the Ethics Committee of the relevant university (Decision no: 2022/25). Informed consent was obtained from the students before the study. In addition, there was no information in the study forms that would reveal the identities of the students.

Statistical Analysis

Descriptive analyzes (percentage, arithmetic mean, etc.), mean and standard deviation, t-test, Anova test and correlation analysis were used for the data obtained from the Personal Information Form.

RESULTS

Within the scope of the study, there are departments of Medical Documentation and Secretarial (34.4%), Operating Room Technician (10.4%), Physical Therapy Technician (23.4%) and Elderly Care (31.8%). The mean age of the students was 20.06 ± 1.75 years; 22.4% are male, 77.6% are female. The income level of 83.3% of the students is medium. 75.5% of the students are in the nuclear family type and more than half (58.3%) have 4-6 siblings. The place where the students spend their lives is mostly (79.2%) stated as the

city. The rate of students who stated that they were exposed to any trauma in their childhood is 74%. The majority of students reported physical (28.2%) and emotional abuse (33.1%) (Table 1).

The CTQ and DASS-21 Average Scores of the students are shown in Table 2. CTQ Physical abuse sub-dimension mean score was 11.10 ± 4.69; Emotional abuse sub-dimension mean score was 11.57 ± 4.79; The mean score of the sexual abuse sub-dimension was 8.96 ± 3.59; The mean score of the physical neglect sub-dimension was 11.44 ± 4.86; Emotional neglect sub-dimension mean score was 12.10 ± 4.62; The mean CTQ total score is 55.19 ± 11.58. DASS-21 subscale mean scores were 12.89 ± 4.70 for depression; it was determined as 10.94 ± 5.02 for anxiety and 11.58 ± 4.87 for stress (see Table 2). Those who were exposed to childhood traumas had high DASS-21 scale anxiety and depression subscale scores, and the difference between them was statistically significant (Table 3).

When the correlations between the scales are examined; A positive and very weak correlation was determined between DASS-21 depression sub-dimension and CTQ total and physical abuse sub-dimension. There was a weak and positive correlation between DASS-21 anxiety sub-dimension and CTQ total, physical abuse and emotional abuse sub-dimensions. The relationship between DASS-21 anxiety sub-dimension and emotional neglect sub-dimension is positive and very weak (Table 4).

Table 1. Distribution of characteristics identifying students

Age (year), Mean ± SD, (min-max)	**20.06 ± 1.75 (18-31)**	
Department	**n**	**%**
Medical documentation and secretarial	66	34.4
Operating room technician	20	10.4
Physiotherapy technician	45	23.4
Elderly care	61	31.8
Gender		
Male	43	22.4
Female	149	77.6
Income status		
Income less than expenses	25	13.0
Income equals expense	160	83.3
Income more than expenses	7	3.6
Number of sibling		
1-3	59	30.7
4-6	112	58.3
7 and above	21	10.9
Family type		
Nuclear family	145	75.5
Extended family	47	24.5
Where lives		
Village	32	16.7
Town	8	4.2
City	152	79.2
Childhood trauma exposure		
Yes	142	74
No	50	26
Trauma type (n = 142)		
Physical abuse	40	28.2
Emotional abuse	47	33.1
Sexual abuse	12	8.5
Neglect	28	19.7
Abuse and neglect	15	10.6
Total	**192**	**100.0**

DISCUSSION

The child's perspective on life and human relations is shaped by his traumatic experience and its consequences. These people carry the traces of this trauma in the relationships they establish throughout their lives and the roles they take in society. They may experience difficulties in fulfilling their responsibilities, inability to establish healthy relationships, and may turn to potential crimes [6]. Problems related to establishing close relationships and attachment can also be encountered by individuals in the later stages of life as difficulties in social, familial and romantic relationships [7].

The present study investigated the interaction of childhood traumas on depression, anxiety and stress in a sample of university students. Entering college life involves adapting at various levels. Being a university student brings with it the challenges of transitioning to an independent environment and coping with new responsibilities. Academic pressures and financial difficulties are the most common problems students face [8]. Childhood traumas added to these problems can cause psychological problems to increase.

In our study, the rate of students who stated that they were exposed to trauma in childhood was deter-

Table 2. Mean scores of Childhood Mental Trauma Scale (CTQ) and Depression-Anxiety-Stress Scale (DASS-21)

Scales	Min - Max	Mean ± SD
CTQ Total	28-92	55.19 ± 11.58
Physical Abuse	5-22	11.10 ± 4.69
Emotional Abuse	5-23	11.57 ± 4.79
Sexual Abuse	5-22	8.96 ± 3.59
Physical Neglect	5-23	11.44 ± 4.86
Emotional Neglect	5-23	12.10 ± 4.62
DASS-21		
Depression	1-21	12.89 ± 4.70
Anxiety	1-21	10.94 ± 5.02
Stress	1-21	11.58 ± 4.87

CTQ = Childhood Mental Trauma Scale, DASS-21 = Depression-Anxiety-Stress Scale

Table 3. Comparison of Childhood Trauma Exposure and DASS-21 Mean Scores

	DASS-21		
Childhood trauma exposure	**Depression**	**Anxiety**	**Stress**
Yes	13.61 ± 4.66	11.37 ± 5.14	11.86 ± 4.86
No	10.86 ± 4.23	9.72 ± 4.49	10.78 ± 4.84
Test and *p* value	t = 3.672 ***p* = 0.001**	t =2.018 ***p* = 0.045**	t =1.359 *p* = 0.176

DASS-21 = Depression-Anxiety-Stress Scale

Table 4. The Relationship Between Childhood Mental Trauma Scale (CTQ) and Depression-Anxiety-Stress Scale (DASS-21)

Scales	DASS-21		
CTQ	**Depression**	**Anxiety**	**Stress**
Physical Abuse	r = .195 ***p* = 0.007**	r = .211 ***p* = 0.003**	r = -.032 *p* = 0.656
Emotional Abuse	r = .113 *p* = 0.117	r = .230 ***p* = 0.001**	r = .019 *p* = 0.798
Sexual Abuse	r = .073 *p* = 0.315	r = -.103 *p* = 0.154	r = .049 *p* = 0.497
Physical Neglect	r = .094 *p* = 0.194	r = .111 *p* = 0.125	r = -.020 *p* = 0.782
Emotional Neglect	r = .065 *p* = 0.369	r = .171 ***p* = 0.018**	r = .101 *p* = 0.162
CTQ Total	r = .159 ***p* = 0.027**	r = .318 ***p* = 0.001**	r = .042 *p* = 0.564

CTQ = Childhood Mental Trauma Scale, DASS-21 = Depression-Anxiety-Stress Scale

mined as 74%. In another study, the rate of exposure to trauma in childhood was high (44.7%) [9]. The high results in our study may be due to cultural differences and social changes. The fact that physical (for example, violent behavior) and emotional (for example, bad words) abuse is used as a common discipline method in our country may have increased this rate. Our results show that behaviors used by parents as a discipline method are perceived as physical and emotional abuse by students. The use of physical or verbal violence as a discipline method is one of the biggest mistakes made in our country. Parents should be taught that children can only be disciplined with love and attention [10].

According to the World Health Organization (WHO), child maltreatment means abuse and neglect [11]. Physical, sexual, emotional abuse and neglect are difficult experiences before the age of 18 [8]. Physical abuse is generally defined as "any non-accidental physical injury to a child" and can include hitting, kicking, burning or biting a child, or any act that causes a child's physical impairment [12]. Emotional abuse is one of the most common types of abuse in daily life. The psychological harm of the child is the basis of emotional abuse [13]. Emotional abuse includes behaviors such as not accepting the child, discriminating, leaving alone, humiliating, intimidating, provoking, pretending to be absent, and showing no emotion [14]. The effects of childhood traumas do not only remain in childhood, their effects continue for a long time. These children may experience severe emotional disturbances and disturbances when faced with subsequent stressful events [15, 16]. Studies show that common childhood traumas cause an increase in physical and mental disorders in childhood and adulthood [17-19].

The majority of students in our study reported physical (28.2%) and emotional abuse (33.1%). In a study, it was found that 36.4% of those who were exposed to child neglect and abuse cases faced physical abuse, 16.4% emotional abuse, 16.4% sexual abuse and 20% neglect cases [20]. A recent systematic review of the international lifetime prevalence of childhood traumas revealed emotional abuse rates of approximately 21.7% in Europe and 23.9% in North America [21]. It has been suggested that exposure to adversities in early childhood is associated with psychiatric problems in adolescence or adulthood [22]. There is evidence of a relationship between child maltreatment (ie, physical abuse, emotional abuse, and/or neglect) and depression and anxiety [9]. The high rates of depression in our study are remarkable. It is known that certain experiences related to negativities in childhood significantly increase the risk of depression in adulthood [23, 24]. Even repeated traumas may cause more severe psychopathology symptoms [25]. In our study, both the depression and anxiety mean scores of the students who reported that they had childhood trauma were significantly higher. Studies have reported that depression, anxiety and stress are common among university students [26, 27]. In one study, students who reported negative events such as exposure to trauma during childhood were found to be approximately three times more likely to report current depression [22]. Our study findings are consistent with the literature.

In a national study, it was found that child abuse was positively associated with depression, anxiety and stress [28]. In other studies, a positive relationship has been reported between childhood traumas and depression [23, 29]. It is predicted that childhood traumas may trigger the onset, recurrence and worse outcomes (more comorbidity and chronicity) of these disorders [3]. In one study, childhood trauma was found to be associated with depression/anxiety symptoms in adulthood [30]. A meta-analysis also found strong evidence that exposure to childhood or adolescence trauma causes adverse mental health outcomes in adulthood [31]. In our study, it was found that childhood traumas were positively associated with depression and anxiety, in line with previous studies. Our study findings support the literature.

College years are associated with an increased risk for depression and anxiety. It is therefore necessary to make help with mental health problems accessible and available as early as possible. Every individual who takes care of children is obliged to know the basics of how to protect children from harm [34]. The World Health Organization states that nurses have an important place in the prevention of child neglect and abuse. In this context, it is recommended that nurses make home visits to parents. Nurses should provide training to families on the development of child-rearing skills [35].

Increasing individual resilience can be a helpful approach to prevent the negative consequences of

childhood traumas observed in adulthood. Resilience is of paramount importance in promoting health and well-being, especially when faced with challenges [8, 36]. For this purpose, it is important to raise awareness about mental health issues and intervention strategies to increase students' levels of resilience. In addition, it is recommended to raise awareness by planning trainings in order to provide psycho-social support to students and develop coping strategies.

Research findings show that there is a relationship between childhood traumatic experiences and depression and anxiety. This result is important in terms of awareness of childhood trauma experiences in students. It may be beneficial to add trainings such as awareness, resilience, and coping with stress to the curriculum of these health-related departments. In addition, it may be useful to conduct retrospective and prospective studies investigating the effects of childhood traumatic experiences.

Children are frequently exposed to trauma. Psychiatric nurses have important roles in the early detection and treatment of the effects of these traumas. Addressing the symptoms that develop after trauma in childhood can reduce the psychiatric symptoms seen in adolescence and adulthood.

Limitations

The small sample size and cross-sectionality limit the generalization of the results to the entire population. Our study was designed based on self-report. For this reason, they may have reported their own experiences differently, since the data obtained were based on students' reports. In addition, measurements of childhood distress do not adequately measure the severity or duration of traumatic experiences.

CONCLUSION

In summary, it can be said that the effects of childhood traumas do not remain only in childhood, but their consequences have negative effects on mental health throughout life. Childhood traumas are one of the important risk factors especially for depression and anxiety disorders [3]. In addition to the current occupational stresses of university students, the presence of childhood traumas can complicate both their personal and professional lives [32, 33]. Considering that difficulties such as abuse and neglect in childhood are associated with an increased probability of suicide in adults [4], it is thought that urgent measures should be taken regarding mental problems.

Authors' Contribution

Study Conception: AB; Study Design: AB; Supervision: AB; Funding: AB; Materials: AB; Data Collection and/or Processing: AB; Statistical Analysis and/or Data Interpretation: AB; Literature Review: AB; Manuscript Preparation: AB and Critical Review: AB.

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

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The relationship between serum uric acid levels and severity of addiction in individuals with substance use disorders

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ABSTRACT

Objectives: This study aimed to compare serum uric acid levels in patients with methamphetamine and synthetic cannabinoid use disorders pre- and post-detoxification treatment with healthy controls. Secondly, to determine the relationship between the serum uric acid levels of the cases and the severity of the addiction.

Methods: Fifty methamphetamine, 50 synthetic cannabinoids, and 40 healthy controls were evaluated on the specified dates. Venous blood samples were taken from the participants to measure serum uric acid and creatinine levels. The arrangement was made by taking the Uric acid/creatinine ratio to neutralize the confounding effect of kidney functions. The Addiction Profile Index was applied to determine the severity of substance abuse in the case group.

Results: A statistically significant difference was found between the uric acid values of all three groups when the One-way ANOVA test was performed ($p < 0.001$). UA values were significantly lower in the case groups than in the healthy control group. When the substance use characteristics of the case groups were compared, a statistically significant difference was found in the duration of substance use ($p < 0.010$) and motivation ($p = 0.031$) subtests. Duration of substance use and craving were higher in the synthetic substance group, and motivation was higher in the methamphetamine group. According to the Pearson analysis, the severity of addiction was deduced not to be correlated with serum uric acid and uric acid/creatinine levels in both case groups.

Conclusions: Serum uric acid and uric acid/creatinine levels were found to be statistically significantly lower in individuals with methamphetamine and synthetic cannabinoid exposure compared to healthy controls. In this study, it was thought that the lower uric acid levels in the case group compared to the control group may be due to the use of uric acid as an antioxidant or a decrease in purinergic transformation. Future studies may focus on making this distinction.

Keywords: Methamphetamine, synthetic cannabinoid, uric acid, severity of addiction

Uric acid (UA), the end product of the purinergic system, is converted from two nucleotides, adenosine monophosphate, and guanosine monophosphate, as a result of the oxidation of purines [1, 2]. The purinergic system includes adenosine and adenosine triphosphate (ATP) receptors and is commonly found in the central nervous system [3]. UA is involved in the transmission of adenosine [3]. Adenosine A1 and

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A2 sub-receptors exert their inhibitory effects on the central nervous system and heart by secreting neurotransmitters. High UA decreases this inhibitory effect by slowing down adenosinergic transmission [4].

Extracellular UA can have both antioxidative and prooxidative functions. UA constitutes an important part of the antioxidants in the plasma and acts as an antioxidant at normal serum levels. However, high serum UA levels show prooxidative and proinflammatory effects [5, 6]. It provides antioxidant activity in neurological diseases by scavenging free radicals [5, 7, 8]. UA has a pro-oxidative effect inside the cell [9]. UA has been reported to maybe cause low-grade systemic inflammation by producing proinflammatory cytokines [10]. High production of UA leads to increased oxidative stress and systemic inflammation [11].

Some studies have been carried out to examine the uric acid level in psychiatric disorders. UA blood serum levels were found to be high in manic and depressive periods of bipolar disorder [12-14]. UA elevation has been found to be associated with impulsivity, disinhibition, aggression, and sensation seeking behavior [15]. Impulsivity, hostility, aggression, irritability, and sensation-seeking behavior are known to be common in patients with substance use disorder [16, 17]. There are not enough studies in the literature evaluating the serum UA level in these patients.

The aim of this study was to evaluate whether serum UA levels were different from healthy controls the pre- and post-detoxification treatment in patients with methamphetamine and synthetic cannabinoid use disorders. Secondly, to determine the relationship between serum UA levels and the severity of addiction.

METHODS

Study Population

This study was carried out in the Alcohol and Substance Addiction Treatment Clinic of a Training and Research Hospital. The patient group was formed from male participants diagnosed with substance use disorder according to DSM-5 (Diagnostic and Statistical Manual of Mental Disorders) diagnostic criteria, between the ages of 18-65, and have been using substances for at least 1 year. Same-sex participants were included in the study to control for the confounding effect of gender. Those with a comorbid psychiatric disease, multiple substance use, those using antidepressant and antipsychotic drugs, and those with gout, hypertension, inflammatory diseases, chronic renal failure, hypertriglyceridemia, hyperglycemia, or other medical conditions, causing hyperuricemia were excluded from the study.

The study sample consisted of 140 participants in equal numbers from 3 groups: methamphetamine use disorder, synthetic cannabinoid use disorder, and control groups. The case group consisted of hospitalized patients. The control group consisted of hospital personnel and their relatives whose demographic characteristics were similar to the study group. All of the participants in both groups were smokers.

All participants reviewed the informed consent form and their written consent was obtained. Illegal substance use of the patients was confirmed by measuring urine samples with Siemens Advia 1800 chemistry analyzer using the Enzyme Multiplied Immunoassay Technique (EMIT).

This study was designed in accordance with the 2013 version of the Declaration of Helsinki and was approved by the local ethics committee (dated 17.11.2021 and decision number 2011-KAEK-25 2021/11-23). Good clinical practice principles were followed throughout the study.

Biochemical Parameters

One tube of venous blood sample was obtained from all participants at the time of enrollment to measure serum uric acid and creatinine levels. Venous blood samples were taken again to measure the same values 1 month after the detoxification treatment from the patient group. After the blood samples were centrifuged at 300 rpm for 15 minutes, plasma and serum samples were separated. Serum samples were stored at -80 °C. Uric acid was analyzed using the Abbott Architect c800 device. The normal range of UA was determined as 3.5-7.2 mg/dl. The arrengement was made by taking the Uric acid/creatinine (UA/Cr) ratio to neutralize the confounding effect of kidney functions. Peripherally measured blood plasma levels of UA are highly correlated with cerebrospinal fluid levels in the central nervous system (CNS) [18]. In this study, serum uric acid levels in peripheral blood were analyzed to determine oxidative stress in the CNS.

Assessment Tools

Sociodemographic Data Form

This form, prepared by the researchers, consists of questions about the participants' sociodemographic characteristics, substance use patterns, and past illnesses.

Addiction Profile Index (API)

The scale developed by Ögel *et al*. [19] to determine the severity of the addiction is a self-report questionnaire consisting of 37 items and 5 subscales. The subscales measure the characteristics of substance use, the diagnosis of addiction, the effect of substance use on one's life, craving, and motivation to quit the substance. Below 12 points are considered as low addiction severity. Ögel *et al*. [19] conducted a validity and reliability study in Turkish.

Statistical Analysis

Demographic and clinical characteristics of the cases evaluated in the study were clarified with descriptive statistical methods such as number, percentage, mean, and standard deviation. Independent samples t-test and chi-square test were used to compare substance use characteristics between the two groups. Non-parametric Mann-Whitney U test was used to compare continuous variables. One-way ANOVA and LSD post hoc tests were performed to evaluate the difference between groups. Pearson analysis was used to identify the relationship between variables. The conformity of the data to the normal distribution was evaluated according to the kurtosis and skewness coefficients (±1.5). IBM SPSS 22.0 program was benefitted for the analysis.

RESULTS

In this study, the absence of divorce in marital status in the healthy control group created a significant difference. There is no statistically significant difference in terms of other sociodemographic data (Table 1).

Considering the normality test results, other variables except motivation values were evaluated with parametric tests. When the substance use characteristics of the two groups of substance users were compared, a statistically significant difference was found in the duration of substance use ($p < 0.010$), and motivation (Mann Whitney U, $p < 0.031$) subtests. Duration of substance use and craving were higher in the synthetic substance group, and motivation was higher in the methamphetamine group (Table 2).

When the One-way ANOVA test was performed between the pre-detoxification uric acid values of all

Table 1. Sociodemographic data of sample groups

	MA Group (n = 44)	SC Group (n = 46)	HC Group (n = 40)	*p* value
Age (years) (mean ± SD)	32.59 ± 5.89	5.89 ± 8.29	32.67 ± 7.56	0.943
Educational background, n (%)				0.664
Primary school graduate	8 (18.2)	8 (17.4)	6 (15.0)	
High school graduate	29 (65.9)	34 (73.9)	26 (65.0)	
University graduate	7 (15.9)	4 (8.7)	8 (20.0)	
Marital status, n (%)				**< 0.050**
Married	19(43.2%)	17(37.0%)	24(60.0%)	
Single	18(40.9%)	21(45.7%)	16(40.0%)	
Widow/Divorced	7(15.9%)	8(17.3%)	0(0%)	
Working status, n (%)				0.526
Has a regular job	19 (43.2)	19 (41.3)	20 (50.0)	
No regular job	25 (56.8)	27 (58.7)	20 (50.0)	

SD = Standard deviation, MA = Methamphetamine, SC = Synthetic cannabinoid, HC = Healthy Control *$p < 0.05$,

Table 2. Comparison of substance use characteristics between two groups (with t-test and chi-square)

	MA Group (n = 44)	SC Group (n = 46)	*p* value
Age at onset of substance use	27.38 ± 7.85	25.46 ± 8.38	0.263
Duration of substance use	4.88 ± 2.81	6.78 ± 3.93	**0.010**
Substance use dose per a day, n (%)			0.538
Between 0.5-1 gr	17 (38.6)	15 (32.6)	
Over 1 gr	27 (61.4)	31 (67.4)	
Suicide attempt, n (%)	9 (20.5)	15 (30.4)	0.278
Substance use characteristics	2.26 ± 1.32	2.03 ± 1.39	0.431
Diagnosis	14.25 ± 4.99	13.71 ± 4.74	0.597
Effect of substance use on one's life	28.91 ± 9.70	28.70 ± 7.83	0.909
Craving	9.14 ± 3.96	9.70 ± 3.67	0.489
Motivation	10.82 ± 1.93	10.09 ± 2.12	**0.031**
API total score (Severity of addiction)	12.29 ± 3.04	11.96 ± 2.52	0.577

Data are shown as mean ± standard deviation or n (%). MA = Methamphetamine, SC = Synthetic cannabinoid

three groups, a statistically significant difference was found at the level of $p < 0.001$. It is seen that the group that made a significant difference in LSD posthoc tests was the healthy control group. The uric acid values of the healthy control group were statistically significantly higher than both drug user groups. There is no statistically significant difference between the pre-detoxification uric acid values of methamphetamine and synthetic substance users ($p = 0.431$), but there is a statistically significant difference between the methamphetamine group and the healthy control group ($p < 0.001$). There is a significant difference between uric acid values of synthetic substance users and healthy controls ($p < 0.001$). There was no statistically significant difference between pre-and post-detoxification the uric acid, creatinine values, and uric

Table 3. Uric acid, creatinine values, uric acid/creatinine ratios the pre-and post-detoxification

	MA Group (n = 44)	SC Group (n = 46)	HC Group (n = 40)	*p* value
Pre-detoxification uric acid	5.48 ± 0.97	5.14 ± 1.15	6.21 ± 1.10	**< 0.001**
Post-detoxification acid	5.36 ± 1.00	5.11 ± 1.47	-	0.371
***p* value**	0.467	0.870		
Pre-detoxification Creatinine	0.87 ± 0.12	0.83 ± 0.13	0.80 ± 0.10	0.064
Post-detoxification Creatinine	0.83 ± 0.13	0.81 ± 0.14		
***p* value**	**0.022**	0.447		
Pre-detoxification UA/Crea ratio	6.39 ± 1.15	6.29 ± 1.59	7.82 ± 1.58	**< 0.001**
Post-detoxification UA/Crea ratio	6.54 ± 1.34	6.24 ± 1.83		
***p* value**	0.361	0.830		

Data are shown as mean ± standard deviation or n (%). MA = Methamphetamine, SC = Synthetic cannabinoid, HC = Healthy Control, UA/Crea = Uric acid/creatinine

Table 4. Comparison of pre-and post-detoxification values of uric acid, creatinine, and API subtest scores in the methamphetamine group according to Pearson Correlation Analysis

	UA-1	UA-2	Crea-1	Crea-2	Characteristics of SU	Diagnosis	Effect of SU on one's life	Craving	Motivation	API Total
UA-1	1	r = 0.49	r = 0.42	r = 0.08	r = 0.05	r = 0.13	r = 0.05	03	r = -0.06	r = 0.09
		***p* < 0.001**	***p* = 0.040**	*p* = 0.622	*p* = 0.712	*p* = 0.388	*p* = 0.713	*p* = 0.828	*p* = 0.678	*p* = 0.554
UA-2	r = 0.49	1	r = 0.12	r = 0.34*	r = 0.27	r = 0.26	r = 0.22	r = 0.05	r = 0.19	r = 0.26
	***p* = 0.001**		*p* = 0 .449	***p* = 0.034**	*p* = 0.081	*p* = 0.096	*p* = 0.159	*p* = 0.717	*p* = 0.238	*p* = 0.096
Crea-1	r = 0.42	r = 0.12	1	r = 0.62**	r = 0.15	r = 0.32	r = 0.12	r = 0.19	r = 0.23	r = 0.27
	***p* = 0.040**	*p* = 0.449		***p* < 0.001**	*p* = 0.332	***p* = 0.030**	*p* = 0.402	p=0.198	p=0.134	p=0.070
Crea-2	r = 0.08	r = 0.34	r = 0.62**	1	r = 0.27	r = 0.25	r = 0.04	r = 0.05	r = 0.17	r = 0.20
	p = 0.622	***p* = 0.034**	***p* < 0.001**		*p* = 0.092	*p* = 0.122	*p* = 0.793	*p* = 0.729	*p* = 0.285	*p* = 0.215

UA = Uric acid, UA-1 = Pre-detoxification uric acid, UA-2 = Post-detoxification uric acid, Crea-1 = Pre-detoxification creatinine, Crea-2 =Post-detoxification creatinine SU = Substance use, API = Addiction Profile Index

acid/creatinine ratios in methamphetamine and synthetic substance users. The pre-detoxification creatinine values were statistically different between the methamphetamine and healthy groups ($p = 0.021$).

The pre-detoxification uric acid/creatinine ratio was statistically different between the three groups. While the healthy group created a statistically significant difference from the substance user groups, there was no significant difference between the methamphetamine and synthetic substance user groups (Table 3).

In the methamphetamine group, pre-detoxification uric acid, post-detoxification uric acid (r = 0.49, $p < 0.001$), and pre-detoxification creatinine (r = 0.42, $p = 0.040$) values were positively correlated. The post-detoxification uric acid value correlated with the post-detoxification creatinine value (r = 0.34, $p = 0.034$). There was also a positive correlation between the pre- and post-detoxification creatinine values (r = 0.62, $p < 0.001$). The pre-detoxification creatinine value was correlated with the API diagnostic criteria subscale (r = 0.32, $p = 0.030$) (Table 4).

In the synthetic substance group, the pre-detoxification uric acid was positively correlated with post-detoxification uric acid values (r = 0.67, $p < 0.001$). There was also a positive correlation between the pre- and post-detoxification values of creatinine (r < 0.69, $p < 0.001$). (Table 5).

DISCUSSION

In this study, serum UA values in pre-detoxification were found to be statistically significantly lower in MAG and SCG compared to CG. This low level suggests that purinergic transformation due to substance use may decrease in case groups. Future studies may focus on the evaluation of the purinergic system components in individuals with substance use disorders (SUD).

In the literature, the use of MA and SC has been reported to increase oxidative stress and inflammation in the CNS. Increased oxidative stress and proinflammatory cytokines in MA abusers cause inflammation in the CNS [20]. The most effective of the compounds in SC is tetrahydrocannabinol [21]. Cannabis use has been clarified to increase oxidative stress and inflammation [22].

Table 5. Comparison of pre-and post-detoxification uric acid and creatinine values in the synthetic substance group with API sub-test scores according to Pearson Correlation Analysis

	UA-1	UA-2	Crea-1	Crea-2	Characteristics of SU	Diagnosis	Effect of SU on one's life	Craving	Motivation	API Total
UA-1	1	r = 0.67 ***p* < 0.001**	r = 0.28 *p* = 0.054	r = 0.09 *p* = 0.540	r = -0.13 *p* = 0.361	r = 0.11 *p* = 0.453	r = -0.01 *p* = 0.900	r = 0.05 *p* = 0.743	r = - 0.09 *p* = 0.515	r = 0.03 *p* = 0.805
UA-2	r = 0.67 ***p* < 0.001**	1	r = 0.21 *p* = 0.168	r = 0.23 *p* = 0.139	r = -0.21 *p* = 0.165	r = -0.05 *p* = 0.744	r = -0.27 *p* = 0.076	r = -0.09 *p* = 0.553	r = 0.00 *p* = 0.979	r = 0.19 *p* = 0.220
Crea-1	r = 0.28 *p* = 0.054	r = 0.21 *p* = 0.168	1	r = 0.69 ***p* < 0.001**	r = 0.04 *p* = 0.752	r = 0.19 *p* = 0.186	r = 0.08 *p* = 0.583	r = 0.25 *p* = 0.086	r = 0.08 *p* = 0.583	r = 0.21 *p* = 0.144
Crea-2	r = 0.21 *p* = 0.16	r = 0.23 *p* = 0.139	r = 0.69 ***p* < 0.001**	1	r = 0.19 *p* = 0.232	r = 0.13 *p* = 0.395	r = -0.03 *p* = 0.819	r = 0.21 *p* = 0.174	r = -0.00 *p* = 0.986	r = 0.16 *p* = 0.310

UA = Uric acid, UA-1 = Pre-detoxification uric acid, UA-2 = Post-detoxification uric acid, Crea-1 = Pre-detoxification creatinine, Crea-2 = Post-detoxification creatinine SU = Substance use, API = Addiction Profile Index

Oxidation products formed as a result of substance use are cleared by uric acid, a powerful antioxidant of plasma [14]. In this study, the low serum uric acid levels in case groups compared to CG were thought to maybe related to their consumption as an antioxidant. In this study, the fact that serum uric acid levels did not show a statistically significant difference in both case groups compared to the control group during the withdrawal period was thought to be related to the decrease in UA consumption in plasma as a result of the decrease in oxidative stress and plasma oxidation products after treatment.

In our study, pre- and post-detoxification uric acid and creatinine levels were positively correlated in the case groups. Both uric acid and creatinine are antioxidant substances in plasma [14, 23]. It is thought that there may be a positive correlation due to the decrease in consumption of uric acid and creatinine levels in the tissues during the period of substance withdrawal.

It has been stated that uric acid can prevent neuronal death by being a strong antioxidant of plasma, reducing systemic inflammation and decreasing permeability in the blood-brain barrier [24]. In SUD, a low uric acid level can lead to an increase in oxidation products, especially free radicals, and neuron damage in the plasma. Our findings suggest that antioxidant defense may be decreased due to uric acid reduction in SUD. As a result, the low uric acid level may contribute to oxidative damage.

In our study, the serum uric acid levels of the case group before detoxification were figured out not to be related to the severity of the addiction. Studies on the relationship between low serum uric acid levels and oxidative stress and inflammation status are limited in the literature. New studies are needed to support our findings in the study.

In the comparison of the case groups in our study, motivation in MAG, craving, and duration of substance use in SCG were high. Although high motivation is a positive factor for treatment, it is accepted that it increases the severity of the addiction. Craving increases the API scores and the severity of addiction [19].

It was thought that the higher craving in the SCG group compared to MG might be related to multiple substance use. As anticholinergic, synthetic opioid, etc. substances are added to strengthen the effect of synthetic cannabinoids, SC can be considered as a

multi-substance in practice. When using synthetic cannabinoids, substance users are also exposed to multi-substance intoxication due to the many chemicals sprayed into them [25]. Individuals with multiple substance use experience more severe withdrawal symptoms and have higher relapse rates [26]. In the CNS substance exposure has been reported to cause craving and impairment in cognitive functions [27]. Therefore, treatment of this inflammation can reduce craving [28, 29].

Systemic inflammation has been mentioned to maybe persist for years in individuals using substances during the withdrawal period [30].

In our clinical practice, we observe that craving and impairment in cognitive functions during the withdrawal period of SUD patients complicate the treatment of patients. Our findings suggest that antioxidant defense may be decreased due to reduced uric acid in methamphetamine use disorders. Evaluation of serum uric acid levels may be useful in the treatment of patients with SUD.

Low uric acid levels are can be corrected with diet and pharmacological intervention. In order to support the findings of our study, new studies evaluating serum uric acid levels in patients with SUD are required.

Limitations

This study has some limitations. First, the relatively small number of male subjects did not evaluate uric acid levels in the long term after withdrawal. The female gender was excluded from the study. A large sample study involving both genders can be done in the future. Although a certain diet was recommended for hospitalized patients, a light diet was recommended for healthy controls 1 day before blood collection. However, the dietary compliance of the groups may be different. In this study, the levels of purine metabolites other than uric acid were not evaluated and their relationship with each other could not be understood. Finally, not evaluating the oxidative stress products and oxidant/antioxidant balance in the participants is among the limitations of our study.

CONCLUSION

SUA levels were found to be statistically significantly different in individuals with MA and SC exposure compared to healthy controls. These results did not change when we consider the glomerular filtration rate. In this study, it could not be evaluated whether the lower uric acid levels in the case group compared to the control group were due to the use of uric acid as an antioxidant or to a decrease in purinergic transformation. Future studies may focus on making this distinction.

Authors' Contribution

Study Conception: ÇT; Study Design: ÇT, SÜ; Supervision: ÇT, SÜ; Funding: ÇT; Materials: ÇT, SÜ; Data Collection and/or Processing: ÇT; Statistical Analysis and/or Data Interpretation: ÇT, SÜ; Literature Review: ÇT, SÜ; Manuscript Preparation: ÇT, SÜ and Critical Review: SÜ.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The European Research Journal 2023;9(4):800-810 Original Article

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Association between self-reported menstrual disorders and occupational exposures in female healthcare workers: a university hospital experience from Turkey

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ABSTRACT

Objectives: This study aims to demonstrate the relationship between menstrual disorders and occupational exposures in female healthcare workers, and to contribute to the regulation and improvement of working conditions of female healthcare workers.

Methods: A cross-sectional study among 503 female healthcare workers at university hospital in Turkey was conducted during December 2020-April 2021. The questionnaire prepared using the Google Forms program was sent electronically.

Results: Prevalence of dysmenorrhea was 59%, abnormal amount of menstrual bleeding 48.7%, abnormal menstrual duration 32%, and abnormal menstrual cycle length 154 30.6% in 503 participants. Dysmenorrhea risk was 0.79-fold (95% CI = 0.64-0.83) lower in those with advanced age and 1.56-fold (95% CI = 1.02-2.37) higher in smokers; risk of abnormal amount of menstrual bleeding was 3.91-fold (95% CI = 1.24-12.30) higher in those with total employment time of ≥ 20 years and 1.56-fold (95% CI = 1.07-2.26) higher in those who worked with display screens for > 20 hours a week; risk of abnormal menstrual cycle length was 3.46-fold (95% CI = 1.41-8.43) higher in technicians, 2.86-fold (95% CI = 1.24-6.61) higher in nurses, 2.63-fold (95% CI = 1.19-5.79) higher in other healthcare workers, and 2.14-fold (95% CI = 1.42-3.21) higher in those who were unsatisfied with their job.

Conclusions: It was found that occupational exposures may increase the risk of menstrual disorders. A plan of action is needed to reduce the frequency of menstrual disorders by preventing occupational exposures experienced by healthcare workers.

Keywords: Healthcare workers, menstrual disorders, occupational exposures

Women employed in health and social service jobs in Turkey constitute 7.3% of the total female workforce [1]. Feminization of the workforce has occurred in field of health, as half or even over half of the general workforce is comprised of women. The traditional acceptance of nursing and midwifery professions as women's work has an important role in the high number of women in the health sector. Due to the

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increase in maternal age and decline in fertility rates in recent years, the preservation of female reproductive health and fertility has become as important as maintaining pregnancy and safe birth [2]. Menstrual cycle regularity is a primary indicator of female reproductive health [3]. Irregular menstrual cycle may cause anovulation and infertility [4]. The menstrual cycle varies according to the balance between the hypothalamus-pituitary-ovarian axis and endogenous hormones. Changes in this hormonal axis can affect menstrual cycle pattern, length, and amount of bleeding [5, 6]. Dysmenorrhea and abnormal bleeding are common problems among young women and may negatively affect quality of life, work productivity, or access to healthcare [7]. Changes in the menstrual cycle have been associated with several factors such as age, smoking, body weight, exercise, race, life events, perceived stress, physiological state (such as cold), work environment (work shift, noise/ vibration/ exposure to organic solvents) [8]. Although occupational hazards associated with the menstrual cycle have been categorized as chemical, biological, psychosocial, and physical hazards, many studies have focused on chemical hazards [9, 10].

There is limited available evidence on the association between occupational exposures and menstrual disorders, and it is unclear how working conditions affect the menstrual cycle. However, chronic inflammation caused by occupational exposures is the most likely mechanism in the relationship between work-related factors and irregular menstrual cycle. Many studies have shown that occupational hazards are associated with reproductive health in female nurses [11, 12]. In studies on nurses in China, the prevalence of irregular menstrual cycles wasreported as 41%, and the prevalence of dysmenorrhea was reported as 70.7% [13, 14]. In Turkey, the prevalence of dysmenorrhea was reported as 69.9% [15]. Occupational exposure to antineoplastic drugs is reportedly associated with menstrual irregularity in nurses working in the United States [16]. Long working hours, and overtime and night shifts are common occurrences among health workers. Working night shifts can disrupt the circadian rhythm, which is effective in ovulation, and as a result, it may cause an irregularity in the menstrual cycle. In the largest epidemiological study comprising of 71.011 nurses in the USA, it was reported that there was a relationship between shift work and menstrual cycles lasting 40 days or more, and a 25% increased risk of change in cycle duration for every 12-month shift work [17]. The number of studies that include all female health workers and investigate the relationship with occupational exposures is limited. This study aims to demonstrate the relationship between menstrual disorders and occupational exposures in female health workers, and to contribute to the regulation and improvement of working conditions of female health workers.

METHODS

Study Design

This cross-sectional study was planned to to evaluate the relationship between menstrual disorders and occupational exposures in female healthcare workers at one of the largest public university hospital in Turkey.

Measurement Tools

A questionnaire designed by occupational diseases specialists was administered to the participants. The questionnaire form consists of three parts. The first part of the questionnaire consisted of 8 items related to sociodemographic data (age, marital status, education level, occupation, smoking and alcohol use, past pregnancies and age of menarche). Participants were classified and evaluated in 4 groups according to occupation: nurse, technician, doctor, others (medical secretary, dentist, dietician, administrative staff, etc.). Age of menarche was classified as 9-11, 12-13, and ≥ 14 years.

The second part of the questionnaire was composed of 18 questions related to working conditions and occupational hazards. To evaluate working conditions, total working time, institution (public, private), work pattern (shift work, permanent day shift, permanent night shift), weekly working hours (< 40 hours and ≥ 40 hours), perceived workload, perceived job stress, perceived job satisfaction, use of personal protective equipment, working time with display screens, long periods of standing, carrying heavy loads (manually carrying loads of ≥ 20kg) were questioned. Workload was defined as the stress perceived by the person due to intense tasks in a professional capacity [18]. A 5-point Likert scale was used to measure work stress. A score of 1-2 was considered mild, 3 as mod-

erate, and 4-5 as intense work-related stress. Working with display screens was defined as working with a computer at least 20 hours a week [19], while long periods of standing was defined as standing for at least 8 hours a day [20]. To assess occupational hazards in the workplace, they were asked whether they were exposed to noise, ionizing radiation, antineoplastic drugs, anesthetic gases, and whether they used vibrating tools, disinfectants, and sterilizing agents.

The third part of the questionnaire consisted of 4 questions to evaluate data related to the menstrual cycles of the participants. Participants who experienced back/abdominal pain that prevented them from doing routine work or pain that required medication in the last 3 months were evaluated as the dysmenorrhea group [21]. Participants were asked to describe the amount of menstrual blood bleeding as low, moderate, heavy, or very heavy for the last three months. Those who described their menstrual bleeding as low, heavy, or very heavy were considered participants with abnormal amount of menstrual bleeding. As for duration of menstrual bleeding of the last three months, 3-7 days was considered normal, while < 3 days and > 7 days was considered abnormal menstrual duration [22]. According to menstrual cycle length of the last three months, 21-35 days was considered normal, < 21 days or >35 days was considered irregular menstrual cycle length [23].

Data Collection

No sample was selected in this study. Between December 2020 and April 2021, the questionnaire prepared using the Google Forms program was sent electronically to 2462 female healthcare workers working at one of the largest public university hospital in Turkey. After clicking on the link sent to them via mobile phone, tablet or computer, the participants consent to participate in the questionnaire and were able to voluntarily complete it out. 556 employees completed the questionnaire completely (22.5%). Of the 556 people who participated in the questionnaire, five (0.9%) participants were excluded from the study due to incomplete responses, three (0.5%) due to menopause, 10 (1.8%) due to history of hysterectomy operation, 24 (4.3%) due to contraceptive use and 11 (1.9%) due to pregnancy. In total, 503 people were included in the evaluation.

Ethical Considerations

The study was approved by Istanbul University-Cerrahpaşa, Cerrahpaşa Medical Faculty Hospital Ethics Committee (Date: 17.11.2020, No. 151643). Informed consent was obtained via a form on the survey website.

Statistical Analysis

SPSS v21.0 (SPSS Inc., Chicago, IL, USA) package program was used to evaluate and analyze the data. Numerical variables were presented as mean and standard deviation, while categorical data were presented as number and percentage tables. Chi-square test was used to analyze the relationship between groups for categorical data. Student's t-test was applied to compare mean values between two groups. Univariate logistic regression analysis was used to calculate the odds ratios (OR) and 95% confidence intervals (CI) of risk factors associated with menstrual disorders. Variables that were found to be statistically significant ($p < 0.05$) as a result of this analysis were subjected to multivariate logistic regression analyzes. *P* value of < 0.05 was considered statistically significant.

RESULTS

Mean age of the participants was 35.04 ± 7.92 years. In total, 334 (66.4%) of the participants were married, 335 (66.6%) had undergraduate or higher diploma, and 242 (48.1%) were nurses; 162 (32.2%) of the participants were smokers, and 176 (35%) used alcohol; 305 (60.6%) had history of previous pregnancy and 318 (63.2%) had age of menarche of 12-13. Prevalence of dysmenorrhea was 297 (59%), prevalence of abnormal amount of menstrual bleeding was 245 (48.7%), prevalence of abnormal menstrual duration was 161 (32%), and prevalence of irregular menstrual cycle length was 154 (30.6%) (Table 1). Working conditions and occupational hazards of female health workers are shown in Table 2.

Bivariate Analysis between Demographic Data and Menstrual Disorders

Participants with dysmenorrhea had younger mean age than those without dysmenorrhea ($p < 0.01$). Dys-

Table 1. Distribution of demographic data and rates of menstrual disorders in female healthcare workers

Variables	Total[×] (n = 503)	Dysmenorrhea[×] (n = 297) (59%)	Abnormal amount of menstrual bleeding[××] (n = 245) (48.7%)	Abnormal menstrual duration[××] (n = 161) (32%)	Abnormal menstrual cycle length[××] (n = 154) (30.6%)
Age (years) ǂ	35.04 ± 7.92	33.66 ± 7.59 **< 0.001**	36.78 ± 7.89 **< 0.001**	37.40 ± 8.09 < 0.001	36.19 ± 8.59 **0.040**
Marital status⁋					
Married	334 (66.4)	192 (57.5)	170 (50.9)	120 (35.9)	102 (30.5)
Single	169 (33.6)	105 (62.1)	75 (44.4)	41 (24.3)	52 (30.8)
p value		0.317	0.167	**0.008**	0.958
Education⁋					
High school and below	30 (6)	18 (60)	20 (66.7)	13 (43.3)	10 (33.3)
Undergraduate	138 (27.4)	91 (65.9)	67 (48.6)	43 (31.2)	46 (33.3)
Graduate and above	335 (66.6)	188 (56.1)	158 (47.2)	105 (31.3)	98 (29.3)
p value		0.142	0.131	0.407	0.645
Occupation⁋					
Nurse	242 (48.1)	151 (62.4)	122 (50.4)	90 (37.2)	83 (34.3)
Technician	130 (25.8)	83 (63.8)	72 (55.4)	41 (31.5)	39 (30)
Doctor	60 (11.9)	25 (41.7)	23 (38.3)	12 (20)	9 (15)
Other	71 (14.1)	38 (53.5)	28 (39.4)	18 (25.4)	23 (32.4)
p value		**0.014**	0.054	**0.038**	**0.027**
Smoker⁋					
Yes	162 (32.2)	109 (67.3)	84 (51.9)	56 (34.6)	59 (36.4)
No	341 (67.8)	188 (55.1)	161 (47.2)	105 (30.8)	95 (27.9)
p value		**0.010**	0.331	0.396	0.052
Alcohol use⁋					
Yes	176 (35)	97 (55.1)	84 (47.7)	47 (26.7)	54 (30.7)
No	327 (65)	200 (61.2)	161 (49.2)	114 (34.9)	100 (30.6)
p value		0.188	0.747	0.061	0.981
Previous pregnancy⁋					
No	198 (39.4)	133 (67.2)	77 (38.9)	47 (23.7)	61 (30.8)
Yes	305 (60.6)	164 (53.8)	168 (55.1)	114 (37.4)	93 (30.5)
p value		**0.003**	**< 0.001**	**0.001**	0.940
Menarche age⁋					
9-11	90 (17.9)	60 (66.7)	43 (47.8)	28 (31.1)	28 (31.1)
12-13	318 (63.2)	184 (57.9)	149 (46.9)	104 (32.7)	97 (30.5)
≥14	95 (18.9)	53 (55.8)	53 (55.8)	29 (30.5)	29 (30.5)
p value		0.251	0.305	0.905	0.994

[×] Column percentage, [××] Row percentage, ǂMean ± SD, ⁋ n (%)

Table 2. Distribution of working conditions, occupational hazards and menstrual disorders in female healthcare workers

Variables	Total [×]	Dysmenorrhea[××]	Abnormal amount of menstrual bleeding[××]	Abnormal menstrual duration[××]	Abnormal menstrual cycle length[××]
Total years of employment[±]					
1-5 years	107 (21.3)	74 (69.2)	35 (32.7)	20 (18.7)	33 (30.8)
5-10 years	102 (20.3)	68 (66.7)	42 (41.2)	28 (27.5)	25 (24.5)
10-20 years	175 (34.8)	95 (54.3)	88 (50.3)	56 (32)	45 (25.7)
≥ 20 years	119 (23.7)	60 (50.4)	80 (67.2)	57 (47.9)	51 (42.9)
p value		**0.006**	**< 0.001**	**< 0.001**	**0.008**
Institution[±]					
Public health institution	443 (88.1)	261 (58.9)	221 (49.9)	142 (32.1)	132 (29.8)
Private health institution	60 (11.9)	36 (60)	24 (40)	19 (31.7)	22 (36.7)
p value		0.984	0.150	1.000	0.350
Work shifts[±]					-
Rotating shifts	236 (46.9)	151 (64)	114 (48.3)	69 (29.2)	75 (31.8)
Permanent day	236 (46.9)	124 (52.5)	116 (49.2)	82 (34.7)	69 (29.2)
Permanent night	31 (6.2)	22 (71)	15 (48.4)	10 (32.3)	10 (32.3)
p value		**0.016**	0.983	0.439	0.818
Weekly work hours[±]					
< 40 hours	309 (61.4)	104 (53.6)	86 (44.3)	55 (28.4)	45 (23.2)
≥ 40 hours	194 (38.6)	193 (62.5)	159 (51.5)	106 (34.3)	109 (35.3)
p value		**0.049**	0.120	0.164	**0.004**
Perceived work-related stress[±]					
Mild	84 (16.7)	39 (46.4)	37 (44)	4 (28.6)	27 (32.1)
Moderate	293 (58.3)	184 (62.8)	139 (47.4)	91 (31.1)	88 (30)
Heavy	126 (25)	74 (58.7)	69 (54.8)	46 (36.5)	39 (31)
p value		**0.027**	0.251	0.417	0.930
Perceived workload[±]					
Yes	475 (94.4)	282 (59.4)	237 (49.9)	154 (32.4)	144 (30.3)
No	28 (5.6)	15 (53.6)	8 (28.6)	7 (25)	10 (35.7)
p value		0.683	**0.046**	0.542	0.696
Perceived job satisfaction[±]					
Yes	237 (47.1)	132 (55.7)	109 (46)	74 (31.2)	55 (23.2)
No	266 (52.9)	165 (62)	136 (51.1)	87 (32.7)	99 (37.2)
p value		0.149	0.250	0.722	**0.001**
Use of PPE[±]					
Yes	469 (93.2)	280 (59.7)	227 (48.4)	153 (32.6)	143 (30.5)
No	34 (6.8)	17 (50)	18 (52.9)	8 (23.5)	11 (32.4)
p value		0.352	0.739	0.364	0.972
Work with display screens[±]					
< 20 hours	214 (42.5)	126 (58.6)	93 (43.3)	67 (31.2)	67 (31.2)
≥ 20 hours	289 (57.5)	171 (59.4)	152 (52.8)	94 (32.6)	87 (30.2)
p value		0.862	**0.035**	0.726	0.818

Table 2 contunied. Distribution of working conditions, occupational hazards and menstrual disorders in female healthcare workers

Variables		Total [×]	Dysmenorrhea[××]	Abnormal amount of menstrual bleeding[××]	Abnormal menstrual duration[××]
Long periods of standing[±]					
< 8 hours	165 (32.8)	87 (52.7)	79 (47.9)	50 (30.3)	44 (26.7)
≥ 8 hours	338 (67.2)	211 (62.4)	166 (49.1)	111 (32.8)	110 (32.5)
p value		**0.038**	0.795	0.567	0.179
Carrying heavy loads[±]					
Yes	137 (27.2)	91 (66.4)	70 (51.1)	54 (39.4)	37 (27)
No	366 (72.8)	207 (56.6)	175 (47.8)	107 (29.2)	117 (32)
p value		**0.045**	0.512	**0.029**	0.283
Exposure to vibration[±]					
Yes	78 (15.5)	44 (56.4)	42 (53.8)	28 (35.9)	20 (25.6)
No	425 (84.5)	254 (59.8)	203 (47.8)	133 (31.3)	134 (31.5)
p value		0.579	0.323	0.503	0.366
Exposure to noise[±]					
Yes	291 (57.9)	179 (61.5)	147 (50.5)	96 (33)	92 (31.6)
No	212 (42.1)	119 (56.1)	98 (46.2)	65 (30.7)	62 (29.2)
p value		0.225	0.342	0.580	0.569
Exposure to ionizing radiation[±]					
Yes	208 (41.4)	132 (63.5)	107 (51.4)	71 (34.1)	67 (32.2)
No	295 (58.6)	166 (56.3)	138 (46.8)	90 (30.5)	87 (29.5)
p value		0.106	0.303	0.391	0.515
Exposure to antineoplastic drugs[±]					
Yes	31 (6.2)	22 (71)	15 (48.4)	9 (29)	12 (38.7)
No	472 (93.8)	276 (58.5)	230 (48.7)	152 (32.2)	142 (30.1)
p value		0.237	1.000	0.867	0.419
Exposure to anesthetic gas[±]					
Yes	76 (15.1)	47 (61)	37 (48.1)	23 (29.9)	
No	427 (84.9)	251 (58.9)	208 (48.8)	138 (32.4)	123 (28.9)
p value		0.728	0.900	0.761	0.063
Exposure to disinfectants[±]					
Yes	435 (86.5)	265 (60.9)	216 (49.7)	146 (33.6)	139 (32)
No	68 (13.5)	32 (47.1)	29 (42.6)	15 (22.1)	15 (22.1)
p value		**0.031**	0.282	0.080	0.132
Exposure to sterilizing agents[±]					
Yes	152 (30.2)	96 (63.2)	79 (52)	56 (36.8)	46 (30.3)
No	351 (69.8)	201 (57.3)	166 (47.3)	105 (29.9)	108 (30.8)
p value		0.217	0.335	0.126	0.910

[×]Column percentage, [××]Row percentage, [±]n (%), PPE = personal protective equipment

menorrhea was more prevalent among technicians compared to other occupational groups ($p = 0.014$), among smokers ($p = 0.010$) and among those without history of pregnancy compared to those who had history of pregnancy ($p = 0.003$). Participants who had an abnormal amount of menstrual bleeding had a significantly older mean age than participants who did not ($p < 0.001$) and abnormal amount of menstrual bleeding was more prevalent among those with a history of pregnancy ($p < 0.001$). Participants who had abnormal menstrual duration had an older mean age than those with normal menstrual duration ($p < 0.001$). Abnormal menstrual cycle length was more prevalent among those who were married compared to those who were not married ($p = 0.008$), among nurses compared to other occupations ($p = 0.038$), and among those with a history of pregnancy ($p < 0.001$). Participants who had abnormal menstrual cycle length had an older mean age than those who did not ($p = 0.040$), and abnormal menstrual cycle length was more prevalent among nurses than other occupational groups ($p = 0.027$).

Bivariate Analysis between Working Conditions/Occupational Hazards and Menstrual Disorders

Dysmenorrhea was prevalent among those with total working experience of 1-5 years ($p = 0.006$), those who worked permanent night shifts ($p = 0.016$), those who worked 40 hours or more per week ($p = 0.049$), those who experienced moderate workrelated stress ($p = 0.027$), those who worked stood for ≥ 8 hours a day (p = 0.038), those who lifted heavy loads ($p = 0.045$), and those who were exposed to disinfectants in the workplace ($p = 0.031$). Abnormal amount of menstrual bleeding was more prevalent among those with total working experience of over 20 years ($p < 0.001$), those who perceived workload ($p = 0.046$), and those who worked with display screens for at least 20 hours a week ($p = 0.035$). Abnormal menstrual duration was more prevalent among those with total working experience of over 20 years ($p < 0.001$) and those who carried heavy loads ($p = 0.029$). Abnormal menstrual cycle length was more prevalent among those with total working experience of over 20 years ($p = 0.008$), those who worked over 40 hours a week ($p = 0.004$), and those were unsatisfied with their job ($p = 0.001$) (Table 2).

Logistic Regression Analysis between Demographic Variable, Occupational Exposures and Menstrual Disorders

Univariate logistic regression analysis was performed between menstrual disorders and demographic, occupational exposure variables of the participants who demonstrated a statistically significant difference. Multivariate logistic regression analyzes were applied to the variables that were statistically significant ($p < 0.05$) as a result of this analysis. A significant correlation was found between the rate of dysmenorrhea and age ($p = 0.007$) and smoking ($p = 0.037$). The risk of dysmenorrhea was found to be 0.79-fold (95% Cl = 0.64-0.83) lower in the elderly and 1.56-fold (95%CI = 1.02-2.37) higher in smokers. The rate of abnormal amount of menstrual bleeding significantly correlated with total years of employment ($p = 0.02$) and working long periods with display screens ($p = 0.018$). Risk of abnormal amount of menstrual bleeding was 3.91-fold (95% CI = 1.24-12.30) higher in those with total work experience of 20 years of more and 1.56-fold (95% CI = 1.07-2.26) higher in those who worked with display screens for over 20 hours a week. Prevalence of abnormal menstrual cycle length was significantly associated with occupation ($p = 0.016$, $p = 0.014$, $p = 0.006$, $p < 0.001$) and job satisfaction ($p < 0.001$). Risk of abnormal menstrual cycle length in technicians was found as 3.46-fold (95% Cl=1.41-8.43), in nurses 2.86-fold (95% Cl = 1.24-6.61), in other occupations 2.63-fold (95% Cl = 1.19-5.79), and in those who were unsatisfied with their job 2.14-fold (95% Cl = 1.42-3.21) (Table 3).

DISCUSSION

This study demonstrates the relationship between menstrual disorders and occupational hazards in female health workers in Turkey. Menstrual disorders included dysmenorrhea, abnormal amount of menstrual bleeding, abnormal menstrual duration, and abnormal menstrual cycle length. In our study, rates of dysmenorrhea, abnormal amount of menstrual bleeding, abnormal menstrual duration, and abnormal menstrual cycle length in female health workers were 59%, 48.7%, 32%, and 30.61%, respectively.

Table 3. Multivariate regression analysis of risk factors for menstrual disorders in female healthcare workers

	Dysmenorrhea		Abnormal amount of menstrual bleeding		Abnormal menstrual duration		Abnormal menstrual cycle length	
	AOR	95% CI	AOR	95% CI	AOR	95% CI	AOR	95%CI
Age	**0.79**	0.64-0.83	0.99	0.94-1.04	1.04	0.98-1.10	1.03	0.98-1.09
Occupation								
Doctor	Ref		**NS**		Ref		Ref	
Other	1.81	0.83-3.93			0.63	0.26-1.49	**2.63**	1.19-5.79
Nurse	0.70	0.37-1.32			1.20	0.62-2.29	**2.86**	1.24-6.61
Technician	0.70	0.36-1.36			1.14	0.57-2.26	**3.46**	1.41-8.43
Total years of employment								
1-5	1.75	0.50-6.13	Ref		Ref		1.81	0.91-3.61
5-10	1.37	0.50-3.71	1.38	0.73-2.61	1.23	0.59-2.56	Ref	
10-20	1.19	0.60-2.35	1.82	0.80-4.14	1.23	0.49-3.12	0.89	0.44-1.77
≥20	Ref		**3.91**	1.24-12.30	1.51	0.43-5.30	1.50	0.54-4.11
Smoking								
Yes	**1.56**	1.02-2.37	**NS**		**NS**		**NS**	
No	Ref							
Job satisfaction								
Yes	**NS**		**NS**		**NS**		Ref	
No							**2.14**	1.42-3.21
Work with display screens								
< 20 hours	**NS**		Ref		**NS**		**NS**	
≥ 20 hours			**1.56**	1.07-2.26				

AOR = Adjusted Odds Ratio, CI = Confidence Interval, *Significant (p value < 0.05), Ref = Reference (lowest prevalence), NS = Nonsignificant

In a study conducted on nurses in China, the rate of menstrual disorders was reported as 41% [13]. Menstrual disorders vary among women with different sociodemographic characteristics such as marital status, birth, and age. Significant differences have been found between married and unmarried women in terms of menstrual characteristics [24]. In a study conducted in China, it was found that women in the 45-50 age group were associated with general menstrual disorders and irregular menstrual cycles, especially heavy menstrual bleeding or hypomenorrhea formation [13]. This may be due to menopausal effects and decreased ovarian function, estrogen, and progesterone levels during the perimenopausal period [25]. In our study, it was observed that rates of abnormal menstrual bleeding, abnormal menstrual duration, and abnormal menstrual cycle length increased with age. Studies conducted in Taiwan and Thailand reported dysmenorrhea prevalence as 70.2% and 70.7%, respectively [14, 26]. A study on nurses in our country reported a dysmenorrhea prevalence of 69.9% [15]. Dysmenorrhea emerged as the most common menstrual disorder in our study. Dysmenorrhea prevalence was relatively lower in our study (59%) compared to other studies, while, in terms of occupation, dysmenorrhea was most common among technicians (63.8%).

According to the literature, prevalence of dysmenorrhea may vary according to study population and definition of dysmenorrhea [7, 26]. In our study, women with dysmenorrhea had higher mean age than women without dysmenorrhea, which was consistent with the literature [27-29]. This result may be related to the fact that young women are unaware of the factors that increase or cause dysmenorrhea and therefore do not use effective coping methods [30]. Smoking is among the modifiable risk factors for dysmenorrhea. In our study, a significant relationship was observed between smoking and dysmenorrhea, and the prevalence of dysmenorrhea was found to be higher in smokers, in line with the literature [31]. Previous studies in the literature have not presented clear evidence of a relationship between dysmenorrhea and childbirth. There are studies reporting that dysmenorrhea is less prevalent among women who have given birth [32], more prevalent among women who have given birth [7, 15, 33] and unrelated [28]. In our study, prevalence of dysmenorrhea was observed to be lower among health workers with history of pregnancy, which was consistent with the literature.

The menstrual cycle is reflective of circadian rhythm in women. Shift work can disrupt the circadian rhythm. An impaired sleep/wake cycle and an impaired circadian rhythm can alter the pattern of the menstrual cycle [34]. One meta-analysis reported increased prevalence of abnormal menstrual cycle length in shift workers, even after adjustment for confounders [35]. In addition, in our study, dysmenorrhea prevalence was found to be higher in those who worked at night shifts permanently and in those who work at rotating shifts. This finding is consistent with the results of the study by Chiu *et al*. [14] and inconsistent with the results of the study by Chuamoor *et al*. [26]. These conflicting results may be due to differences in perceived stress levels, mean ages of the study groups, pain perception, and methods of coping with pain.

It is reported that heavy working conditions and work-related stress disrupt the menstrual cycle and cause dysmenorrhea [33]. There is a theory that stress affects the menstrual cycle through the hypothalamic–pituitary–adrenal (HPA) axis. When stress levels are high, HPA activity is disrupted. Therefore, women who experience significant stress may experience more irregular menstruation than those who are not under stress [36]. Stress causes disruption of follicular development by preventing the release of follicle stimulating hormone and luteinizing hormone. This can alter progesterone synthesis and release, which can affect prostaglandin activity. Besides progesterone, stress-related hormones such as adrenaline and cortisol also appear to affect prostaglandin synthesis and/or its binding in the myometrium [7]. In line with this theory, in our study, it was observed that rates of dysmenorrhea was higher in healthcare workers who experienced moderate and severe work-related stress, and prevalence of abnormal menstrual bleeding were increased among those who perceived workload and among those who worked with display screens for more than 20 hours per week. Long working hours and short rest periods increase the employee's anxiety and dissatisfaction, which can affect the menstrual cycle [37]. In our study, prevalence of abnormal amount of menstrual bleeding was higher among those who were unsatisfied with their job. In a study by Lawson *et al*. [38], prevalence of abnormal menstrual cycle length was 19% in nurses, and even higher among nurses who worked night shifts, ≥ 40 hours a week, and carried heavy loads. Intense physical activity has been identified as a risk factor for menstrual disorders and a significant association was found between intensity of physical exercise (hours/week) and menstrual disorders [39]. In our study, there was more frequent dysmenorrhea and abnormal menstrual cycle length in participants who worked ≥ 40 hours a week, and a higher prevalence of dysmenorrhea and abnormal menstrual duration in healthcare workers carrying heavy loads [15].

Disinfectants are widely used among all health institutions. It is reported that 78% of nurses in the United States are exposed to disinfectants in their daily work through inhalation and dermal absorption [40]. The use of disinfectants has been listed among the risk factors for menstrual disorders. Although studies suggest that disinfectants may cause menstrual disorders by affecting the hypothalamic-pituitary-gonadal axis and causing changes in reproductive hormone profiles, there is insufficient evidence that disinfectants can affect menstrual disorders [13]. In our study, prevalence of dysmenorrhea was significantly higher among healthcare workers exposed to disinfectants.

Strenghts and Limitations

Our study had several strenghts and limitations. Studies investigating menstrual disorders in nurses have been conducted by other researchers. However, to the best of our knowledge, this is the first study that has included female healthcare workers of different occupations and investigated the relationship between occupational hazards and menstrual disorders. Studies on menstrual disorders in healthcare workers worldwide have generally been conducted among nurses, and very few studies have included female healthcare workers of different occupations. We used a self-report questionnaire to reach as many participants as possible and minimize face-to-face interviews during pandemic conditions. Recall bias from self-reporting of exposure and outcomes may influence odds ratios among participants who believe that occupation is the primary contributor to their menstrual disorders. The cross-sectional design of our study in unable to provide a causal relationship. Personal factors such as sleep and obesity, which may also be related to menstrual disorders, were not investigated.

CONCLUSION

Menstrual disorders are a significant indicator of existing and potential health problems. Therefore, it is necessary to evaluate factors related to menstrual disorders in order to determine the necessary strategies for prevention and treatment. Our study revealed that occupational exposures may increase the risk of menstrual disorders. The underlying mechanisms of menstrual disorders due to occupational exposures are still unclear. The results of our study demonstrate the need for further research in this field in order to reduce the negative effects of menstrual disorders among female healthcare workers in Turkey and worldwide. A plan of action is needed to reduce the frequency of menstrual disorders by preventing occupational exposures experienced by healthcare workers.

Authors' Contribution

Study Conception: NG, SK; Study Design: NG, SK; Supervision: NG, SK; Funding: N/A; Materials: NG, SK; Data Collection and/or Processing: NG, SK; Statistical Analysis and/or Data Interpretation: NG, SK; Literature Review: NG, SK; Manuscript Preparation: NG, SK and Critical Review: NG, SK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The role of FIGO-PALM pathologies in heavy menstrual bleeding resistant to levonorgestrel-releasing intrauterine system (Mirena®) treatment

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ABSTRACT

Objectives: To evaluate of the indications requiring surgical intervention after Levonorgestrel-Releasing Intrauterine System (LNG-IUS) (Mirena®) treatment due to heavy menstrual bleeding.

Methods: This retrospectively designed study was created with 72 patients who applied to the university hospital gynecology outpatient clinics between January 2018 and April 2019 and were diagnosed with heavy menstrual bleeding and received LNG IUS (Mirena®) for treatment.

Results: Surgery was not performed in 60 (83.33%) patients who had heavy menstrual bleeding and underlying organic pathology (FIGO-PALM group) These patients were treated with LNG-IUS. However, in 12 (16.67%) patients, LNG-IUS was removed and surgery was performed due to resistance to treatment within an average of 10.2 ± 8.0 months. There was no difference between the groups in terms of age, gravida, parity, body mass index and endometrial thickness ($p > 0.05$ for all parameters), but the mean hemoglobin value in the surgical group was found to be significantly lower than the group without it (8.9 ± 1.2 g/dL vs 11 ± 1.6 g/dL, $p = 0.03$) In the surgical group, the median diameters of leiomyoma and myoma compressing the endometrium were found to be significantly higher (44 mm vs 34 mm, $p = 0.03$ and 42 mm vs 33 mm, $p = 0.04$; respectively).

Conclusions: LNG-IUS (Mirena®) is a popular and effective treatment option for heavy menstrual bleeding. The necessity of surgical intervention due to resistance to LNG-IUS revealed that the underlying organic pathologies in these patients should be determined precisely and effective treatment options should be carefully selected before LNG-IUS is inserted.

Keywords: FIGO-PALM-COEIN, heavy menstrual bleeding, levonorgestrel-releasing intrauterine system

The term heavy menstrual bleeding is used to describe irregular uterine bleeding that is excessive in duration, frequency and amount. Heavy menstrual bleeding is the most common symptom of conditions that lead to abnormal uterine bleeding [1]. Abnormal uterine bleeding undermines a woman's physical health, as well as her quality of life and impact on society. In the treatment of heavy menstrual bleeding, the underlying pathologies should be determined first and treatments should be considered [2, 3].

The process to reach a common consensus on menstrual terminology has evolved to include a system called the International Federation of Gynecology and Obstetrics (FIGO) PALM-COEIN classification.

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This system describes possible known causes or contributors to symptoms. Various treatment options are available for the management of anatomical disorders (PALM [polyps, adenomyosis, leiomyoma, malignancy]) and non-anatomical disorders (COEIN [coagulation disorders, ovulation dysfunction, endometrial, iatrogenic, not otherwise classified]) that causes symptoms of heavy menstrual bleeding [4, 5].

However, since the side effects that develop during treatment generally limit patient compliance and effectiveness, it is imperative that treatment options are chosen based on the cause, effectively [6]. Surgical procedures such as endometrial ablation, myomectomy and hysterectomy can be applied when hormonal and non-hormonal medical treatment options do not respond or are insufficient [7]. In general, 80% of women receiving treatment for abnormal uterine bleeding do not have an anatomical pathology; therefore, the uterus was found to be anatomically normal in one third of the women who underwent hysterectomy. For this reason, medical treatment is a remarkable option to prevent unnecessary surgery [8].

The levonorgestrel-releasing intrauterine system (LNG-IUS) (Mirena®; Schering AG, Berlin, Germany) was originally produced in Finland in 1990 as a long-acting contraceptive agent and was approved by the Food and Drug Administration (FDA) in 2000. At the same time, in addition to this effectiveness, studies have shown that LNG-IUS releases 20mcg of levonorgestrel per day to the endometrial environment with a local effect, preventing endometrial proliferation and thus providing effective treatment in menorrhagia and dysmenorrhea [9]. Similarly, the use of LNG-IUS was found to be effective in reducing pain and uterine volume in women with adenomyosis. [10, 11].

In this study we aimed to evaluate indications requiring surgical intervention after LNG-IUS (Mirena®) treatment due to heavy menstrual bleeding.

METHODS

In our retrospective study, a total of 72 patients who were admitted to the University Hospital's gynecology clinic between January 2018 and April 2019 and who underwent LNG-IUS (Mirena®) with the diagnosis of heavy menstrual bleeding were included and their demographic and clinical characteristics were recorded. Inclusion criteria for the study; the patients were between 30 and 55 years old and had an LNG-IUS (Mirena®) inserted due to HMB. Study exclusion criteria; endometrial intraepithelial lesion (EIN) as a result of endometrial biopsy, presence of hematological comorbidity, liver disease, and use of anticoagulant drugs. The total number of patients who underwent LNG-IUS (Mirena®) between the dates determined in our hospital was 80. Eight of these patients in the study group were excluded from the study (anticoagulant use [n =1, 12.5%], LNG-IUS dislocation [n = 4, 50%], endometrial biopsy result reported as EIN [n = 1, 12.5%], and LNG-IUS decline in the early period [n = 2, 25%]).

Before LNG-IUS (Mirena®) is inserted in our hospital, procedures such as transvaginal utrasonography (TV-USG) or endometrial biopsy or office hysteroscopy (H/S) are routinely performed for exclusion of endometrial hyperplasia, neoplasia, polyp or submucous myoma. Informed consent form is obtained about participation. Our study was approved by the Local Ethics Committee with the date 2019/05-21 and issue number 2011-KAEK-25.

Statistical Analysis

The statistical analysis of the study was carried out with IBM SPSS 21.0 (IBM Corp.) program. The normal distribution for each continuous variable was checked with Kolmogorov Smirnov and Shapiro-Wilk tests. All numerical data were expressed as a median (minimum-maximum) or mean and standard deviation. The chi-square test was used to compare qualitative data and these data were expressed as frequency and percentages. Student-t-test and Mann-Whitney U tests were used to compare normally distributed and undistributed variables in the two-group analysis. The statistical significance level was considered $p < 0.05$.

RESULTS

Surgery was not required in 60 (83.33%) patients with heavy menstrual bleeding who had underlying organic pathology (FIGO-PALM group) while In 12 (16.67%) patients, LNG-IUS (MIRENA®) was removed and surgical operation was performed due to resistance to treatment within an average of 10.2 ± 8.0 months.

Table 1. Demographic, laboratory and clinical characteristics of patients with heavy menstrual bleeding and who underwent LNG-IUS (Mirena®)

Variables	Surgical treatment (-) n = 60 (83.33%)	Surgical treatment (+) n = 12 (16.67%)
Age (year)	42.3 ± 6.1	44.7 ± 3.91
BMI (kg/m^2)	29.1 ± 4.6	30.4 ± 4.9
Gravida	2.9 ± 1.6	2.7 ± 1.5
Parity	2.1 ± 1.0	1.9 ± 0.8
Hb level (g/dL)	11 ± 1.6	8.9 ± 1.2
Endometrial thickness (mm)	8.5 (7.0-9.2	8.6 (7.2-9.4)
Leiomyom diameter (mm)	34 (21-45)	44 (23-57)
Fibroid diameter pressing on the endometrium (mm)	33 (19-35)	42 (34-45)
Endometrial polyp size (mm)	12 (9-16)	15 (10-18)
Oral – IV antianemi treatment		
Yes	44 (73.3%)	11 (91.6%)
No	16 (26.6%)	1 (8.4%)

BMI = Body mass index, Hb = hemoglobin, IV = intravenous

[a] Independent Samples t Test (Mean ± SD), [b] Chi-Square Test [n (%)] [c] Mann-Whitney U Test (Median (Min-Max)

There was no difference in age, gravida, parity, BMI and endometrial thickness in the surgical and non-surgical groups ($p > 0.05$ for all parameters). However, the mean hemoglobin value in the surgical group was found to be significantly lower than in the non-surgical group (8.9 ± 1.2 g/dL vs 11 ± 1.6 g/dL, $p = 0.03$) In the surgical group, the median diameters of leiomyoma and myoma compressing the endometrium were found to be significantly higher (44 mm vs 34 mm, $p = 0.03$ and 42 mm vs. 33 mm, $p = 0.04$; respectively) (Table 1) In this study, for heavy menstrual bleeding patients who underwent LNG-IUS, when we evaluated the ultrasound findings and pathology results according to the PALM- etiology, there were leiomyoma was found with the highest (n = 5 [41.6%]) while submucous myoma with the least (n = 1 [8.3%]) (Table 2).

DISCUSSION

The primary outcome of the study was revealed by the number of patients who inserted LNG-IUS (MIRENA®) and then underwent surgery, surgical procedures, ultrasound findings and pathology results. There are many effective treatment options in modern medicine, from non-invasive to invasive, conservative to non-conservative, non-hormonal to hormonal. Surgical methods have emerged, blended with new technological developments in modern treatment methods. The patient's personal preference, age, desire for a child, future fertility demand, and the variety of personal symptoms affect different treatment modalities. Any surgical intervention, even minor, carries a risk of multiple complications such as bleeding, possible need for transfusion, risk of infection, bladder, bowel or ureter injury, postoperative adhesion formation, anesthetic complications, and hospitalization in general. Traditionally, the goal of heavy menstrual bleeding treatment has been surgical removal of the uterus/hysterectomy. Although hysterectomy has been an effective treatment for heavy menstrual bleeding, it has only been in the last 2-3 decades that the focus has gradually shifted towards the non-surgical treatment of heavy menstrual bleeding. That is not only cost effective but also preserves the uterus and fertility for patients whenever possible [12, 13]. LNG-IUS is a minimally invasive treatment method that reduces heavy menstrual bleeding up to 90% with its progestogenic effect on the uterine endometrium. Since its introduction, published data indicate that it is a cost-effective and safe non-surgical treatment modality for heavy men-

Table 2. Ultrasonographic imaging and pathology results of patients who underwent LNG-IUS (Mirena®) and surgery due to resistance to treatment

Variables	Surgical treatment (+) (n = 12)
Ultrasound Finding	
Leiomyoma	6 (50%)
Submucous fibroids	1 (8.3%)
Endometrial polyp	3(33.3%)
Adenomyosis	2 (16.6%)
Time to surgical treatment (month)	10.2 ± 8.0
Surgical procedure	
OP H/S polypectomy	3 (25%)
OP H/S polypectomy + myomectomy	1 (8.3%)
Myomectomy	2 (16.6%)
Hysterectomy	6 (50%)
Pathology result	
Leiomyoma	5 (41.6%)
Polyp	2 (16.6%)
Adenomyosis	2 (16.6%)
Adenomyosis + leiomyoma	2 (16.6%)
Adenomyosis + leiomyoma + endometrial hyperplasia	1 (8.3%)

OP H/S = Operative hysteroscopy

strual bleeding treatment. Compared with other pharmacological agents used in the treatment of menorrhagia, LNG-IUS has been shown to be an effective and well tolerated method [14].

Irvine *et al*. [15] in their study of 22 patients with menorrhagia, 14 (64%) of 22 women using LNG-IUS reported their satisfaction with the treatment method as good or very good (77%) and continued treatment, 3 (33%) discontinued treatment. Durga *et al*. [16] stated in their study that 83.3% of the patients had better compliance with LNG-IUS after 1 year of use, 9.5% of them were dislocated and fell and only 7.1% had to undergo hysterectomy. Although LNG-IUS is a well-tolerated treatment, its cost and placement partially limit its use. LNG IUS should be inserted by clinicians who are experienced in administration and have received adequate training for administration because it is different from other intrauterine systems. Malposition can be caused by anatomical causes, lack of skill and inexperience of the practitioner, but the main cause is probably attributable to the disparity between the IUS and the uterine cavity [17].

In a similar study by Günay *et al*. [18], LNG-IUS was continued in 90% (n = 42) patients, and 9.5% (n = 4) patients did not continue the treatment (n = 2 patients had dislocation (5%) and 2 patients had surgical procedure (5%) because LNG-IUS was removed. In our study, in 5% (n = 4) patients, the device was removed due to dislocation and the patient continued her treatment with oral gestagen. In our study, the rate of continuation of treatment in the group of patients who underwent LNG-IUS for the treatment of heavy menstrual bleeding was found to be slightly lower than in the literature (83.33% vs. 91%). It is seen that our LNG-IUS removal rate due to organic pathology requiring surgical procedure is higher with 16.67% compared to the literature, and the rates of removal due to dislocation are similar. This increase in going to surgery emphasizes the need to increase the diagnosis and surgical treatment of organic pathologies before LNG-IUS insertion [19]. In current study, endometrial biopsy was performed to rule out intrauterine organic pathologies and cancers before the use of Levonorgestrel active substance product in patients with acute heavy menstrual bleeding, and it was performed after the pathology result was found to be normal [20].

This study, for in heavy menstrual bleeding patients who underwent LNG-IUD, when we classified the ultrasound findings according to the etiology of PALM-COEIN, the most leiomyoma was 50% and the least adenomyosis and polyp (for both 16.6%). Ni *et al*. [21], in which they classified the etiology of PALM-COEIN for heavy menstrual bleeding according to ultrasound and histopathology, the findings revealed as leiomyoma (45.7%), polyp (16.5%), adenomyosis (9.6%) and malignant lesions (2.9%), respectively [21].

Chaturverdi *et al*. [22] in heavy menstrual bleeding, in the clinicopathological studies based on the FIGO PALM category, found that the pathological distribution of the cases was mostly leiomyoma (71%), leiomyoma+adenomyosis (59%), adenomyosis (8%),

polyp (8%), endometrial hyperplasia (1.3%) and cervical intraepithelial neoplasia (1%) [22]. The most common pathology finding was leiomyoma, which was similar to the literature in this study (58.2%). When our study is compared with the literature, it is seen that the rates of leiomyomas are similar, and the rates of adenomyosis and polyp rates are higher.

The National Institute for Health and Care Excellence (NICE) guideline supports the use of LNG-IUS as first-line therapy in women with fibroids smaller than 3 centimeter in the management of heavy menstrual bleeding [1]. More invasive treatments such as uterine artery embolization or surgery should be first-line treatment options in women with fibroids of this size [23]. In current study, the mean fibroid diameter of patients who underwent surgery was 44 mm, which was consistent with the NICE guideline. At the same time, the fact that the mean value of myoma diameter that can compress the endometrium was 42 mm higher in the surgical group, emphasizes the importance of considering the size, number and location of fibroids and the severity of symptoms before treating these women with LNG-IUS.

Limitations

This study has some limitations. It had a small sample size arising from the same centre from a local region.

CONCLUSION

LNG-IUS seems to be a popular and effective option in heavy menstrual bleeding. However, in patients with known organic etiology (FIGO PALM), the main goal should be to correct the detected pathologies that cause heavy menstrual bleeding, and it should not be ignored that the success rate of LNG-IUS may be lower in this patient group.

Authors' Contribution

Study Conception: LÖ; Study Design: LÖ; Supervision: LÖ; Funding: N/A; Materials: LÖ; Data Collection and/or Processing: LÖ; Statistical Analysis and/or Data Interpretation: LÖ; Literature Review: LÖ; Manuscript Preparation: LÖ and Critical Review: LÖ.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Evaluation of umbilical cord blood hematological parameters in pregnant women with fetal growth restriction

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ABSTRACT

Objectives: The present study attempted to evaluate the relationship between fetal growth restriction (FGR) and the platelet-to-lymphocyte ratio (PLR) and neutrophil-to-lymphocyte ratio (NLR) in singleton term pregnancy.

Methods: We carried out this prospective, case-control study at a Training and Research Hospital in Türkiye between May 2021 and January 2023. We utilized ultrasonography to assess singleton pregnancy with FGR between 37-40 weeks of gestation and considered the patients' last menstrual periods (LMD).

Results: We recruited 250 pregnant women in the study and divided them into the FGR (n = 125) and healthy control (n = 125) groups. Our findings revealed no significant difference between the groups by age, body mass index (BMI), stillbirth history, abortion, tobacco use, and regular pregnancy monitoring ($p > 0.05$). Fetal birth weights and hospitalization in the neonatal intensive care unit (NICU) were also similar between the groups. Yet, 1- and 5-minute APGAR scores were significantly lower in the FGR group than in the control group. In addition, compared to the healthy subjects, the FGR group had significantly increased white blood cell, lymphocyte, neutrophil, platelet counts and NLR and PLR values, but mean platelet volume (MPV) remained similar.

Conclusions: In a nutshell, our findings suggested that two noteworthy inflammatory markers, NLR and PLR, are likely to elevate in the presence of FGR.

Keywords: Fetal growth restriction, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), hematologic parameters, cord blood

Fetal growth restriction (FGR) persists to be among the significant causes of morbidity and mortality in both intrauterine and neonatal periods and complicates approximately 4-6% of pregnancies [1]. It is defined as the estimated fetal weight (EFW) of a healthy fetus by chromosomal or karyotyping analysis or the fetal abdominal circumference by ultrasonography below the 10th percentile [2]. Uteroplacental insufficiency, leading to insufficient blood flow along the umbilical cord, may be considered the most prominent cause of FGR, and impaired umbilical blood flow always accompanies the disorder [3, 4].

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Impaired trophoblast invasion explains the early pathophysiology of this failure [5]. Causing the fetus to be deprived of sufficient oxygen and nutrients, this situation results from the deterioration of the transformation of spiral arteries to low-resistance veins [6].

In general, the parameters of the complete blood count e.g., neutrophil and lymphocyte counts, platelet distribution width (PDW), red blood cell distribution width (RDW), and mean platelet volume (MPV) are considered the indicators of the severity and prognosis of many diseases, particularly malignant and infectious ones [7]. It was previously proposed that MPV, platelet-to-lymphocyte (PLR), and red blood cells (RBC) can be used as a simple, inexpensive method to predict early pregnancy loss in obstetric evaluation [8]. In their meta-analysis, Kang *et al*. emphasized that the neutrophil-to-lymphocyte ratio (NLR) can be a useful marker in determining the clinical prediction and severity of preeclampsia [9]. It was also determined that white blood cell counts (WBC) and NLR, PLR, and MPV values may be considered independent parameters to predict gestational diabetes mellitus (GDM) [10]. In this sense, the present study aimed to investigate the role of MPV, PDW, NLR, RDW, plateletcrit (PCT), and PLR values obtained from the cord blood of pregnant women with intrauterine growth retardation in predicting newborn outcomes.

METHODS

Research Design and Sample

After obtaining ethical approval from the Ethics Committee of Kayseri City Hospital (No.: 383 dated 04.29.2021), we carried out this prospective, case-control study at a training and research hospital in Türkiye between May 2021 and January 2023. We recruited 250 pregnant women in the study and divided them into the FGR (n = 125) and healthy control (n = 125) groups. However, we excluded patients below 37 weeks and over 40 weeks pregnant, those with multiple pregnancies, those with concomitant maternal (preeclampsia, diabetes mellitus) or fetal disease (oligohydramnios, fetal anomaly), patients with missing prenatal complete blood count, and those not administered any pregnancy-related tests (Fig. 1). We utilized ultrasonography to assess singleton pregnancy with FGR between 37-40 weeks of gestation and considered the patients' last menstrual periods (LMD). Besides, we adopted the Delphi consensus criteria for the diagnosis of FGR [11, 12].

Measurements

We initially noted down the maternal characteristics of the patients [Age, obstetric history (gestational age, gravida, parity, live births), gestational week ac-

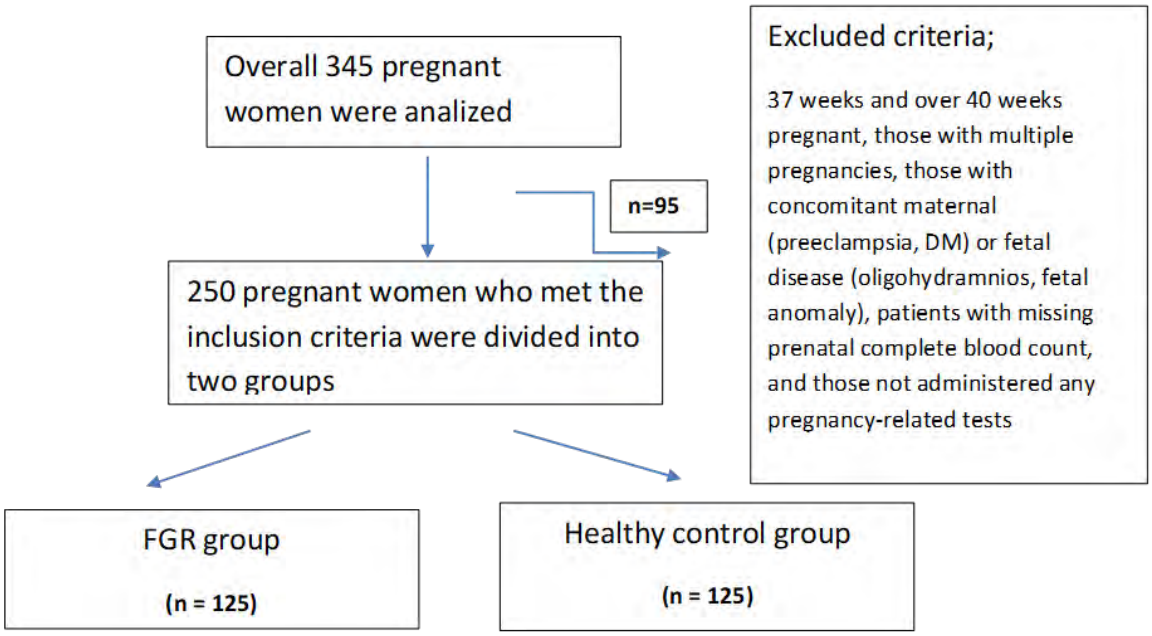

Fig 1. Patient flow chart.

cording to the LMP, tobacco use, regular pregnancy monitoring, type of delivery, birth weight, maternal complications (bleeding, dysuria, etc.), 1- and 5-min. Apgar scores, and hospitalization in the neonatal intensive care unit (NICU)]. Then, we stored about 2 ml of cord blood obtained from each patient at delivery in hemogram tubes with ethylenediaminetetraacetic acid (EDTA) to measure leukocyte, neutrophil, and lymphocyte counts, MPV, PDW, and RDW.

Statistical Analysis

The data are presented descriptively, and we utilized the Kolmogorov-Smirnov test to check whether the data showed a normal distribution. While the normally-distributed data were compared pair-wise using independent samples t-test, we utilized the Mann-Whitney U test to compare non-normally distributed data. The categorical variables were compared using the Chi-square and Fischer's exact tests. We performed all statistical analyses on the SPSS 26.0 program and accepted a p value of < 0.05 as statistically significant.

RESULTS

Our findings showed no significant difference between the FGR and healthy control groups by age ($p = 0.142$), BMI ($p = 0.54$), stillbirth ($p = 0.625$), abortion ($p = 0.56$), tobacco use ($p = 0.92$), and regular pregnancy monitoring ($p = 0.88$). While we found that the control group had significantly higher gravida ($p = 0.01$), parity ($p = 0.003$), gestational week ($p = 0.003$), birth weight ($p = 0.002$), and the number of surviving children ($p = 0.005$) than the FGR group. The number of male newborns was significantly higher ($p < 0.001$), while 1- and 5-minute Apgar scores were significantly lower in the FGR group ($p < 0.001$ for both). (Table 1).

Besides, NLR and PLR values ($p = 0.008$ and 0.001) and neutrophil and platelet counts ($p = 0.002$ and 0.001) were found to be significantly increased in the FGR group. Nevertheless, leukocyte ($p = 0.95$) and lymphocyte counts ($p = 0.08$), MPV ($p = 0.33$), PCT ($p = 0.88$), and PDW ($p = 0.64$) values were similar between the groups (Table 2).

Table 1. Patients' demographic characteristics and obstetric findings

	FGR group	Control group	*p* value
Age (years)	27.5 ± 6.3	26.3 ± 5.1	0.142
BMI (kg/m^2)	24.5 ± 4.5	25.6 ± 4.3	0.540
Gravida	2.2 ± 1.5	2.4 ± 1.3	**0.010**
Gestational week	37.1 ± 2.1	39.6 ± 1.1	**0.003**
Parity	0.9 ± 1.2	1.2 ± 1.3	**0.003**
Surviving children	0.8 ± 1.2	1.1 ± 1.1	**0.005**
Previous stillbirth	1.6 ± 1.1	2.1 ± 1.0	0.625
Previous abortion	0.9 ± 1.0	1.2 ± 1.1	0.560
Tobacco use	13 (10.4%)	12 (9.6%)	0.920
Regular pregnancy monitoring	110 (88%)	108 (86.4%)	0.880
Birth weight (g)	2,350 ± 450	3,240 ± 400	**0.002**
1-min. Apgar score	6.5 ± 0.7	8.9 ± 0.6	**< 0.001**
5-min. Apgar score	8.1 ± 0.6	9.2 ± 0.7	**< 0.001**
Gender			**< 0.001**
Female	45 (36%)	65 (52%)	
Male	80 (64%)	60 (48%)	
NICU admission (days)	11 (8.8%)	7 (5.6%)	0.260

Data are shown as mean ± standard deviation or n (%). FGR = Fetal growth restriction, BMI = Body mass index, NICU = Neonatal intensive care unit

Table 2. Patients' hematological and biochemical findings

	FGR group	Control group	*p* value
Leukocyte (×10^3 /mm^3)	34.7 ± 1.5	36.2 ± 1.3	0.950
Neutrophil (×10^3/mm^3)	7.5± 0.9	8.4 ± 1.1	**0.002**
Lymphocyte (×10^3/mm^3)	1.9 ± 0.6	1.8 ± 0.7	0.080
Platelet (×10^3/mm^3)	197 ± 51	278 ± 43	**0.001**
NLR	3.94 ± 1.1	4.66 ± 0.9	**0.008**
PLR	103.6 ±1.7	154.4 ± 1.2	**0.001**
MPV	9.2 ± 0.8	9 ± 0.9	0.330
PCT	0.35 ± 0.04	0.34 ± 0.03	0.880
PDW	11.7 ± 2.1	11.5 ± 1.9	0.640

Data are shown as mean±standard deviation. FGR = Fetal growth restriction, NLR = neutrophil-to-lymphocyte ratio, PLR = platelet-to-lymphocyte ratio, MPV = mean platelet volume, PCT = plateletcrit, PDW = platelet distribution width

DISCUSSION

Described as the inability of the fetus to reach its growth potential, FGR may be the most accurate description of the fetus [13]. Although a plethora of research on the subject adopted different criteria to describe it, there is still no consensus on a standard definition of FGR. Yet it is defined as a birth weight below 3%, 5%, or 10% or 2SD of mean gestational age or a birth weight of 2500 g or less in gestational age of 37 weeks and above [14]. In this study, we included those whose birth weight was 3% lower than the gestational age.

Overlapping with previous findings, our findings showed significantly lower gravida and parity and higher male fetuses among the patients with fetal distress. Pregnancy is likely to bring multiple changes in the hematological system. For example, hematological findings of the pregnant may yield an increase in blood volume by 30-40%, erythrocyte, and plasma levels by 30%, and reticulocyte erythropoietin levels by 2-3 times. While the absolute lymphocyte count is within normal limits, platelet half-life is shortened, and MPV and PDW values are increased [15]. It was previously reported that whole blood parameters can be adopted to predict specific diseases during pregnancy and reveal their prognosis. In this regard, many studies previously attempted to predict preeclampsia and its severity through some whole blood parameters [16-19]. It was documented that first and second-trimester NLR values may be considered a helpful marker to predict preeclampsia [20, 21]. Accordingly, NLR and PLR are often calculated to be higher among pregnant women with preeclampsia [16-22]. Moreover, the previous research showed that increased second-trimester NLR, PLR, and MPV values may be independent risk factors for GDM [23]. Moreover, it is known that pregnant women with hyperemesis gravidarum are likely to have elevated PLR and NLR values secondary to metabolic changes and inflammation [24]. The relevant research also concluded high MPV and RBC values and low PLR values among those with early pregnancy loss and miscarriage threat in the first trimester [8]. The literature also hosts studies reporting significantly higher NLR and PLR values in ectopic pregnancy cases with tubal abortion [25]. In contrast, Bullens *et al*. could not conclude any relationship between maternal hemoglobin levels and fetal distress and neonatal outcomes [26]. In our study, MPV values and hematocrit and lymphocyte counts were significantly lower, while neutrophil counts and PDW, NLR, and PLR values were significantly higher among patients with FGR. Meconium aspiration and infections, in particular, may have caused fetal and maternal systemic inflammatory response, leading to elevated neutrophil counts and NLR, PDW, and PLR values.

To our knowledge, this is the first study to explore the relationship between umbilical cord NLR and PLR values and FGR. The previous research that traced maternal inflammation with NLR indicated that inflammation causes disorganization of the placental vascular bed. In these studies, the mother's existing

inflammatory response was found to be related to low birth weight in the ongoing process [15-27].

Limitations

It should be noted that the present study is not free of a few limitations. The small sample size may be considered a significant limitation of our findings. In addition, the study did not include the findings of small-for-gestational-age (SGA) infants since we preferred to exclude the effects of SGA on inflammatory processes.

CONCLUSION

In our study, our findings suggested that NLR and PLR, two notable inflammatory markers, would likely be elevated in the presence of FGR. It can be used daily as screening biomarkers in the detection of FGR. These results should be supported by studies using larger samples.

Authors' Contribution

Study Conception: MA, ŞÇ; Study Design: MA, MBD; Supervision: MA, CRC, MBD; Funding: ŞÇ, MBD; Materials: CRC, MBD; Data Collection and/or Processing: MA, CRC; Statistical Analysis and/or Data Interpretation: MA, CRC; Literature Review MA, ŞÇ, CRC, MBD; Manuscript Preparation: MA, CRC, and Critical Review: MA, ŞÇ, CRC, MBD.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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The European Research Journal 2023;9(4):823-827 Case Report

DOI: 10.18621/eurj.1032176 *Pathology*

Paraganglioma of the urinary bladder mimicking urothelial carcinoma: a case report

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ABSTRACT

Paragangliomas of the urinary bladder are rare tumors and can mimic urothelial carcinomas due to some features and can be misdiagnosed. A 71-year-old female was seen for hematuria and there was a solid lesion that measured 3 cm on the urinary bladder at cystoscopy. Treatment approaches for paraganglioma and urothelial carcinoma are very different from each other; therefore differential diagnoses should be made carefully. Although immunohistochemical studies are helpful in differential diagnosis, they may cause misdiagnosis in some cases. In this article, we will discuss the clinical, histomorphological, and immunohistochemical differences between paraganglioma and urothelial carcinomas under their differential diagnosis.

Keywords: Paraganglioma, urothelial carcinoma, GATA-3, tyrosine hydroxylase

Paraganglioma of the urinary bladder is a neuroendocrine neoplasm that is rare and accounts for 0.06% to 10% of all bladder tumors that develops from Chromaffin cells located in the bladder wall. It is divided into functional (chromaffin) or nonfunctional (non-chromaffin) according to catecholamine expression in the whole body. In the urinary bladder, findings related to catecholamine secretion can be seen in patients with functional paraganglioma. While some of the patients may be asymptomatic, hematuria and lower urinary tract symptoms may develop rarely [1].

Paragangliomas of the urinary bladder can mimic urothelial carcinomas and misdiagnose. In this article, we will discuss the important morphological, clinical, and immunohistochemical studies in differential diagnosis.

CASE PRESENTATION

A 71-year-old female was seen in the urology clinic for hematuria for over a week duration. She had a history of thyroidectomy for papillary carcinoma 5 years ago and well-controlled hypertension for 10 years with drugs. Her blood pressure values were around 120/80 before the operation. For further assessment cystoscopy was performed and there was a solid, invasive lesion on the right lateral wall of the urinary bladder. Macroscopically, the tumor which measured 3 cm, infiltrated the detrusor muscle. Transurethral resection was performed.

Microscopically, the tumor was composed of nests of cells with monotonous nuclei and large granular amphophilic cytoplasm. Nests were surrounded by a

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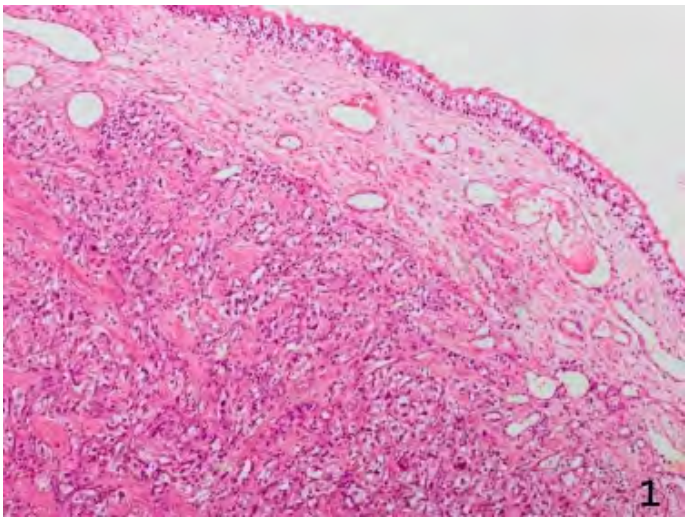

Fig. 1. Neoplastic proliferation below the normal urothelial epithelium (HE×40).

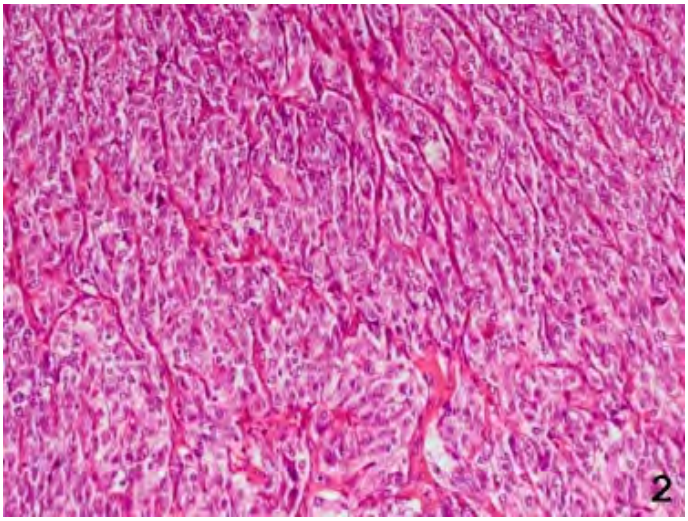

Fig. 2. Classic Zellballen configuration consisting of nests surrounded by thin fibrovascular connective tissue (HE×200).

thin, fibrovascular connective tissue in a classic Zellballen configuration. The tumor invaded through the muscularis propria. No angioinvasion, lymphatic and perineural invasion was observed in the tumor and no mitotic activity and necrosis were seen. The overlying urothelium was normal (Figs. 1, 2, and 3). The tumor cells were strongly positive for neuroendocrine markers Chromogranin A, Synaptophysin and INSM-1 on immunohistochemistry. S100 protein high lights sustentacular cells. The tumor was negative for cytokeratins. Tyrosine hydroxylase was positive and there was no loss in succinate dehydrogenase B immunohistochemically. The Ki67 proliferation index was about 2-3%. Additionally, the tumor was positive for GATA-3 (Fig. 4). The patient was evaluated postoperatively by an endocrinologist. No pathology was found on physical examination. Laboratory results were normal reference ranges. 18 FDG Positron emission tomography (PET-CT) was done on the patient for metastasis no metastases were detected. The patient's hypertension has been well controlled in her follow-up and was evaluated as essential hypertension.

DISCUSSION

Paragangliomas of the urinary bladder are extremely rare tumors and numerous cases of paraganglioma misdiagnosed as urothelial carcinoma have been reported in the literature [1]. The distinction between paraganglioma and urothelial carcinoma is extremely important because of the different treatments. Even when paraganglioma invades the muscularis propria, partial cystectomy is sufficient; muscle-invasive urothelial carcinomas are treated with radical cystectomy. Patients with paraganglioma may be differentiated from patients with urothelial carcinoma by their age and clinical characteristics [2]. Paraganglioma is seen 1-2 decades younger than urothelial carcinoma. If paraganglioma is functional, symptoms due to cat-

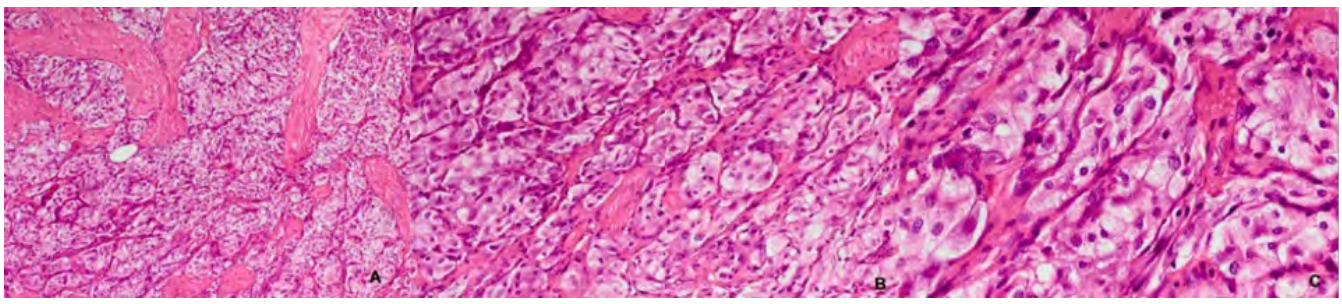

Fig. 3. Monotonous tumor cells with large granular amphophilic cytoplasm invading the muscularis propria (A:100×HE, B:200×HE, and C:400×HE).

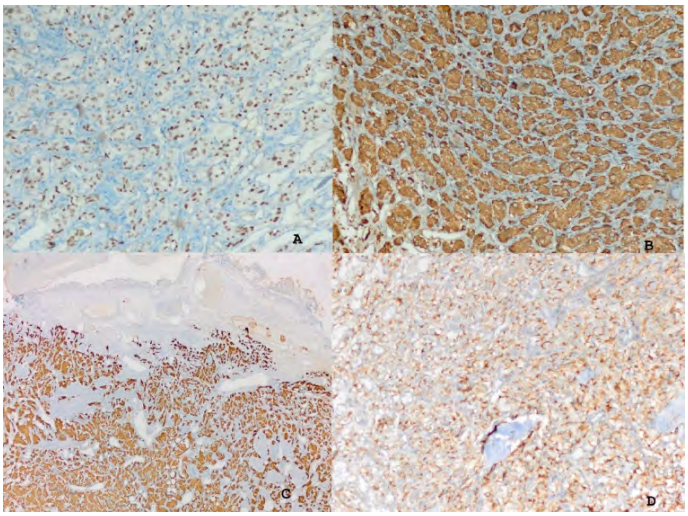

Fig. 4. (A) and (B) Immunohistochemically GATA-3 and Synaptophysin positivity in tumor cells (Anti GATA-3×200, Anti Synaptophysin×200), (C) Strong tyrosine hydroxylase positivity in tumor cells beneath the urothelium (Anti Tyrosine Hydroxilase×40), and (D) No loss of SDHB was seen by immunohistochemistry in tumor cells (Anti SDHB×200).).

echolamine secretion can be seen in patients. Macroscopically, paragangliomas are lobulated and well-circumscribed, while urothelial carcinomas show an infiltrative appearance [3]. Our case was a 71-year-old patient and urothelial carcinoma was considered during the cystoscopic examination.

Paraganglioma may be confused with nested variant urothelial carcinoma because of their nest structures; therefore, zellballen structures in the histopathological examination are very helpful in diagnosis. The majority of tumors have the characteristic zellballen pattern consisting of nest structures separated by thin fibrovascular septa at least focally [4].

More than half of the cases with paraganglioma have muscularis propria invasion without a desmoplastic reaction. In urothelial carcinomas, a stromal reaction is expected to accompany muscle invasion, even if it is focal. Our case was also invasive to muscularis propria, but there was no accompanying stromal response, it consisted of zellballen structures in large areas. Tumor cells in paraganglioma always have large basophilic and granular amphophilic cytoplasm and uniform smooth chromatin. Sometimes pleomorphic or bizarre cells that are considered neuroendocrine atypia can be observed. These cells can be confusing for urothelial carcinoma, but the absence of mitosis supports the diagnosis of paraganglioma [5].

Immunohistochemical studies are very useful in the differential diagnosis of paraganglioma and urothelial carcinoma. Whereas urothelial carcinomas are always positive for various keratins such as CK7 and CK20, paragangliomas are not stained with keratins. Insulinoma-associated protein 1 (INSM-1), a nuclear transcription factor used in the detection of neuroendocrine differentiated cells and tumors, may be helpful in the differential diagnosis between paragangliomas and urothelial carcinomas [6]. In our case, INSM-1 was positive in tumor cells, while it was negative in urothelial cells.

GATA-3 which demonstrate urothelial differentiation has been reported to be positive in many tumors and urinary bladder paragangliomas as well as urothelial carcinomas. Therefore, using GATA-3 may cause a misdiagnosis [7]. Similar to the cases reported in the literature, GATA-3 positivity was observed in our case. Therefore, in the differential diagnosis of urothelial carcinoma, other immunohistochemical studies, and all morphological and clinical findings should be evaluated together.

Another lesion that is important in the differential diagnosis with paraganglioma is metastatic neuroendocrine tumors. Since neuroendocrine markers are positive in both paraganglioma and neuroendocrine tumors, it may cause misdiagnosis if additional immunohistochemical studies are not performed. A useful immunohistochemical marker in this subject is tyrosine hydroxylase which plays role in the biosynthetic pathway of catecholamines [8]. Immunohistochemically staining with tyrosine hydroxylase proves that the tissue is paraganglioma and pheochromocytoma. The negativity of tyrosine hydroxylase in neuroendocrine tumors is very helpful in differential diagnosis, but it should be kept in mind that it may be negative in parasympathetic paragangliomas [9]. In our case, the possibility of neuroendocrine tumor metastasis was excluded with the keratins negativity and tyrosine hydroxylase positivity.

Histopathological characteristics of the tumors can be determined using the GAPP scoring system in determining the metastasis risk of paraganglioma. GAPP criteria consist of architectural patterns, cellularity, presence of comedo necrosis, presence of vascular/capsular invasion, Ki67 labeling, and biochemical evidence of disease [10]. Although the GAPP scoring system shows high accuracy in deter-

mining the metastasis risk of the cases, it has been argued that the succinate dehydrogenase (SDH) gene mutation should be considered in addition to the GAPP criteria in recent studies [11]. Investigation of the status of SDHB immunohistochemically provides common information for all SDHx related diseases. Because any mutation involving one of the SDH subunits and assembly factors leads to destabilization of the protein complex and loss of immunoreactivity [9]. SDHB mutation was studied by immunohistochemical study, which is an easy and practical method in our case, and staining was observed with this marker and it was concluded that there was no mutation in the SDHB gene. When our case was evaluated according to the GAPP criteria, her score was found to be low, but since paraganglioma was not considered clinically at the beginning, biochemical measurement of catecholamine metabolites could be made 10 days after transurethral resection. In this case, the catecholamine level may have decreased to normal limits biochemically due to the resection of the tumor.

In addition to mutation status and GAPP criteria, tumor size and localization are also important in determining the poor clinical course [9]. In our case; no metastasis was detected according to the 18FDG-PET CT result. The functional status of the tumor is not known, but it can be said that the risk of metastasis is low due to the SDHB expression and small tumor size. The 2017 World Health Organization no longer classified paragangliomas as benign, even without metastasis as multifocal and progressive diseases have significant morbidity and mortality [12]. Therefore, how long these patients will be followed is a controversial issue, cases are presenting with metastasis even 20-40 years after diagnosis. For this reason, it is recommended that patients with paraganglioma should be followed for life with catecholamines and their metabolites and imaging methods [13].

More than 40% of patients with paraganglioma have germline mutations involving one of more than 20 genes. A genetic examination is recommended for determining disease progression because it enables early diagnosis and treatment of other family members before complications develop. Our case is an elderly patient, and sporadic mutation was thought to be more likely. A genetic examination was recommended for the patient, but could not be performed due to the patient's socioeconomic conditions.

CONCLUSION

Treatment approaches for paraganglioma and urothelial carcinoma are very different from each other; therefore differential diagnoses should be made carefully. Patients should be evaluated together with all morphological, clinical findings, and immunohistochemical study results. For a correct diagnosis, immunohistochemical studies should be performed in combination with neuroendocrine markers, keratins, and tyrosine hydroxylase.

Authors' Contribution

Study Conception: ÇÖ, HG; Study Design: ÇÖ, HG, SÇA; Supervision: HG, OO; Funding: ÇÖ, OO; Materials: ÇÖ, SÇA; Data Collection and/or Processing: ÇÖ, HG; Statistical Analysis and/or Data Interpretation: ÇÖ, SÇA; Literature Review: ÇÖ, HG; Manuscript Preparation: ÇÖ, SÇA and Critical Review: HG, OO.

InformedConsent

Written informed consent was obtained from the patient for publication of this case and any accompanying images or data.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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The European Research Journal 2023;9(4):828-830 Case Report

DOI: 10.18621/eurj.1027864 *Surgery*

Giant gastrointestinal stromal tumor of the duodenum mimicking a pancreas head tumor: a case report

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ABSTRACT

Objectives: Duodenal gastrointestinal stromal tumor (GIST) is rarely observed in the gastrointestinal system. It can be confused with pancreas pathologies as it shows an extramural growth pattern. The case is here presented of a 65-year-old male who presented with the complaints of abdominal pain, nausea and vomiting. On contrast abdominal tomography, a centrally hypodense, necrotic mass, 140×115×100 mm in size, was observed in the pancreas head. With a preliminary diagnosis of neuroendocrine tumour/serous cystic neoplasia, a Whipple + partial vena cava resection + primary repair operation was performed. The pathology was reported as duodenal GIST. The patient was discharged with medication and referred to the oncology clinic.

Keywords: Duodenum, gastrointestinal stromal tumor, pancreas, Whipple

Gastrointestinal stromal tumor (GIST) originates from the interstitial cells of Cajal and is found in the submucosa of the gastrointestinal system and the myenteric plexus. There are 3 histological types: spindle cell type, which is the most common, epitheloid type, and mixed type [1]. GISTs are specific mesenchymal neoplasia, which can be observed in the digestive system from the mouth to the anus. They are generally seen in the gastric (60%-70%), small intestine (30%) and colon-rectum (10%), and are only seen in the duodenum at the rate of 3%-5%. Duodenal stromal tumors are rarely seen. Tumors with an extramural growth pattern which can clinically and radiologically mimic pancreas head cancer are extremely rare [2]. The case is here presented of a patient with a spindle cell duodenal GIST which appeared as a mass on the pancreas head.

CASE PRESENTATION

A 65-year-old male presented at our clinic with complaints of abdominal pain, nausea and vomiting. On physical examination a palpable mass and associated sensitivity was determined in the epigastric region. The laboratory values and tumor markers were normal. Triphasic abdominal computed tomography (CT) with intravenous contrast was applied to the patient. A centrally hypodense, necrotic, mass lesion was observed showing exotic development at the level of the pancreas uncinate process, measuring approximately 140×115×100 mm, which showed significant peripheral contrast in the arterial phase after contrast injection, a relative decrease in contrast in the venous phase, and washout in the late phase. The mass lesion was surrounding the aorta from the anterolateral at ap-

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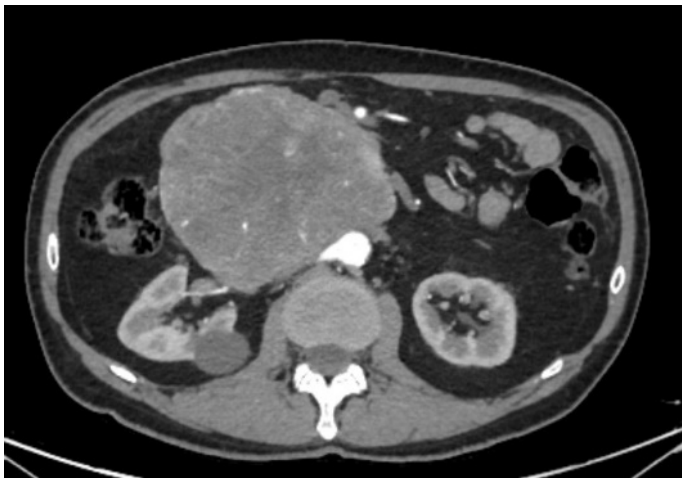

Fig. 1. **Triphasic abdominal computed tomography (CT).**

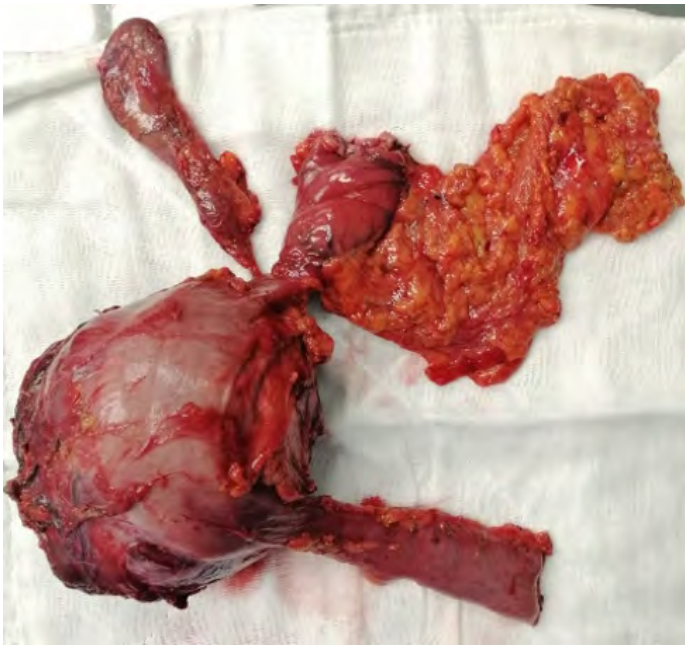

Fig. 2. **Whipple prosedure.**

proximately 45°. The mass was compressing the vena cava inferior and right ureter. The superior mesenteric artery (SMA) was bowed and pushed towards the anterior by the mass lesion. In the preliminary diagnosis, neuroendocrine tumour showing exotic development and serous cystic neoplasia of the pancreas were first considered (Fig 1). Endoscopic Ultrasonography (EUS) and biopsy was not performed. Because GIST was not considered in the first stage and an operable mass was observed in the head of the pancreas. A Whipple procedure and partial vena cava resection + primary suture repair were performed (Fig. 2). As a result of pathology examination, the mass was reported as a duodenal GIST, 12 cm in diameter, formed of spindle cells, with mitosis ratio 9/5 mm2, DOG1: +++, CD34:-, and Desmin Ki67: >10% high risk. Six lymph nodes dissected from around the pancreas and small intestine were reactive. No complications developed postoperatively and the patient was discharged on day 12 with medication, and was referred to the oncology clinic. Treatment of imatinib was started by the oncology department. On the follow-up tomography at postoperative 18 months, irregular tissue of 25×15 mm was observed adjacent to the SMV. No involvement was observed on PET-CT. The patient was seen by the oncology council and it was decided to continue follow up with a doubling of the imatinib dose.

DISCUSSION

The first definitive immunohistochemical diagnosis of GIST was made by showing over-expression in KIT (CD117) marker. Nowadays, almost 100% of diagnoses are made from CD117 and DOG 1 positivity. There are known correlations between CD34 and the tumour region, and between CD117 and tumour morphology [3].

The annual incidence of GIST is approximately 10 in 1 million. Although there are a greater number of asymptomatic GIST, determination is difficult. In autopsy series, asymptomatic GISTs < 1 cm have been observed at the rate of 25%. Symptomatic GISTs mostly manifest with dyspepsia, bleeding and abdominal mass [4]. The current patient had dyspeptic complaints and there was an abdominal mass.

Duodenal GIST is uncommon but is mostly located in the second section of the duodenum, followed by the third, fourth, and first sections. In many duodenal GISTs, giant ulcers can be observed in the mucosa due to the pressure of the submucosal mass [5]. Endoscopy, tomography, or MRI can be used in the diagnosis of duodenal GIST. Giant GISTs show a heterogenous structure on tomography. Together with areas of cystic degeneration, there is hemorrhage and necrosis in the centre. This can therefore lead to incorrect evaluation of giant duodenal GIST as a mass on the pancreas head [6]. Especially in the arterial phase or late venous phase, GIST can be seen as a hypervascular exophytic or endophytic mass [7]. In the current case, there was central necrosis in a giant mass com-

pressing the vena cava and bowing the SMA. In the arterial phase, the mass showed significant peripheral contrast and in the late venous phase, there was washout. In the initial radiological diagnosis, it was thought to be a neuroendocrine tumour (NET) of the pancreas or serous adenocarcinoma.

For duodenal GISTs, just as for other GISTs, the only curative treatment is surgery. Optimal surgery is the removal of the mass with clean surgical margins to include invaded organs. There is no lymph node metastasis in GISTs. They are well-encapsulated tumours showing a submucosal growth pattern. Local excision or duodenectomy is associated with long-term disease-free survival. However, when necessary, pancreatoduodenectomy is applied at rates of 20%-86% [7]. In the current case, a pancreatoduodenectomy operation was performed.

Curative surgery applied with complete tumour resection increases survival. If negative surgical margins are obtained, recurrence can be observed. Pidhorecky *et al* reported the development of recurrence at 76% in the stomach and 64% in the small intestine despite clean surgical margins [8]. On the follow-up tomography of the current case at postoperative 18 months, irregular tissue of 25×15mm was observed adjacent to the SMV. No involvement was observed on PET-CT. The patient was seen by the oncology council and it was decided to continue follow up with a doubling of the imatinib dose.

CONCLUSION

In conclusion, duodenal GISTs can mimic pancreas head masses becausee of the extramural growth pattern. This must not be ignored in the differential diagnosis of tumours located in the duodenum and pancreas.

Authors' Contribution

Study Conception: EA, MAÜ; Study Design: EA, MAÜ; Supervision: OA; Funding: N/A; Materials: EA; Data Collection and/or Processing: OA; Statistical Analysis and/or Data Interpretation: MAÜ, EBB; Literature Review: EBB; Manuscript Preparation: EA, MAÜ and Critical Review: OA, EBB.

Informed Consent

Written informed consent was obtained from the patient for publication of this case and any accompanying images or data.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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