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The European Research Journal 2026;12(4):407-421 Original Article

DOI: https://doi.org/10.18621/eurj.1760073 *Cell Physiology*

Intracellular Angiotensin-II Measurement in Streptozotocin-Induced Rat Vascular Smooth Muscle Cells and Its Relationship with Angiotensin-II Receptors

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ABSTRACT

Objectives: Angiotensin II (Ang-II) is vital constituent of renin angiotensin aldosterone system and increases in some cardiovascular diseases such as diabetes. However, there are not enough studies related to intracellular and extracellular Ang-II levels and its interaction with Ang-II type 1 and 2 receptors (ATR1, ATR2) in vascular smooth muscle cells (VSMCs). We aimed to investigate healthy and diabetic rat model of VSMCs (H-VSMCs, D-VSMCs) proliferation and Ang-II levels.

Methods: VSMCs were isolated from the aorta of healthy and diabetic Wistar rats. Diabetic model was achieved by intravenous administration of 45 mg/kg streptozotocin. Firstly, different Ang-II (0-1000 µM) concentration was performed for dose study. Ang-II (0.1 µM), Ang-II type 1 receptor (ATR1) antagonist (Olmesartan, 1 µM) and Ang-II type 2 receptor (ATR2) antagonist (PD123,319, 1 µM) were practiced together, and thereafter cell proliferation was evaluated by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) method. Intracellular and extracellular Ang-II levels were measured by ELISA kit.

Results: While H-VSMCs proliferation increased in Ang-II 0.1, 0.01, 0.001 and 0.0001 µM, D-VSMCs proliferation increased Ang-II 0.1 and 0.01 µM applications ($P<0.05$). Olmesartan 1 µM inhibited proliferation in H-VSMCs. Ang-II detected intracellular and extracellular groups of VSMCs, but no significant difference was found between H-VSMCs and D-VSMCs groups ($P>0.05$).

Conclusions: Ang-II enhances proliferation of H-VSMCs and D-VSMCs. There is no relationship that could be established between intracellular and extracellular Ang-II levels, H-VSMCs and D-VSMCs proliferation and Ang-II receptors.

Keywords: Angiotensin II, Diabetes, Smooth Muscle Cell, Vascular

One of the most important components of the vascular structure is vascular smooth muscle cells (VSMCs). Numerous physiological (growth, vasoconstriction, extracellular matrix formation, vasodilatation) and pathological processes (hypertrophy, fibrosis, migration, proliferation, inflammation) occur in the media layer of the vascular tissue. Moreover, proliferation of VSMCs causes

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pathologies such as atherosclerosis, hypertension and diabetes mellitus [1, 2].

Primary cell culture of the VSMCs is constantly used in vitro studies by researchers. Different techniques are also used to obtain primary VSMCs culture. However, achieving pure VSMCs can be quite difficult, and it has some complex stages [3, 4].

VSMCs may also undergo phenotypic changes in vascular diseases [5, 6]. Many specific proteins are expressed in early (SMα-actin, myocardin, SM22-α, h-caldesmon and SM-calponin) and late (desmin, meta-vinculin, SM-1, SM-2 and smoothelin) phases from the early stages of the differentiation of VSMCs [7, 8].

The systemic or extracellular renin angiotensin aldosterone system (RAS) has various roles in regulating water and electrolyte homeostasis, blood pressure, proliferation and growth of the VSMCs, migration and inflammation in cardiovascular system [9, 10].

Local or intracellular RAS components have been identified in many different types of tissues and cells. But, role of the nuclear components of intracellular RAS is still unknown [11]. The major components of RAS are (1-) Angiotensinogen synthesized from the liver, (2-) Renin, produced from the juxtaglomerular apparatus (JGA) cells in kidneys and converts angiotensinogen to Angiotensin I (Ang-I), (3-) Angiotensin-converting enzyme (ACE), converts Ang-I to Ang-II, synthesized in lung capillaries (4-) Ang-II receptors (ATR1s, ATR2s) are responsible for Ang-II's cellular effects [12-14].

It is also known that the component of RAS is synthesized in different cells and secreted to the outside of the cell membrane [11, 15]. The secretion of Ang-II from vascular layer can be inhibited by ACE, ATR and renin inhibitors. Some studies demonstrate local production was controlled by secretion of Ang-II [11].

RAS is directly involved in physiology and physiopathology of the cardiovascular system. However, some of RAS components, signaling pathways, and cellular effects of local RAS are cited as unexplained aspects of this system [16].

Ang-II is locating center of RAS and has physiological and pathological effects in the cardiovascular system. While, Ang-II has acute effects such regulation blood pressure by regulating water, salt homeostasis and vasoconstriction, its chronic effects cause hyperplasia, hypertrophy and migration in vascular smooth muscle cells [17].

Ang-II shows its effects on VSMCs through ATR1 and ATR2. Inositol-3 phosphate (IP3) and diacylglycerol (DAG) are formed by binding to ATR1 and activate number of signaling pathways that induce vasoconstriction, cell proliferation, growth, fibrosis and inflammation [14, 18]. The formation of mitogen activated protein kinase (MAPK), protein kinase C (PKC), reactive oxygen products (ROS) and NADPH oxidase increase with the activation of tyrosine kinase receptors [19]. But binding to ATR2 results in increased nitric oxide (NO) release, leading to vasodilatation and decreased cell proliferation [9, 20].

It has been suggested that local RAS components can be selectively activated in many specific conditions and cell types that induce various cardiovascular pathological problems [12, 14]. Local RAS components also increased in diseases such as diabetes mellitus (DM), atherosclerosis and hypertension emerge in cardiovascular and many other tissues [21]. For all that, it is understood that it may play a key role in its development and progress of these diseases. However, the intracellular effects of local RAS components on cardiovascular tissue and the mechanism of these pathways have not been fully elucidated [15, 22]. When its effects on VSMCs mechanisms are figured out, intracellular RAS can be used to treat cardiovascular diseases [23].

Many in vivo and in vitro studies have been performed to compare the effects of systemic and local RAS components in normal and diabetic disease conditions. It has been indicated that VSMCs may have changes in their individual behaviors and interaction with each other, under physiological and pathological conditions. But there are differences in the cellular effects (proliferation, migration) of Ang-II and ATR in pathological conditions such as diabetes and hyperglycemia and it may lead several differences in cellular responses to ATR blockers that constantly used in the treatment of hypertension [24-26].

In the light of these aspects, we aim to investigate the effects of Ang-II, ATR1 antagonist (Olmesartan) and ATR2 antagonist (PD123,319) on proliferation, and determine the intracellular and medium Ang-II levels in healthy and streptozotocin (STZ)-induced

diabetic rat model VSMCs (H-VSMCs and D-VSMCs) in this study.

METHODS

Animals

We used Wistar albino male rats (8 weeks old, body weight 180-200 g) in the present study. Healthy and diabetic animals were housed under a 12-hour light/12-hour dark cycle in care rooms, fed with standard rat chow (MBD Feed Trade, protein 19%, Türkiye) and were allowed to water with ad libitum. Rats were anaesthetized with ketamine/xylazine (Ketasol 10%, richterpharma ag, Rompun 2%, Bayer, 100/10 mg/kg) was administered intraperitoneally. The diabetic rat model (n=5) was performed by intravenous injection of 45 mg/kg streptozotocin (STZ) (Cat-No: S0130, Sigma-Aldrich) in citrate buffer (10 mM, pH 4.5) through tail vein and the animals in the control group (n=5) were administered the same amount of citrate buffer. The blood glucose levels of the animals were measured with a glucometer (Gluco Dr). Healthy and diabetic rats blood glucose level evaluated before and three days after STZ application. Animals with blood glucose levels above 250 mg/mL were included in the diabetic model group (Figure 1). Primary cell culture of VSMCs were done 8 weeks after induction of diabetes.

Isolation of VSMCs from Healthy and Diabetic Model Rats

Firstly, abdomen of the anaesthetized rat was opened from the upper middle region under sterile conditions. The thoracic aorta was removed and transferred to a culture dish with cold transfer medium (Hank's Balanced Salt Solution-HBSS, Cat-No: L2055, Biochrom Merck, Calcium chloride dihydrate, Cat-No: C7902, Sigma-Aldrich, Penicillin-streptomycin-PSA, Cat-No: P4333, Sigma-Aldrich) (Figure 2).

The isolated tissue was cleaned from connective tissue under a stereo microscope and endothelial layer was scraped slowly and lightly along the vessel. After

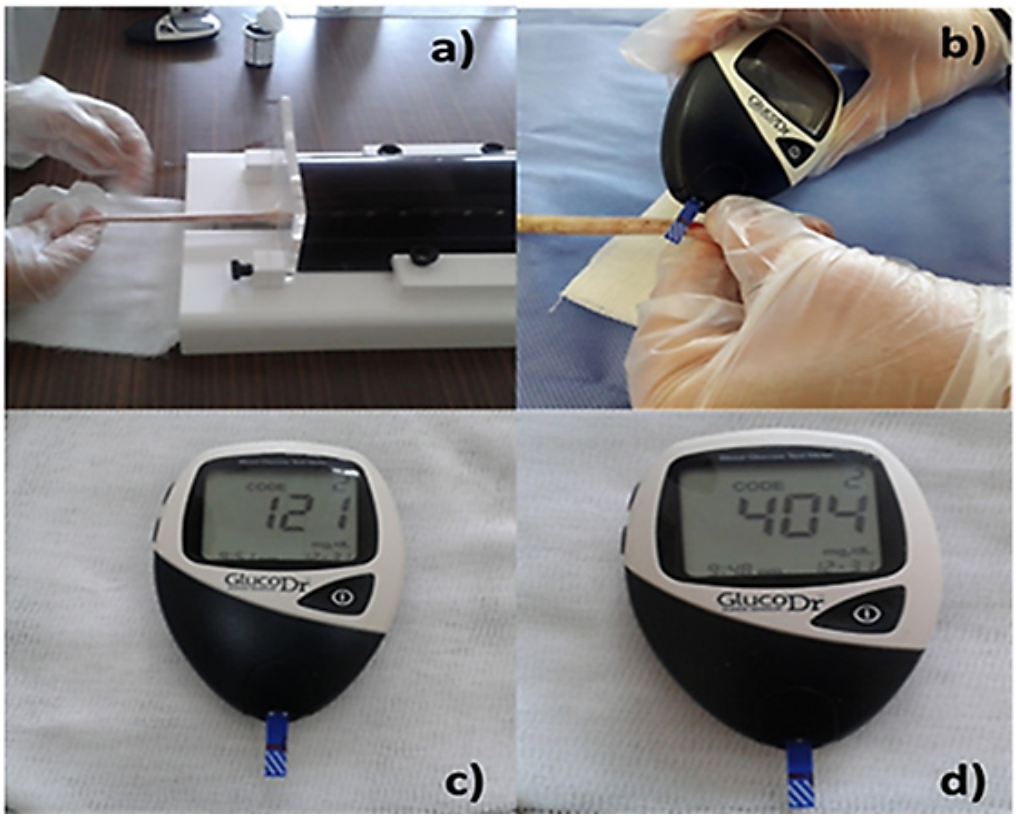

FIGURE 1. Diabetic animal. a) Placing the animal in the handle, b) Measurement of blood glucose levels with glucometer, c) Normal rat blood glucose, and d) Diabetic rat blood glucose.

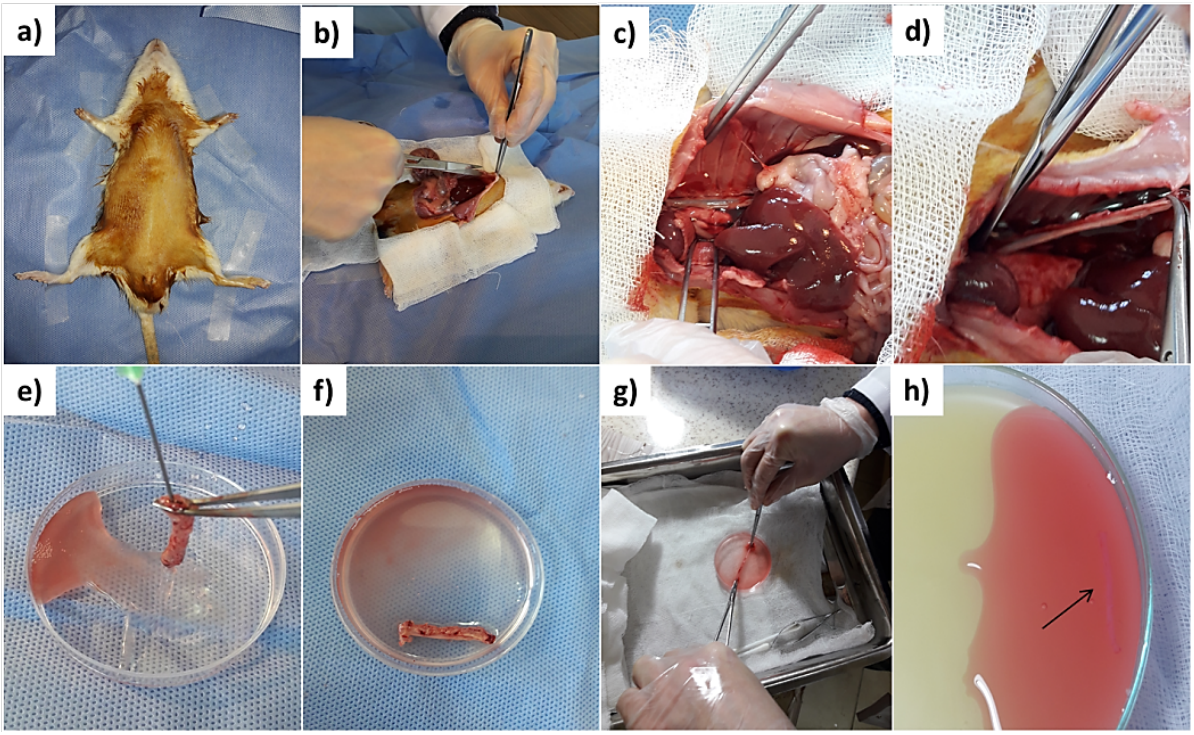

FIGURE 2. Isolation of the aorta. a) Sterilizing the abdomen, b) Cutting the midline of the abdomen, c) Isolation of the thoracic aorta, d) Holding the end of the vein with forceps, e) Cleaning the inner section of aorta with transfer solution, f) Isolated aorta, g) Cleaning the tissue surrounding the vessel, and h) Isolated aorta indicated by arrow.

separating the media layer from the adventitia, it was taken into a petri dish containing enzyme dissociation solution (HBSS, HEPES, Bovine serum albumin (BSA, Cat-No: A3156, Sigma-Aldrich), Trypsin inhibitor from glycine (STI, Cat-No: T6522, Sigma-Aldrich), Elastase (Cat-No: E7885, Sigma-Aldrich), Collagenase (Cat-No: C2674, Sigma-Aldrich), Calcium chloride dihydrate. It was cut into small pieces and incubated 45 min at 37°C in Dulbecco's Modified Eagle Medium (DMEM, Cat-No: D6046, Sigma-Aldrich) containing 20% Fetal Bovine Serum (FBS, Gibco, Thermo Fisher Scientific) and 1% Penicillin-Streptomycin (PSA, Cat-No: P4343, Sigma-Aldrich). It was centrifuged at 300 g for 5 min (Figure 3). The pellet was seeded in a 25 cm2 cell culture dish with medium. When the cells were examined with inverted microscope on day 5, it was seen that the cells adhered to the culture plate basement and started to proliferate. The culture cell medium was changed every 72 hours and incubated in a 37°C incubator with 5% CO_2, 95% air mixture and humidity. When cells were grown to 70 to 80% confluence passaging was performed.

The primary culture of VSMCs exhibited typical spindle-shaped appearance with characteristic 'hill and valley' patterns [27, 28]. All experiments were performed in VSMCs between passages 4 and 8. It was observed that in our study isolated thoracic aorta of the diabetic animals was very fragile and tended to fragmentation. The probability of culturing VSMCs from diabetic rats was at a very low rate compared to healthy rats. While rate of successful culture from diabetic animals is ~10%, in which vascular cells are obtained and attached to the base, ~90% in healthy animals.

Cell Purity and Identification

Immunocytochemistry (IHC) method performed to show expression of SMα-actin, caldesmon and calponin proteins. Firstly, VSMCs are seeded in polylysine-coated slides. They were placed in petri dishes containing cell media and incubated for 3-5 days. IHC staining confirmed positive for α-SMA, caldesmon and calponin. Our primary VSMCs culture method showed higher purity incidence and morphology similarity.

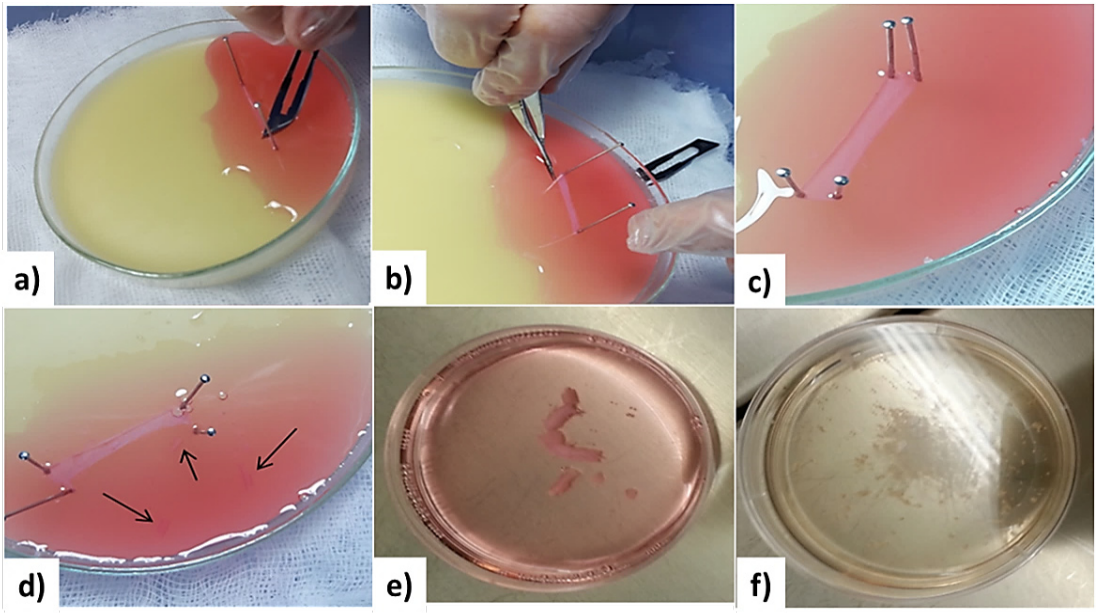

FIGURE 3. VSMCs isolation. a) Cleaning the adventitia layer with scalpel, b) Cutting the aorta with microsurgical scissors, c) Stretching the aorta from the inner surface with the needles, d) Isolated media layer indicated black arrows. Enzyme dissociation stage, e) Isolated smooth muscle layer, and f) Smooth muscle layer divided into pieces. VSMCs, vascular smooth muscle cells.

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) Assay

H-VSMCs and D-VSMCs were seeded at a density of 5×103-10x103 cells/100 µL per well and incubated for 48 hours before drug treatment. Then, cells were incubated for 24-48 hours with Ang-II concentrations (0-Control-1000 µM, [Val5]-Angiotensin II acetate salt hydrate, Cat-No: A2900, Sigma-Aldrich) for dose study. Maximum proliferative Ang-II dose was determined for 24 hours and administered with Olmesartan (1 µM, Cat-No: SML1394, Sigma-Aldrich) and PD123,319 (1 µM, Cat-No: P186, Sigma-Aldrich). After removing solutions 100 uL fresh medium and 10 µL Thiazolyl Blue Tetrazolium Bromide-5 mg/mL (MTT, Cat-No: M5655, Sigma-Aldrich) was added to each well and incubated 3-4 hours. Then, MTT solution is removed and 100 µL dimethyl sulfoxide (DMSO, Cat-No: M116743100, Merck) was added for dissolving formazan. After 20 min of incubation, the absorbance values of the wells at 570 nm wavelength were measured on the plate reader (Eon, Biotek). The average absorbance value of each group was determined, and the percentage viability of the cells was calculated.

Preparation of Vsmcs and Medium Extracts

H-VSMCs and D-VSMCs (~0.5×106) were seeded on 6-well plates. Then, Ang-II, (0.1 µM), Olmesartan, (1 µM) and PD123,319, (1 µM) were applied for 24 hours. Mediums in wells were collected and centrifuged at 300 g for 10 min. Cell extracts prepared with RIPA lysis buffer (Cat-No:SC-24948A, Santa Cruz Biotechnology) according to protocol. RIPA buffer-450 µL added to wells. Cells were removed from the surface with a plastic cell scraper and centrifuged at 10.000 g for 15 min at +4°C. Supernatant was stored at -80°C until the study carried out.

Total Protein Measurement by Lowry Method

The protein amount of samples between 0.01-1.0 mg/mL can be determined with this method [29]. BSA doses (0-4 mg/mL), blank and unknown samples (50 µL) were pipetted into wells and incubated at room temperature for 45 min. C reagent (150 µL, 100:1 mixture of A (2% Na2CO3, 0.4% NaOH, 0.16% Na-tartrate) and B (4% CuSO4.5H2O) reagents) incubated 20 min and following Folin-Ciocalteau's reagent (3 µL) was added and incubated 30 min. Absorbance values of the samples and standards were measured at

660 nm wavelength. The standard curve created with BSA absorbance values and protein concentration of the cell extracts were calculated.

ELISA

Ang-II levels in H-VSMCs, D-VSMCs and mediums was measured with a competitive ELISA kit (Angiotensin II EIA Kit, Cat-No: RAB0010, Sigma-Aldrich). Standards were prepared and other experimental steps were performed in accordance with the specified protocols. The absorbance values of the samples at 450 nm were measured. The medium Ang-II level was determined in pg/mL. H-VSMCs and D-VSMCs Ang-II level pg Ang-II/mg protein was calculated based on the protein amount of the samples.

Statistical Analysis

The data were analyzed using SPSS statistics 21.0 program (SPSS Inc, Chicago, 9 Illinois, USA) and expressed as mean±SE. One-way ANOVA was used as a parametric test in the case of homogeneous distributions. Post hoc Dunnett's and Tukey test were used to show the difference between groups. Mann Whitney U test was used as non-parametric Kruskal Wallis Post Hoc for data that were not homogeneously distributed. $P<0.05$ value was considered as significant.

RESULTS

Animal Blood Sugar and Urine Levels

Blood glucose levels of healthy and diabetic animals were monitored by glucometer before and on the 3rd day after STZ administration. Measurement of blood glucose levels followed for eight weeks. The urine of healthy and diabetic animals was measured with a strip. It was observed that levels of blood glucose in healthy rats were between 120-140 mg/dL, STZ-induced rats glucose changed between 330-430 mg/dL in 8 weeks. While urine glucose levels were <50 mg/dL in healthy rats, it was measured in diabetic

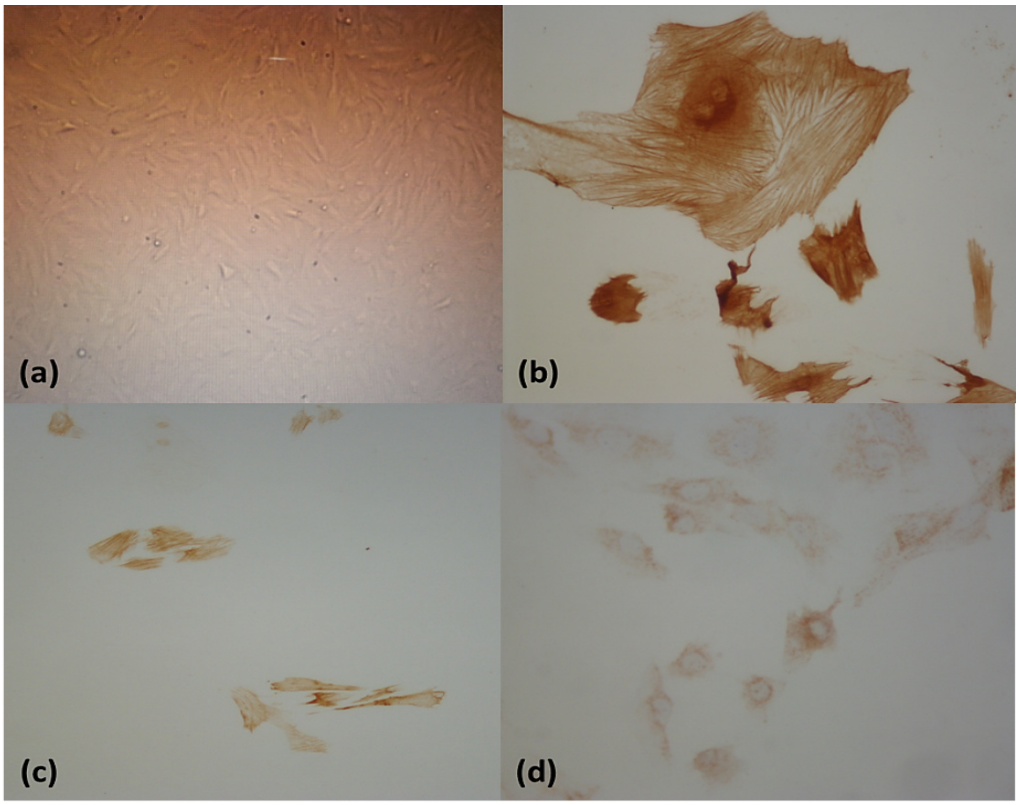

FIGURE 4. Evaluation of VSMCs. (a) View of VSMCs in light microscopy before being stained (×200), (b) VSMCs stained with α-SMA (×400), (c) VSMCs stained with caldesmon (×200), and (d) VSMCs stained with calponin (×400). VSMCs, vascular smooth muscle cells; α-SMA, α-smooth muscle actin.

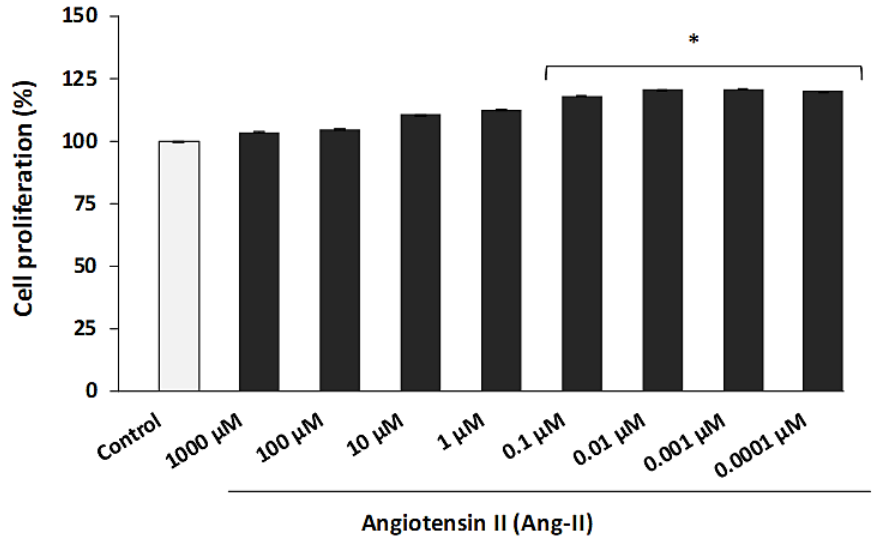

FIGURE 5. Effect of Ang-II doses on H-VSMCs proliferation for 24 h. *Compared with the control group (P<0.05). Results shown as mean±SE for the three experimental replicates (n=6-12). H-VSMCs= healthy rat model vascular smooth muscle cells, SE=standard error.

rats as 500-1000 mg/dL. However, proteinuria was detected in diabetic model rats, while proteinuria was not observed in healthy rats with urine strips.

Evaluation of VSMCs

The VSMCs were adhered to the glass coverslip surface and multiplied before the staining and VSMCs proliferation was evaluated with the inverted microscope (Figure 4a). After VSMCs covered the slide surface entirely, the specimens were incubated with α-SMA, caldesmon and calponin antibodies by IHC (immunohistochemical) method protocol (Figures. 4b, 4c and 4d). The cells used in the study were verified to be VSMCs.

Effect of Ang-II on H-VSMCs Proliferation for 24 and 48 Hours

It was determined that the Ang-II doses (0-1000

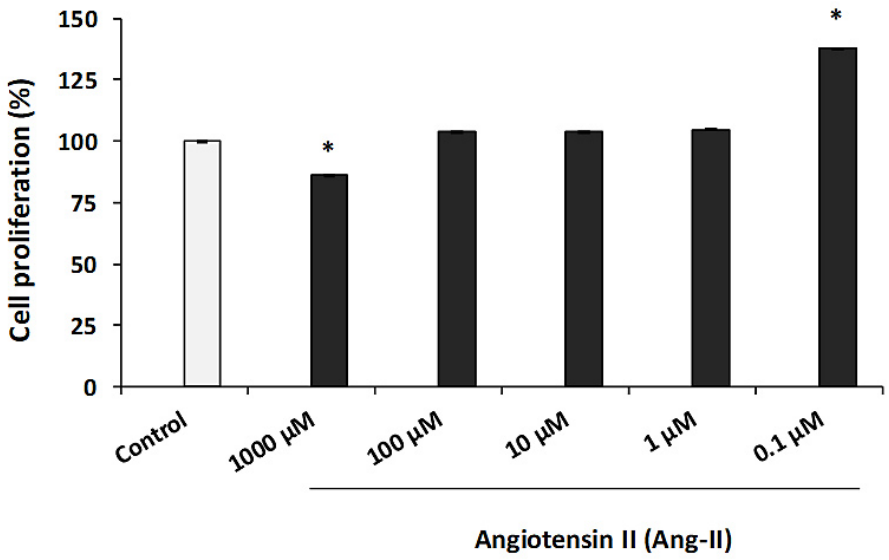

FIGURE 6. Effect of Ang-II doses on H-VSMCs proliferation for 48 h. *Compared with the control group (P<0.05). Results shown as mean±SE for the three experimental replicates (n=12-15). H-VSMCs, healthy rat model vascular smooth muscle cells; SE, standard error.

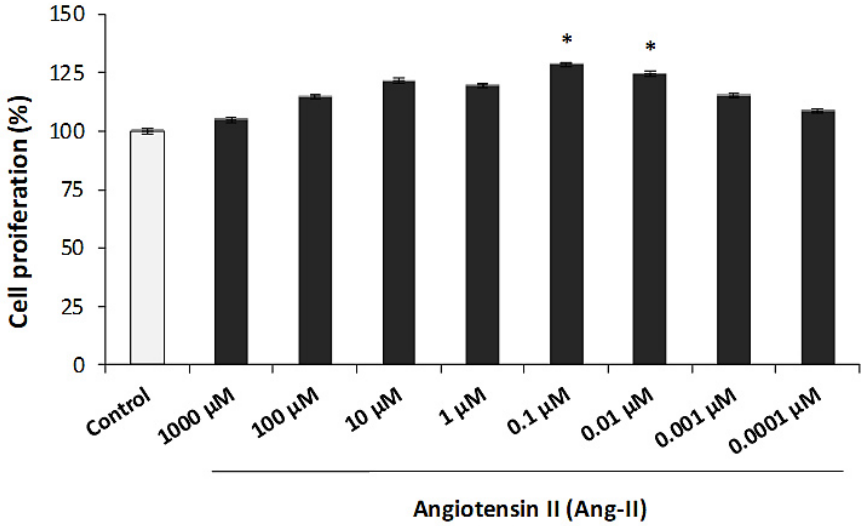

FIGURE 7. Effect of Ang-II doses on D-VSMCs proliferation for 24 h. *Compared with the control group (P<0.05). Results shown as mean±SE for the three experimental replicates (n=6-7). D-VSMCs, diabetic rat model vascular smooth muscle cells; SE, standard error.

µM) effect H-VSMCs proliferation for 24 h and 48 h. The H-VSMCs proliferation increased in Ang-II (0.1 µM) by 18%, Ang-II (0.01 µM) by 20%, Ang-II (0.001 µM) by 20% and Ang-II (0.0001 µM) by 20% (P<0.001). It was observed that Ang-II elevated H-VSMCs proliferation in a dose-dependent manner. Maximum cell proliferation was seen in Ang-II (0.1 µM) dose. Consequently, it was selected for subsequent combined applications (Figure 5).

While Ang-II (0.1 µM) increased cell proliferation in H-VSMCs for 48 h by 37%, Ang-II (1000 µM) dose reduced cell proliferation by 14% (P<0.001) (Figure 6).

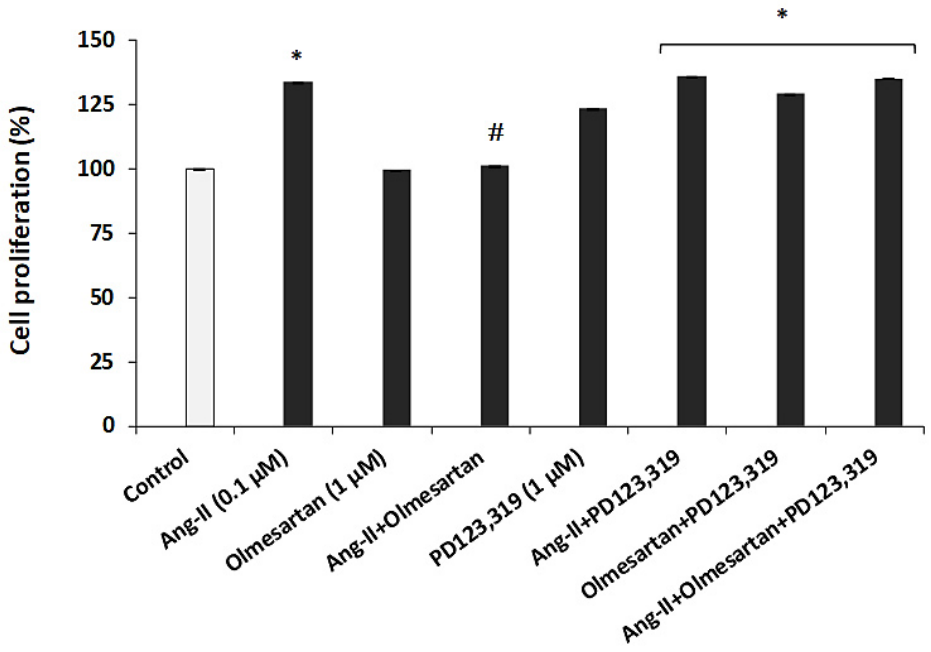

FIGURE 8. Effect of Angiotensin II (Ang-II), Olmesartan and PD123,319 on H-VSMCs proliferation for 24 h. *Compared with the control group, ✓Compared with the Ang-II group (P<0.05). Results shown as mean±SE for the three experimental replicates (n=12). H-VSMCs, healthy rat model vascular smooth muscle cells; SE, standard error.

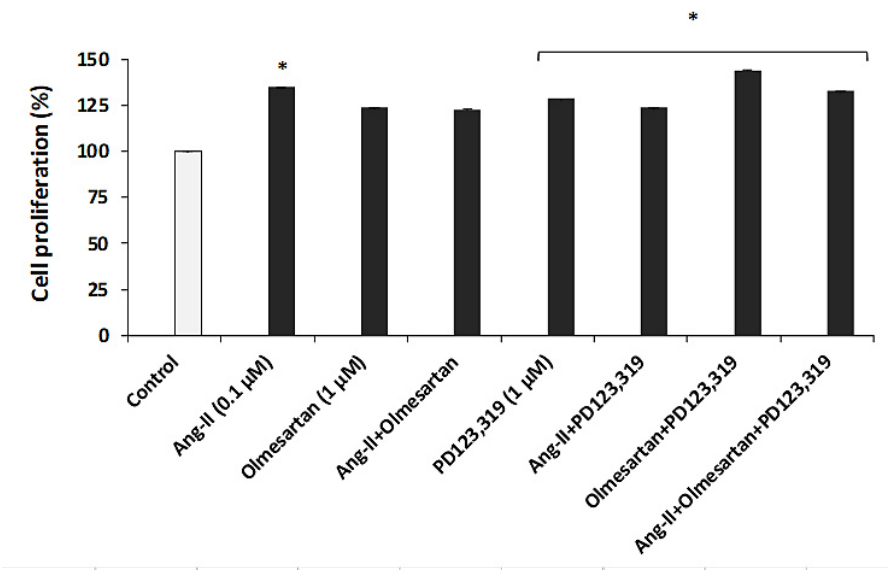

FIGURE 9. Effect of Angiotensin II (Ang-II), Olmesartan and PD123,319 on D-VSMCs proliferation for 24 h. *Compared with the control group (P<0.05). Results shown as mean±SE for the three experimental replicates (n=12). D-VSMCs, diabetic rat model vascular smooth muscle cells; SE, standard error.

Effect of Ang-II on D-VSMCs Proliferation for 24 Hours

D-VSMCs proliferation increased in the application of Ang-II (0.1 µM) by 28% (P=0.010) and Ang-II (0.01 µM) by 24% (P=0.030) (Figure 7).

Effect of Angiotensin II, Olmesartan and PD123,319 on H-VSMCs Proliferation for 24 Hours

There was a significant difference between the groups of H-VSMCs in single and combined applications of Ang-II (1 µM), Olmesartan (1 µM) and

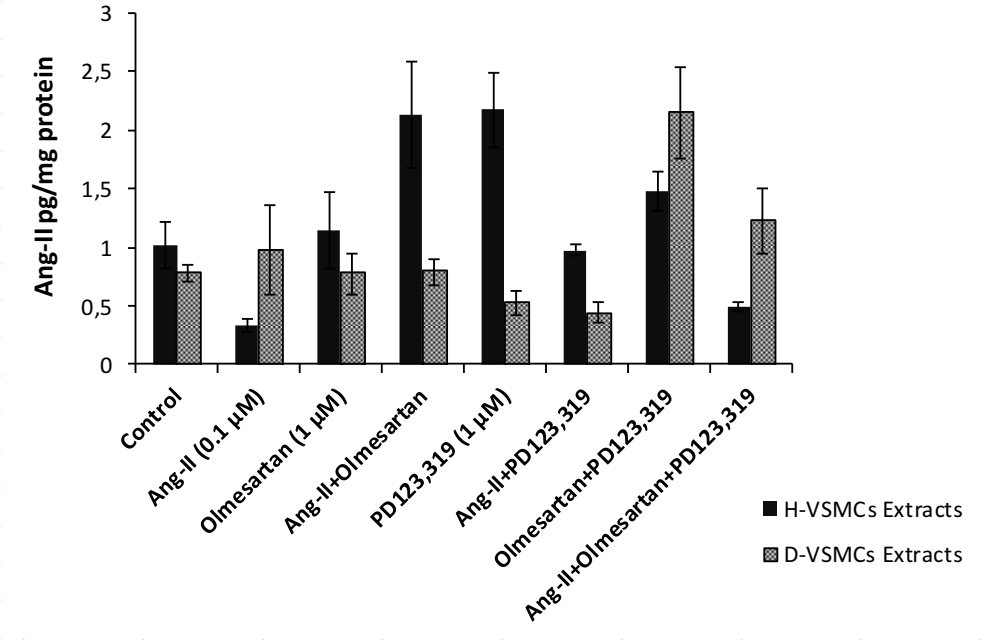

FIGURE 10. Ang-II levels in H-VSMCs and D-VSMCs extracts groups. Results shown as mean±SE for the experimental replicates (n=4). H-VSMCs, healthy rat model vascular smooth muscle cells; D-VSMCs, diabetic rat model vascular smooth muscle cells; SE, standard error.

PD123,319 (0.1 µM). Cell proliferation increased in Ang-II (0.1 µM) by 33% (P=0.007), Ang-II+PD123,319 by 36% (P=0.001), Olmesartan +PD123,319 by 29% (P=0.008), Ang-II+Olmesartan +PD123,319 by 35% (P=0.001) compared with the control group. But no significant difference was observed when Olmesartan (P=1.000) and PD123,319 compared with the control group (P=0.071). When Ang-II+Olmesartan group compared with the Ang-II (0.1 µM) group it was determined that cell proliferation decreased by 32% (P=0.028) (Figure 8).

Effect of Angiotensin II, Olmesartan and PD123,319 on D-VSMCs Proliferation for 24 Hours

Significant differences were found in D-VSMCs proliferation in single and combine treatment of Ang-II (0.1 µM), Olmesartan (1 µM) and PD123,319 (1 µM). Cell proliferation increased significantly in Ang-II (0.1 µM) by 34% (P=0.002), PD123,319 by 28% (P=0.011), Ang-II+PD123,319 by 23% (P=0.049), Olmesartan+PD123,319 by 44% (P<0.001), Ang-II+Olmesartan+PD123,319 groups by 32% (P<0.001) compared with the control group (Figure 9).

Ang-II Levels in H-VSMCs and D-VSMCs Extracts

Ang-II levels in H-VSMCs and D-VSMCs extract (intracellular) groups expressed as pg/mg protein. We didn't find significant difference between the groups of H-VSMCs and D-VSMCs extracts groups. Intracellular Ang-II levels decreased in Ang-II (0.1 µM) (P=0.338), Ang-II+PD123,319 (P=0.999) and Ang-II+Olmesartan+PD123,319 groups in H-VSMCs compared with the control group (P=0.953). However, it was detected that Ang-II increased in Olmesartan (1 µM), Ang-II+Olmesartan, PD123,319 (1 µM) and Olmesartan+PD123,319 groups compared with the control group in H-VSMCs (P=0.766).

Contrary to these findings, it was determined that Ang-II levels increased in Ang-II (0.1 µM) (P=1.000), Ang-II+Olmesartan (P=0.998), Olmesartan+PD123,319 (P=0.089) and Ang-II+Olmesartan+PD123,319 groups compared with the control group in D-VSMCs (P=0.945). Ang-II levels declined in Olmesartan (1 µM) (P=1.000), PD123,319 (1 µM) (P=0.926) and Ang-II+PD123,319 groups in compared with the control group in D-VSMCs (P=0.954) (Figure 10).

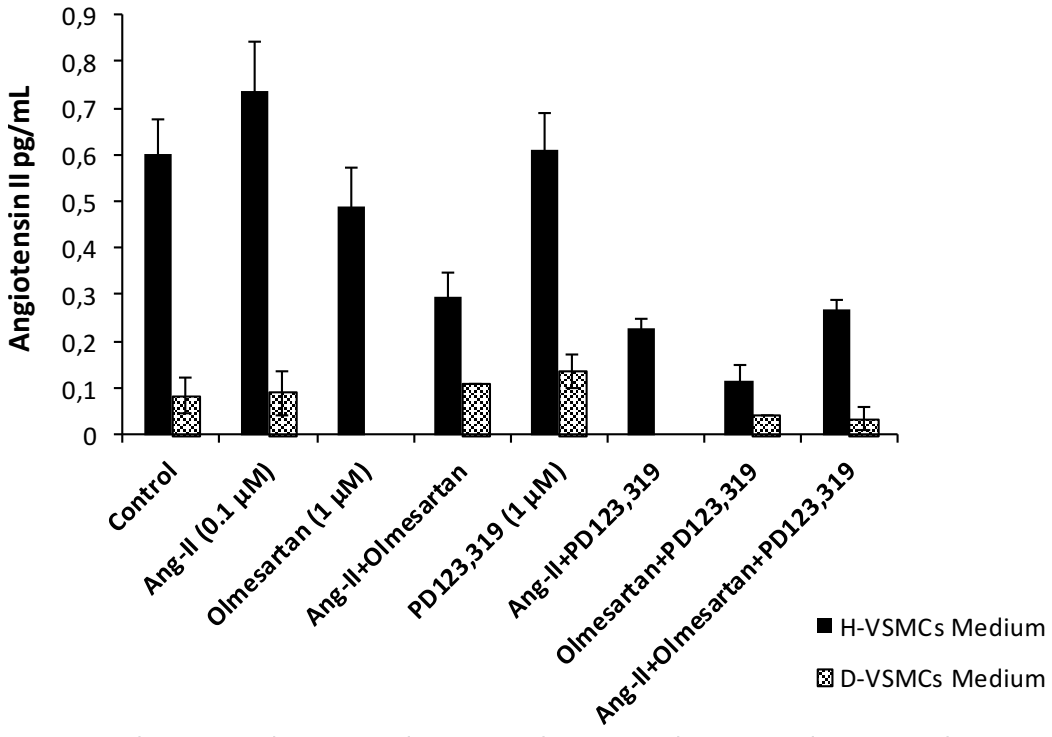

FIGURE 11. Ang-II levels in H-VSMCs and D-VSMCs medium groups. Results shown as mean±SE for the experimental replicates (n=4). H-VSMCs, healthy rat model vascular smooth muscle cells; D-VSMCs, diabetic rat model vascular smooth muscle cells; SE, standard error.

Ang-II Levels in H-VSMCs and D-VSMCs Medium

Ang-II levels in H-VSMCs and D-VSMCs medium (extracellular) groups were presented as pg/mL. Ang-II levels in H-VSMCs groups higher than all the D-VSMCs groups. But there is no significant difference between the groups of H-VSMCs and D-VSMCs.

Ang-II levels increased in Ang-II (0.1 µM) (P=0.822) and PD123,319 (1 µM) groups compared with the control group in H-VSMCs (P=0.992). On the other hand, Ang-II levels attenuated in Olmesartan (1 µM), Ang-II+Olmesartan, Ang-II+PD123,319, Olmesartan+PD123,319 and Ang-II+Olmesartan+PD123,319 groups compared with the control group in D-VSMCs (P=0.438).

We could not detect Ang-II in Olmesartan and Ang-II+PD123,319 groups in D-VSMCs (Figure 11). Ang-II levels increased in Ang-II+Olmesartan and PD123,319 (1 µM) groups compared with the control group in D-VSMCs. However, Ang-II levels decreased in Olmesartan+PD123,319 and Ang-II+Olmesartan+PD123,319 groups compared with the control group in D-VSMCs (P=0.825).

DISCUSSION

Intracellular components of RAS and its effects of signaling pathways have many undisclosed aspects. Local RAS can have also selective effects in many specific conditions and cell types, that lead to inducing various cardiovascular pathological problems. It has been stated that local RAS components as well as systemic RAS are increased in cardiovascular and many other tissues where diseases such as diabetes, atherosclerosis and hypertension occur [22].

The intracellular effect of local RAS components on cardiovascular tissue and the mechanism of these pathways has not been fully elucidated. It may play a key role in the development and progression of cardiovascular diseases [30]. However, there are differences in the cellular effects (proliferation, migration) of Ang-II and ATRs in pathological conditions such as diabetes and hyperglycemia [31]. These changes may lead to differences in cellular responses to agents such as ATR1 blockers being used in the treatment of cardiovascular pathologies [30].

In the dose study of Ang-II in H-VSMCs and D-VSMCs, we found a dose-dependent response curve. The highest proliferating dose of Ang-II in 24- and 48-hours applications was determined as 0.1 µM and this dose was used in combined applications. Ang-II (1000 µM) application also significantly reduced cell proliferation. Our result showed that, high doses of Ang-II caused cell death. In a study of primary VSMCs, 100 nM Ang-II application for 24 hours showed an increase of 80% in protein synthesis and 45% in cell proliferation. In the same study, it was stated that 100 nM Ang-II was the most increased dose of hypertrophy in VSMCs [32]. This dose is like the Ang-II dose (0.1 µM) used in our study.

Moreover, Ang-II has been reported to be a potent stimulant for VSMCs and has been investigated to cause an increase in cell volume and protein content [33]. But in another study, Ang-II (100 µM) administration for 24 and 48 hours significantly increased cell proliferation. The proliferative dose of Ang-II was 1000 times higher than our dose [34]. It was considered that may be occurred due to the use of a different cell culture line such as A7r5 is the embryonic rat smooth muscle cell line. Moreover, A7r5 cells may have the ability to reproduce more rapidly and actively due to their embryonic origin. For this reason, changes in the proliferation responses of different doses of Ang-II can be seen.

In current study, Ang-II is a potent hypertrophic agent but has no sufficient evidence regarding with its mitogenic activity compared D-VSMCs to H-VSMCs. When the groups were compared in terms of proliferation in Ang-II applications, D-VSMCs proliferation tended to increase compared to H-VSMCs. It has also been suggested that the cells undergo hypertrophy along with proliferation under pathological conditions. When we examined H-VSMCs and D-VSMCs under inverted microscope, some morphological changes (increase in diabetic cell size) between H-VSMCs and D-VSMCs were observed. These morphological changes in VSMCs may clarify why we cannot see significant proliferation increase in our study.

In a study, administration of Ang-II to rats for 2 weeks has been shown to cause hypertension and hypertrophy of the vascular smooth muscle layer [35]. In other study has indicated that activation Ang-II

synthesis increases vascular wall and intima thickness of large arteries in diseases such as atherosclerosis, hypertension and diabetes. In addition, it may cause hypertrophy, hyperplasia and migration in VSMCs and it has been known that Ang-II has these effects mainly on ATR1 and ATR2 [6].

It was observed that in our study, Ang-II, Ang-II+PD123,319, Olmesartan+PD123,319 and Ang-II+Olmesartan+PD123,319 groups caused an increase in cell proliferation, while a decline was seen in Ang-II+Olmesartan group in H-VSMCs. Olmesartan 1 µM also inhibits cell proliferation in H-VSMCs but not detected a significant decrease in D-VSMCs. This may be thought to be due to Olmesartan dose not being sufficient to protect against vascular dysfunction and proliferation caused by diabetes mellitus. But in an in vivo study, 0.1 µM Ang-II showed that cell proliferation in both obese and non-obese Wistar rats and it was significantly attenuated with application of 0.1 µM Ang-II+0.1 µM Olmesartan. In this study, the dose of 0.1 µM Olmesartan was sufficient to decrease cell proliferation [36]. However, higher dose of Olmesartan was also used to inhibit proliferation and migration in another study [37]. It has been reported in the literature that ATR blockers inhibit VSMCs proliferation both in vitro and in vivo research [24]. In addition, it has been shown that, 10 µmol/L and 100 µmol/L Losartan application in A7r5 cells significantly reduced Ang II-induced cell proliferation in co-administration with 100 µmol/L Ang-II. It was stated that dose of 100 µmol/L Losartan significantly attenuated cell proliferation. This finding had been associated with intracellular Ang-II production by researchers [34].

Likewise, it was determined that, while the number of cells increased, application of 0.1 µM PD123,319 with 0.1 µM Ang-II attenuate apoptosis significantly in VSMCs. It has been reported that ATR2 blockade has increased cell proliferation [38]. However, the effect of ATR2 on cell proliferation could not be determined in our study when 0.1 µM Ang-II and 1 µM PD123,319 were applied together.

We have thus shown in our study that rat aortic smooth muscle cells synthesize Ang-II. And we found that intracellular Ang-II levels in H-VSMCs extracts and D-VSMCs extracts were higher than the extracellular Ang-II levels in H-VSMCs and D-VSMCs medium. But we did not detect any differences between groups in intracellular and extracellular Ang-II levels compared in H-VSMCs and diabetic D-VSMCs.

There are also some studies in which the Ang-II level is measured differently. In a review study, it is indicated that the plasma value of Ang-II in humans has been measured as 5-35 fmol/mL and 8.7 fmol/mL [17]. Ang-II levels in human arterial blood were determined by Catt *et al*. [39] 0.5-4.7 mµg/100 mL, Boyd *et al*. [40] 0.8-5.6 mµg/100 mL in venous plasma, Gocke *et al*. [41] (1968) stated it as 1.8-11.0 mg/100 mL [42]. Campbell *et al*. [43] measured Ang-II level in plasma as 50 fmol/mL [43]. It is stated that the plasma levels of RAS components in different species such as rats may be lower than humans [17].

RAS components are found in small amounts inside of the cells, which can be changed in pathological conditions. Due to the increase of Ang-II levels in various cardiovascular pathologies, it has been seen that the determination of Ang-II levels is very substantial. It is also necessary to fully analyze both the intracellular and extracellular molecular mechanism linkages of this system.

Strengths and Limitations

It was crucial to isolate and then produce smooth muscle cells from aorta of diabetic rats. Our chances of success were low because of the fragility of diabetic animal aorta. The process of isolating vascular smooth muscle cells had become very sensitive due to the damage caused by diabetes in the vascular structure. Ang-II measurement was quite difficult in consequence of low intracellular Ang-II levels among species and different tissues. Furthermore, short half-life and instability of Ang-II were also among the limitations of our study.

CONCLUSION

In current study, Ang-II increased healthy and diabetic VSMCs proliferation. When Ang-II and Olmesartan, an ATR1 antagonist, were applied together, proliferation decreased in healthy VSMCs. A decrease was also seen in diabetic VSMCs, but it was not significant. However, when Ang-II and PD123,319, an

ATR2 antagonist were administered together to healthy and diabetic VSMCs, no effect on proliferation was determined. In addition, since there was no meaning difference in intracellular and extracellular Ang-II levels compared in healthy and diabetic VSMCs, no relationship could be established between Ang-II levels, cell proliferation and Ang-II type 1 and 2 receptors in diabetes. Furthermore, it has been considered that further studies and methods with high accuracy and sensitivity were needed to measure Ang-II levels. Thus, comparisons of Ang-II levels between groups can be made more clearly and accurately. Additionally, it may enable the determination of the relationship between Ang-II levels and their effect on vascular smooth muscle cells proliferation.

Ethics Approval and Consent to Participate

The present study was approved by Çukurova University Animal Experiments Local Ethics Committee (Decision No: 2016/11-1 and date: 22.12.2016, Adana, Türkiye). All experimental procedures were carried out accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

Data Availability

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available on request from the corresponding author, upon reasonable request.

Authors' Contribution

Study Conception: ZÇ, KA, ZGY, AD; Study Design: ZÇ, KA, ZGY, AD; Supervision: ZÇ, KA, ZGY, AD; Funding: CÜBAP-TTU No. 2017-8071; Materials: N/A; Data Collection and/or Processing: ZÇ, KA, ZGY; Statistical Analysis and/or Data Interpretation: ZÇ, KA; Literature Review: ZÇ, KA, AD; Manuscript Preparation: ZÇ, KA, AD; and Critical Review: ZÇ, KA, AD.

Conflict of Interest

The author(s) disclosed no conflict of interest during the preparation or publication of this manuscript.

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Generative Artificial Intelligence Statement

The author(s) declare that no artificial intelligence-based tools or applications were used during the preparation process of this manuscript. The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

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The Role of Epicardial Adipose Tissue in Systemic Sclerosis: A Clinical and Radiological Analysis

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ABSTRACT

Objectives: Systemic sclerosis (SSc) is a chronic autoimmune disorder characterized by progressive fibrosis, vasculopathy, and immune dysregulation, leading to multi-organ involvement. Cardiopulmonary complications are major determinants of morbidity and mortality in SSc. Epicardial adipose tissue (EAT), a metabolically active adipose tissue, has been implicated in systemic inflammation and cardiovascular risk. However, its role in SSc remains poorly understood. This study aims to evaluate the relationship between EAT and SSc patients.

Methods: This retrospective cross-sectional study was performed at Rheumatology department between 2020 and 2021. Patients were classified according to the 2013 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) criteria for SSc. Laboratory and radiology results were obtained from the electronic registration database. Data were analyzed and compared between groups. EAT was measured using non-contrast computed tomography (CT), and its correlations with clinical features, inflammatory markers, and thoracic CT abnormalities were evaluated.

Results: A total of 229 participants, 157 with SSc and 72 age-matched healthy controls, were included in the study. The mean EAT volume was 171.02±81.84 cm^3. Correlation analysis revealed a significant positive correlation between EAT volume and interstitial lung disease (ILD) ($r=0.260$, $P=0.001$), C-reactive protein (CRP) levels ($r= 0.250$, $P=0.002$), disease duration ($r=0.205$, $P=0.010$), and age ($r=0.528$, $P<0.001$). ROC analysis showed a moderate discriminatory power of EAT volume with an area under the curve (AUC) of 0.647 (95% CI: 0.564-0.729, $P<0.001$).

Conclusions: Higher EAT volume was observed in SSc patients with ILD, suggesting a possible role of epicardial fat in pulmonary involvement in SSc.

Keywords: Epicardial Adipose Tissue, Inflammation, Interstitial Lung Disease, Systemic Sclerosis

Systemic sclerosis (SSc) is a chronic autoimmune disorder (AID) characterised by disruption of the immune system, microvasculature alterations and matrix substances accumulation in the skin and internal organs. A recent meta-analysis estimated the pooled prevalence of SSc to be 17.6 per 100,000 and the pooled incidence at 1.4 per 100,000 person-years. SSc is most commonly diagnosed in women aged 40–

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60 years, with reported female-to-male ratios between 1.5:1 and 17:1. There are two main subtypes of SSc: limited cutaneous SSc (lcSSc) and diffuse cutaneous SSc (dcSSc), which have differences in disease progression, severity, and survival. The disease primarily affects the skin but can also involve internal organs, including the lungs, heart, gastrointestinal tract, and kidneys. Studies confirmed that pulmonary cardiac, and renal SSc manifestations are associated with a poor survival prognosis. Interstitial lung disease (ILD) and pulmonary arterial hypertension (PAH) remain the most common causes of death for patients with SSc. Cardiac manifestations are frequently subclinical and may result from direct myocardial involvement, or arise secondarily due to complications such as PAH, ILD or renal disease. Clinically evident cardiac involvement in SSc significantly worsens prognosis and a the leading cause of mortality, with a five-year mortality rate of 70%. A contemporary study recently demonstrated that 26% of SSc-related deaths were attributed to PAH, and another 26% were attributed to cardiac etiologies such as heart failure and arrhythmia. Even though improvements in understanding the pathogenesis of SSc, predicting disease progression and organ involvement remains a challenge. One of the major concerns in SSc is the high prevalence of cardiopulmoner complications, which significantly contribute to morbidity and mortality [1].

Epicardial adipose tissue (EAT) located between the myocardium and visceral pericardium. EAT is highly vascularized and possesses endocrine and paracrine functions. Unlike subcutaneous fat, EAT has direct interactions with the myocardium and coronary arteries, secreting pro-inflammatory cytokines and adipokines. EAT has been identified as a metabolically active tissue with a role in inflammation and cardiovascular risk [2, 6]. EAT volume can be measured with echocardiography, computed tomography (CT), or magnetic resonance imaging (MRI) among them CT is a trustworthy and replicable method for quantification of EAT independent of cardiac cycle phase [3-5].

Increased EAT has been associated with AID, such as systemic lupus erythematosus (SLE), SSc, psoriasis and rheumatoid arthritis (RA) with cardiometabolic disease [6-11]. Prior research has indicated that EAT might contribute to inflammatory processes and endothelial dysfunction, which are key mechanisms in SSc pathogenesis. The potential role of EAT in SSc has not been thoroughly researched. These researches focused on cardiovasculer disease (CVD) using echocardiography in SSc patients. The present study investigates to research the connection between EAT and clinical, laboratory, radiological findings and other involvement in SSc patients using CT. Evaluating the connection between EAT and disease characteristics is crucial for understanding its potential role as a landmark for disease severity and organ involvement.

METHODS

Study Design and Population

This retrospective cross-sectional study was conducted at a single, tertiary care center Rheumatology department between 2020 and 2021. The study population was categorized into two groups: patients and healthy controls over 18 years. Demographic characteristics, clinical features, and laboratory findings were reviewed using electronic medical records. Patients who fulfilled the 2013 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria for SSc were included in the study. The control group included individuals with normal hematological and biochemical blood tests, no known chronic diseases, and no pathological findings on elective thoracic CT, performed for evaluation of cough and dyspnea in the internal medicine outpatient clinic.

Exclusion criteria included the presence of other AID, a history of malignancy, severe renal or hepatic impairment, history of CVD (coronary artery disease, heart failure, arrhythmia, cardiomyopathy), and prior cardiac surgery or interventions. Additionally, patients with poor-quality imaging that prevented accurate measurement of EAT volume were excluded.

Clinical and Laboratory Assessments

Baseline demographic characteristics (age, sex, disease duration, and body mass index [BMI]) were recorded from medical records. Clinical data on pulmonary, joint, gastrointestinal involvement, Raynaud's phenomenon, skin manifestations and digital ulcers were also documented. Pulmonary hypertension was determined based on echocardiographic findings (estimated systolic pulmonary artery pressure >35

mmHg) or right heart catheterization (mean pulmonary artery pressure ≥25 mmHg). Findings of nailfold capillaroscopy were recognized as the presence of neoangiogenesis, capillary elongation/tortuosity, decreased capillary density, avascular area, hemorrhage, giant loops, and abnormal blood flow.

Complete blood count and biochemical analyses were performed using peripheral venous blood samples. The erythrocyte sedimentation rate (ESR; 0-20 mm/hour) and C-reactive protein (CRP; 0-8 mg/L) and autoantibodies [Antinuclear antibodies (ANA) titer; anti-topoisomerase I antibody (Anti-Scl-70); anti-centromere (ACA); anti-PM/Scl; anti-RNP; anti-Ro52; and rheumatoid factor (RF;0-20IU/mL)] of the patient group were recorded. ANA and anti-ENA antibodies measured by indirect immunofluorescence assay (IFA), RF measured by ELISA.

Non-contrast-enhanced CT was used to asses EAT (Somatom Scope 16 or Somatom Definition Flash, Siemens Healthcare, Germany). The patient was placed in the supine position and CT scans were taken at full inspiration. EAT volume was measured following a standardized protocol using axial CT slices. EAT was defined as the fat surrounding the myocardium, bounded externally by the visceral pericardium using semi-automated volumetric analysis. Typical chest CT image acquisition parameters included a pitch of 1-1.5, 1.2-mm collimation, 3-mm slice thickness, 2-3 mm reconstruction interval, 80-130 kVp, and 100-250 mAs. All imaging assessments were conducted independently by two experienced radiologists who were blinded to the patients' clinical and laboratory data with inter-observer variability calculated.

Thoracic CT scans were also evaluated for ILD, ground-glass opacities, honeycombing sign, and other pulmonary abnormalities. Those without a specific diagnosis of ILD were considered to have no ILD. Patterns of ILD on high-resolution computed tomography (HRCT) classified as non-specific interstitial pneumonia (NSIP) and usual interstitial pneumonia (UIP) patterns. These patterns classified limited or extensive disease according to algorithm proposed by Goh *et al*. [12].

The study protocol was approved by the institutional ethics committee (Approval Number: 2021/296), and all procedures were performed in line with the principles of the Declaration of Helsinki.

TABLE 1. Clinical Characteristics and Laboratory Findings of Systemic Sclerosis Patients (n=157)

Variable	Data
Neutrophils (×10³/µL)	4.46±1.88 (0.78-13.21)
Lymphocytes (×10³/µL)	1.97±0.72 (0.25-4.50)
Monocytes (×10³/µL)	0.56±0.18 (0.18-1.26)
Platelet (×10³/µL)	278.67±83.40 (58.00-775.00)
Hemoglobin (g/dL)	12.71±1.60 (2.12-17.50)
MPV (fL)	8.45±0.95 (6.30-11.60)
RDW (%)	15.21±2.28 (12.20-28.60)
CRP (mg/L)	7.12±8.89 (1.00-67.00)
ESR (mm/h)	22.97±18.20 (1.00-96.00)
PAP (mmHg)	30.92±10.98 (15.00-110.00)
Disease duration (years)	6.03±3.64 (1.00-17.00)

Data are shown as mean±standard deviation (minimum-maximum). ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; MPV, Mean Platelet volume; RDW, red cell distribution width; PAP, pulmonary artery pressure.

Statistical Analysis

The Statistical Package for Social Sciences software was utilized for all procedures (IBM SPSS Statistics 21.0, IBM Corporation, Armonk, NY, USA). The Kolmogorov–Smirnov test was employed to assess the normality distribution of scale variables. For continuous numerical variables, descriptive statistics are presented as mean standard deviation. Categorical variables are represented by the number of cases and percent. The Chi-square test was utilized to compare categorical variables, and the student's t test was used to compare continuous numerical variables. Receiver operating characteristic (ROC) curve analysis was conducted to assess diagnostic performance. If the area under the curve was found to be significant, the Youden index was conducted to identify the best cut-off point. The sensitivity and specificity of diagnostic performance indicators were calculated. To determine the relationship between EAT volume and laboratory values, Pearson correlation analysis was used. Results were considered statistically significant at $P<0.05$.

TABLE 2. Demographic and Clinical Characteristics of Systemic Sclerosis Patients and Control Group

	Systemic sclerosis patients (n=157)	Healthy control (n=72)	P-value
Gender (female), n (%)	157/167 (93.6)	58/72 (80.6)	**0.005***
Age (years)	52.06±14.09	51.13±11.47	0.621**
Weight (kg)	56.26±6.68	57.55±9.69	0.341**
Height (cm)	160.42±5.24	158.50±3.43	0.055**
BMI (kg/m^2)	21.97±3.25	22.96±4.15	0.073**
EAT volume (cm^3)	171.99±81.01	134.20±91.11	**0.002****

Data are shown as mean±standard deviation. BMI, body-mass-index; EAT, epicardial adipose tissue.
*Chi-Square Tests, data are presented as counts, with percentages in brackets.
**Independent sample *t*-test. Statistically significant P-values are shown in bold.

RESULTS

A total of 229 participants, 157 with SSc and 72 age-matched healthy controls, were included in the study. The study comprised 157 SSc patients, with 6.4% males and 93.6% females, a mean age of 47.58±10.98 years, and a mean disease duration of 7.52±5.68 years (range, 1- 20 years).

Diffuse cutaneous form was in 71 (45.2%) and limited pattern in 80 (51%). Raynaud phenomenon was found in 143 (91.1%), whereas digital ulcers were noted in 28 (17.8 %) of patients. Nailfold capillaroscopy was performed on all patients. SSc capillaroscopy pattern was present (n=126, 80.3 %). Renal involvement was detected only one patient and common organ involvement were GIT (n=102, 65 %), ILD (n=73, 46.5 %) and joint involvement (n=22, 14%). Patients with ILD had NSIP pattern (n=56, 35.7%) and UIP patterns (n=16, 10.2%). The most common imaging pattern observed on HRCT is NSIP. Lung involvement with >20% was 35 (22.3%) patient and extent of fibrotic ILD≥10% on HRCT was present in 10 patients (6.4%). ILD was not detected on HRCT in 84 (53.5%) patients.

Positives for ANA (n=150, 95.5%), ACA (n = 54, 34.4%), anti-Scl70 (n = 48, 30.6%), anti-Ro52 (n=23, 14.6%), anti-PM/Scl (n=17, 10.8%), RF (n = 14, 8.9%) and anti-RNP (n=5, 3.2 %) positivities were detected, listed by frequency. Dyspnea was reported by (n=65, 41.4 %) of patients.

Table 1 shows the laboratory and clinical characteristics of SSc patients. The Mean PAP was 30.92±10.98 with a range of 15.00-110 (mmHg). Cardiac catheterization was performed on one patient.

Table 2 presents a comparison demographic and clinical characteristics between the SSc patients and control group. The groups showed no significant differences regarding age, height, weight, or BMI (P>0. 05). There was a difference in gender among the groups (P=0.005).

The mean EAT volume in SSc patients was 171.02±81.84 cm³, with a range of 2.12 - 17.50 cm³.The EAT volume was observed to be markedly

TABLE 3. ROC Analysis Results of Epipericardial Fat Tissue Volume Used for Diagnosis of Systemic Sclerosis Patients

	AUC (95% CI)	P-value	Cut-off	Sensitivity (%)	Specificity (%)
EAT volume	0.647 (0.564-0.729)	**0.001**	> 117.97	73.9	52.8

AUC, area under the curve; CI, confidence interval; EAT, epicardial adipose tissue; ROC, receiver operating characteristic. Statistically significant P-value is shown in bold.

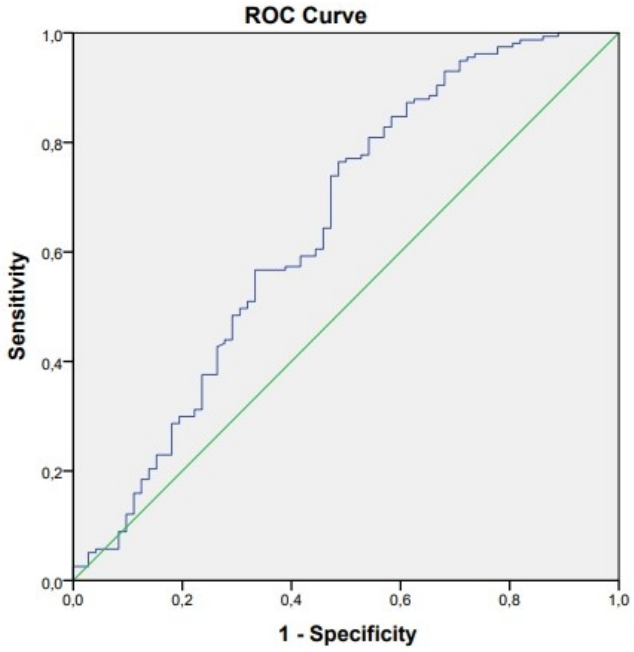

FIGURE 1. ROC analysis results of epipericardial fat tissue used for diagnosis of systemic sclerosis patients.

greater in the SSc patient group compared to the healthy control group (P=0.002) (Table 2). Within the SSc patient group, EAT volume did not differ significantly between patients with and without antibody positivity (P>0.05).

Table 3 shows the results of the ROC analysis. The ROC curve analysis assessed the discriminatory power of EAT volume (Figure 1). The area under the curve (AUC) was 0.647 (P=0.001), with a sensitivity of 73.9% and a specificity of 52.8% at a cut-off value of 117.97.

Pearson correlation analysis was performed to detect the relationships between the EFV and laboratory values of SSc patients (Table 4). ILD precense exhibited a weak positive correlation with EAT volume (r=0.260, P=0.001). CRP exhibited a weak positive correlation (r=0.250, P=0.002). Disease duration was weakly correlated with EAT volume (r=0.205, P=0.010). Age had a moderate positive correlation with EAT volume (r=0.528, P<0.001. PAP showed no significant correlation with EAT volume (r= -0.002, P= 0.983).

Patients were stratified based on key clinical features and EAT volume was compared across subgroups (Table 5). EAT volume was notably greater in patients with ILD than in those without ILD (P=0.004). ILD also demostrated a notable difference (P=0.035), reinforcing the correlation observed in the correlation analysis. There was no significant difference in EAT volume between UIP and NSIP patterns (P=0.784). Raynaud's phenomenon, joint involvement, ulcer presence, gastrointestinal involvement, and renal involvement did not show significant differences in EAT volume (P>0.05). Dyspnea showed a notable connection with increased EAT (P<0.001).

DISCUSSION

The role of EAT as a pro-inflammatory and pro-fibrotic mediator in CVD is increasingly recognized, yet its implications in SSc remain underexplored. Our study expands on this concept, demonstrating a significant correlation between increased EAT volume, systemic inflammatory markers, and pulmonary involvement in SSc. These results underscore the potential role of EAT as an inflammatory and pulmonary-related factor in SSc.

EAT is an ectopic fat depot with endocrine and paracrine functions, secreting a range of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), leptin, IL-1B and monocyte chemoattractant protein-1 (MCP-1).

TABLE 4. Correlation of Epipericardial Fat Tissue Volume with Disease Activity, Duration and Laboratory Findings in Systemic Sclerosis Patients (n=157)

	rs	P-value
EAT volume–Age	**0.528**	**<0.001**
EAT volme–BMI	**0.039**	0.558
EAT volume–Disease duration	0.205	**0.010**
EAT volume–PAP	0.047	0.561
EAT volume–Hemoglobin	0.025	0.754
EAT volume–Platelets	0.046	0.570
EAT volume–Neutrophil	0.060	0.458
EAT volume–Monocyte	0.131	0.101
EAT volume–Lymphocyte	0.055	0.495
EAT volume–RDW	0.152	0.058
EAT volume–MPV	0.043	0.594
EAT volume–CRP	0.250	**0.002**
EAT volume–ESR	0.157	0.050
EAT volume–ILD presence	0.260	**0.001**
NSIP	0.221	**0.005**
UIP	0.119	0.138

BMI, body mass index; CRP, C-reactive protein; EAT, epicardial adipose tissue; ESR, erythrocyte sedimentation rate; ILD, ınterstitial lung disease, MPV, mean platelet volume; NSIP, non-specific interstitial pneumonia; RDW, red cell distribution width, rs, pearson's Rho correlation coefficients; UIP, usual interstitial pneumonia.

Statistically significant P-values are shown in bold.

These cytokines support the local and systemic inflammatory milieu, promoting endothelial dysfunction and tissue fibrosis hallmarks of SSc pathology. Mazurek *et al*. [13] compared EAT and subcutaneous adipose tissue, they observed that EAT produced more inflammatory cytokines. They hypothesized that the presence of proinflammatory mediators in the tissues surrounding the coronary arteries can cause an enhancement in vascular inflammation and neovascularization via apoptosis.

TABLE 5. Epicardial Fat Tissue Volume Compared Across Subgroups

Clinical finding	Mean difference	P-value
Raynaud's phenomenon	22.73	0.318
Joint involvement	-22.86	0.221
Skin involvement	-8.72	0.503
Digital ulcer	16.16	0.34
Gastrointestinal involvement	3.05	0.823
Renal involvement	1.75	0.983
Dyspnea	-46.21	**0.01**
Pulmonary involvement	43.73	**0.035**
Capillaroscopy finding	17.89	0.272

Statistically significant P-values are shown in bold.

Packer *et al*. [14] proposed that EAT acts as an inflammatory reservoir, exacerbating systemic autoimmune conditions. It is recognized as an active metabolic and inflammatory tissue increase in AID [15-17]. Similar to our study previous studies demonstrated that several AID, including RA, SSc, SLE, and psoriasis had increase EAT, is consistent with duration and severity of the underlying disease. Previous research showing that EAT is linked to increased cardiovascular risk in AID such as RA and SLE [18-20]. Lipson *et al*. [20] identified elevated EAT thickness in SLE, linking adipose-driven inflammation to heightened cardiovascular risk. Karpouzas *et al*. [21] found that RA patients with higher EAT volumes had increased coronary atherosclerosis, independent of traditional cardiovascular risk factors. Other cross-sectional studies showed greater EAT thickness in RA patients [21-25]. Increased EAT volume is related with endothelial dysfunction in spondyloarthritis [26, 27]. Systematic review and meta-analysis also show that Familial Meditterian Fever patients and psoriasis patients have a higher EAT volume compared to control subjects [28, 29].

Similar to our study, several studies observed increased EAT volume in SSc patients, even in the lack of overt cardiac disease [15, 30]. The present study showed a significant correlation between EAT volume and systemic inflammatory markers in SSc patients. This association between EAT volume and CRP levels supports the hypothesis that EAT volume is a marker of systemic inflammation in SSc. According to Wang *et al*. [30], higher EAT was linked to higher CRP and NT-proBNP levels, lower eGFR and diffusing capacity of the lungs for carbon monoxide (DLCO), and a longer delay to diagnosis. This suggests a link between EAT and the severity of SSc and additional organ damage [30]. Mahabadi *et al*. [31] demonstrated that increased EAT volume is an independent predictor of adverse CV events in the general population, reinforcing the need to consider EAT as a key factor in SSc-related cardiac risk. Temiz-Karadag *et al*. [15] reported that SSc patients with pulmonary hypertension had noticeably greater EAT thickness using echocardiograpy, supporting the notion that EAT may contribute to pulmonary vascular remodeling. EAT thickness was positively correlated with age, ESR, CRP, insulin, hemoglobin A1c and cholesterol. Long *et al*. [6] showed increased EAT is linked with severity of SSc, independent of cardiovascular risk factors and ILD. The EAT is larger SSc patients with PAH than without PAH [6]. Yılmaz *et al*. [7] have found increased EAT thickness in SSc and it is correlated with disease severity scale and digital ulcer [7].

Previous researches have indicated that EAT might reflect systemic inflammation and be associated with disease activity. However, its association with pulmonary involvement, a hallmark of SSc, remains unclear. Pulmonary complications, including ILD and PH are prevalent in SSc and are major determinants of patient outcomes. Our study demonstrated a significant association between EAT volume and ILD in SSc. Additionally, patients with dyspnea showed higher EAT volume, suggesting a link between EAT accumulation and respiratory impairment in SSc. The pathophysiological link between EAT and pulmonary involvement is likely multifactorial. Increased EAT volume may contribute to pulmonary vascular remodeling through the release of inflammatory mediators that promote endothelial dysfunction and fibrosis. Shi *et al*. [19] highlighted that EAT in AID contributes to vascular stiffening and pulmonary vascular remodeling, leading to increased pulmonary artery pressures. Studies such as Masson *et al*. [16] have reported a link between increased EAT volume and vascular inflammation, suggesting a common pathophysiological mechanism underlying pulmonary and cardiovascular involvement in AID. The exact mechanisms linking increased EAD to disease severity and organ involvement in SSc remain to be fully elucidated. However, several potential pathways can be considered: Inflammatory cytokines secreted by EAT are known to contribute to pulmonary fibrosis in SSc. Additionally, EAT is closely related to coronary arteries, allowing for direct interactions that may cause vascular dysfunction and fibrosis, a key feature of SSc. Oxidative stress and hypoxia within EAT may promote endothelial dysfunction in the pulmonary circulation, further exacerbating ILD progression. These mechanisms provide a biological rationale for considering EAT as a potential therapeutic target in SSc. Besides they highlight the need for further investigation into the role of EAT as a biomarker for pulmonary complications in SSc.

Our study suggests that EAT volume assessment

using non-invasive imaging modalities, such as CT, could provide valuable insights into risk in SSc. Given the growing evidence linking EAT to systemic inflammation, pulmonary complications, and cardiac dysfunction, integrating EAT assessment into routine clinical evaluations may help identify high-risk patients at an earlier stage.

Interventions aimed at reducing EAT volume and its inflammatory activity may be a new therapeutic approach for managing SSc-related cardiopulmonary complications. Packer *et al*. [14] proposed that targeting EAT through pharmacological or lifestyle modifications could mitigate its detrimental effects on the myocardium. Weight loss and physical activity have been shown to reduce EAT volume and recover cardiac function [32]. Further research should also explore the potential of lifestyle modifications, pharmacological interventions, and targeted therapies to mitigate the effects of EAT accumulation in autoimmune diseases.

The advantages of our research consist of a thorough assessment of EAT volume in relation to clinical, laboratory, and radiological parameters. Unlike prior research that mainly focused on cardiovascular disease, our study examined the connection between EAT volume and lung-related issues. In the context of SSc, study have demonstrated that patients without overt cardiac disease exhibit increased EAT volume. Increased EAT volume is linked to disease severity and systemic inflammation in SSc patients. These results indicate that EAT volume may be used as a non-invasive marker for identifying patients at higher risk for pulmonary complications.

Strengths and Limitations

A significant strength of the present study resides in its investigation of the relationship between EAT and pulmonary involvement, a predominant factor contributing to morbidity in SSc patients. This specific aspect differentiates this study from earlier research in this field, which has largely focused on cardiac issues.

There are some limitations to the study such as the lack of anti-RNP measurement, absence of EAT comparison between dSSc and lSSc subtypes, failure to assess the relationship between EAT and modified Rodnan score, and missing DLCO data. A retrospective and cross-sectional design with a small samples size is a limitation in terms of generalizability of the study. Future research should focus on longitudinal assessments of EAT, exploring its potential as a prognostic marker and a therapeutic target in SSc management.

In addition, the variability in EAT measurement techniques across different imaging modalities and analysis methods could introduce inconsistencies. We didn't calculate the intraclass correlation coefficient (ICC) for EAT volume measurements to validate measurement reproducibility. Another limitation is the lack of functional assessment of cardiac performance. Although we identified associations between EAT volume, inflammation, and pulmonary involvement, we did not evaluate the impact of increased EAT on clinical outcomes such as heart failure, arrhythmias, or myocardial fibrosis. Besides, we have a few patients with PAH. Furthermore, potential confounders such as concomitant medications, metabolic factors, and genetic predispositions were not extensively evaluated.

CONCLUSION

In conclusion, the present study showed that increased EAT volume is related to systemic inflammation, pulmonary involvement, in SSc patients. Incorporating EAT assessment into routine clinical practice may enhance risk stratification and facilitate early intervention, ultimately improving long-term prognosis in this high-risk patient population.

Ethics Approval and Consent to Participate

This study was approved by the Selçuk University Faculty of Medicine Hospital Clinical Research Ethics Committee (Decision No: 2021/296; date: 26.05.2021). All procedures were conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from all individual participants included in the study.

Authors' Contribution

Study Conception: DT, HÖ; Study Design: DT,

HÖ; Supervision: DT, HÖ, ÖFT, AK; Funding: HÖ, ÖFT, AK, SY; Materials: DT, SY, SH; Data Collection and/or Processing: DT, ÖFT, SH; Statistical Analysis and/or Data Interpretation: HÖ, DT; Literature Review: DT; Manuscript Preparation: DT, HÖ; and Critical Review: DT.

Conflict of Interest

The author(s) disclosed no conflict of interest during the preparation or publication of this manuscript.

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Generative Artificial Intelligence Statement

The author(s) declare that no artificial intelligence-based tools or applications were used during the preparation process of this manuscript. The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

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Effect of Pressure Wound Dressing Using Elastic Bandage Following Axillary Dissection Compared to Standard Wound Dressing on Postoperative Seroma Development and Surgical Complications

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ABSTRACT

Objectives: Postoperative seroma is a common complication following breast surgery and axillary dissection. This issue persists despite attempts to prevent seroma, including intraoperative fibrin isolation, application of thrombin spray, fascia treatment techniques, and shoulder immobilization. This study aims to evaluate the impact of pressure wound dressings on the development of postoperative seroma after axillary dissection.

Methods: This is a randomized, controlled prospective study involving 63 patients. Pressure wound dressings were applied to patients in the intervention group (n=32), while standard dressings were applied to those in the control group (n=31). Both groups were compared regarding the postoperative length of stay for the drain catheter, the amount of drainage, duration of hospital stay, postoperative pain levels on the 1st, 3rd, and 10th days, as well as rates of reoperation and rehospitalization.

Results: The study indicated that the daily and total fluid drainage was significantly lower in the pressure wound dressing group. Moreover, postoperative pain scores on the 1st, 3rd, and 10th days, as well as the duration of hospital stay for patients after surgery, were notably lower in this group.

Conclusions: The use of pressure wound dressings significantly reduces fluid drainage and postoperative pain in patients undergoing axillary dissection. This approach holds promise for decreasing postoperative complications.

Keywords: Axillary Dissection, Pressure Dressing, Seroma, Mastectomy

Breast cancer is the most common cancer in women. Mastectomy and lymph node dissection are treatment methods used in necessary cases of breast cancer treatment [1-3]. In surgical treatment, sentinel node biopsy is used for staging the axilla. Personalized approaches minimize the need for axillary dissection in women with positive sentinel nodes [4]. However, axillary dissection remains the mainstay of treatment in patients who have undergone mastectomy with at least one metastatic sentinel lymph node and in patients who are eligible for breast-conserving surgery with three or more positive sentinel lymph nodes [5].

Complications such as lymphedema, nerve injury,

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pain, lymphangiosarcoma, and seroma formation can be seen in patients after breast surgery and axillary dissection [6, 7]. Seroma is the most common complication of breast cancer surgery [7, 8]. Although the exact cause of seroma formation is unclear, it is thought to develop due to surgical dead space. A study reported that the content of postoperative drainage fluid resembles inflammatory exudate, which can be reduced by changing the surgical technique and eliminating the dead space [8].

Although many methods have been tried to prevent the development of seroma, such as intraoperative fibrin glue, thrombin spray, application of a closed/open drainage system, use of multiple drains, fascia suture technique, postoperative shoulder immobilization, and late initiation of shoulder-arm physiotherapy, this issue remains a problem today [7, 9-12].

This study aims to determine the effects of pressure wound dressing (PWD) applied to the axilla after surgery on the length and amount of drainage, the length of hospital stay, postoperative pain level, reoperation, rehospitalization, wound infection, flap necrosis, and hematoma in patients who underwent mastectomy and axillary dissection (MAD).

METHODS

Study Type and Setting

This prospective, randomized, controlled study was conducted with patients who underwent breast cancer surgery by general surgeons. The study population included patients who received MAD between March 1, 2022, and September 1, 2022, in a tertiary hospital. Before the initiation of this study, it received approval from the Ethics Committee (Approval date: December 23, 2021; Number: 0537).

Study Population

Written consent was obtained from patients who were planned to be included in the study after they were informed about the study. Patients with ASA 1-2, without cognitive impairment, not using anticoagulant or anti-aggregant drugs, those without bleeding diathesis, and patients who knew at least one of the Turkish or English languages, did not have hearing or vision problems, and volunteered to participate were included in the study. Patients under 18 and over 80, those with acute infections, individuals experiencing severe complications during

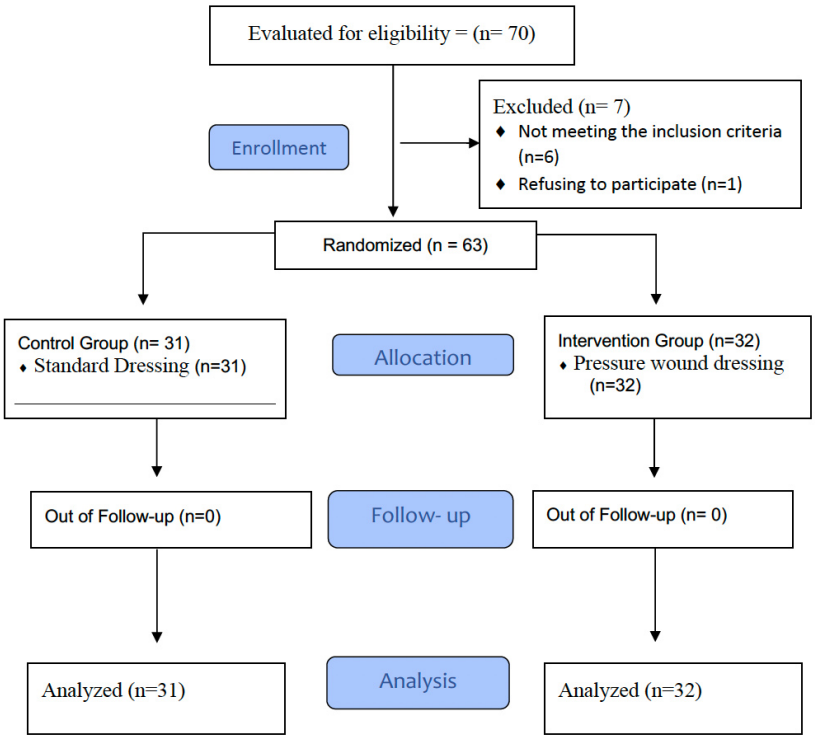

FIGURE 1. Research flow diagram based on Consort Statement 2010.

the intraoperative period, those who received chemotherapy or radiotherapy before surgery, and patients who did not agree to participate were excluded from the study. Patients were randomly divided into two groups of 31 and 32. A total of 63 patients' data was analyzed (Figure 1).

Interventions

Immediately after the operation, PWD was administered to the patients in the intervention group (n=31) (Figure 2), while standard wound dressing (SWD) was given to the patients in the control group. All patients in both groups remained dressed for five days postoperatively.

Standard Wound Dressing

While applying the SWD, the incision line was initially wiped with Providan iodine. Then, a 20 cm × 12 cm sterile cotton-filled pad was placed on both the mastectomy area and the axillary region and secured with a plaster. No standard or elastic bandages were used.

Pressure Wound Dressing

After cleaning the incision line with povidone-iodine, four cotton-filled sterile pads measuring 20 cm × 12 cm were placed, two on the axilla and two on the mastectomy area, as seen in Fig. 2. Subsequently, a 20 cm-wide sterile bandage was wrapped around the pads, covering the entire thorax. Finally, a 12 cm wide elastic bandage was applied over the sterile dressing, and pressure was applied to the wound to eliminate any dead space. The pressure from the elastic bandage was adjusted to the maximum level the patient could tolerate, ensuring no discomfort or disruption of blood circulation.

Follow-up

The patients were evaluated for the length of postoperative drain catheter stay, the amount of drainage from the axillary drain catheter, the length of hospital stay, pain levels on the 1st and 2nd postoperative days, and during the 1st week (VAS: Visual Analogue Scale). Additionally, reoperation, rehospitalization, and

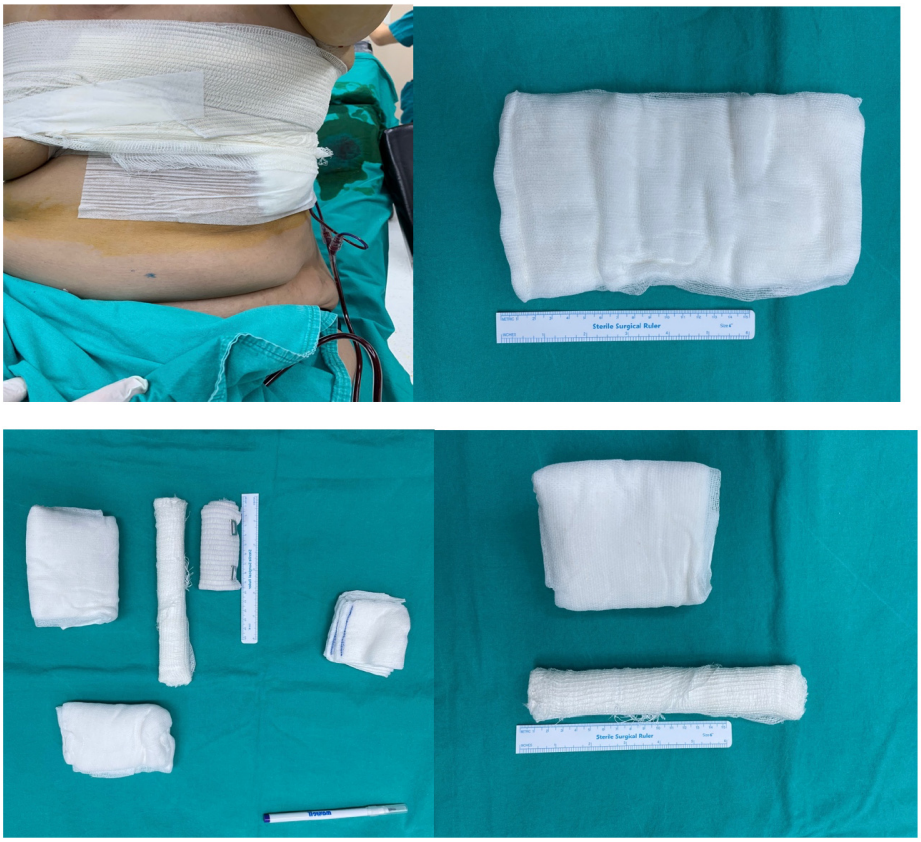

FIGURE 2. Application of pressure wound dressing and materials used.

other surgical complications such as wound infection, flap necrosis, and hematoma were monitored.

At the end of the postoperative 1st week and 1st month after discharge, Surgical site infection (SSI) was evaluated in terms of flap necrosis and pain experienced during outpatient control. The diagnosis of SSI was classified according to the definition of the US Centers for Disease Control and Prevention (CDC) criteria as superficial, deep incisional, or involving Organ/Space [12]. A single SSI was recorded in case of more than one primary incisional SSI from the same operation.

Operational Data

Operations were performed under general anesthesia. A single physician performed all surgical interventions. The dead space was reduced by suturing the flap to the chest wall. The subcutaneous tissues were sutured with an absorbable polyglactin multiflament suture. The skin was sutured subcutaneously with an insoluble synthetic, monofilament, nonabsorbable polypropylene suture. A total of 2 removal drains, 1 to the flap area and 1 to the axilla, were placed. The drain catheters were removed when the fluid drained decreased below 40 ml in 24 hours. The patient was discharged if no complications developed within 1-2 days after removal of the drain catheter. The skin suture was removed on the 10th day if the wound was healing well. All dressings were applied by the same surgeon and the same nurse at the end of the operation. The patient was asked not to use his arm actively for the first week and to keep it immobile. The patients were called for outpatient control on the 10th day and the 1st month.

Patient Information Form

The researcher prepared a 14-question form to record the patients' demographic characteristics and data about the study.

Visual Analogue Scale (VAS)

VAS consists of numbers from “0” to “10” (0: no pain, 10: I have unbearable pain), which was used to determine the pain level of the patients.

Statistical Analysis

Data were analyzed using IBM SPSS 26.0 (SPSS Inc., Chicago, IL, USA) for Windows software. Descriptive statistics were expressed as frequency, percentage, mean, and standard deviation. Histogram curves, kurtosis, skewness values, and the Shapiro-Wilk test were used to determine whether or not data were normally distributed. Normally distributed parameters were expressed as the mean plus standard deviation, and the Chi-square test was used to compare independent groups for categorical variables. In the comparison of continuous variables, when comparing the means of two independent groups, the Student-t test was used for normally distributed variables, and the Mann-Whitney U test was used for non-normally distributed variables. The results were given at a 95% confidence interval, and the $P<0.05$ value was considered statistically significant.

RESULTS

The study included 63 subjects, 31 in the SWD group and 32 in the PWD group. The mean age of the subjects was 53 ± 9 years. Table 1 shows that the subjects' general characteristics were similar in the SWD and PWD groups.

When the length of hospital stay and the length of stay of the axillary drain catheter were compared between the SD group and the PWD group, it was observed that the length of hospital stay and the length of stay of the axillary drain catheter were significantly less in the PWD group ($P<0.001$ and $P=0.002$ respectively). When the SD group and PWD groups were compared in terms of the daily amount of fluid drained and the total amount of fluid drained, both the amount of fluid drained daily and the total amount of fluid drained in the first 5 days, including the day of surgery were found to be significantly lower in the PWD group. ($P<0.001$, $P<0.001$, $P=0.031$, $P=0.012$, $P=0.002$ and $P<0.001$; respectively) (Table 2).

When the pain scores of the SWD and PWD groups were compared, the pain scores on the 1st, 3rd, and 10th days were significantly lower in the PWD group ($P=0.043$, $P=0.016$, and $P=0.040$; respectively) (Table 2).

None of the subjects included in the study required reoperation due to postoperative bleeding and hematoma. None of the subjects developed surgical site infection or flap necrosis. None of the subjects required hospitalization after discharge.

TABLE 1. Patients' General Characteristics

		Standard wound dressing (n=31)	Pressure wound dressing (n=32)	P-value
Age (years)		52.6±9.4	53±9.3	0.847*
BMI (kg/m²)		24.5±2.6	24.6±3.1	0.855*
Operation time (min)		120±20	120±16	0.950*
Comorbid disease	**No**	4 (12.9%)	2 (6.3%)	0.368**
	Yes	27 (87.1%)	30 (93.8%)	
	Total	31 (100%)	32 (100%)	
Smoking	**No**	23 (74.2%)	20 (62.5%)	0.319**
	Yes	8 (25.8%)	12 (37.5%)	
	Total	31 (100%)	32 (100%)	
Alcohol	**No**	17 (54.8%)	21 (65.6%)	0.382**
	Yes	14 (45.2%)	11 (34.4%)	
	Total	31 (100%)	32 (100%)	

Data are shown as mean±standard deviation or n (%). BMI=Body mass index
*Student-t test, **Chi-square test.

DISCUSSION

In this randomized controlled study, PWD and standard dressing were compared in patients who underwent MAD for breast cancer. Compared to standard dressing, PWD significantly reduced the postoperative length of stay of the drain, total drainage amount, hospital stay, and postoperative pain level.

TABLE 2. Comparison of Hospital Stay, Rainage Duration, Amount of Drainage, and Pain Scores Between Groups

	Standard wound dressing (n=31)	Pressure wound dressing (n=32)	P-value
Hospital stay (days)	4±1	3±0.8	**<0.001**
Drain stay (days)	4.1±0.8	3.5±0.6	**0.002**
Drain 1st day (mL)	114±27	28±20	**<0.001**
Drain 2nd day (mL)	83±15	58±18	**<0.001**
Drain 3rd day (mL)	47±13	40±13	**0.031**
Drain 4th day (mL)	27±21	14±17	**0.012**
Drain 5th day (mL)	10±14	1±4	**0.002**
Total drain (mL)	280±52	192±42	**<0.001**
Pain score 1st day	7.5±1.5	6.7±1.4	**0.043**
Pain score 2nd day	6.3±1.4	5.5±1.1	**0.016**
Pain score 10th day	5.6±1.4	5±1.1	**0.040**

Data are shown as mean±standard deviation. Student-t test was used.
Statistically significant P-values are shown in bold.

In a study by O'Hea *et al*. [13] in which PWD applied after axillary dissection and standard dressing were compared, it was found that PWD did not reduce the amount of fluid from the drain and the length of stay of the drain and was even associated with a statistically significant increase in seroma development after removal of the drain. In the present study, it was observed that PWD significantly reduced the daily amount of fluid from the drain and the total amount of fluid from the drain in the first 5 days, including the day of surgery, when compared to SD. In addition, it was found that PWD significantly shortened the mean length of stay of axillary drain. O'Hea *et al*. [13], while considering the drainage from both the axillary and mastectomy sites, only the amount of axillary drainage and the length of stay of the axillary drain were followed in our study. This may explain the differences in the results of the study. In addition, when the study of O'Hea *et al*. [13]. was examined, it was seen that surgibra was used for SD. In our study, as the DS, the sterile pad placed on the incision site was fixed with a plaster. Due to the structure surrounding the thorax, Surgibra may exert more pressure than the SD we used in our study. The result in our study differs from the study of Brian J *et al*. may be due to the possible superiority of surgibra over the SD we used in our study. In another study, Kottayasamy Seenivasagam *et al*. [14] found that pressure dressing significantly shortened the drainage time but did not affect the total drainage. However, since Kottayasamy Seenivasagam *et al*. [14] applied only pressure dressing without using a fixation suture in this study, it may have failed to reduce the amount of drainage compared to the pressure dressing applied with the fixation suture in our study.

Post-MAD pain is a condition that results from both tension due to the developing seroma and the drainage catheter placed, which impairs patient comfort [15, 16]. To our knowledge, this is the first study to compare the effect of PWD and SD on postoperative pain. This study observed that the pain scores of the patients who underwent PWD were significantly lower compared to the postoperative 1st, 3rd, and 10th days. Conditions such as the fact that PWD does not allow tissue tension with the pressure applied and shortens the length of stay of the axillary drain may be the reason for the low postoperative pain scores.

Strengths and Limitations

The primary strength of this study lies in its prospective, randomized, and controlled design, which enables the generation of reliable evidence by establishing causal relationships and minimizing the risk of bias. Moreover, the performance of all surgical procedures by the same team of surgeons and nurses ensured consistency in clinical practice, thereby enhancing the internal validity of the study.

Also the study has some limitations. While a sterile pad fixed with plaster was used as SD in this study, it is known that more effective products, such as surgibra, can be used for the same purpose. Surgibra, with its structure fully encompassing the body, may have been superior to the method we applied, which may have affected the result. Studies comparing the SD method we used with surgibra may illuminate this issue.

CONCLUSION

In this study, PWD decreased the amount of fluid drained from the catheter, length of hospital stay, and postoperative pain in patients undergoing MAD compared to SD. Thus, PWD is a promising treatment for reducing postoperative complications in these patients.

Ethical Statement

The study was approved by the Izmir Katip Çelebi University Non-Interventional Clinical Research Ethics Committee (Decision no.: 0537 and date: 23.12.2021).

Data Availability

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available on request from the corresponding author, upon reasonable request.

Authors' Contribution

Study Conception: KA, YYB; Study Design: YYB, KA; Supervision: YYB; Funding: KA; Materials: YYB, KA; Data Collection and/or Processing: KA, YYB; Statistical Analysis and/or

Data Interpretation: YYB; Literature Review: KA; Manuscript Preparation: KA; and Critical Review: KA, YYB.

Conflict of interest

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

Financing

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Generative Artificial Intelligence Statement

The author(s) declare that no artificial intelligence-based tools or applications were used during the preparation process of this manuscript. The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

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The European Research Journal 2026;12(4):439-446 Original Article

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An Investigation of the Relationship Between e-Health Literacy and Telerehabilitation Awareness, Knowledge, Attitudes, and Skills of Physiotherapists in Türkiye

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ABSTRACT

Objectives: The aim of this study was to determine the e-health literacy levels of physiotherapists in Türkiye and to examine their relationship with telerehabilitation awareness, knowledge, attitudes, and skills.

Methods: This descriptive and cross-sectional study included 106 physiotherapists who participated through an online survey between January and March 2024. E-health literacy was evaluated using the E-Health Literacy Scale, while telerehabilitation competencies were assessed with the Telerehabilitation Awareness, Knowledge, Attitudes and Skills Scale. Statistical analyses were conducted using multiple regression and ordinal regression models.

Results: The median e-health literacy score was 30 (min: 12-max: 40), the mean (±SD) was (28.79±6.44). A statistically significant relationship was found between e-health literacy and all subdimensions of telerehabilitation: awareness (η^2_p=0.153, P<0.001), knowledge (η^2_p=0.041, P=0.038), attitudes (η^2_p=0.299, P<0.001), and skills (η^2_p=0.178, P<0.001). E-health literacy explained 34.7% of the variance in these domains. Additionally, telerehabilitation awareness significantly predicted the frequency of telerehabilitation use (β=0.143, P<0.001).

Conclusions: E-health literacy plays a critical role in the readiness of physiotherapists to adopt telerehabilitation practices. Educational programs aimed at improving digital literacy and awareness may enhance the effective integration and implementation of e-health and telerehabilitation services in physiotherapy.

Keywords: E-HealthLiteracy, Telerehabilitation, Physiotherapist, Digital Health

Advancements in digital health technology have improved the accessibility of healthcare services, enabling a broader patient demographic to obtain treatment. These developments significantly help individuals facing challenges in accessing healthcare due to financial or geographical constraints [1]. The digitalization of health systems strengthens communication between patients and healthcare professionals and supports the delivery of healthcare services independent of time and location [2].

The 2020 report by the World Health Organization (WHO) indicated that telehealth became the most

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widely adopted method of healthcare delivery among countries during the pandemic. According to the report, the use of telehealth services has increased proportionally with income levels. However, even in low-income countries, 42% of individuals who experienced interruptions in healthcare services during the COVID-19 crisis reported benefiting from this technology [3].

Digital health technologies such as electronic health records (EHR), health information systems (HIS), digital supply and supply chain systems, delivery of medical supplies via drones, and patient-reported outcomes contribute to making healthcare services more efficient and accessible [4, 5]. The growing use of digital health applications necessitates the adaptation of healthcare professionals, particularly physiotherapists, to these technologies. The increased usage of mobile devices and the replacement of face-to-face conversation with digital communication have caused healthcare applications to move towards digital interactions [6].

Telerehabilitation is the remote delivery of rehabilitation services using telecommunications technologies. This concept has developed as a subfield of telemedicine, allowing healthcare professionals to assess, diagnose, and manage treatment processes without seeing patients face-to-face [7]. Telerehabilitation can be applied through synchronous and asynchronous models, includes components such as video consultations, treatment planning through digital health applications, exercise prescriptions, and visual-video-based assessments [8]. Synchronous rehabilitation is a model in which the patient and therapist interact live at the same time; asynchronous rehabilitation is a model in which the patient does the exercises in his or her own time and the therapist evaluates them later [9]. However, telerehabilitation faces difficulties including patient privacy, data security, and technological obstacles; in addition, problems related to technological competence have been reported and found to be associated with patients' health literacy. Moreover, the belief that face-to-face communication and direct patient contact are necessary, along with the absence of a treatment environment, are considered to reduce the effectiveness of telerehabilitation [10].

Digital health literacy is defined as the ability of individuals to find, understand, and use health information via the internet and mobile devices. Online video interviews, digital health records, social networks, and mobile health applications are among the key components of digital health literacy [11]. The expansion of e-health applications increases patient access, reduces transportation and waiting times, and optimizes the workload of healthcare professionals, thereby improving operational efficiency. The OECD report emphasizes that the widespread adoption of telehealth practices is directly related to the level of e-health literacy among individuals and healthcare workers [12].

The technical capacity of digital health systems in Turkey is sufficient to accommodate telerehabilitation [13]. The effectiveness of telerehabilitation is closely linked not only to technical infrastructure but also to patient acceptance and health literacy [14]. This effectiveness depends not only on the knowledge, awareness, and attitudes of patients but also of physiotherapists [15]. For effective implementation of telerehabilitation, physiotherapists must possess not only technical knowledge but also digital health literacy [16].

This study aims to assess the digital health literacy levels of physiotherapists, along with their awareness, knowledge, attitudes and skills towards telerehabilitation.

METHODS

Sample size was determined using GPower 3.1.9.7 A priori power analysis for a multiple linear regression (fixed model, R^2 deviation from zero) with a medium effect size ($f^2 = 0.15$), $\alpha = 0.05$, power = 0.85, and 5 predictors indicated that a minimum sample of 102 participants was required. We evaluated 112 actively working physiotherapists in this cross-sectional study. We collected the study's data online using Google Forms. We shared the survey link with physiotherapists through professional groups and social media platforms. Prior to participation, informed consent was obtained from all participants.

To be included in the study, participants had to be practicing physiotherapists in Türkiye, agree to participate. Participants who provided incomplete or

incorrect information during the data collection process, individuals who were not working as physiotherapists, were excluded from the study.

Measurement Tools

The data used in this study were collected online using a survey method via Google Forms. Participants were invited to the study through the LinkedIn platform, and the survey link was shared within professional physiotherapist groups and through individual connections. Participants were informed about the purpose of the study and completed the survey after approving the online informed consent form.

A demographic data form was prepared to collect information about participants' age, gender, professional experience, field of work, and frequency of telerehabilitation use.

To measure the e-health literacy levels of the participants, the e-Health Literacy Scale (eHEALS), developed by Norman and Skinner [17] and adapted into Turkish by Uskun *et al*. [18] was used. The scale consists of 8 items and is answered in a 5-point Likert format (1 = Strongly disagree, 5 = Strongly agree). The total score range of the scale varies between 8 and 40, with higher scores indicating higher electronic health literacy [17].

We used the Telerehabilitation Awareness, Knowledge, Attitudes, and Skills Questionnaire [19] to assess the participants' awareness, knowledge level, attitudes, and skills regarding telerehabilitation. This scale was developed to evaluate different dimensions of telerehabilitation, and its Turkish validity and reliability study has been completed [20]. The scale consists of four subdimensions, each with specific scoring criteria. The scale consists of four subdimensions. Awareness subsection includes 12 items assessing participants' awareness of telemedicine. Each item is rated on a 3-point scale ranging from 0 to 2 (0 = don't know, 1 = heard of it, 2 = know about it), yielding a total possible score between 0 and 24. Knowledge subsection consists of 11 items measuring participants' level of telemedicine knowledge. Each item is rated on a 2-point scale (0 = no, 1 = yes), with a total possible score ranging from 0 to 11. Attitude subsection contains 11 items evaluating participants' attitudes toward telemedicine. Each item is scored on a 5-point Likert scale ranging from 0 to 4 (0 = strongly disagree, 1 = disagree, 2 = undecided, 3 = agree, 4 = strongly agree). The total possible score ranges from 0 to 44. Skills subsection includes 13 items assessing participants' computer and information technology skills relevant to telemedicine. Each item is scored on a 4-point scale (0 = inadequate, 1 = moderate, 2 = good, 3 = very good), with a total score ranging from 0 to 39 [19].

The Ethics Committee of the relevant university granted approval for this study. We explained the study's purpose to all participants and obtained their informed consent. The participants' data were kept confidential and used solely for scientific purposes.

Statistical Analysis

The statistical analyses of the study were performed using the SPSS 24.0 software. The normality of the data distribution was assessed using the Kolmogorov-Smirnov test and histogram graphics. Descriptive statistics were presented as frequency and percentage for categorical variables and as median, interquartile range (IQR), range (minimum-maximum) values for continuous variables that did not follow a normal distribution. Multiple linear regression analysis was applied to evaluate the effect of independent continuous variables on the dependent variable while ordinal regression analysis was used to assess the effect of categorical variables. A significance level of $P<0.05$ was considered statistically significant.

RESULTS

A total of 106 physiotherapists completed the study, including 48 males (mean ages: 33.00±6.45, 23-47 years) and 58 females (mean ages: 31.48±6.15, 21-45 years). The number and percentage distribution of the participants according to their demographic characteristics are presented in Table 1.

It was observed that 35 (33.0%) physiotherapists reported never using e-health applications, 56 (52.8%) reported using them occasionally, and 15 (14.2%) reported using them regularly. Regarding telerehabilitation experience, 58 (54.7%) physiotherapists reported never using telerehabilitation, 41 (38.7%) reported using it occasionally, and 7 (6.6%)

TABLE 1. Demographic Characteristics of the Participants

		Freqency (n)		Percentage (%)
Gender	Female	58		54.7
	Male	48		45.3
Marital status	Single	59		55.7
	Married	47		44.3
Degree	Bachelor's	72		67.9
	Master's	25		23.6
	Doctorate	9		8.5
Field of specialization	Orthopedic rehabilitation	35		33.0
	Sports rehabilitation	21		19.8
	Neurological rehabilitation	21		19.8
	Cardiopulmonary rehabilitation	3		2.8
	Pediatrics rehabilitation	16		15.1
	Obstetric rehabilitation	7		6.6
	Geriatric rehabilitation	3		2.8
Working experience as a physiotherapist	0-5 Years	50		47.2
	5-10 Years	26		24.5
	10-15 Years	19		17.9
	15+ Years	11		10.4
		Median (IQR)	**Mean±SD**	**Min-Max**
E-health literacy		30 (7)	28.79±6.443	12-40
AKAS of telemedicine	Awareness	13 (11)	13.44±6.802	0-24
	Knowledge	9 (4)	8.37±2.727	0-11
	Attitude	30 (11)	28.32±8.518	0-44
	Skills	23 (15)	20.52±10.044	0-39

AKAS, Awareness, knowledge, attitude, and skills; IQR, Iinterquartile range; Min, minimum; Max, maximum; SD, standard deviation.

reported using it regularly.

The number of physiotherapists who had received training in e-health and telerehabilitation was 30 (28.3%), while 76 (71.7%) physiotherapists had not received any training. There was no significant effect of professional experience on e-health literacy (F=1.494, η^2_p=0.042, P=0.221), telerehabilitationawareness (F = 0.216, η^2_p=0.006, P=0.885), knowledge (F=1.441, η^2_p=0.041, P=0.235), attitudes (F=0.933, η^2_p=0.027, P=0.428), skills (F = 0.002, η^2_p= 0.000, P=1.000).

E-health literacy significantly affected the sub-dimensions of telerehabilitation awareness (P<0.001), knowledge (P=0.038), attitude(P<0.001), and skills (P<0.001). E-health literacy explained 34.7% of the variance in telerehabilitation awareness, knowledge, attitude, and skills.The results of the multiple linear regression analysis between e-health literacy and telerehabilitation awareness, knowledge, attitude, and skills are presented in Table 2.

A moderate relationship was found between e-health literacy and telerehabilitation awareness (F = 18.802, η^2_p=0.153, P<0.001), a low-level relationship with telerehabilitation knowledge (η^2_p=0.041), a high-level relationship with telerehabilitation attitude (F=4.411, η^2_p=0.041, P=0.038), and a moderate relationship with telerehabilitation skills (F=44.386, η^2_p= 0.299, P<0.001) (Table 2).

The relationship between physiotherapists'

TABLE 2. The Effect Of E-Health Literacy on Telerehabilitation Awareness, Knowledge, Attitudes, and Skills

	E-Health Literacy		
	F	η^2_p	P-value
Telerehabilitation awareness	18.802	0.153	**0.000**
Telerehabilitation knowledge	4.411	0.041	**0.038**
Telerehabilitation attitudes	44.386	0.299	**0.000**
Telerehabilitation skills	22.464	0.178	**0.000**

η^2_p, Partial Eta Squared. Statistically significant P-values are shown in bold.

telerehabilitation experience and their telerehabilitation awareness, knowledge, attitudes, and skills was evaluated using ordinal regression analysis, and the results are presented in Table 3.

There was a significant relationship between physiotherapists' telerehabilitation experience and their levels of telerehabilitation awareness, knowledge, attitudes, and skills ($\chi^2 = 22.44$, P<0.001). (Table 3).

Telerehabilitation awareness, knowledge, attitudes, and skills had a moderate power in explaining the frequency of telerehabilitation use ($R^2 = 0.231$, P<0.001).

As telerehabilitation awareness increased, the frequency of telerehabilitation use showed a moderate and significant relationship (β = 0.143, P<0.001). However, telerehabilitation knowledge level (β = –0.067, P=0.401), attitudes (β = 0.015, P=0.634), and skills (β = –0.003, P=0.916) did not significantly affect the frequency of telerehabilitation use. (Table 3)

DISCUSSION

The primary aim of this study was to examine the relationship between the e-health literacy levels of physiotherapists working in Türkiye and their levels of awareness, knowledge, attitudes, and skills regarding telerehabilitation. The findings of the study indicated that e-health literacy was significantly associated with the sub-dimensions of telerehabilitation awareness and skills at a moderate level, with attitudes at a high level, and with knowledge at a low level.

Most of the physiotherapists participating in the study reported that they had never used or only occasionally used e-health and telerehabilitation applications. Although the frequency of internet use among the participants was high, their limited experience with telemedicine and e-health applications suggests restricted integration of physiotherapists into digital health services. Norman and Skinner [17] emphasized that e-health literacy is a fundamental

TABLE 3. The Relationship Between Telerehabilitation Experience and Telerehabilitation Awareness, Knowledge, Attitudes, and Skills

	Estimate (β)	SE	95% Confidence interval	P-value
Never used - occasionally used telerehabilitation	2.007	0.890	(0.262, 3.752)	**0.024**
Occasionally used - regularly Use telerehabilitation	4.821	1.014	(2.833, 6.809)	**0.000**
Telerehabilitation awareness	0.143	0.041	(0.063, 0.224)	**0.000**
Telerehabilitation knowledge	-0.067	0.080	(-0.223, 0.089)	0.401
Telerehabilitation attitudes	0.015	0.032	(-0.047, 0.077)	0.634
Telerehabilitation skills	-0.003	0.024	(-0.050, 0.045)	0.916

SE, Standart Error. Statistically significant P-values are shown in bold.

component of active participation in healthcare services. Similarly, in our study, lower e-health literacy was associated with lower frequency of e-health and telerehabilitation use.

Mair *et al*. [21] examined studies on patient satisfaction with telehealth services and demonstrated that perceived benefit, reliability, and ease of use were the main factors influencing telehealth services. In our study, e-health literacy was found to have an effect on telerehabilitation awareness, knowledge, and attitudes, with the strongest effect being on attitudes. This may suggest that participants' approaches and motivation towards health technologies were more influential than their knowledge level. Another study indicated that the sustainability of telerehabilitation applications depends not only on individual skills but also on professional guidance, systematic support, and technological infrastructure [22]. Consistent with these findings, our study showed that telerehabilitation experience had a significant effect on telerehabilitation awareness. The lack of a significant effect of knowledge level indicates that having knowledge alone is not sufficient for practical implementation.

Professional experience did not have a significant effect on e-health literacy or telerehabilitation attitudes. This suggests that telehealth services are more related to motivation, interest, and digital literacy skills rather than seniority. This highlights the importance of providing training in telehealth to all healthcare professionals, regardless of age or seniority. Although the COVID-19 pandemic accelerated the adoption of telerehabilitation, the sustainability of these services requires overcoming structural and educational barriers [23]. Bonet-Collantes *et al*. [24] suggested that even short-term e-health literacy interventions could increase awareness. Saeed *et al*. [25] reported that physiotherapists generally had a positive attitude towards telerehabilitation but recommended targeted training programs to improve competence with digital platforms. These findings emphasize the importance of educational strategies in this field. Our study found that professional experience did not significantly influence attitudes toward telerehabilitation, which may reflect increased familiarity with and interest in digital health technologies in recent years. In contrast, studies conducted in Nigeria reported that age and professional experience were significant factors, though no gender differences were observed [26]. These variations may be influenced by socioeconomic factors such as cultural acceptance, internet accessibility, and awareness of the benefits of telerehabilitation.

In line with these observations, the attitudes of healthcare professionals constitute a prerequisite for the integration of e-health and telemedicine concepts into contemporary healthcare systems. Thus, efforts to increase the knowledge level and awareness of physiotherapists should become an essential component of the education provided to students in this field as well as to practicing professionals.

Strengths and Limitations

This study's strength is its assessment of the e-health literacy levels of physiotherapists in Türkiye, along with their attitudes, knowledge, abilities, and awareness of telerehabilitation procedures. This study significantly contributes to the field of physiotherapy and rehabilitation by particularly examining the digital health attitudes of physiotherapists, in contrast to prior work that generally focusses on the other healthcare professionals or patients.

This study has some limitations. One of the main limitations is the small sample size. Although the sample size met the minimum requirement according to the power analysis, this may limit the generalizability of the findings. Additionally, the use of self-reported online surveys may have led to partial bias. If this study had been conducted with a larger sample group through face-to-face surveys, the results might have been more generalizable.

CONCLUSION

Study results show a significant relationship between physical therapists' e-health literacy and telerehabilitation awareness, attitudes and skills. In line with these findings, it is recommended to include digital health literacy and telerehabilitation in physiotherapy undergraduate and graduate programs. In addition, due to the limited sample size of the study, it is necessary to conduct research with more extensive and representative samples from different regions and fields of study in the future. It is recommended that participants use qualitative research methods so that

perception, experience, and barriers to telerehabilitation can be studied in depth.

Ethics Approval and Consent to Participate

This study was approved by the Mardin Artuklu University Non-Interventional Clinical Research Ethics Committee (Decision No: 2025/2-12; date: 18.02.2025). All procedures were conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. All participants provided informed consent before inclusion, confirming their understanding and willingness to participate under clearly defined conditions. The participants' data were kept confidential and used solely for scientific purposes.

Data Availability

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available on request from the corresponding author, upon reasonable request.

Authors' Contribution

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

Conflict of Interest

The author(s) disclosed no conflict of interest during the preparation or publication of this manuscript.

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Generative Artificial Intelligence Statement

The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles. During the preparation of this work, the authors used ChatGPT to improve language and readability. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Editor's Note

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Could Ratio-Based Morphometric Analysis of Subcortical Limbic Structures Assist in Alzheimer's Disease Diagnosis?

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ABSTRACT

Objectives: Alzheimer's disease is a neurodegenerative disorder that primarily affects subcortical limbic structures. This study aimed to assess volumetric differences in subcortical limbic structures and to compare the relative volumes of surrounding brain regions - such as the telencephalon, diencephalon, and brainstem subdivisions - between individuals with Alzheimer's disease and healthy controls.

Methods: This study involved 24 patients with Alzheimer's disease and 16 healthy controls. Subcortical structures were segmented automatically using MRICloud on 3D T1-weighted magnetic resonance imaging scans. To minimize individual anatomical variability, volume ratios relative to neighboring brain regions were also calculated.

Results: Significant volume reductions were found in the amygdala (left: P=0.004, right: P=0.005, total: P=0.004), hypothalamus (left: P=0.005, right: P>0.05, total: P=0.007), diencephalon (left: P=0.001, right: P=0.012, total: P>0.05), and mammillary bodies (left: P=0.002, right: P=0.003, total: P=0.003) in the Alzheimer's disease group compared to healthy controls. Although most volume ratios - particularly those involving the amygdala and mammillary bodies - were higher in the Alzheimer's disease group, they did not reach statistical significance (P>0.05).

Conclusions: This study confirms prominent atrophy in subcortical limbic structures in Alzheimer's disease. While diencephalon volume was reduced, its ratio to the amygdalae changed minimally, likely reflecting more severe atrophy of the amygdalae. Similarly, the mesencephalon-to-hypothalamus ratio showed no significant difference, suggesting parallel atrophy. These findings support the combined use of absolute and ratio-based analyses and demonstrate the potential of MRICloud to identify Alzheimer's disease-related neuroanatomical changes.

Keywords: Alzheimer's Disease, Amygdala, Hypothalamus, Mammillary Bodies, Subcortical Structures, Magnetic Resonance Imaging

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Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative disorder that primarily affects older adults, including both elderly individuals and those with early-onset forms. It is characterized by cognitive decline, memory impairment, and behavioral disturbances, and its prevalence is increasing as the global population ages [1, 2]. The number of individuals affected is projected to reach 13.8 million by 2060 [3]. Therefore, accurate and reliable risk prediction is critical for the development of disease-modifying therapies aimed at mitigating the devastating impact of AD.

Several studies based on magnetic resonance imaging (MRI) have demonstrated volume reductions in cortical structures, such as the hippocampus, in individuals with Alzheimer's disease [4]. However, subcortical limbic system structures - including the mammillary bodies, amygdala, thalamus, and hypothalamus - have been less extensively studied. Motor symptoms, which reflect pathological changes in the extrapyramidal system, tend to emerge in the later stages of Alzheimer's disease [5]. Therefore, it is commonly assumed that degeneration in the hippocampus and cortex precedes that in frontal–subcortical circuits [6]. Nevertheless, this widely accepted sequence of degeneration may not fully reflect the underlying pathophysiology. It is often overlooked that other regions of the Papez circuit are affected concurrently with the hippocampus [7], and that the hippocampus constitutes only one component of this interconnected system [8]. Given the possibility that pathological changes in subcortical structures may begin earlier than those in the hippocampus and progressively worsen, volumetric analyses of the amygdala, hypothalamus, and mammillary bodies - structures that have received comparatively less attention in the literature - may offer valuable insights as potential biomarkers for the prediction of AD.

However, relying exclusively on the absolute volumes of these structures may limit diagnostic accuracy due to genetic and structural variability among individuals. For instance, a study by Gerritsen *et al*. [9] demonstrated that, in non-depressed controls, a higher amygdala-to-hippocampus (AH) volume ratio was associated with a stronger bias toward negative memories - a key cognitive marker of depression - whereas weaker associations were observed when the amygdala and hippocampus were assessed separately. Such discrepancies may contribute to suboptimal clinical decision-making or misdiagnosis. Considering the structural and functional interconnectivity of brain regions, evaluating the amygdala, hypothalamus, and mammillary bodies in relation to their adjacent structures may yield more meaningful diagnostic insights than assessing each region independently.

To address these limitations, recent developments in neuroimaging have led to the emergence of automated tools that can analyze both absolute and relative brain volumes. In parallel, web-based platforms for evaluating neuroanatomical structures have gained traction and increasingly supplanted traditional manual measurement methods. MRICloud is one such platform that performs automated volumetric analysis of T1-weighted MRI data and generates comprehensive neuroanatomical profiles. It is suitable for multicenter clinical validation studies, and its reliability in predicting future cognitive decline has been previously validated [10]. Although several studies have used automatic segmentation tools such as volBrain, Computational Anatomy Toolbox (CAT), BrainSuite, and FreeSurfer to evaluate the volumes of the amygdala, hypothalamus, and mammillary bodies in AD, to the best of our knowledge, no study has assessed both the volumes and volume ratios of these structures using the web-based MRICloud platform in AD.

In this study, we analyzed the volumes of the left amygdala (AmygL), right amygdala (AmygR), total amygdala (TotAmyg), left hypothalamus (HypoThL), right hypothalamus (HypoThR), total hypothalamus (TotHypoTh), left mammillary body (MamL), right mammillary body (MamR), and total mammillary body (TotMam). Additionally, we evaluated the volume ratios of surrounding brain regions - including the right and left telencephalon (TelenR and TelenL), total telencephalon (TotTelen), left and right diencephalon (DiencL and DiencR), total diencephalon (TotDienc), and brainstem components such as the mesencephalon (Mesenc), metencephalon (Metenc), and myelencephalon (Myelenc) - relative to these limbic structures using MRICloud. We aimed to explore their potential utility as volumetric biomarkers to enhance diagnostic accuracy in AD.

METHODS

Ethics Approval

This study was approved by the Non-Interventional Clinical Research Ethics Committee of Istanbul Medipol University (February 19, 2020; No: 170).

Study Design

Patients over the age of 50 who presented with complaints of amnesia to the Neurology Outpatient Clinic of Istanbul Medipol University Hospital between January 1, 2017, and December 31, 2019, were retrospectively evaluated. All patients had completed at least primary school education and were diagnosed with AD according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria. Mini-Mental State Examination (MMSE) scores and T1-weighted MRI scans were retrieved from electronic medical records.

Participants

This study included 24 patients diagnosed with AD and 16 healthy controls (HC), selected from the Neurology Outpatient Clinic of Istanbul Medipol University Hospital between January 2017 and December 2019. All participants met the diagnostic criteria outlined in the DSM-5. The demographic characteristics of the participants are presented in Table 1.

Inclusion and Exclusion Criteria

Participants included individuals aged 55–84 years who underwent 3T MRI and were diagnosed with AD or classified as HC. The AD and HC groups had comparable age and sex distributions. Exclusion criteria included age below 50, illiteracy, space-occupying brain lesions, global brain atrophy, cerebrovascular disease, and other neurodegenerative or structural brain disorders such as Parkinson's disease, amyotrophic lateral sclerosis, essential tremor, and primary or metastatic brain tumors. From the initial sample, 65 patients without 3D T1-weighted MRI images, 33 with mismatched MMSE and MRI dates, 20 with inconsistencies between MMSE scores and clinical findings, and 19 who met other exclusion criteria were excluded. Ultimately, 24 patients with AD (10 males, 14 females) were included in the study. The final sample also included 16 healthy controls (7 males and 9 females).

Structural MRI Protocol

MRI was performed using a 3-T Philips Achieva TX scanner (Philips Healthcare, Best, Netherlands) equipped with a 20-channel head coil. The imaging protocol consisted of 3D T1-weighted and FLAIR sequences. Sagittal T1-weighted images were used for volumetric analysis. Imaging parameters for the T1-weighted Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) sequence were as follows: repetition time (TR) = 2400 ms, echo time (TE) = 3.54 ms, field of view (FOV) = 250 × 250 mm (sagittal plane), slice thickness = 1 mm, voxel size = 1 × 1 × 1 mm, turbo field echo (TFE) factor = 97, flip angle = 8°, and matrix size = 228 × 227 pixels. MRIcron software was used to convert the MRI data into a format suitable for analysis. The preprocessed data were then uploaded to the MRICloud platform.

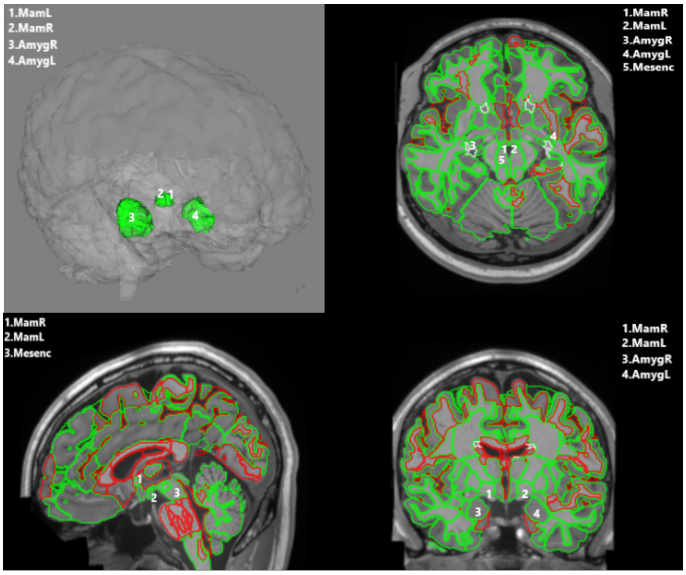

FIGURE 1. Parcellation of MPRAGE images based on the Multi-Atlas Likelihood Fusion algorithm. 3D and cross-sectional views (axial, coronal, and sagittal) illustrate bilateral segmentation of limbic system subcortical structures and mesencephalon: 1. Left Mammillary Body (MamL), 2. Right Mammillary Body (MamR), 3. Right Amygdala (AmygR), 4. Left Amygdala (AmygL), 5. Mesencephalon (Mesenc). Green areas represent the segmented regions overlaid on anatomical MRI sections.

Automatic Segmentation

Magnetic Resonance Imaging Cloud (MRICloud)

MRICloud provides a fully automated segmentation service for MPRAGE images using the Multi-Atlas Likelihood Fusion algorithm, the JHU multi-atlas inventory with 286 labeled structures, and Ontology Level Control [11]. The segmentation atlas used in this study was "adult_286labels_11atlases_V5L" (Figure 1).

DICOM files were converted directly to the Analyze format (.hdr and .img extensions) using the dcm2analyze tool, as recommended by the official MRICloud documentation. This step ensured compatibility with the MRICloud platform, which requires input files in Analyze format. After conversion, the resulting .hdr and .img files were archived and uploaded to MRICloud for automated segmentation and volumetric analysis.

Finally, the archived Analyze format files (.hdr and .img) were uploaded to MRICloud via a web browser. Following the entry of demographic information such as age and gender, volumetric measurement results were made available in the "My Job Status" section as a downloadable .zip file containing the "corrected_stats.txt" table. Segmentation results were visualized in 3D using ITK-SNAP and MRIcroGL, two commonly used neuroimaging tools for anatomical visualization and surface rendering (Figure 2).

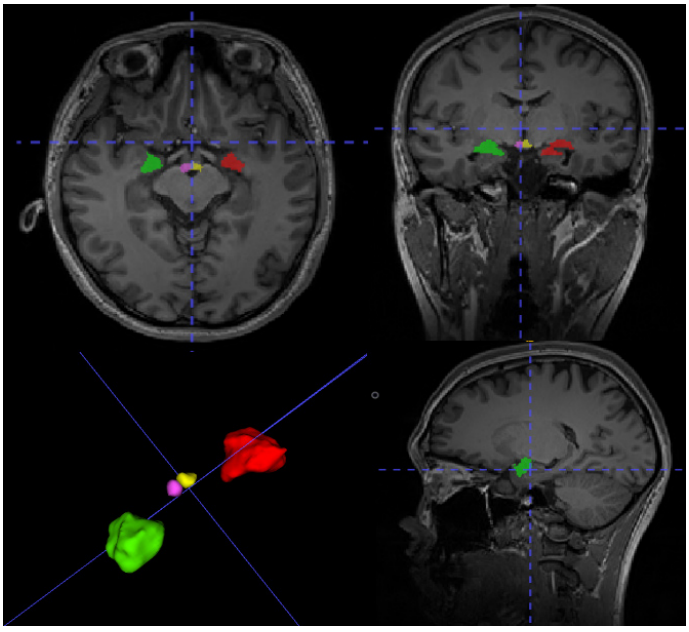

FIGURE 2. Colorization and three-dimensional modeling of MRICloud data using ITK-SNAP. Axial, coronal, and sagittal T1-weighted MR images with 3D volume renderings showing the bilateral segmentation of subcortical limbic structures using MRICloud and visualized in ITK-SNAP. Colored areas represent automatically segmented limbic structures.

Statistical Analysis

Descriptive statistics included mean, standard deviation, median, minimum, maximum, frequency, and percentage values. The distribution of variables was assessed using the Kolmogorov-Smirnov test. For the analysis of independent quantitative variables, the independent-samples t-test and the Mann-Whitney U test were used, depending on the distribution. The chi-square test was used to analyze independent categorical variables. All statistical analyses were performed using IBM SPSS Statistics version 27.0, and a significance level of $P<0.05$ was considered statistically significant.

RESULTS

The volumes of 18 subcortical brain structures were evaluated using MRICloud segmentation derived from 3D T1-weighted MRI scans. Descriptive statistics and group comparisons between patients with AD and HC are presented in Table 2.

Statistical analysis revealed that the volumes of the AmygL, AmygR, TotAmyg, HypoThL, TotHypoth, MamL, MamR, and TotMam were significantly lower in the AD group compared with the healthy control (HC) group ($P<0.05$). Additionally, the diencephalic structures - DiencL, DiencR, and TotDien - were also significantly reduced in the AD group ($P=0.001$, $P=0.012$, and $P=0.003$, respectively).

No significant volumetric differences were observed in the telencephalon (TelenL, TelenR, TotTelen), mesencephalon (Mesenc), metencephalon (Metenc), or myelencephalon (Myelenc) ($P>0.05$; Table 2). In addition to absolute volumes, inter-regional volume ratios were calculated to assess proportional relationships between adjacent brain regions. These comparisons are presented in Table 3.

The ratios of TelenL/AmygL, TelenR/AmygR,

TABLE 1. Demographic Characteristics of Alzheimer's Disease and Healthy Control Groups

Variable	Min-Max	Median (Overall)	Mean±SD (Overall)	Mean±SD (AD)	Median (AD)	Mean±SD (HC)	Median (HC)	P-value
Age (years)	55.0-84.0	70.5	70.9 ± 7.7	72.3 ± 7.7	72.5	68.9 ± 7.5	67.0	0.173[t]
Gender, n (%)								
Female			23 (58%)	14 (58%)		9 (56%)		0.896[x2]
Male			17 (43%)	10 (42%)		7 (44%)		

Data are shown as mean±standard deviation or median (minimum-maximum) or n (%). AD, alzheimer's disease; HC, healty control.

t=Independent-samples t-test, χ^2=Chi-square test

TABLE 2. Descriptive Statistics and Volumetric Comparisons of Brain Structures Between Alzheimer's Disease and Healthy Control Groups

Structure	AD group (Mean±SD)	AD group (Median)	HC group (Mean±SD)	HC group (Median)	P-value
TelenL (mm³)	438.1±47.6	444.8	454.1±43.0	466.0	0.298[t]
TelenR (mm³)	440.9±43.3	436.8	455.1±44.1	460.8	0.298[t]
TotTelen(mm³)	879.1±86.6	888.3	909.2±86.9	926.8	0.298[t]
DiencL (mm³)	6863.9±515.9	6887.5	7499.8±655.9	7428.0	**0.001[m]**
DiencR (mm³)	6965.9±578.1	6862.0	7428.9±577.3	7504.0	**0.012[m]**
TotDienc (mm³)	13.8±1.0	13.8	14.9±1.2	14.9	**0.003[t]**
Mesenc (mm³)	12.7±2.1	8.6	9.1±1.0	9.5	0.534[t]
Metenc (mm³)	141.4±21.1	136.4	136.5±21.8	131.7	0.384[t]
Myelenc (mm³)	5208.0±723.5	5364.0	5028.2±594.4	4955.0	0.414
AmygL (mm³)	1076.0±239.1	1018.0	1396.8±232.6	1322.0	**<0.001[t]**
AmygR (mm³)	1259.1±235.1	1194.0	1544.9±276.7	1528.5	**<0.001[m]**
TotAmyg(mm³)	2335.1±433.4	2211.5	2941.7±489.4	2866.5	**<0.001[m]**
HypoThL (mm³)	494.6 ± 47.3	487.0	528.4 ± 36.4	529.0	**0.020[t]**
HypoThR (mm³)	587.6±37.6	579.0	606.2±61.5	587.0	0.456[m]
TotHypoth(mm³)	1082.2±75.4	1067.0	1134.6±84.4	1131.0	**0.047[t]**
MamL (mm³)	67.9±13.2	65.0	85.2±12.5	86.0	**<0.001[t]**
MamR (mm³)	72.8±18.0	70.0	91.0±15.0	87.0	**0.002[m]**
TotMam (mm³)	140.7±28.2	135.0	176.2±24.0	176.5	**<0.001[t]**

Data are shown as mean±standard deviation or median. AD, Alzheimer's disease; HC, healty control; TelenL, left telencephalon; TelenR, right telencephalon; TotTelen, total telencephalon; DiencL, left diencephalon; DiencR, right diencephalon; TotDienc, total diencephalon; Mesenc, mesencephalon; Metenc, metencephalon; Myelenc, myelencepalon; AmygL, left amygdala; AmygR, right amygdala; TotAmyg, total amygdala; HypoThL, left hypothalamus; HypoThR, right hypothalamus; TotHypoTh, total hypothalamus; MamL, left mammillary body; MamR, right mammillary body; TotMam, total mammillary body. All volumetric data are presented in mm³, as directly obtained from MRICloud.

[t]Independent-samples t-test, [m]Mann–Whitney U test. Statistically significant P-values are shown in bold.

TABLE 3. Volume Ratios of Adjacent Brain Structures to the Amygdala, Hypothalamus, and Mammillary Body in Alzheimer's Disease and Healthy Control Groups

Ratio (Structure/Region)	AD group (Mean±SD)	AD group (Median)	HC group (Mean±SD)	HC group (Median)	P-value
TelenL/AmygL	417.3±71.9	414.5	332.4±59.2	316.3	**<0.001[m]**
TelenR/AmygR	361.0±68.0	359.4	302.7±60.1	281.5	**0.008[t]**
TelenL/HypoThL	890.3±102.6	898.7	861.5±86.2	845.8	0.360[t]
TelenR/HypoThR	751.4±70.1	750.8	756.2±94.0	744.2	0.659[m]
TelenL/MamL	6773.5±1897.0	6320.4	5415.4±788.4	5341.6	**0.009[m]**
TelenR/MamR	6425.8±1813.1	5824.1	5112.0±866.4	5046.7	**0.004[m]**
DiencL/AmygL	6.59±1.25	6.1	5.47±0.77	5.29	**0.003[t]**
DiencR/AmygR	5.71±1.04	6.1	4.94±0.88	4.84	**0.020[t]**
DiencL/HypoThL	14.0±1.9	13.9	14.2±1.9	14.0	0.662[t]
DiencR/HypoThR	11.9±1.0	11.9	12.3±1.2	11.8	0.197[t]
DiencL/MamL	105.2±23.8	103.0	89.3±13.3	84.3	**0.017[t]**
DiencR/MamR	101.7±28.1	93.2	83.5±13.7	83.3	**0.026**
Mesenc/TotAmyg	5.3±0.9	3.8	3.2±0.6	3.0	**<0.001[t]**
Mesenc/TotHypoth	11.5±1.6	8.2	8.0±0.7	7.9	0.269[t]
Mesenc/TotMam	62.9±12.9	67.2	51.6±4.3	51.6	**<0.001[t]**
Metenc/TotAmyg	62.2±13.0	63.2	47.4±10.2	45.0	**<0.001[t]**
Metenc/TotHypoth	130.9±18.9	129.4	120.7±19.5	117.5	0.106[t]
Metenc/TotMam	1047.2±296.8	954.0	783.4±131.7	769.5	**<0.001[t]**
Myelen/TotAmyg	4.83±0.9	4.60	4.45±0.6	4.19	**0.086[t]**
Myelen/TotHypoth	130.9±18.9	129.4	120.7±19.5	117.5	0.106[t]
Myelen/TotMam	140.7±28.2	135.0	176.2±24.0	176.5	**<0.001[t]**

Data are shown as mean±standard deviation or median. AD, alzheimer's disease; HC, healty control; TelenL, left telencephalon; TelenR, right telencephalon; TotTelen, total telencephalon; DiencL, left diencephalon; DiencR, right diencephalon; TotDienc, total diencephalon; Mesenc, mesencephalon; Metenc, metencephalon; Myelenc, myelencepalon; AmygL, left amygdala; AmygR, right amygdala; TotAmyg, total amygdala; HypoThL, left hypothalamus; HypoThR, right hypothalamus; TotHypoTh, total hypothalamus; MamL, left mammillary body; MamR, right mammillary body; TotMam, total mammillary body. [t]Independent-samples t-test, [m]Mann-Whitney U test. Statistically significant P-values are shown in bold.

TelenL/MamL, and TelenR/MamR; as well as DiencL/AmygL, DiencR/AmygR, DiencL/MamL, and DiencR/MamR, were all significantly higher in the AD group compared with HC (P<0.05), suggesting relatively greater volume loss in the amygdala and mammillary bodies compared with their adjacent structures. Conversely, ratios involving the hypothalamus - such as TelenL/HypoThL, TelenR/HypoThR, DiencL/HypoThL, and DiencR/HypoThR - were not statistically significant (P>0.05), indicating proportionate atrophic changes between the hypothalamus and its adjacent structures.

Moreover, when brainstem structures were evaluated relative to limbic structures, significant differences were observed in ratios such as Mesenc/TotAmyg, Mesenc/TotMam, Metenc/TotAmyg, Metenc/TotMam, and Myelenc/TotMam (P<0.001), indicating structural disproportions in these regions in the AD group (Table 3). However, the ratios of Mesenc/TotHypoth, Metenc/TotHypoth, and Myelenc/TotHypoth were not statistically significant (P>0.05), suggesting that the hypothalamus and brainstem structures undergo parallel volumetric changes in AD.

DISCUSSION

This study compared the volume ratios of selected brain structures between individuals with AD and HC. Specifically, the volume ratios of the HypoThL and TotHypoth were significantly higher in the AD group. Although the diencephalon-to-hypothalamus ratios (DiencL/HypoThL and DiencR/HypoThR) were also higher in the AD group, these differences did not reach statistical significance. This is likely because both the diencephalon (DiencL, DiencR) and the hypothalamus (HypoThL, HypoThR) exhibited similar degrees of volume reduction, resulting in comparable ratios. Moreover, the findings in Table 2 and Table 3 are consistent, as the reduction in diencephalon volume is evident both when assessed independently (Table 2) and relative to the hypothalamus (Table 3).

Recent studies emphasize that the hypothalamus plays an increasingly central role in the pathophysiology of AD, challenging earlier assumptions that primarily focused on cortical regions. Neuroimaging evidence indicates that early neurodegenerative changes may disrupt hypothalamic circuits involved in sleep, thermoregulation, and endocrine signaling - all of which are commonly impaired in AD. In particular, recent high-resolution volumetric analyses suggest that hypothalamic atrophy can occur early in the disease process, independent of global brain atrophy, making it a promising target for early diagnostic approaches [12]. This aligns with our findings, in which both absolute and ratio-based reductions in hypothalamic volume were observed in patients with AD compared with healthy controls.

Similarly, although no significant volume change was observed in the mesencephalon (Table 2), its ratio to the hypothalamus was likewise not statistically significant (Table 3). This further supports the idea that, in AD, the mesencephalon is affected to a similar extent as the hypothalamus, suggesting potential involvement of brainstem structures in disease pathology.

Adding to the existing evidence, Qu *et al*. [13] assessed the volumes of amygdala subfields using FreeSurfer software in individuals diagnosed with AD, those with mild cognitive impairment (MCI), and HC. Their results revealed significantly larger subfield volumes in the HC group compared with the AD group. Similarly, Copenhaver *et al*. [14] reported pronounced reductions in mammillary body volume in AD patients compared with individuals with MCI, other cognitive disorders, and HC.

Although mammillary bodies are integral components of the limbic circuitry, they have received relatively limited research attention over the past decade. Recent studies have begun to address this gap. For instance, Huang *et al*. [15] demonstrated that neuronal hyperactivity within the lateral mammillary nuclei may contribute to memory deficits in AD, emphasizing the region-specific vulnerability of these structures. Likewise, Salman *et al*. [16] reported heterogeneous atrophy patterns in the amygdala, with tau pathology predominantly affecting the central, medial, and accessory basal nuclei.

These findings underscore that both the amygdala and mammillary bodies are not only structurally compromised in AD but may also serve as region-specific biomarkers that reflect distinct pathological processes. Given their role in the Papez circuit, further investigation into mammillary body degeneration may offer novel insights into memory-related symptoms in Alzheimer's disease.

This study addresses this critical gap by presenting an up-to-date volumetric assessment of the mammillary bodies using an automated segmentation approach. This methodological precision enhances the reliability of volumetric measurements and contributes valuable data to an underexplored area.

In contrast to the extensive literature on hippocampal volumetry, research on other subcortical components of the limbic system has remained relatively limited. Our findings demonstrated that the volumes of the AmygL, AmygR, TotAmyg, HypoThL, TotHypoth, MamL, MamR, and TotMam were significantly lower in the AD group compared with HC.

Moreover, although the TelenL/HypoThL and TelenR/HypoThR ratios were higher in the AD group, the differences did not reach statistical significance. These findings suggest that the telencephalon undergoes volumetric changes that parallel those of the hypothalamus in AD, suggesting a broader pattern of subcortical structural degeneration associated with the disease.

Adding further context, Raji *et al*. [17] performed a volumetric comparison between bilingual and

monolingual individuals diagnosed with Alzheimer's disease (AD), examining 45 subcortical and cortical brain structures. Their findings demonstrated that monolingual individuals with AD exhibited significantly smaller volumes in the ventral diencephalon compared to their bilingual counterparts.

Consistent with these findings, our study also revealed a significant reduction in diencephalon volume when evaluated independently in AD patients compared to HC. However, when the diencephalon was analyzed relative to the hypothalamus (DiencL/HypoThL and DiencR/HypoThR), no statistically significant differences were detected between the groups. This suggests that the diencephalon undergoes atrophy parallel to that of the hypothalamus in the context of Alzheimer's disease.

By extending the current understanding of subcortical involvement, our findings provide valuable contributions to the relatively sparse literature on the role of diencephalic structures in AD pathology.

Expanding on the role of the brainstem in AD, Lee *et al*. [18] conducted an in vivo neuroimaging study that revealed significant reductions in total brainstem volume and notable structural deformities, particularly in the midbrain, in patients with AD compared to healthy individuals.

In contrast, our study did not reveal statistically significant reductions in the volumes of the mesencephalon, metencephalon, or myelencephalon when assessed individually (Table 2). Similarly, their volume ratios relative to the hypothalamus (Table 3) also showed no significant differences between groups. This pattern suggests that brainstem structures may undergo atrophy in parallel with the hypothalamus, reinforcing the hypothesis that brainstem degeneration is an integral component of AD pathology.

By combining absolute volume assessments with inter-regional ratio analyses, our study offers a refined perspective on brainstem involvement in AD, drawing attention to a structurally and functionally critical region that has been relatively underexplored in neuroimaging literature.

Strengths and Limitations

This study has several limitations that should be acknowledged. First, the relatively small sample size (24 patients with Alzheimer's disease and 16 healthy controls) may limit the generalizability of the findings. Although statistically significant differences were observed in several volumetric and ratio-based parameters, larger samples would provide greater statistical power and may reveal additional group differences not detected in this study.

Second, due to the retrospective design and reliance on previously acquired MRI scans, certain factors such as scanner variability, image quality, and acquisition protocols could not be fully standardized across all subjects. Although all images were obtained using the same 3T MRI scanner, minor technical variations may have influenced the segmentation outcomes.

Lastly, the cross-sectional nature of this study precludes conclusions about the longitudinal progression of subcortical atrophy in Alzheimer's disease. Future longitudinal studies are needed to monitor dynamic volumetric changes over time and to better evaluate the predictive value of these structural alterations as potential biomarkers.

One of the main limitations of the present study is the relatively small sample size. The data collection period coincided with the onset of the COVID-19 pandemic, and institutional safety measures implemented during this period limited researchers' access to the hospital environment, thereby restricting the number of eligible patients who could be included in the study. Nevertheless, a major strength of the present study is that it goes beyond the commonly emphasized hippocampal alterations in Alzheimer's disease and demonstrates significant volumetric changes in other components of the subcortical limbic system, particularly the amygdala, hypothalamus, and mammillary bodies. Furthermore, the combined evaluation of absolute volumetric measurements and regional volume ratios contributes to a more comprehensive characterization of proportional atrophy patterns among subcortical structures in Alzheimer's disease.

CONCLUSIONS

In conclusion, our study demonstrated significant atrophy in key subcortical limbic structures - including the bilateral amygdala, left and total hypothalamus,

and mammillary bodies - in individuals with AD. In contrast, volume ratios between diencephalic and brainstem regions and the hypothalamus showed no significant differences, suggesting a parallel pattern of atrophic change. These findings reinforce the view that AD impacts a broader subcortical network beyond the traditionally emphasized hippocampus, underscoring the importance of including these regions in future diagnostic and research frameworks.

Volumetric ratio analysis offers enhanced sensitivity in detecting inter-regional structural differences and may serve as a valuable complement to absolute volumetric assessments in future research. In this context, MRICloud stands out as a reliable and user-friendly automated platform, not only for mapping disease-associated neuroanatomical alterations but also for identifying novel subcortical biomarkers that could support early diagnosis, personalized risk stratification, and improved prognostic accuracy in clinical settings.

Ethics Approval and Consent to Participate

The study was approved by the Istanbul Medicol University Non-Interventional Clinical Research Ethics Committee (Decision no.: 170 and date: 19.02.2020). All experimental procedures involving animals were conducted in accordance with the ethical standards of the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Data Availability

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available on request from the corresponding author, upon reasonable request.

Authors' Contribution

Study Conception: MED, MŞ; Study Design: MED, MŞ; Supervision: MŞ; Funding: MED, NY; Materials: MED, NY; Data Collection and/or Processing: LH, CE, GEA; Statistical Analysis and/or Data Interpretation: NY, MED; Literature Review: MED; Manuscript Preparation: MED; and Critical Review: MED.

Conflict of interest

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

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Generative Artificial Intelligence Statement

The author(s) declare that no artificial intelligence-based tools or applications were used during the preparation process of this manuscript. The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

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A Transdiagnostic Perspective on Unwanted Intrusive Thoughts in Eating Disorders and Obsessive-Compulsive Disorder and Their Relationship with Early Maladaptive Schemas: A Path Modeling Analysis

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ABSTRACT

Objectives: Unwanted intrusive thoughts (UITs) are sudden, repetitive, ego-dystonic cognitions commonly observed across psychological disorders, including obsessive-compulsive disorder (OCD) and eating disorders (EDs). Despite disorder-specific content, their shared features - such as intrusiveness and suppression urges - suggest a transdiagnostic cognitive function. Early maladaptive schemas (EMSs), as deep cognitive structures, may shape how individuals interpret and respond to UITs, thereby influencing symptom severity. While some EMSs broadly relate to psychopathology, others appear disorder-specific. However, studies jointly examining UITs and EMSs across OCD and EDs within the same sample remain scarce. This study aims to explore the transdiagnostic and disorder-specific roles of UITs and EMSs in relation to OCD and ED symptoms by analyzing their shared and distinct associations in a non-clinical sample.

Methods: A total of 364 university students (270 females, 94 males) completed self-report measures assessing UITs related to EDs and OCD, EMSs, and ED/OCD symptomatology. Path analysis was conducted in Analysis of Moment Structures (AMOS) to test the theoretical model.

Results: EMS domains were differentially associated with UITs and symptom severity in OCD and EDs. Other-Directedness predicted OBQ (β=0.32) and indirectly OCI-R (β=0.06). Impaired Autonomy was linked to both OBQ (β=0.23) and EDITs (β=0.48), while Impaired Limits was associated only with OCI-R (β=0.18). OBQ predicted OCI-R (β=0.20) and EDITs predicted both EDE-Q (β=0.65) and OBQ (β=0.17).

Conclusions: UITs and specific schema domains may jointly contribute to EDs and OCD symptoms, suggesting targets for early intervention in subclinical populations.

Keywords: Unwanted Intrusive Thoughts, Early Maladaptive Schemas, Transdiagnostic, Obsessive-Compulsive Disorder, Eating Disorders

Unwanted Intrusive Thoughts (UITs), which are a universal and common human experience, are typically sudden and unexpected thoughts, images, or impulses that individuals experience as difficult to control. These intrusions are often repetitive and involve

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unacceptable content [1]. Cognitive-behavioral models propose that such dysfunctional beliefs—particularly perceptions of danger, responsibility to prevent harm, or catastrophic interpretations—can lead to obsessions. Thus, it is not the presence of UITs per se, but their interpretation that contributes to psychopathology [2]. From this perspective, UITs have been a main focus of interest in the cognitive conceptualization of Obsessive-Compulsive Disorder (OCD), showing that obsessions are the extreme variants of normal UITs [3]. Nonetheless, recently these intrusions have also been described in other disorders such as Body Dysmorphic Disorder (BDD) [4], Hypochondriasis [5] and Eating Disorders (EDs) [6]. For example, BDD patients experience UITs related to perceived appearance flaws [4], while hypochondriacal patients report UITs about illnesses and death [5]. Similarly, both patients with EDs and individuals from nonclinical populations experience recurrent cognitions about food, diet, physical exercise, and appearance [7]. Therefore, although UITs observed in OCD, BDD, EDs, and hypochondriasis differ in terms of content, they show similar functional characteristics such as urges to suppress or control them, ego-dystonic quality, and intrusiveness. These shared features suggest that UITs may reflect a transdiagnostic structure and contribute to a better understanding of the common cognitive mechanisms underlying different psychopathologies [8]. These research findings raise the question of whether UIT may function as a transdiagnostic construct across psychopathologies and also their potential role in the development of these disorders [9].

To address this question, this study aims to examine the relationship between UITs in OCD and EDs, given their high comorbidity and overlapping cognitive, behavioral, and clinical features [6, 7]. For instance, lifetime OCD prevalence in individuals with EDs ranges from 9.5% to 62%, while ED prevalence in those with OCD ranges from 11% to 42% [10]. Investigating their shared mechanisms is thus essential for understanding common underlying processes. Since the content of UITs differs, both disorders exhibit similar patterns. For example, althought EDs related intrusive thoughs (EDITs) often center on food and body image, while OCD related UITs (OCDITs) involve control and repetition [8]. Some EDs symptoms, like checking, reassurance seeking, and ritualized eating, may reflect obsessive-compulsive traits. Thus, obsessive thoughts about body and food in EDs resemble obsessions, and behaviors such as avoidance or excessive exercise may parallel compulsions [6-8]. Similarly, post-treatment ED patients show comparable UITs levels to healthy controls, supporting their etiological role in psychopathology [11].

Moreover, although unwanted intrusive thoughts (UITs) are recognized as a common etiological risk factor across various psychopathologies, ongoing discussions focus on how the content of these thoughts and the coping strategies employed differ depending on the specific mental disorder [3]. A key factor in this context is early maladaptive schemas (EMSs), which act as cognitive filters shaping how individuals interpret and process their experiences [12]. From a theoretical standpoint, EMSs may influence how individuals interpret and respond to intrusive thoughts. For instance, schemas such as Defectiveness and Punitiveness may cause individuals to perceive UITs as threatening or unacceptable, thereby increasing distress and reinforcing symptom persistence. Similarly, schemas like Unrelenting Standards and Emotional Inhibition may prompt suppression attempts, which paradoxically increase the frequency of these thoughts [13]. Thus, EMSs influence both cognitive patterns and behavioral responses, increasing vulnerability to psychological disorders. They are typically formed in response to adverse early life events. Therefore, as emphasized by Bär *et al*. [14], EMSs function as transdiagnostic mechanisms but may also develop into hybrid patterns specific to particular disorder. The substantial number of robust associations between EMSs and clinical disorders further highlights their importance throughout the development and progression of psychopathology. For example, the abandonment schema has been found to be more prevalent in individuals with schizophrenia and OCD, whereas the vulnerability to harm schema has shown strong associations with both panic disorder and OCD [14]. These findings align with schema theory, which posits that early cognitive structures influence the appraisal and regulation of internal experiences, including intrusive cognitions.

When EMSs are examined in the context of both EDs and OCD, findings suggest that while some schemas are shared across disorders, others may be

disorder-specific [14]. For instance, a recent review found that the unrelenting standards schema was consistently observed across all forms of EDs [15], whereas the failure schema was more specific to OCD [16]. Similarly, a separate meta-analysis on EDs identified defectiveness/shame, abandonment/instability, dependence/incompetence, failure, and enmeshment /undeveloped self as the schemas most strongly and consistently associated with ED symptomatology [17]. In parallel, a recent meta-analysis examining the relationship between OCD and EMSs found that among 22 studies, the schemas most strongly linked to OCD symptoms were dependence/incompetence, vulnerability to harm or illness, and negativity/pessimism [18]. Taken together, these findings support the view that while EMSs may function as transdiagnostic indicators of general vulnerability to psychopathology, specific EMS constellations may also point to vulnerabilities unique to particular disorders. Therefore, although the literature has predominantly examined disorder-specific schema patterns, the present study aims to evaluate EMSs in relation to both EDs and OCD symptom severity, allowing for a comparative analysis of their potential transdiagnostic role across these disorders.

In summary, although UITs are suggested to operate transdiagnostically across OCD and EDs, existing research has largely addressed OCDITs and EDITs separately [3, 6, 8]. The present study aims to examine both UITs and EDITs within the same individuals to explore their associations with OCD and EDs symptomatology. Additionally, the study investigates how EMSs may relate to these intrusions and whether they influence the severity and content of the associated symptoms.

Hypotheses

H1. Higher levels of OCDITs will be associated with increased symptom severity in both OCD and EDs.

H2. Higher levels of EDITs will be associated with increased symptom severity in both EDs and OCD.

H3. While some EMSs will be transdiagnostically associated with both OCD and EDs symptom severity, others will demonstrate disorder-specific associations, being linked exclusively to either OCD or ED symptoms.

METHODS

Participants

In accordance with the inclusion criteria, 12 individuals who had a current psychological disorder diagnosis and were undergoing treatment were excluded out of 376 participants. A total of 364 participants aged 18-35 years were included in the study. Among them, 270 were female (20.62±2.34 years) and 94 were male (21.53±3.23 years). Based on the Body Mass Index (BMI) classification, 14.3% (n=52) of the participants were classified as underweight, 67.6% (n=246) as normal weight, 15.6% (n=57) as overweight, and 2.5% (n=9) as obese.

Demographic Information Form

Participants provided data on age, education, BMI, parental education, current mental-health status, socio-economic level and longest-lived location.

Eating-Related Intrusive Thoughts Inventory (EDITs)

Developed by Perpiñá *et al*. [19], this inventory assesses the frequency and nature of intrusive thoughts, images, and urges related to eating, body image, and exercise, along with associated control strategies. It comprises three sections; however, only Section I - which specifically measures intrusive thoughts - was used in the present study (50 items; 6-point Likert scale) [20]. In the Turkish version, Cronbach's α was .98 (Section I) [20]. In the current study, Cronbach's α of the total score was .98.

Young Schema Questionnaire–Short Form–3 (YSQ-SF3)

This 90-item short form (6-point Likert scale), adapted from Young *et al*. [12], assesses 18 early maladaptive schemas across five domains. Higher scores reflect greater schema severity. In the Turkish version, 14 distinct schema structures were identified and categorized under five schema domains: Impaired Autonomy (enmeshment/dependence, abandonment, failure, pessimism, vulnerability to Harm), Disconnection (emotional deprivation, emotional inhibition, social isolation/mistrust, and defectiveness), Unrelenting Standards (enrelenting standards, approval-seeking), Other-Directedness, and

Impaired limits (entitlement /insufficient self-control). The Cronbach's alpha coefficients ranged from .63 to .80 for individual schemas, and from .53 to .81 for the schema domains [21]. In the current study, Cronbach's α of the individual schemas ranged from .78 to .90.

Obsessive Beliefs Questionnaire (OBQ)

An instrument intended to capture intrusive thoughts characteristic of OCD, as well as those commonly shared with other forms of psychopathology. It contains 44 items rated on a 7-point Likert scale and covers three domains: estimation, perfectionism /certainty, and importance/control of thoughts [22]. In the Turkish version, Cronbach's α ranged from .80 to .92 [23]. In the current study, Cronbach's α of the total score was .95.

Eating Disorder Examination Questionnaire (EDE-Q)

Developed by Fairburn *et al*. [24], the EDE-Q is a 28-item scale rated on a 6-point Likert scale, assessing four subdomains: restraint, eating concern, shape concern, and weight concern. Higher scores indicate greater eating disorder symptomatology. In the Turkish version Cronbach's α .93 [25]. In the current study, Cronbach's α of the total score was .91.

Obsessive-Compulsive Inventory-Revised

The OCI-R is an 18-item self-report scale that evaluates distress associated with obsessive-compulsive symptoms. Items are rated on a 5-point Likert scale and represent six dimensions: washing, checking, obsessions, neutralizing, hoarding, and ordering. Each subscale includes three items, and total scores range from 0 to 72 [26]. The Turkish version Cronbach's α ranged from .64 to .84 for subscales, and .90 for the total scale [27]. In the current study, Cronbach's α of the total score was .91.

Process

The study was approved by the Clinical Research Ethics Committee of Akdeniz University with the decision number 243. Data were collected online via a survey announced through the university's email system. Participants, aged 18-35 with no current psychiatric diagnosis, accessed the Google Forms survey after signing up and gave informed consent. Questionnaire order was counterbalanced every 50 participants.

Statistical Analysis

With SPSS 23 program, outlier and missing data analyses were conducted, and missing values were replaced with mean scores. Skewness, kurtosis, and Levene's test statistics were calculated to assess normality and homogeneity of variances. To investigate the proposed hypotheses modeling of structural equations based on path analisis in Analysis

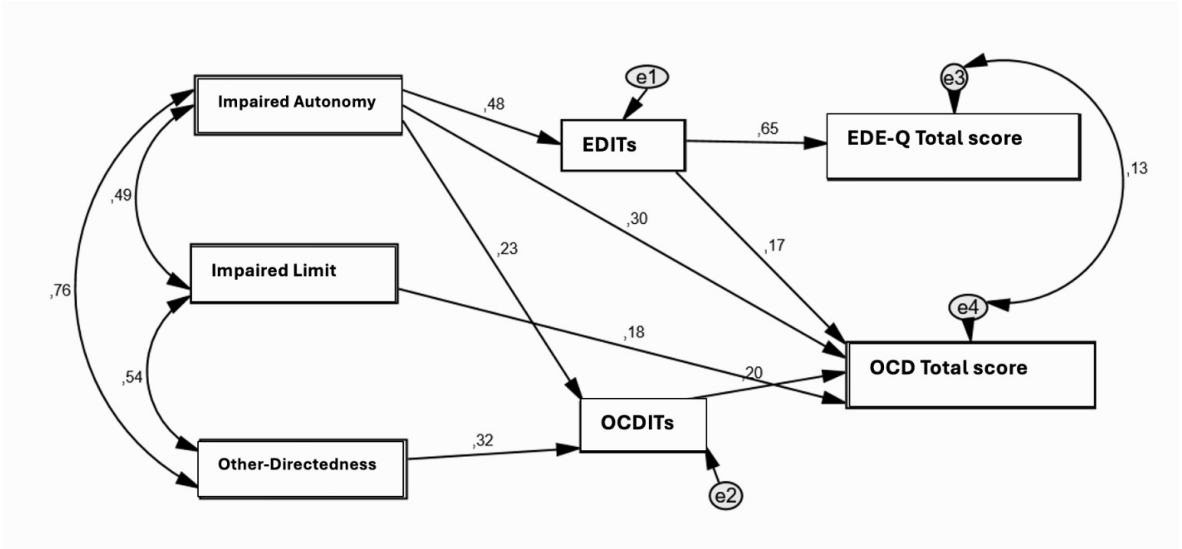

FIGURE 1. The tested theoretical model with standardized path coefficients. EDITs, eating-related intrusive thoughts inventory total score; OCDITS, obsessive beliefs questionnaire (OBQ) total score; OCD Total score, obsessive-compulsive inventory-revised (OCI-R) total score; EDE-Q, eating disorder examination questionnaire total score.

of Moment Structures (AMOS) software version 24 has been used and the model's parameters have been estimated through Maximum Likelihood (ML). The model fit was evaluated based on fit indices to determine how well the proposed model explained the data [28]. Multiple indices of goodness-of-fit were described: the Relative Chi-square (χ^2 /df) (cut-of values: <2-5), the Root Mean Square Error of Approximation (RMSEA) (close and acceptable ft are considered for values <0.05 and <0.11 respectively), the Tucker Lewis Index (TLI) and the Comparative Fit Index (CFI) (acceptable values are ≥0.90) [28].

RESULTS

Use of the proposed model tested whether the five schema domains formed by EMSs would predict dysfunctional eating attitudes and OCD symptom severity both directly and indirectly through EDITS and OCDITs. Model tested during the structural equation modeling is summarized in Figure 1, relationship between variables are summarized in Table 1 and the coefficients with standard errors and P-values of the direct, indirect and total efects of variables on each other are summarized in Tables 2 and 3.

The findings suggest that the hypothesized model demonstrates a good fit to the data ($\chi^2(3)=5.01$, P=0.171), CFI=0.999, TLI=0.986, IFI=0.999, NFI=0.997, RMSEA=0.043, confidence interval (CI) [0.000, 0.107]). After removal of the other nonsignificant paths, the final model with standardized parameter estimates was obtained, as seen in Table 2. The goodness-of-fit statistics indicated excellent model fit indices, ($\chi^2(9)=11.26$, P=0.259, CFI=0.998, TLI=0.995, IFI=0.998, NFI=0.989, RMSEA=0.026, CI [.000, .068]).

According to results. the standardized total effects indicated that Impaired Autonomy had the strongest total effect on EDITs (β=0.48) and OCI-R (β=0.43), as well as a moderate effect on OBQ (β=0.23) and EDE-Q (β=0.31). Other-Directedness showed a moderate effect on OBQ (β=0.32) and a small effect on OCI-R (β=0.06). OBQ (β=0.20). Impaired Limits (β=0.18) and EDITs (β=0.17) also had meaningful total effects on OCI-R. Notably, EDITs had the highest total effect on EDE-Q (β=0.65). while all other predictors had no total effect on this outcome. Among the schema dimensions only Other-Directedness was found to directly predict OBQ (β=0.32). Impaired Autonomy on the other hand had significant direct effects on OBQ (β=0.23). EDITs (β=0.48) and OCI-R (β=0.30). When examining the indirect effects among variables both Other-Directedness (β=0.06) and

TABLE 1. Correlations Amongst the Variables

	1	2	3	4	5	6	7	8	9	10	11
1.EDITs	1										
2. OBQ	**0.29****	1									
3. OCI-R	**0.40****	**0.44****	1								
4. EDE-Q	**0.65****	**0.14****	**0.33****	1							
5.SD1	**0.48****	**0.48****	**0.57****	**0.33****	1						
6.SD2	**0.39****	**0.44****	**0.53****	**0.30****	**0.83****	1					
7.SD3	**0.31****	**0.42****	**0.46****	**0.24****	**0.63****	**0.60****	1				
8.SD4	**0.17****	**0.32****	**0.42****	**0.10***	**0.49****	**0.50****	**0.65****	1			
9.SD5	**0.40****	**0.50****	**0.49****	**0.24****	**0.76****	**0.68****	**0.67****	**0.54****	1		
10.Age	-0.02	0.05	-0.08	-0.03	-0.01	0.03	0.10	0.003	0.09	1	
11.BMI	**0.15****	-0.02	-0.01	-0.02	-0.04	-0.01	0.04	0.06	0.04	**0.26****	1

EDITs, Eating-Related Intrusive Thoughts Inventory Total Score; OBQ, obsessive beliefs questionnaire total score (OCDITs); OCI-R, obsessive-compulsive inventory–revised total score; EDE-Q, eating disorder examination questionnaire total score; SD1, impaired autonomy; SD2, disconnection; SD3, unrelenting standards; SD4, impaired limits; SD5, other-directedness, BMI, body mass index.

**P<0.001, *P<0.05

TABLE 2. Unstandardized and Standardized Regression Coefficients From the Model

		B	Beta	SE	Z-value	P-value***
Impaired Autonomy	EDITs	4.72	0.48	0.46	10.31	**<0.001**
	OBQ	2.57	0.23	0.76	3.40	**<0.001**
Other directedness	OBQ	8.57	0.32	1.83	4.70	**<0.001**
EDITs	EDE-Q	0.37	0.65	0.02	16.44	**<0.001**
	OCI-R	0.05	0.17	0.01	3.74	**<0.001**
Impaired autonomy	OCI-R	0.81	0.30	0.15	5.34	**<0.001**
Impaired limits	OCI-R	2.32	0.18	0.59	3.92	**<0.001**
OBQ	OCI-R	0.05	0.20	0.01	4.29	**<0.001**

EDITs, eating-related intrusive thoughts inventory total score; OBQ, obsessive beliefs questionnaire total score (ocdits); OCI-R, obsessive-compulsive inventory-revised total score; EDE-Q, eating disorder examination questionnaire total score; SE, standard error.

***P<0.001. Statistically significant P-values are shown in bold.

Impaired Autonomy (β=0.13) showed indirect effects on OCI-R. Additionally Impaired Autonomy had a significant indirect effect on EDE-Q (β=0.31). The standardized direct effects indicated that Impaired Autonomy had significant direct paths to OBQ (β=0.23), EDITs (β=0.48) and OCI-R (β=0.30), while Other-Directedness directly predicted only OBQ (β=0.32). Additionally. OBQ (β=0.20). Impaired Limits (β=0.18) and EDITs (β=0.17) showed direct effects on OCI-R. The only significant direct predictor of EDE-Q was EDITs (β=0.65). Regarding indirect effects, Impaired Autonomy had notable indirect effects on OCI-R (β=0.13) and EDE-Q (β=0.31), suggesting mediation through OBQ and EDITs. Respectively Other-Directedness also exhibited a small indirect effect on OCI-R (β=0.06).

DISCUSSION

This study contributes to the growing body of research examining OCD and EDs symptomatology in relation to UITs within the same sample, enabling a comparative analysis of how UITs may contribute to each condition. To the best of our knowledge, this is the first study in Turkey to investigate the role of UITs in both OCD and EDs within a shared sample framework. Furthermore, the study uniquely contributes to the literature by examining the differential content of EDITs and OCDITs and their associations with EMSs. The inclusion of EMSs in this transdiagnostic model positions the study as one of the first to explore the cognitive underpinnings that may influence both the severity and thematic characteristics of UITs across OCD and EDs.

Findings from the analysis indicated that as the severity of EDITs and OCDITs increased, the symptom levels of both OCD and EDs also rose. This aligns with the existing literature. In EDs, negative automatic thoughts and cognitive distortions about eating and weight are linked to symptom escalation [6-9]. Similarly, in OCD, UITs - central to the disorder - lead to distress when misinterpreted, predicting symptom severity [9-11]. Individuals with EDs and OCD often experience repetitive, distressing UITs and engage in compensatory behaviors to alleviate the resulting emotions [6, 7]. The literature thus highlights a cyclical relationship between UITs and both disorders [7, 28]. However, in the present study, the relationship between UITs and both EDs and OCD symptom severity was evaluated concurrently, offering a more in-depth examination of the transdiagnostic nature of UITs. Analyses exploring whether UITs function as a transdiagnostic construct revealed that both EDITs and OCDITs significantly predicted OCD symptoms, whereas only EDITs predicted ED symptoms. This may reflect cognitive specificity within each disorder. The finding that EDITs predict OCD symptoms may be explained by the anxiety-provoking content of these thoughts and

TABLE 3. Standardized Estimates of Total, Direct, and Indirect Effects Among Study Variables

		SD5	SD1	OBQ	SD4	EDITs
Direct effects	OBQ	0.32	0.23	0.000	0.000	0.000
	EDITs	0.000	0.48	0.000	0.000	0.000
	OCI-R	0.000	0.30	0.20	0.18	0.17
	EDE-Q	0.000	0.000	0.000	0.000	0.65
Indirect effect	OBQ	0.000	0.000	0.000	0.000	0.000
	EDITs	0.000	0.000	0.000	0.000	0.000
	OCI-R	0.06	0.13	0.000	0.000	0.000
	EDE-Q	0.000	0.31	0.000	0.000	0.000
Total effect	OBQ	0.32	0.23	0.000	0.000	0.000
	EDITs	0.000	0.48	0.000	0.000	0.000
	OCI-R	0.06	0.43	0.20	0.18	0.17
	EDE-Q	0.000	0.31	0.000	0.000	0.65

EDITs, eating-related intrusive thoughts inventory total score; OBQ, obsessive beliefs questionnaire total score; OCI-R, obsessive-compulsive inventory-revised total score; EDE-Q, eating disorder examination questionnaire total score; SD1, impaired autonomy, SD2, disconnection; SD3, unrelenting standards; SD4, impaired limits; SD5, other-directedness.

their tendency to trigger a heightened need for control. These thoughts often center on weight gain, body shape, or eating behaviors, and may initiate compensatory or avoidance behaviors, thereby activating a cycle similar to the obsession–compulsion loop observed in OCD [7, 8, 29].

However, OCD-specific UITs (e.g., contamination, harm, or religious themes) are typically not directly related to the core concerns underlying ED symptoms, such as body image, caloric intake, and weight control. Therefore, OCDITs may be insufficient in predicting disordered eating attitudes. In contrast, EDITs - due to their frequency, perceived threat, and tendency to elicit avoidance behaviors - may be potent enough to trigger OCD symptoms, whereas OCDITs may fail to activate the same cognitive and behavioral mechanisms within the context of eating disorders [8, 29]. Kinkel-Ram *et al*. [29] support this view; using network analysis, they found that food- and calorie-related UITs linked EDs and OCD symptoms but did not serve as direct bridges between disorders. Similarly, García-Soriano *et al*. [6] reported that UITs occur with similar frequency and distress in both disorders, though their relationship was not directly assessed. Consistent with the current study's findings, it has been suggested that individuals with ED and OCD share similarities in cognitive components such as obsessive beliefs, thought-action fusion, and magical thinking; however, compulsions may differ between the two disorders [30]. Furthermore, the authors suggest that while the content of UITs may be disorder-specific across different psychopathologies, the dysfunction stems not from the mere presence of these thoughts, but from how individuals cope with them. For example, it was found that although EDITs were not directly associated with OCD, the attentional disruption caused by these thoughts was related to OCD symptoms [29]. Finally, in this context, the lack of association between EDITs and OCDITs may also be influenced by the methodology of the present study. In this study, only the frequency and content of OCD- and ED-related intrusive thoughts were assessed, while appraisals and control strategies related to these thoughts were not examined. Therefore, future research should not only consider the presence and content of intrusive thoughts but also investigate how individuals cope with them. This approach may offer a more comprehensive understanding of the transdiagnostic nature of these thoughts.

Secondly, findings revealed the presence of both shared and disorder-specific EMSs as across EDs and OCD, thereby supporting Hypothesis 2 (H2). According to the results, the Impaired Autonomy schema domain (Enmeshment/Dependence,

Abandonment, Failure, Pessimism, Vulnerability to Harm) directly predicted EDITs, OCDITs, and OCD symptom severity, and also indirectly predicted ED symptom levels. Therefore, the Impaired Autonomy schema domain appears to represent a transdiagnostic structure linking EDs and OCD. The Impaired Limits schema domain (Entitlement/Insufficient Self-Control) was found to directly predict OCD symptom severity, while the Other-Directedness schema domain (Self-Sacrifice, Punitiveness) directly predicted OCDITs and had an indirect effect on OCD symptom severity.

Overall, research has not yielded consistent findings regarding whether different ED subgroups and OCD differ in EMS patterns or whether disorder-specific schemas exist [3, 18]. While many findings align with previous literature, some studies have reported divergent outcomes. For instance, Atalay *et al*. [31] identified the Unrelenting Standards, Emotional Inhibition, Pessimism, and Punitiveness schemas as being associated with OCD; Kizilagac and Cerit [32] reported associations with failure, insufficient self-control, self-sacrificing schemas; and Voderholzer *et al*. [33] found that the Unrelenting Standards, Punitiveness, Emotional Inhibition, Emotional Deprivation, and Vulnerability to Harm or Illness schemas were related to OCD. Similarly, studies using non-clinical samples have shown inconsistent associations between EMSs and dysfunctional eating attitudes. According to the findings of a meta-analysis, the early maladaptive schemas most strongly and consistently associated with eating disorders are Defectiveness/Shame, Abandonment/Instability, Dependence/Incompetence, Failure, and Enmeshment/Undeveloped Self [34].

However, it has also been noted that schema patterns may differ across ED subtypes. For example, individuals with anorexia nervosa (AN) tend to score higher on schemas such as Insufficient Self-Control, Self-Sacrifice, Unrelenting Standards, and Punitiveness, whereas Emotional Inhibition is more prominent in individuals with bulimia nervosa (BN) [35, 36]. These findings are consistent with cognitive models and Young's Schema Theory. According to Schema Theory, the reason why different schemas become prominent across various mental disorders or subtypes of the same disorder lies not only in the individual's developmental history, but also in the cognitive sensitivities and coping mechanisms shaped in response to disorder-specific stimuli [12]. For instance, the defectiveness schema has been found to be associated with efforts to control physical appearance in EDs and with perfectionism and excessive need for order in OCD. In the case of borderline personality traits, this schema is thought to lead to excessive attachment behaviors in response to fear of abandonment [12]. These findings suggest that the same schema may manifest through different cognitive and behavioral pathways across distinct psychopathologies, and that schemas influence not only symptoms but also individuals' coping strategies. Moreover, according to Young's model, schemas are not only reflections of one's individual history but also serve as the cognitive foundations of coping strategies in response to environmental stressors. Therefore, certain schemas may become activated in a more "maladaptive yet functional" manner in specific disorders. For instance, in AN, the unrelenting standards and insufficient self-control schemas may be employed to meet the individual's need for self-control and adequacy; whereas in OCD, the punitiveness schema may function as a cognitive defense against perceived mistakes or moral failure [37]. These findings indicate that the same schema may manifest through different cognitive and behavioral pathways across distinct psychopathologies and that schemas influence not only the expression of symptoms but also the coping strategies individuals adopt. In this context, EMSs have been shown to play a role not only in the development of symptoms but also in treatment response. In addition to their role in symptom development, certain EMSs may also interfere with treatment outcomes. For instance, the effectiveness of ERP in OCD treatment may decrease in the presence of high levels of Emotional Inhibition and Failure schemas, indicating that these schemas can hinder therapeutic response and complicate symptom reduction [38].

In conclusion, individual differences such as EMSs may contribute to the onset and persistence of UITs, which increase vulnerability to EDs and OCD. These findings support the need for transdiagnostic treatment approaches for these disorders. In this context, identifying schema profiles specific to each clinical condition holds clinical significance both for understanding psychopathology and for guiding the intervention process.

Limitations and Future Studies

This study has several limitations. First, as it was conducted with a non-clinical sample, the findings may not be fully generalizable to clinical populations. Additionally, its cross-sectional design precludes any causal interpretations. Future research should employ longitudinal designs to investigate the long-term effects of EMSs and to clarify whether these constructs function as causes or consequences of EDs and OCD in clinical populations. Secondly, at this point, we do not know whether schemas or symptoms come first in psychological development. Alternatively, maladaptive schemas may well be an epiphenomenon of a specific disorder. Thirdly reliance on self-report measures may introduce social desirability bias. To reduce this risk, no identifying data were collected, and part of the data was gathered online to encourage honest responses. Finally, the sample consisted solely of university students, the majority of whom were women, which limits the generalizability of the findings. Future studies should include diverse age groups, educational levels, and cultural backgrounds to better explore intrusive thoughts, EMSs and well-being. Also while UITs are observed universally, the way they are appraised, their frequency and the emotional impact they generate appear to be shaped by cultural context. For example, in the Turkish sample, UITs related to OCD and Illness Anxiety Disorder were reported to occur more frequently and were experienced as more distressing. This has been associated with the influence of religious and moral norms in Turkey, particularly themes related to thought control and guilt. In contrast, EDITs were found to be less distressing, which may reflect the cultural internalization of the thin-ideal body image [39]. These findings underscore the importance of integrating culturally sensitive constructs - such as values, belief systems, and social norms - into cognitive models in order to enhance their cross-cultural applicability. Future studies should further investigate the role of culture in shaping the expression, appraisal, and consequences of UITs across diverse populations [39, 40]. Lastly, in the present study, analyses were conducted based on the frequency of UITs. Future research could further elucidate the transdiagnostic nature of UITs by examining their emotional, cognitive, and behavioral consequences, as well as the coping strategies individuals employ to manage or neutralize these intrusions.

CONCLUSION

In summary, the results highlight that although UITs are common experiences, individuals at high risk for OCD and EDs report them more frequently and with greater intensity. UITs appear to reflect shared cognitive mechanisms underlying both disorders, while also capturing disorder-specific features. Furthermore, EMSs are hybrid constructs that may function transdiagnostically, y*et al*so exhibit specificity for certain disorders. Conceptualizing early maladaptive schemas and UITs as transdiagnostic constructs is vital for developing effective interventions. Schema therapy targeting EMSs may alleviate symptoms in both EDs and OCD. Targeting these mechanisms may reduce symptom severity, relapse risk, and enhance long-term well-being. Additionally, the study highlights the value of examining OCD and EDs in non-clinical populations. Evidence that UITs severity in non-clinical groups is comparable to clinical samples suggests such data can inform clinical interventions. Identifying transdiagnostic mechanisms holds practical benefits, enabling more efficient treatments with reduced duration, cost, and effort - while improving therapeutic outcomes.

Ethics Approval and Consent to Participate

This study was approved by the Akdeniz University Faculty of Medicine Clinical Research Ethics Committee (Decision no. 2019-243, date: 06.03.2019). All subjects gave written informed consent in accordance with the Declaration of Helsinki (2013). All experimental procedures involving animals were conducted in accordance with the ethical standards of the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Data Availability

The data that support the findings of this study are available on request from the corresponding author, upon reasonable request.

Authors' Contribution

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

Conflict of interest

The author(s) disclosed no conflict of interest during the preparation or publication of this manuscript.

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Generative Artificial Intelligence Statement

The author(s) declare that no artificial intelligence-based tools or applications were used during the preparation process of this manuscript. However, the authors only received English language editing support for grammar and style improvement. The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

Editor's note

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Could Altered Red Cell Indices Reflect Oxidative Stress in Pediatric Atopic Dermatitis?

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ABSTRACT

Objectives: To investigate whether alterations in red blood cell indices - particularly mean corpuscular volume (MCV), red cell distribution width-coefficient of variation (RDW-CV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) - reflect systemic oxidative stress and metabolic disturbances in pediatric patients with atopic dermatitis (AD).

Methods: A retrospective, cross-sectional study was conducted involving 250 pediatric patients diagnosed with AD and 163 healthy controls. Hematological and biochemical parameters were obtained from hospital records, including complete blood count variables and serum levels of urea, creatinine, AST, ALT, uric acid, TSH, free T4, and vitamin B12. Data were analyzed using distribution assessments, intergroup comparisons, and Spearman correlation tests.

Results: Pediatric AD patients exhibited significant alterations in red cell indices. RDW-CV was markedly elevated and showed extreme positive skewness, indicating anisocytosis and disrupted erythropoiesis. RDW-CV strongly correlated with AST and ALT levels. Additionally, MCH positively correlated with vitamin B12 and TSH, while MCHC showed an inverse correlation with ALT.

Conclusions: Altered red blood cell indices, especially RDW-CV and MCH, may serve as accessible and cost-effective surrogate markers for systemic oxidative and metabolic stress in pediatric atopic dermatitis. These hematologic parameters could be integrated into routine assessments to support individualized treatment approaches and endotype-specific care strategies in pediatric populations.

Keywords: Atopic Dermatitis, Oxidative Stress, Red Cell Indices, Red Cell Distribution Width, Pediatric Dermatology, Erythropoiesis, Inflammation

Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disease that predominantly affects infants and children, with a prevalence reaching up to 20% in developed countries [1]. Clinically, AD is characterized by intense pruritus, xerosis, and eczematous lesions that vary with age and disease stage [2]. Pathophysiologically, AD is recognized as a complex, multifactorial disease involving epidermal barrier dysfunction, genetic predisposition, environmental triggers, and immune dysregulation [3-5]. While early studies emphasized the role of filaggrin mutations and barrier impairment [6], recent research has increasingly focused on the immunological underpinnings, particularly the

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dominant T helper 2 (Th2) skewing, accompanied in children by heightened Th17 and Th22 responses [7, 8].

Despite advances in understanding AD's immunopathogenesis, therapeutic strategies still largely follow a one-size-fits-all model, with limited biomarker-guided stratification [9]. Most current biomarkers under investigation, such as thymus and activation-regulated chemokine (TARC), periostin, and interleukin (IL)-13, aim to reflect disease activity or predict therapeutic response [10-12]. However, these markers are not always routinely available in clinical settings, especially in pediatric practice. In this regard, attention has recently turned toward more accessible surrogate markers - particularly hematological parameters - for their potential role in reflecting systemic inflammation and oxidative stress associated with AD [13].

Red blood cells (RBCs), beyond their oxygen-carrying function, are sensitive indicators of systemic oxidative injury due to their high polyunsaturated fatty acid content and reliance on antioxidant defense systems [14]. Oxidative stress, defined by an imbalance between reactive oxygen species (ROS) production and antioxidant defenses, is increasingly implicated in AD pathogenesis. ROS not only promote keratinocyte damage and barrier disruption but also enhance antigen presentation and inflammatory cytokine production, thereby perpetuating the inflammatory cycle [15, 16].

In pediatric AD, markers of oxidative damage such as malondialdehyde (MDA), 8-hydroxy-2′-deoxyguanosine (8-OHdG), and altered thiol/disulfide homeostasis have been found to be elevated in both serum and exhaled breath condensate [17-19]. These findings suggest that chronic skin inflammation in AD extends beyond local tissue, exerting systemic effects that can be captured through peripheral biomarkers. Given their oxidative susceptibility, erythrocyte indices - including mean corpuscular volume (MCV), red cell distribution width (RDW), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) - may be altered in response to systemic inflammation or redox imbalance [20].

Among these, RDW has gained attention as a nonspecific inflammatory and oxidative stress marker across various pediatric diseases, including asthma, allergic rhinitis, and Kawasaki disease [21, 22]. An elevated red cell distribution width-coefficient of variation (RDW-CV) may reflect anisocytosis due to impaired erythropoiesis, iron metabolism dysregulation, or increased oxidative damage to erythrocyte membranes [23]. Similarly, reduced MCHC or increased MCV has been proposed as indirect indicators of red cell fragility under oxidative conditions [24]. Despite these associations, the relevance of red cell parameters in pediatric AD has been scarcely investigated, and their potential utility as accessible, cost-effective markers of immune or oxidative imbalance remains unclear.

In this study, hemogram values from children diagnosed with atopic dermatitis were compared to those of healthy controls. By evaluating red blood cell parameters, the study aimed to investigate whether subtle changes reflecting oxidative stress could be detected in routine blood tests. The aim of this study was to evaluate whether red blood cell indices, specifically MCV, RDW-CV, MCH, and MCHC, differ between pediatric patients with atopic dermatitis and healthy controls, and to assess their potential role as indirect markers of oxidative stress.

Understanding whether simple hematological parameters reflect oxidative stress in pediatric atopic dermatitis could provide an inexpensive and easily applicable tool for clinical practice. If significant differences are observed, these indices could contribute to early detection of systemic involvement in AD, help monitor disease progression, and possibly support individualized treatment approaches focused on oxidative balance. Furthermore, the findings may stimulate new research directions into the broader systemic effects of atopic dermatitis in children.

METHODS

Study Design and Patient Selection

This retrospective, cross-sectional case-control study was conducted at the Department of Dermatology, Malatya Gozde Hospital. The study included a total of 250 pediatric patients diagnosed with atopic dermatitis (AD) based on the Hanifin and Rajka diagnostic criteria between 11/08/2023 and 11/10/2024. Additionally, a healthy control group was formed, consisting of pediatric patients without any known chronic or allergic diseases who presented for

routine follow-up visits without active infection or acute complaints during the same period.

The age range of all participants was between 1 month and 18 years. Both sexes were included in the study. Patients with concomitant acute infections, chronic systemic diseases, known primary immunodeficiencies, anemia, or those who had received systemic or topical corticosteroid therapy, immunosuppressive treatments, or multivitamin supplementation within the last month were excluded to minimize confounding effects on hematological and biochemical parameters.

Data Collection and Variables

Clinical data were retrieved from the hospital's digital archive system (ENLIL Laboratory Information System). The following variables were systematically recorded and analyzed:

(1) Demographic Data: Age (months/years), sex, and presence of any comorbid conditions (e.g., kyphosis, sinus tachycardia).

(2) Hematological Parameters: White blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), (MCV), RDW-CV, MCH, MCHC, platelet count (PLT), mean platelet volume (MPV), lymphocyte count and percentage, monocyte count and percentage, neutrophil count and percentage, eosinophil count and percentage, basophil count and percentage.

(3) Biochemical Parameters: Serum urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), uric acid, thyroid-stimulating hormone (TSH), free thyroxine (free T4), and vitamin B12 levels.

A detailed summary of laboratory parameters analyzed is presented in Table 1.

Laboratory Measurements

Peripheral blood samples were obtained through venipuncture between 08:00 and 10:00 a.m. following an overnight fasting period. Hematological analyses were performed using the Sysmex XN-1000™ Automated Hematology Analyzer (Sysmex Corporation, Kobe, Japan). Biochemical analyses were conducted using the Roche Cobas® 8000 system (Roche Diagnostics, Basel, Switzerland). All laboratory results were interpreted according to pediatric reference ranges standardized nationally for age and sex.

Ethical Considerations

This study was approved by the Malatya Turgut Ozal University Non-Interventional Clinical Research Ethics Committee (Decision no. 2023/27, date: 25.04.2023). Since the study was retrospective, informed consent was waived. All procedures were in accordance with the Helsinki Declaration and institutional guidelines for medical research involving human subjects.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corporation, Armonk, NY, USA). The distribution of continuous variables was assessed with the Kolmogorov-Smirnov test and by examining skewness, kurtosis, and Q-Q plots. Continuous variables were expressed as mean±standard deviation (SD) or median (interquartile range, IQR), depending on distribution. Categorical variables were presented as frequencies and percentages. Comparative analyses between the atopic dermatitis group and the healthy control group were conducted using the Student's t-test or Mann-Whitney U test for continuous variables, and the chi-square test or Fisher's exact test for categorical variables. A P-value of <0.05 was considered statistically significant. Where applicable, ratios such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), eosinophil-to-lymphocyte ratio (ELR), and other derived hematological indices were calculated.

RESULTS

Descriptive Characteristics of Red Blood Cell Indices

This study included a total of 250 pediatric patients diagnosed with atopic dermatitis and a healthy control group. In both groups, erythrocyte parameters - MCV, RDW-CV, MCH, and MCHC - were analyzed to evaluate potential indicators of oxidative stress and redox imbalance.

The descriptive statistics for these indices in the

TABLE 1. List of Laboratory Parameters Included in the Analysis.

Category	Parameters
Demographic data	Age, sex, comorbidities
Hematological	WBC, RBC, HGB, HCT, MCV, RDW-CV, MCH, MCHC, PLT, MPV
White cell subtypes	Absolute and percentage counts of lymphocytes, monocytes, neutrophils, eosinophils, and basophils
Biochemical	Urea, creatinine, AST, ALT, uric acid
Hormonal/Nutritional	TSH, free T4, vitamin B12

WBC, White blood cell count; RBC, red blood cell count; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; RDW-CV, red cell distribution width-coefficient of variation; MCH, mean corpuscular hemoglobin,; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet count; MPV, mean platelet volume; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TSH, thyroid-stimulating hormone; free T4, free thyroxine.

atopic dermatitis group are summarized in Table 2. As presented in Table 2, the mean MCV was 77.5±10.4 fL, showing a moderately consistent red blood cell volume across the cohort. The RDW-CV displayed substantial variability (mean 19.7±66.6%), primarily driven by extreme outliers, suggesting the presence of marked anisocytosis or analytical errors. MCH and MCHC demonstrated relatively narrow interquartile ranges but still contained high-end outliers, indicating some disturbances in erythrocyte hemoglobin content and concentration.

The D'Agostino-Pearson normality test showed significant deviation from normal distribution for all four indices ($P<0.001$), confirming the need for non-parametric statistical methods in subsequent analyses.

Distributional Pattern and Visualization

Histograms of MCV, RDW-CV, MCH, and MCHC are displayed in Figure 1. As seen in Figure 1, while MCV and MCH distributions were relatively symmetric, RDW-CV demonstrated a marked right tail due to a minority of patients with significantly elevated red cell size variability.

Clinical Interpretation and Relevance to Oxidative Stress

The wide inter-individual variability in RDW-CV, along with skewed distributions of MCH and MCHC, supports the hypothesis that oxidative stress may affect erythropoiesis and red blood cell stability in children with atopic dermatitis. To explore potential systemic influences on red cell indices, Spearman correlation analysis was performed between erythrocyte parameters (MCV, RDW-CV, MCH, MCHC) and selected biochemical markers (urea, creatinine, AST, ALT, uric acid, TSH, free T4, vitamin B12). The results are summarized in Table 3 and visualized in Figure 2. In Figure 2, the strongest positive correlations were found between RDW-CV and AST ($P=0.41$, $P<0.001$), suggesting that RDW-CV may serve as a surrogate marker of systemic oxidative stress and hepatocellular damage. MCH

TABLE 2. Descriptive Statistics of Red Cell Indices in Children with Atopic Dermatitis (n=250).

Parameter	Mean±SD	Min-Max	Median (25th %tile, 75th %tile)	IQR	P (normality)
MCV (fL)	77.5±10.4	6.1-105.6	77.5 (74.4, 81.7)	7.3	1.59×10^{-32}
RDW-CV (%)	19.7±66.6	1.6-1038.0	14.4 (13.5, 15.3)	1.8	5.14×10^{-118}
MCH (pg)	26.5±7.3	17.2-82.2	25.4 (24.1, 27.1)	3.0	6.24×10^{-69}
MCHC (g/dl)	33.7±19.1	27.9-333.0	32.5 (31.5, 33.5)	2.0	5.17×10^{-123}

Data are shown as mean±standard deviation or median (interquartile range, IQR). MCV, mean corpuscular volume; RDW-CV, red cell distribution width-coefficient of variation; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

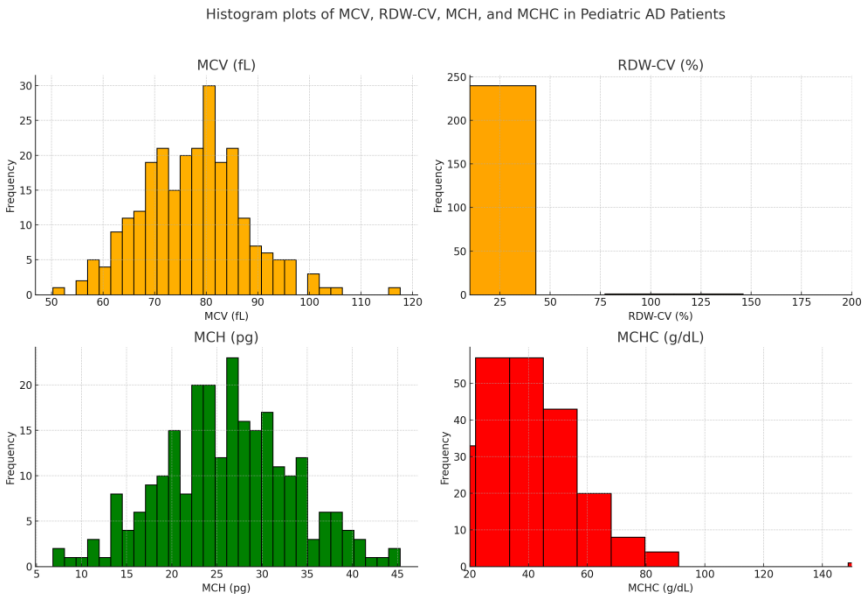

FIGURE 1. Histogram plots showing the distribution of MCV, RDW-CV, MCH, and MCHC in pediatric patients with atopic dermatitis. (a) MCV showed a relatively normal distribution centered around ~77.5 fL. (b) RDW-CV exhibited pronounced right-skewness due to several extremely high values, consistent with a subgroup experiencing severe anisocytosis. (c) MCH had a symmetric and moderately narrow distribution and (d) MCHC displayed mild skewness, with some high outliers reaching up to 333 g/dL. MCV, mean corpuscular volume; RDW-CV, red cell distribution width-coefficient of variation; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

showed positive correlations with TSH (P=0.33) and vitamin B12 (P=0.36), reinforcing the relationship between red blood cell hemoglobin content and metabolic/endocrine balance. Moreover, MCV was positively correlated with free T4 (P=0.21) and vitamin B12 (P=0.23), indicating that thyroid and nutritional status may influence erythrocyte size variability.

Subgroup Analysis Based on Age

Since immune profile and erythrocyte morphology parameters can vary substantially in pediatric patients depending on age, the study population was stratified into two groups: children younger than 4 years (<49 months) and those aged 4 years or older (≥49 months). The subgroup distribution by age category is shown

TABLE 3. Spearman Correlation Coefficients (P) Between Red Cell Indices and Biochemical Parameters in Pediatric Atopic Dermatitis Patients.

Parameter	MCV	RDW-CV	MCH	MCHC
Urea	0.08	0.06	0.14	-0.11
Creatinine	0.03	0.04	0.01	-0.07
AST	0.18	0.41	0.29	-0.23
ALT	0.11	0.32	0.22	-0.29
Uric Acid	0.05	0.14	0.08	-0.12
TSH	0.09	0.19	0.33	-0.06
Free T4	0.21	0.09	0.18	-0.04
Vitamin B12	0.23	0.11	0.36	-0.01

MCV, mean corpuscular volume, RDW-CV, red cell distribution width-coefficient of variation; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TSH, thyroid-stimulating hormone; free T4, free thyroxine.

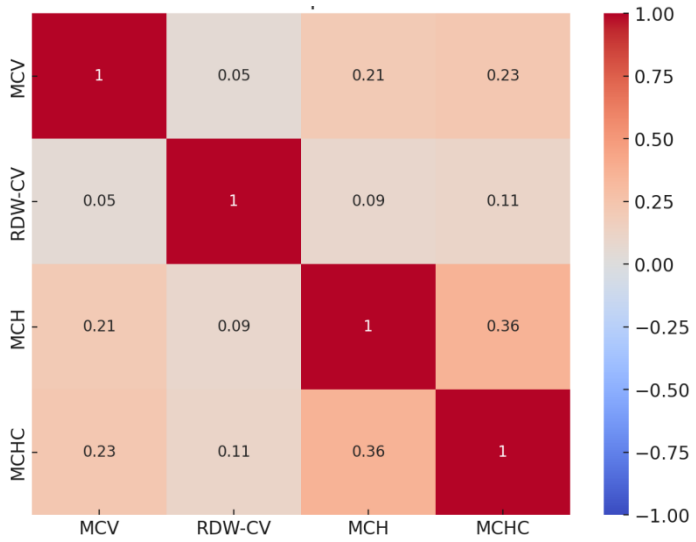

FIGURE 2. Spearman correlation heatmap between RBC indices and biochemical markers in pediatric atopic dermatitis. MCV, mean corpuscular volume; RDW-CV, red cell distribution width-coefficient of variation; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

in Table 4. As seen in Table 4, the majority of the patients (64%) were younger than 4 years old. Comparative analysis revealed that: (1) MCV was significantly lower in patients <49 months compared to older children (P=0.012); (2) RDW-CV was markedly higher in the <49 months group (P<0.001), suggesting higher erythrocyte size variability in younger children; and (3) MCH and MCHC did not show statistically significant differences between age groups (P>0.05).

The comparative distributions are illustrated in Figure 3. These findings suggest that younger children with atopic dermatitis may experience more pronounced oxidative stress-related alterations in erythrocyte morphology.

Subgroup Analysis Based on Sex

The patient cohort included 124 (49.6%) females and 126 (50.4%) males. Sex-based comparisons of red blood cell indices are summarized in Table 5. As indicated in Table 5, there were no statistically significant differences between females and males regarding any of the evaluated erythrocyte indices.

TABLE 4. Age Subgroup Distribution of Pediatric Patients with Atopic Dermatitis (n = 250).

Age Group	n (%)
< 49 months	160 (64%)
≥ 49 months	90 (36%)

Correlation Analysis Between Red Cell Indices and Biochemical Markers

Spearman correlation analyses between erythrocyte indices and biochemical parameters (urea, creatinine, AST, ALT, uric acid, TSH, free T4, vitamin B12) revealed several significant associations: (1) RDW-CV showed strong positive correlations with AST (P=0.41, P<0.001) and ALT (P=0.32, P<0.001); (2) MCH was positively correlated with TSH (P=0.33, P<0.001) and vitamin B12 (P=0.36, P<0.001); and (3) MCV correlated moderately with free T4 (P=0.21, P<0.001) and vitamin B12 (P=0.23, P<0.001). These correlations are visualized in the heatmap shown in Figure 2, and further detailed in Table 3 (refer to 3.3).

The most clinically relevant interpretation from these results is that elevated RDW-CV, linked with hepatic enzymes, may serve as a potential surrogate marker of oxidative stress in children with atopic dermatitis, especially in younger age groups.

DISCUSSION

AD is increasingly recognized not merely as a

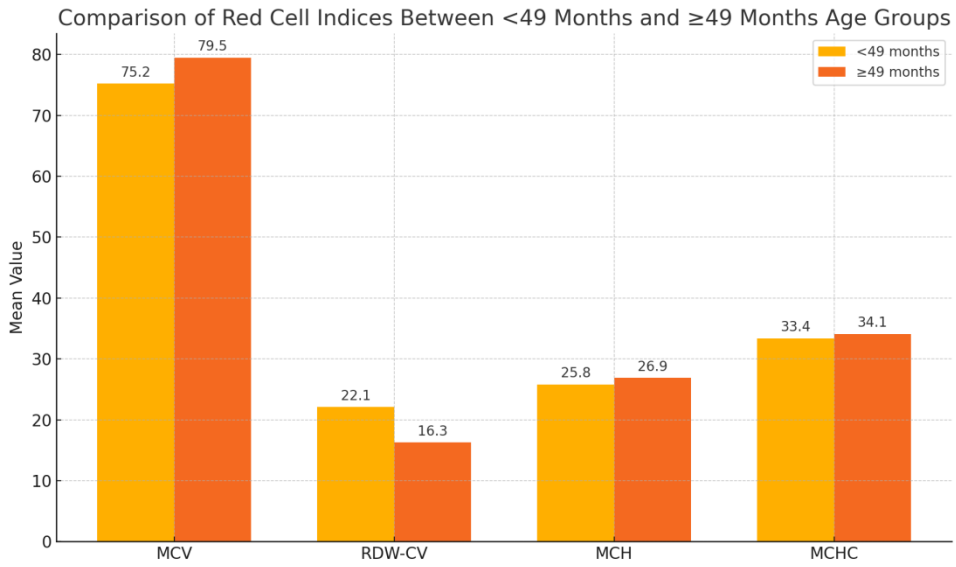

FIGURE 3. Comparison of MCV, RDW-CV, MCH, and MCHC between age subgroups (<49 months vs. ≥49 months) in pediatric atopic dermatitis. (a) MCV was lower in younger patients. (b) RDW-CV was significantly higher in younger patients. (c) MCH and (d) MCHC showed similar distributions across both age groups. MCV=mean corpuscular volume, RDW-CV, red cell distribution width-coefficient of variation; MCH, mean corpuscular hemoglobin; MCHC;mean corpuscular hemoglobin concentration.

cutaneous disorder but as a systemic, immune-mediated disease characterized by heterogeneous endotypes and clinical phenotypes, particularly in pediatric populations [1-3]. Although advances in precision medicine have shifted focus toward molecular and cellular biomarkers, clinical management of AD still predominantly relies on severity scoring systems such as the SCORAD index [4, 5]. In this context, our study aimed to investigate whether routinely available hematological indices - specifically red cell parameters - could serve as accessible, cost-effective markers reflecting systemic oxidative stress and immune dysregulation in children with AD.

Our findings demonstrated that red blood cell indices, particularly RDW-CV, MCH, and MCHC, were markedly altered in pediatric AD patients compared to healthy controls (Table 2, Figure 1). The RDW-CV parameter, in particular, exhibited an extremely wide distribution with notable positive skewness, including values exceeding 1000%. This pattern suggests a high degree of erythrocyte size variability, which is consistent with previous reports linking elevated RDW to chronic inflammation and oxidative stress in systemic diseases such as asthma, Kawasaki disease, and allergic rhinitis [21-23].

In pediatric AD, chronic skin inflammation is thought to contribute to systemic oxidative burden, impairing erythropoiesis and destabilizing erythrocyte membranes [13, 14, 20]. This mechanism may explain

TABLE 5. Sex-Based Comparison of Red Cell Indices in Pediatric Atopic Dermatitis (n=250).

Parameter	Female (n=124)	Male (n=126)	P-value
MCV (fL)	77.9±10.1	77.1±10.7	0.328
RDW-CV (%)	20.1±68.2	19.3±65.0	0.611
MCH (pg)	26.8±7.1	26.2±7.5	0.404
MCHC (g/dL)	33.9±18.7	33.5±19.5	0.712

MCV, mean corpuscular volume; RDW-CV, red cell distribution width-coefficient of variation; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

the heightened anisocytosis reflected in RDW-CV elevations in our cohort. These observations align with the concept that oxidative stress plays a significant role in the pathophysiology of AD, particularly in children whose antioxidant defense mechanisms are still immature [17-19].

Further reinforcing this link, our correlation analysis (Table 3, Figure 2) revealed significant associations between RDW-CV and hepatic enzymes (AST, ALT), as well as between MCH and metabolic-hormonal parameters such as TSH and vitamin B12. The positive correlation between RDW-CV and transaminases is noteworthy, as elevations in these enzymes have been described in AD patients under systemic inflammatory and oxidative stress conditions [25-27]. Additionally, the association between MCH and vitamin B12 or TSH levels suggests that alterations in erythrocyte hemoglobin content may partially reflect subclinical metabolic-endocrine dysregulation - a phenomenon observed in other chronic inflammatory skin disorders as well [14, 24].

Another intriguing finding was the inverse correlation between MCHC and ALT levels. Reduced MCHC values could signify compromised erythrocyte membrane integrity due to oxidative damage or alterations in red cell hydration states, mechanisms previously implicated in disorders such as psoriasis and chronic urticaria [13, 20, 24]. Notably, these abnormalities were observed across both age subgroups, although RDW-CV variability appeared more pronounced in children under four years of age, as illustrated in Figure 3.

These findings collectively suggest that red cell indices, particularly RDW-CV, may serve as valuable surrogate markers of systemic oxidative imbalance in pediatric AD. Importantly, these parameters are universally accessible through standard complete blood count (CBC) tests, making them practical adjuncts in clinical settings, especially in resource-limited environments where advanced molecular diagnostics are unavailable [10, 11].

The broader implications of these results extend to the ongoing effort to refine pediatric AD endotypes. Emerging literature suggests that early-onset AD in children is characterized by distinct immunological features, including heightened Th17 and Th22 polarization and enhanced eosinophilic signatures [7-9]. Red cell alterations, as indirect reflections of systemic oxidative and immune perturbations, could potentially complement immunophenotypic profiling to achieve a more comprehensive disease characterization.

AD is increasingly understood as a systemic immune-mediated disorder with heterogeneous endotypes and phenotypes, particularly pronounced in pediatric populations [1-3]. Despite growing emphasis on precision medicine, current clinical management still largely follows severity-based scoring systems rather than molecular or cellular endotyping [4, 5]. This study contributes to the expanding landscape of accessible, cost-effective biomarkers by evaluating red cell indices in children with AD as potential reflections of systemic oxidative stress and immune dysregulation.

Our findings indicate that red blood cell parameters - particularly RDW-CV, MCH, and MCHC - are notably altered in pediatric AD patients. RDW-CV demonstrated extreme positive skewness and a wide inter-individual range, including outliers exceeding 1000%, a phenomenon suggestive of significant erythrocyte size variability. Elevated RDW is increasingly recognized as a marker of chronic inflammation and oxidative stress in systemic diseases, including asthma, Kawasaki disease, and allergic rhinitis [21-23]. In AD, where systemic immune activation is sustained by chronic skin inflammation, oxidative mechanisms can impair erythropoiesis and destabilize erythrocyte membranes, thereby increasing anisocytosis [13, 14, 20].

These results are in line with the known role of oxidative stress in AD pathophysiology, particularly in pediatric populations where antioxidant capacity may be immature and redox homeostasis more easily disrupted [17-19]. Furthermore, significant correlations were observed between RDW-CV and liver enzymes (AST, ALT), as well as between MCH and metabolic-hormonal parameters such as TSH and vitamin B12. The hepatic link is particularly interesting; transaminase elevations in AD have been reported in contexts of systemic inflammation or hepatic oxidative stress, while B12 and thyroid hormones are known modulators of erythropoiesis and red cell maturation.

The alterations observed in MCHC, notably its inverse correlation with ALT, may reflect decreased hemoglobin concentration per cell, possibly secondary

to oxidative damage to membrane proteins or red cell hydration states - mechanisms that have been previously discussed in chronic inflammatory skin disorders [14, 24]. Notably, high MCH and RDW have also been described in patients with chronic urticaria and psoriasis, further supporting the role of oxidative injury in systemic manifestations of skin diseases [13, 20].

These findings complement emerging literature that calls for alternative biomarker strategies in AD, particularly for resource-limited settings or pediatric care. Although current AD biomarker research focuses largely on chemokines (e.g., TARC, CCL17), interleukins (e.g., IL-13, IL-22), and barrier proteins (e.g., filaggrin), these are not routinely available in standard clinical laboratories [10, 11]. Hematological indices, on the other hand, are universally available and offer the advantage of reflecting systemic changes beyond the skin. They also intersect with other physiopathological axes in AD, including anemia of inflammation, nutrient malabsorption, and metabolic-endocrine crosstalk.

The significance of these findings is underscored by the current movement toward identifying novel AD endotypes. As recent studies suggest, pediatric AD may show distinctive immune profiles, including enhanced Th17 and Th22 polarization, and even eosinophilic signatures that are not always captured by traditional scoring systems [7-9]. In this context, red cell indices could represent an indirect but clinically meaningful readout of the systemic effects of such immune skewing, especially where oxidative burden is a key driver.

Limitations

This study has several limitations. As a retrospective, cross-sectional analysis, causal relationships between erythrocyte indices and oxidative stress cannot be established. Some parameters, such as RDW-CV, were affected by outliers, which may be due to laboratory or data entry artifacts. Furthermore, direct oxidative biomarkers (e.g., MDA, 8-OHdG, thiol/disulfide ratios) were not available for comparison. Future prospective studies incorporating both red cell indices and validated oxidative stress markers will be essential to strengthen these associations.

Given the limitations of current biomarker availability in routine clinical practice, hematological indices offer a cost-effective, universally available tool that could complement emerging endotype-driven approaches in pediatric AD management. These findings emphasize the potential role of complete blood count parameters not only in disease characterization but also in monitoring systemic inflammation and guiding early personalized interventions. However, the retrospective design and absence of direct oxidative stress markers warrant cautious interpretation. Future prospective studies integrating red cell indices with validated oxidative biomarkers and longitudinal follow-up will be critical to establish their prognostic value and clinical applicability.

Summary of Key Findings

(1) RDW-CV was markedly elevated and skewed, particularly in children under 4 years of age, supporting the hypothesis of systemic oxidative imbalance.

(2) MCV was lower in younger children, suggesting age-related differences in erythrocyte development and oxidative damage susceptibility.

(3) MCH and MCHC showed mild deviations but remained relatively consistent across subgroups.

(4) RDW-CV strongly correlated with AST and ALT, confirming its potential role as a systemic stress marker.

(5) No significant sex differences were observed in any erythrocyte parameters.

CONCLUSIONS

This study demonstrates that red blood cell indices—particularly RDW-CV and MCH—can serve as practical surrogate markers of systemic oxidative stress and metabolic disturbances in pediatric atopic dermatitis. Elevated RDW-CV shows strong correlations with systemic inflammatory and hepatic parameters, highlighting the disease's systemic involvement beyond the skin. Furthermore, associations between MCH, MCHC, and metabolic-hormonal markers like vitamin B12 and TSH indicate a link between erythropoiesis and endocrine-immune dysregulation in these patients.

Given their accessibility and cost-effectiveness, these hematologic indices hold promise as biomarkers in clinical practice. Integrating them into comprehensive panels may improve precision medicine strategies, allowing more individualized and systemic monitoring of pediatric AD, ultimately enhancing patient management and outcomes.

Ethics Approval and Consent to Participate

This study was approved by the Malatya Turgut Ozal University Non-Interventional Clinical Research Ethics Committee (Decision no. 2023/27, date: 25.04.2023). Since the study was retrospective, informed consent was waived. All procedures were in accordance with the Helsinki Declaration and institutional guidelines for medical research involving human subjects.

Data Availability

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available on request from the corresponding author, upon reasonable request.

Authors' Contribution

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

Conflict of Interest

The author(s) disclosed no conflict of interest during the preparation or publication of this manuscript.

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Generative Artificial Intelligence Statement

The author(s) declare that no artificial intelligence-based tools or applications were used during the preparation process of this manuscript. The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles

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The European Research Journal 2026;12(4):479-487 Original Article

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Effect of Prognostic Nutritional Index on Hospital Stay in Patients Undergoing Metallic Prosthetic Mitral Valve Surgery

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ABSTRACT

Objectives: Preoperative malnutrition has been associated with higher rates of morbidity and death, longer hospital admissions, and a worse quality of life after surgery. This study aimed to examine the association between the Prognostic Nutritional Index (PNI) and the duration of hospitalization, in-hospital, and 1-year all-cause mortality among patients having surgery on metallic prosthetic mitral valves.

Methods: The study retrospectively included 90 consecutive patients with metallic prosthetic mitral valve surgery at Dicle University Hospital between January 2021 and December 2023. Patients were split into two groups based on the median length of stay in the hospital (14 days). Those who stayed ≥14 days were included in the longer in-hospital stay group (53 patients), and those who stayed <14 days were included in the shorter in-hospital stay group (37 patients).

Results: The study included patients aged 52.3±15.2 years, 48.9% (n=44) female. Patients with longer hospital stays had wider left atrial diameters, lower albumin levels, and lower PNI values (34.7±6.1 vs. 38±4, P=0.002). Inotropic support was more frequent in this group (18.9% vs. 2.7%, P=0.021). ROC analysis identified PNI <37.2 as predictive of prolonged stays (AUC=0.653, P=0.014). Logistic regression analysis revealed significant associations between prolonged stays and inotropic support, left atrial diameter, albumin, CRP, and PNI values. PNI also predicted in-hospital and 1-year all-cause mortality.

Conclusions: PNI was associated with hospital stay duration, in-hospital, and 1-year all-cause mortality in patients undergoing metallic prosthetic mitral valve surgery. Incorporating PNI into routine preoperative evaluation may enhance perioperative management and outcomes.

Keywords: Prognostic Nutritional Index, Length of Hospital Stay, Prosthetic Mitral Valve Surgery

Patients having cardiac surgery still face a comparatively high risk of mortality and morbidity, even with new surgical procedures and recent technology improvements [1]. Preoperative anemia, advanced age, coronary artery diameter, socioeconomic status, and left ventricular dysfunction are among the variables that affect mortality and morbidity rates [2-6]. To maximize patient outcomes,

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preoperative examination is frequently carried out before elective surgery. In addition to determining a patient's functional ability, a comprehensive assessment often consists of a history and physical examination with an emphasis on risk factors for infectious, pulmonary, and cardiac problems. However, this preoperative evaluation frequently leaves out the patient's nutritional status.

It's also crucial to evaluate the dietary state of people having surgery. Preoperative malnutrition has been associated with higher rates of morbidity and death, longer hospital admissions, and a worse quality of life after surgery [7-9]. It negatively impacts several body systems, including the immunological, gastrointestinal, cardiovascular, and endocrine systems.

As a result, nutritional evaluation is rarely routinely included in preoperative screening, and there are currently no established methods for assessing the nutritional condition of patients having cardiac surgery. A simple prognosis tool, the prognostic nutritional index (PNI), was first created by Buzby et al. [10] and then revised by Onodera et al. [11]. In recent years, it has been associated with adverse outcomes in cardiovascular diseases [12-14].

The purpose of this study was to examine the association between PNI and the duration of hospitalization, in-hospital mortality, and 1-year all-cause mortality among patients having surgery on metallic prosthetic mitral valves.

METHODS

Our analysis retrospectively included 154 consecutive patients who had isolated prosthetic mitral valve surgery at Dicle University Faculty of Medicine Hospital between January 2021 and December 2023. Ninety patients in all were included in the analysis when the exclusion criteria were met. Figure 1 displays the flowchart for patient enrollment. Based on the population's median hospital stay (14 days), patients were split into two groups. The longer in-hospital stay group (≥14 days) consisted of 53 patients, and the shorter in-hospital stay group (<14 days) consisted of 37 patients. Demographic, clinical, and laboratory characteristics of the patients were obtained from hospital records. An estimated glomerular filtration rate (eGFR) of less than 60 mL/min/1.73 m^2 for a minimum of three months [15] was considered chronic kidney disease (CKD). The 10th Revision Codes of the International Classification of Diseases were used to classify heart failure (HF), atrial fibrillation (AF), coronary artery diseases (CAD), type 2 diabetes (DM), and hypertension (HT).

To reduce the risk of thromboembolism,

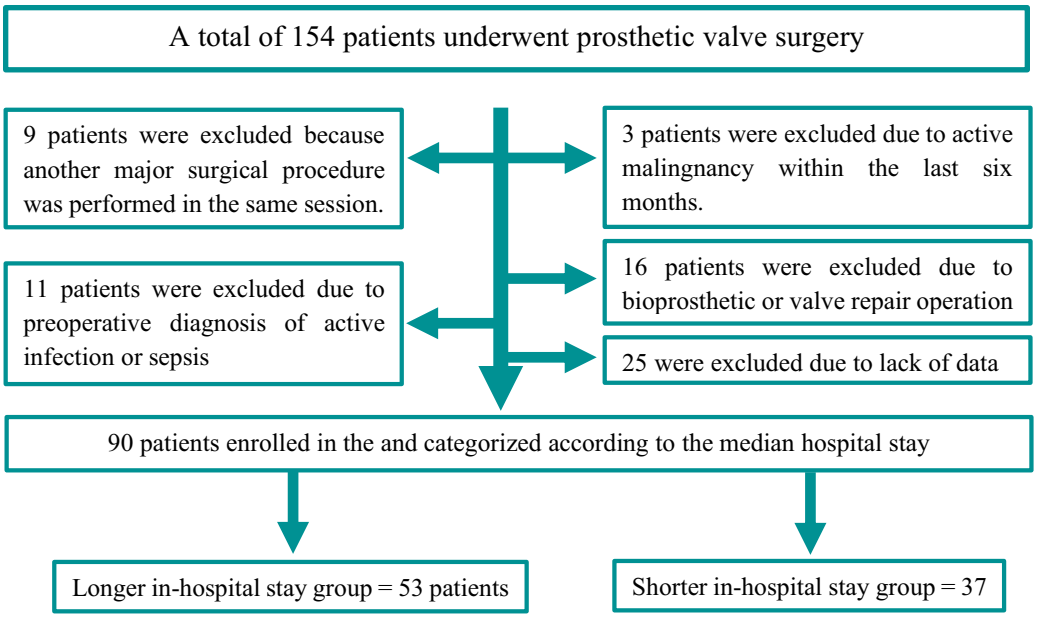

FIGURE 1. Patient enrollment flowchart.

anticoagulant medication was carefully controlled before surgery, shifting patients from warfarin to low-molecular-weight or unfractionated heparin. Valve function was assessed by transthoracic or transesophageal echocardiography, and perioperative stability was ensured by optimizing comorbidities.

Perioperative Anesthesia Management

After the patients were taken to the operating room, standard monitoring was performed (Electrocardiogram (ECG), pulse oximetry (SpO_2), and noninvasive blood pressure). Additionally, all patients received noninvasive brain function monitoring, regional oximetry, and hemodynamic monitoring (Masimo® Sedline®, Masimo Corporation, Irvine, California, USA).

Preoperative sedoanalgesia was provided with midazolam 1-2 mg IV (Zolamid®, Vem İlaç Sanayi ve Tic. A.Ş. Ankara, Turkey) or midazolam (1-2 mg IV) and fentanyl 1-2 µg/kg IV (Talinat®, Vem İlaç Sanayi ve Tic. A.Ş. Ankara, Turkey) according to the clinical status and vital signs. Radial artery cannulation was done for invasive arterial blood pressure measurement following sedoanalgesia. When radial artery cannulation fails, cannulation is performed from the femoral artery. For anesthesia induction, propofol 1-3 mg/kg IV (Propofol® 2% Fresenius®, Fresenius Kabi, Bad Hamburg, Germany) was used as general anesthetic, fentanyl (1-2 µg/kg IV) was used as analgesic, and rocuronium 0.6-0.9 mg/kg IV (Curon®, Mustafa Nevzat İlaç Sanayi A.Ş., Istanbul, Turkey) was used as neuromuscular blocker. In critically ill patients with low ejection fraction (EF), induction was performed with midazolam (0.1 mg/kg IV) and fentanyl (5 µg/kg IV) instead of propofol. The patients were intubated and connected to mechanical ventilators. The right jugular vein was used for central venous catheterization. To maintain anesthesia, 2% sevoflurane (1 MAC, 50% O_2, and 50% air mixture) was used. All interventions during cardiopulmonary bypass were performed by the joint decision of the surgeon, anesthesiologist, and perfusion specialist. All surgery procedures applied in the entire region were managed according to the clinical protocol, and training was provided in a standard manner. Valve surgeries were performed by two different surgeons with similar skills and experience. The anesthesiologists working on the table where the cases were taken were two anesthesiologists with similar skills and experience.

Prioritizing hemodynamic management during surgery involves reducing myocardial depression. Transesophageal echocardiography, central venous access, and invasive arterial pressure were also monitored. Intravenous heparin was used to establish anticoagulation during cardiopulmonary bypass, with an ACT over 480 seconds. This was carefully reversed after the bypass to balance the hazards of thrombosis and hemorrhage. To preserve valve performance and avoid problems, anticoagulation was quickly begun after surgery while being closely monitored. Early mobilization and recovery were supported by customized pain management.

Assessing Nutritional Status

Using the PNI and the following formula, the patient's pre-surgery nutritional status was ascertained:

10 × serum albumin (g/Dl) + 0.005 × total lymphocyte count (/mm^3)

While scores of 35–38 and <35 suggest moderate and severe malnutrition, a score of >38 is regarded as normal. For PNI, there is no "mild" category.

Statistical Analysis

The Statistical Package for Social Science for Windows (SPSS) 27 package tool was used to analyze the data. The Shapiro-Wilks test and histogram were used to confirm the data's normal distribution. Depending on the distribution, continuous parameters have been shown as median, interquartile range (IQR), or mean±SD. The chi-square test was used to compare groups of categorical variables, and categorical results were presented as percentages. The Mann-Whitney U test or Student t-test was used when comparing continuous variables. To determine the factors that contribute to longer hospital stays, univariable logistic regression analyses were conducted. The findings of the univariable logistic regression study were also displayed using a Forest-Plot graph. Receiver operating characteristic (ROC) curve analysis was used to assess the connection between PNI and length of hospital stay. The statistical significance level of the gathered data was ascertained using the P-value. At $P<0.05$, statistics were considered significant.

TABLE 1. Baseline Characteristics of the Total Population

Parameters	Longer in-hospital stay (n=53)	Shorter in-hospital stay (n=37)	Total population (n=90)	P-value
Age (years)	54.1±15	49.6±15.2	52.3±15.2	0.165
Female gender, n (%)	28 (52.8)	16 (43.2)	44 (48.9)	0.371
LVEF (%)	56.8±7.4	58.9±5.4	57.6±6.7	0.147
Mean pulmonary arterial pressure (mmHg)	46±15.8	50±15.2	47.5±15.6	0.301
GFR (ml/dk/1.73 mm^2)	82.6±35.2	90.6±27.4	85.8±32.3	0.255
Left atrial diameter (cm)	5.1±1.1	4.5±1	4.8±1.1	**0.027**
Hypertension, n (%)	26 (49.1)	15 (40.5)	41 (45.6)	0.425
Diabetes mellitus, n (%)	8 (15.1)	5 (13.5)	13 (14.4)	0.834
Coronary artery disease, n (%)	11 (20.8)	5 (13.5)	16 (17.8)	0.377
Cerebrovascular disease, n (%)	4 (7.5)	0 (0)	4 (4.4)	0.087
Re-do operation, n (%)	0 (0)	1 (2.7)	1 (1.1)	0.229
Heart failure, n (%)	10 (18.9)	7 (18.9)	17 (18.9)	0.995
Pulmonary artery disease, n (%)	9 (17.3)	4 (10.8)	13 (14.6)	0.392
Smoker, n (%)	10 (18.9)	5 (13.5)	15 (16.7)	0.502
Inotropic support, n (%)	10 (18.9)	1 (2.7)	11 (12.2)	**0.021**
Atrial fibrillation, n (%)	9 (17)	5 (13.5)	14 (15.6)	0.655
In-hospital mortality, n (%)	6 (11.3)	5 (13.5)	11 (12.2)	0.755
One-year mortality, n (%)	12 (22.6)	7 (18.9)	19 (21.1)	0.670
Laboratory parameters				
Hemoglobin (g/dL)	13.4±1.1	13.3±1.2	13.3±1.2	0.839
Albumin (mg/dL)	3.5±0.6	3.8±0.4	3.6±0.6	**0.002**
Neutrophile ($\times10^3/\mu L$)	5.4±1.7	5.4±2.1	5.4±1.9	0.978
Lymphocyte ($\times10^3/\mu L$)	2.4±0.9	2.2±0.7	2.2±0.8	0.786
White blood cell ($\times10^3/\mu L$)	8.7±2	8.5±2.4	8.6±2.2	0.634
Platelet ($\times10^3/\mu L$)	232±76	245±78	237±76	0.464
CRP (mg/dL)	0.6 (2.1)	1 (1.1)	0.54 (1.2)	**0.041**
Total cholesterol (mg/dL)	171±49	179±41	174±45	0.426
PNI	34.7±6.1	38±4	36±5.5	**0.002**
Medications, n (%)				
Acetyl salicylic acid	22 (41.5)	11 (29.7)	33 (36.7)	0.254
Beta-blocker	30 (56.6)	19 (51.4)	49 (54.4)	0.623
Statin, n (%)	6 (11.3)	1 (2.7)	7 (7.8)	0.133
ACE-I or ARB	20 (37.7)	10 (27)	30 (33.3)	0.484
Calcium channel blocker	7 (13.2)	7 (18.9)	14 (15.6)	0.462
Vitamin K antagonist	11 (20.8)	6 (16.2)	17 (18.9)	0.588
Non-vitamin K oral anticoagulant	3 (5.7)	1 (2.7)	4 (4.4)	0.503

Data are shown as mean±standard deviation or n (%).

ACE-I, angiotensin converting enzyme inhibitör; ARB, aldosterone receptor blocker; GFR, glomerular filtration rate; LVEF, left ventricular ejection fraction; PNI, prognostic nutritional index. Statistically significant P-values are shown in bold.

TABLE 2. Pearson Correlation Analysis of Length of In-Hospital Stay and Other Parameters

Parameters	Correlation coefficient	P-value
PNI	-0.388	**0.002**
Age	0.176	0.172
Left ventricular ejection fraction	-0.215	0.093
Mean pulmonary artery pressure	-0.005	0.967
Left atrial diameter	0.070	0.588
Lymphocyte	0.002	0.991
Monocyte	0.152	0.239
Glomerular filtration rate	0.056	0.666
Albumin	-0.388	**0.002**

PNI, prognostic nutritional index. Statistically significant P-values are shown in bold.

RESULTS

The mean age of our patients was 52.3±15.2 years, and the female-male ratios were similar. The female-patient ratio was 48.9% (n=44). In the longer in-hospital stay group, left atrial diameter (5.1±1.1 cm vs. 4.5±1 cm, P=0.027) was wider, albumin values were lower (3.5±0.6 mg/dL vs. 3.8±0.4 mg/dL, P=0.002), and PNI values were lower (34.7±6.1 vs. 38±4, P=.002) as expected. Inotropic support was more common in the longer in-hospital stay group (10 [18.9] vs. 1 [2.7], P=0.021). Although left ventricular ejection fraction (LVEF), mean pulmonary arterial blood pressure, and GFR values were lower in the longer in-hospital stay group, this was not statistically significant. There was no noticeable distinction in the medication utilized by the groups. Table 1 shows the comparison of baseline characteristics, laboratory parameters, and medication use of the total population between groups.

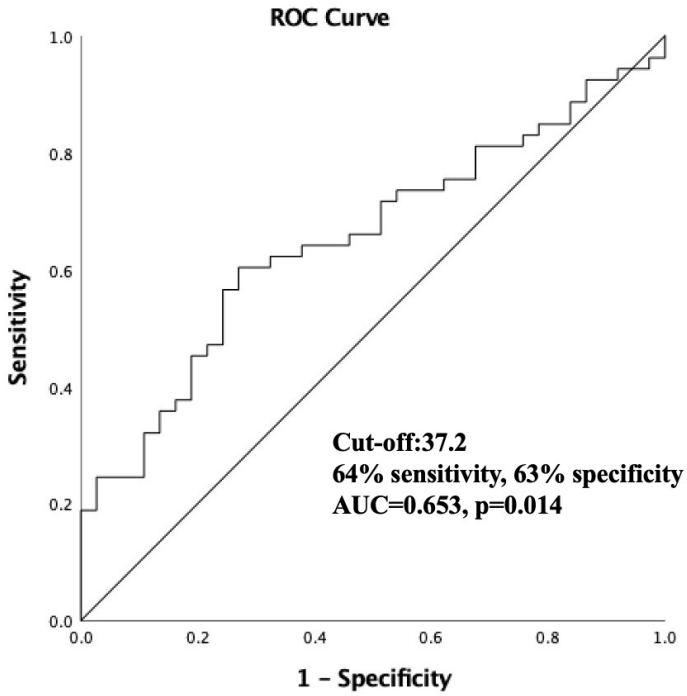

FIGURE 2. ROC curve analysis.

TABLE 3. Logistic Regression Analysis for Predictors 0f Longer In-Hospital Stay

Parameters	OR (95% CI)	P-value
Age	1.020 (0.992-1.050)	0.165
Inotropic support	8.372 (1.022-68.558)	**0.048**
LVEF	0.949 (0.882-1.020)	0.154
Left atrial diameter	1.713 (1.043-2.814)	**0.033**
Albumin	0.288 (0.116-0.715)	**0.007**
Lymphocyte	0.632 (0.351-1.140)	0.128
PNI	0.883 (0.806-0.967)	**0.007**
CRP	1.227 (1.044-1.442)	**0.013**
Regression analyses of the associations of PNI with mortality		
In-hospital mortality	0.875 (0.781-0.981)	**0.022**
1-year all-cause mortality	0.870 (0.789-0.959)	**0.005**

CI, confidence interval; CRP, c-reactive protein; LVEF, left ventricular ejection fraction; OR, odds ratio; PNI, prognostic nutritional index. Statistically significant P-values are shown in bold.

Pearson correlation analyses were performed between the parameters discussed in the literature for their effects on the length of hospital stay. In these comparisons, the strongest relationship between the length of hospital stay and PNI was observed (r=-0.388, P=0.002). Correlation analyses are shown in Table 2.

ROC curve analysis was performed to show the relationship between PNI and longer in-hospital stay groups. In ROC analysis, the cut-off value of PNI 37.2 was statistically significant for longer in-hospital stays with 64% sensitivity and 63% specificity (Area under curve: 0.653, P=0.014). (figure 2).

In univariable logistic regression analysis, statistically significant associations were found between inotropic support (OR=8.372, 95% CI:1.022-68.558, P=0.048), left atrial diameter (OR=1.713, 95% CI:1.043-2.814, P=0.033), albumin (OR=0.288, 95% CI:0.116-0.715, P=0.007), CRP (OR=1.227, 95% CI:1.044-1.442, P=0.013), and PNI (OR=0.883, 95% CI:0.806-0.967, P=0.007) and longer in-hospital stay. In addition, the relationship between PNI and in-hospital (logistic regression) and 1-year all-cause mortality (Cox regression) was investigated by regression analyses. PNI was found to be a predictor of both in-hospital (OR=0.875, 95% CI:0.781- 0.981, P=0.022) and 1-year all-cause mortality (OR=0.870, 95% CI:0.789- 0.959, P=0.005). Logistic regression analyses are shown in Table 3.

DISCUSSION

In this study, we investigated the effect of PNI on longer in-hospital stay in patients who underwent metallic prosthetic heart valve surgery. As a result of the study, we found that PNI had a statistically significant effect on longer in-hospital stay in both logistic regression analysis and correlation analysis. We also found that PNI was associated with postoperative in-hospital and 1-year all-cause mortality.

The results of our study are compatible with the studies in the literature. According to a study by Tasbulak et al. [16], patients undergoing isolated coronary artery bypass graft (CABG) procedures had greater mortality rates and long-term adverse cardiac and cerebrovascular events when compared to the control group when nutritional indicators such as PNI, controlling nutritional status score (CONUT), and geriatric nutritional risk index (GNRI) were present [22]. Recent investigations on cardiovascular illness have also demonstrated a clear correlation between lower PNI levels and greater rates of morbidity and death [17-19]. According to Lee et al. [20], decreased PNI was linked to prolonged hospital and intensive care unit stays and may serve as an independent predictor of early morbidity and death. The results of Hayashi et al. [21] showed a high correlation between a poor prognostic nutrition index and surgical

complications and survival. Almohammadi et al.'s study [22], which was comparable to ours, showed that lower PNI levels were linked to greater rates of mortality and morbidity as well as longer hospital stays. These studies show how PNI and other nutritional indicators may be useful in determining risk and forecasting results for patients undergoing heart surgery.

Our study also shows that serum albumin and CRP levels, left atrial diameter, and intraoperative inotropic support are associated with longer in-hospital stay. Inflammatory indicators, including the level of serum albumin and CRP concentrations, can have a major impact on a patient's likelihood of staying in the hospital for an extended period after having a prosthetic valve operation. Studies show that patients with infective endocarditis had higher long-term death rates when their CRP and albumin levels were raised, indicating that these biomarkers may be predictive of unfavorable outcomes [23, 24]. CRP and albumin levels are considered to be part of the nutritional status and immune response system [25, 26]. It seems to be in line with the literature that they are predictors of poor outcomes in patients undergoing valve surgery, as in many cardiovascular diseases.

We believe that the most important result of our study that distinguishes it from the literature is the lack of a relationship between LVEF and the duration of hospital stay. One study, like previous studies, has shown that low LVEF is a predictor of prolonged hospital stay [27]. In our study, although LVEF was not associated, left atrial diameter, another indicator of ventricular geometry, was associated with prolonged hospital stay. In a study by Augustin P et al., it was shown that the length of hospital stay was not related to mortality. The results of our study were also consistent with this study [28].

The European Society of Parenteral and Enteral Nutrition advises that nutritional optimization for 7-14 days before elective heart valve surgery should be performed on patients with severe malnutrition [29]. Before surgery, nutritional supplements taken orally are believed to improve the outcomes of patients and lessen problems associated with malnutrition by maximizing daily vitamin intake and avoiding excessive fluid and salt [30, 31]. According to these results, nutritional assessment should be performed at the time of admission for every patient, irrespective of age, having heart valve surgery. Perioperative evaluations and multidisciplinary treatment plans should include nutritional screening and nutritional therapy optimization.

Strengths and Limitations

As in other studies, our study has many limitations. Three primary limitations of our study are its retrospective design, small patient population, and single-center design. In addition, many parameters that are likely to affect hospitalization were not available in our data. Hospital-acquired infections, operator experience, illnesses that coexist (like chronic obstructive pulmonary disease), and some laboratory parameters could not be included in the statistical analysis. Due to the retrospective design, bias could not be completely eliminated. Inotropic support was available in only 1 patient in the shorter in-hospital stay group, and this low number also raises doubts about the reliability of the results. Finally, our data regarding patients who underwent intraoperative blood transfusion were not included in the analysis because of the doubts.

Unlike most previous studies, which have mainly focused on mortality or major postoperative complications in heterogeneous cardiac surgery populations, this study specifically evaluates the prognostic significance of the Prognostic Nutritional Index (PNI) in patients undergoing metallic prosthetic mitral valve surgery. This patient population has been relatively less studied in the literature and presents distinct clinical characteristics. In addition, our study comprehensively examines the relationship between preoperative nutritional status and length of hospital stay, in-hospital outcomes, and 1-year mortality, thereby providing a broader evaluation of the clinical impact of malnutrition in this surgical population. Furthermore, demonstrating that the PNI, as a simple, easily accessible, and low-cost biomarker, may predict prolonged hospital stay provides important clinical implications for preoperative risk stratification and perioperative patient management. This may help identify high-risk patients before surgery and facilitate the planning of targeted nutritional or supportive interventions when necessary.

CONCLUSION

According to the results of our study, PNI was associated with the length of hospital stay, in-hospital, and 1-year all-cause mortality in patients undergoing metallic prosthetic mitral valve surgery. Adding PNI, which is not routinely used in preoperative evaluation, to routine evaluation may provide useful information to clinicians in improving perioperative patient management and outcomes.

Ethics Approval and Consent to Participate

This study was approved by the University of Health Sciences Gazi Yaşargil Training and Research Hospital Clinical Research Ethics Committee (Decision no.: 322, date: 17.01.2025). All procedures were conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Informed consent was waived because of the retrospective nature of the study and the analysis used anonymous clinical data.

Data Availability

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available on request from the corresponding author, upon reasonable request.

Authors' Contribution

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

Conflict of interest

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

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Generative Artificial Intelligence Statement

The author(s) declare that no artificial intelligence-based tools or applications were used during the preparation process of this manuscript. The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

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Evaluation of subclinical cardiac dysfunction in rheumatoid arthritis and ankylosing spondylitis using tissue Doppler imaging: Impact of TNF-α inhibitor therapy

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ABSTRACT

Objectives: This study aimed to evaluate left ventricular diastolic function in patients with rheumatoid arthritis and ankylosing spondylitis using the myocardial performance index derived from tissue Doppler imaging. A secondary objective was to assess whether tumor necrosis factor-alpha inhibitor therapy was associated with changes in cardiac performance.

Methods: Patients diagnosed with rheumatoid arthritis or ankylosing spondylitis and age- and sex-matched healthy controls underwent comprehensive transthoracic echocardiography, including tissue Doppler imaging. The myocardial performance index was calculated using isovolumetric contraction time, isovolumetric relaxation time, and ejection time. Clinical disease activity and functional status were assessed. Subgroup analyses were conducted based on the use of tumor necrosis factor-alpha inhibitors.

Results: Patients with rheumatoid arthritis exhibited significantly reduced early/atrial transmitral flow ratios in the lateral, anterior, and inferior walls of the left ventricle ($P<0.05$), as well as elevated pulmonary artery pressure ($P=0.003$), consistent with early diastolic dysfunction. Myocardial performance index values were regionally impaired. In contrast, patients with ankylosing spondylitis demonstrated preserved diastolic function with only a mild increase in pulmonary artery pressure ($P=0.009$). The use of tumor necrosis factor-alpha inhibitors was not significantly associated with differences in echocardiographic parameters.

Conclusions: Subclinical diastolic dysfunction may be present in patients with rheumatoid arthritis even in the absence of overt cardiovascular disease. Diastolic indices appear preserved in ankylosing spondylitis. No significant echocardiographic differences were observed based on tumor necrosis factor-alpha inhibitor use, though further studies are warranted to clarify treatment-related effects.

Keywords: Rheumatoid arthritis, ankylosing spondylitis, echocardiography, diastolic dysfunction, tumor necrosis factor inhibitors

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Ankylosing spondylitis (AS) is a chronic inflammatory rheumatologic disorder that primarily affects the axial skeleton but may also involve extra-articular organs, including the eye (e.g., uveitis), gastrointestinal tract (e.g., inflammatory bowel disease), and the cardiovascular system. Cardiac involvement is heterogeneous and includes atrioventricular conduction abnormalities - the most common manifestation - as well as aortic regurgitation, pericarditis, and left ventricular (LV) dysfunction [1]. Cardiovascular manifestations occur in up to 10% of AS patients, emphasizing the importance of early detection. A recent meta-analysis confirmed a significantly increased risk of cardiovascular events, such as ischemic heart disease and heart failure, in this population [2].

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease mainly affecting synovial joints but often accompanied by extra-articular involvement, including cardiac manifestations. These may include pericarditis, myocarditis, valvular disease, conduction abnormalities, and coronary vasculitis, with pericardial involvement being the most common, frequently detected subclinically [3]. Subclinical cardiac involvement is common in RA, with echocardiographic abnormalities identified in about one-third of patients [4]. Growing evidence shows that RA independently increases cardiovascular risk beyond traditional factors such as hypertension or dyslipidemia, highlighting the role of systemic inflammation [5]. Mendelian randomization studies further support a causal link between RA-related immune dysregulation and the development of coronary artery disease and intracerebral hemorrhage [6].

Nonsteroidal anti-inflammatory drugs (NSAIDs) are recommended as the first-line treatment for locomotor system involvement in AS, and they are also frequently used to control pain and inflammation in RA [7]. NSAIDs may also be preferred for pain palliation in patients with RA [8]. However, long-term NSAID use is associated with elevated cardiovascular risk. Disease-modifying antirheumatic drugs (DMARDs), both synthetic (e.g., methotrexate, leflunomide) and biologic (e.g., TNF-α inhibitors, abatacept, rituximab), exert variable effects on cardiovascular function. While some biologics improve disease control, they may also contribute to hypertension, arrhythmias, or cardiac dysfunction in certain contexts [9, 10]. Biologic therapies have substantially improved outcomes in inflammatory rheumatic diseases. Early initiation can prevent irreversible damage and potentially influence cardiovascular prognosis [11].

Despite these advances, no reliable biomarkers exist to predict cardiac involvement, underscoring the need for routine cardiovascular monitoring in inflammatory arthritis. Echocardiography remains the primary non-invasive method for evaluating cardiac structure and function in these patients.

Although left ventricular ejection fraction (LVEF) and pericardial effusion are commonly assessed, they may not detect early dysfunction. In contrast, tissue Doppler imaging (TDI) has emerged as a more sensitive modality. The myocardial performance index (MPI), derived from TDI, assesses both systolic and diastolic function. MPI changes often precede LVEF decline and has shown utility in detecting subclinical dysfunction, particularly in oncology patients receiving cardiotoxic agents [12, 13].

To our knowledge, this is one of the few studies to evaluate MPI as a screening tool for subclinical cardiac dysfunction specifically in AS and RA cohorts using conventional echocardiography. Despite its potential, data on the prognostic utility of MPI in inflammatory arthritis remain limited. The current study aimed to evaluate whether tissue Doppler-derived MPI, when used alongside conventional echocardiographic parameters, may assist in the early detection of subclinical myocardial dysfunction in patients with RA and AS.

METHODS

Patient Selection

This study included 46 adult patients diagnosed with AS according to the 2009 ASAS axial spondyloarthritis classification criteria and 54 adult patients diagnosed with RA based on the 2010 ACR/EULAR classification criteria.[14, 15] Only patients aged 18 years or older were enrolled. The study was conducted between November 2013 and June 2015 at the Department of Rheumatology of a tertiary-level academic referral center.

Exclusion criteria were systematically defined as

follows: congenital heart disease; known cardiac conditions including reduced ejection fraction, significant valvular pathology, or atrioventricular conduction disorders; systemic diseases such as stroke, malignancy, or chronic kidney disease; and technical factors including poor echocardiographic image quality or atrial fibrillation. Patients with a prior history of acute rheumatic fever with carditis in childhood were also excluded.

After obtaining detailed medical histories, all patients underwent a standardized physical examination to assess the number of swollen and tender joints, limitations in joint movement, and spinal mobility. Disease activity was assessed using the Disease Activity Score-28 (DAS28) for RA and the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) for AS.[16, 17] Functional status was evaluated using the Health Assessment Questionnaire (HAQ) in RA patients and the Bath Ankylosing Spondylitis Functional Index (BASFI) in AS patients. DAS28 and BASDAI scores were assessed by a single experienced rheumatologist (author H.E.).

Measurements

Peripheral venous blood samples were used for laboratory assessments, which included erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), complete blood count, urea, creatinine, estimated glomerular filtration rate (eGFR), fasting blood glucose, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides.

All participants underwent comprehensive transthoracic echocardiographic examination. Parameters measured included LVEF, pulmonary artery systolic pressure, cardiac chamber diameters, LV mass, pericardial effusion, and valvular involvement. Tissue Doppler imaging was used to assess myocardial relaxation, isovolumetric contraction, and ejection times. Myocardial performance index was calculated using the following formula:

MPI=(Isovolumetric contraction time+ Isovolumetric relaxation time) / Ejection time.

All echocardiographic measurements were performed by a single experienced cardiologist (author H.A.), who was blinded to the patients' clinical data.

Ethical Standards

The study was approved by the local ethics committee (Decision No: 2014/81, Date: 28.05.2014). All procedures were conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from all individual participants included in the study.

Statistical Analysis

Continuous variables were presented as mean ± standard deviation or median with interquartile ranges (IQR), and categorical variables as number and percentage. The Kolmogorov–Smirnov test was used to assess data normality. Comparisons between groups were performed using the independent samples t-test or Mann-Whitney U test for continuous variables and the chi-square or Fisher's exact test for categorical variables. A P-value less than 0.05 was considered statistically significant. Patients were stratified into two subgroups according to TNF-α inhibitor use; however, treatment initiation date and cumulative exposure duration could not be ascertained retrospectively from the medical records, and therefore these variables were not included in the multivariable analyses.

RESULTS

Comparison Between Rheumatoid Arthritis Patients and Controls

Table 1 summarizes the clinical and echocardiographic findings of patients with RA and healthy controls. The control group had a significantly higher proportion of male participants (P=0.002) and higher systolic and diastolic blood pressure values (P=0.035 and P=0.021, respectively). The body mass index was significantly lower in the control group than in RA patients (P=0.002). Regarding baseline echocardiographic parameters, LVEF was significantly higher (P<0.001), and pulmonary artery pressure was significantly lower (P=0.003) in the control group. Regarding diastolic function, E/A ratios measured at the lateral, anterior, and inferior walls were significantly higher among controls (P=0.015, P=0.043, and P=0.004, respectively). When MPI values were compared, the control group showed

TABLE 1. Clinical and Echocardiographic Characteristics of Patients with Rheumatoid Arthritis and Healthy Controls

Parameters	Control group (n=45)	Rheumatoid arthritis (n=55)	P-value
Age (years)	54.04±15.41	55.16±11.25	0.453
Male sex	15 (33.3%)	9 (16.4%)	**0.002**
Hypertension	8 (26.7%)	18 (32.7%)	0.629
Diabetes mellitus	7 (23.3%)	5 (9.1%)	0.103
Body mass index (kg/m²)	26.85±6.35	32.00±6.40	**0.002**
Systolic blood pressure (mmHg)	130 (10)	120 (20)	**0.035**
Diastolic blood pressure (mmHg)	80 (0)	80 (10)	**0.021**
Heart rate at rest (beats/min)	75.00 (11.25)	77 (16)	0.797
Lateral E/A	0.89 (0.32)	0.79 (0.32)	**0.015**
Lateral MPI	0.56 (0.07)	0.51 (0.30)	0.096
Septal E/A	0.77 (0.25)	0.70 (0.28)	0.103
Septal MPI	0.60 (0.21)	0.51 (0.16)	**0.039**
Anterior E/A	0.98 (0.47)	0.78 (0.44)	**0.043**
Anterior MPI	0.59 (0.15)	0.52 (0.21)	**0.023**
Inferior E/A	0.84 (0.23)	0.71 (0.30)	**0.004**
Inferior MPI	0.61 (0.12)	0.53 (0.19)	**0.022**
Pulmonary arterial pressure (mmHg)	24.00 (6.25)	27.00 (7.00)	**0.003**
LV end-diastolic diameter (cm)	4.40±0.38	4.55±0.43	0.148
LV end-systolic diameter (cm)	2.50 (0.67)	2.50 (0.70)	0.170
LV ejection fraction (%)	62.50 (5.00)	60.00 (0.00)	**<0.001**
Interventricular septum thickness (cm)	0.93 (0.15)	1.00 (0.20)	0.065
Left atrial diameter (cm)	3.40±0.35	3.52±0.49	0.166

Data are shown as mean±standard deviation or median (IQR) or n (%). E, peak early diastolic mitral inflow velocity; A, late (atrial) diastolic mitral inflow velocity; MPI, myocardial performance index; LV, left ventricle.

Statistically significant P-values are shown in bold.

significantly higher MPI values at the septal, anterior, and inferior walls (P=0.039, P=0.023, and P=0.022, respectively). No significant differences were observed in LV chamber diameters, left atrial diameter, or interventricular septum thickness between the two groups (Table 1).

Association Between Baseline Characteristics and TNF-α Blocker Use in RA Patients

Table 2 compares clinical, biochemical, and echocardiographic parameters between RA patients using and not using TNF-α blockers. The only significant difference between the groups was disease duration, which was longer in the TNF-α blocker group (P=0.004). No statistically significant differences were observed in age, sex, blood pressure, laboratory values, or disease activity scores. No significant differences were found between the two groups regarding echocardiographic parameters, including all MPI measurements. LV dimensions, pulmonary artery pressures, and left atrial diameters were comparable (Table 2).

Comparison Between Ankylosing Spondylitis Patients and Controls

Table 3 compares clinical and echocardiographic

TABLE 2. Comparison of Baseline Clinical, Biochemical, and Echocardiographic Characteristics in Rheumatoid Arthritis Patients According to TNF-α Inhibitor use

Parameters	TNF-α blocker– (n= 46)	TNF-α blocker+ (n= 9)	P-value
Age (years)	55.60±10.76	55.22±11.22	0.923
Gender, male	7 (15.2%)	2 (22.2%)	0.457
Duration of disease (years)	6.00 (12.00)	30.00 (29.00)	**0.004**
Hypertension	13 (28.2%)	5 (55.6%)	0.115
Diabetes mellitus	4 (8.7%)	1 (11.1%)	0.606
Body mass index (kg/m²)	32.00±6.17	28.30±7.80	0.955
Systolic blood pressure (mmHg)	120 (20)	120 (10)	0.972
Diastolic blood pressure (mmHg)	70 (10)	80 (0)	0.239
Heart rate at rest (beats/min)	77.00 (15.50)	81.00 (15.00)	0.972
Hemoglobin (g/dL)	12.44±1.69	13.37±2.71	0.259
White blood cell count (×10³/µL)	7.33±2.77	7.66±2.47	0.738
Platelet count (×10³/µL)	281.00 (102.50)	266.00 (72.00)	0.439
ESR (mm/h)	22.50 (30.75)	20.00 (17.00)	0.546
CRP (mg/dL)	10.57 (15.26)	7.59 (17.35)	0.682
Urea (mg/dL)	31.15± 11.77	28.33± 6.44	0.319
Creatinine (mg/dL)	0.71±0.18	0.79±0.27	0.351
Glomerular filtration rate (mL/min)	100.82± 23.58	96.19±20.85	0.586
LDL (mg/dL)	114.53±30.47	118.54±41.16	0.735
HDL (mg/dL)	49.49±11.28	43.37±8.76	0.131
Triglyceride (mg/dL)	108.50 (61.75)	150.00 (65.00)	0.136
Total cholesterol (mg/dL)	190.84±37.65	191.66±48.29	0.955
Fasting blood glucose (mg/dL)	101.63±39.57	96,11±15.30	0.481
DAS28	3.94 (1.92)	2.33 (1.32)	0.144
HAQ	0.75 (0.50)	0.55 (0.80)	0.881
Lateral MPI	0.50±0.26	0.60±0.30	0.331
Septal MPI	0.51±0.17	0.52±0.13	0.601
Anterior MPI	0.53±0.20	0.51 (0.27)	0.879
Inferior MPI	0.53±0.19	0.50±0.10	0.404
Pulmonary arterial pressure (mmHg)	27.50±7.50	29.00±11.00	0.550
LV end-diastolic diameter (cm)	4.53±0.43	4.57±0.47	0.790
LV end-systolic diameter (cm)	2.50±0.52	2.50±0.70	0.819
LV ejection fraction (%)	60.00 (0.00)	60.00 (0.00)	0.062
Interventricular septum thickness (cm)	1.00 (0.20)	1.00 (0.10)	0.051
Left atrium diameter (cm)	3.52±0.46	3.55±0.45	0.843

Data are shown as mean±standard deviation or median (IQR) or n (%). CRP, c-reactive protein; DAS28, disease activity score 28; ESR, erythrocyte sedimentation rate; HAQ, health assessment questionnaire; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LV, left ventricle; MPI, myocardial performance index.

Statistically significant P-value is shown in bold.

parameters between patients with AS and healthy controls. The control group had a significantly higher mean age (P=0.003), as well as higher systolic and diastolic blood pressures (P=0.001 and P<0.001, respectively). Regarding echocardiographic measurements, LVEF was significantly higher in the control group (P=0.001), whereas LV end-systolic diameter and pulmonary artery pressure were significantly lower (P=0.012 and P=0.009, respectively). No statistically significant differences between the two groups were observed in MPI or E/A ratios (Table 3).

Association Between Baseline Characteristics and TNF-α Blocker Use in AS Patients

Baseline clinical, laboratory, and echocardiographic features of AS patients receiving or not receiving TNF-α blocker therapy are summarized in Table 4. Patients treated with TNF-α blockers had significantly longer disease duration (P=0.014) and higher hemoglobin levels (P=0.008) than those without biologic therapy. No statistically significant differences were found between the groups regarding age, sex, blood pressure, body mass index, or markers of disease activity. Similarly, echocardiographic

TABLE 3. Clinical and Echocardiographic Characteristics of Patients with Ankylosing Spondylitis and Healthy Controls

Parameters	Control group (n= 45)	Ankylosing spondylitis (n= 45)	P-value
Age (years)	54.04±15.41	43.34±11.06	**0.003**
Gender, male	15 (33.3%)	32 (71.1%)	0.089
Hypertension	8 (26.7%)	7 (15.6%)	0.255
Diabetes mellitus	7 (23.3%)	6 (13.3%)	0.353
Body mass index (kg/m²)	26.85±6.35	27.45±6.02	0.882
Systolic blood pressure (mmHg)	130 (10)	120 (10)	**0.001**
Diastolic blood pressure (mmHg)	80 (0)	70 (10)	**<0.001**
Heart rate at rest (beats/min)	75.00 (11.25)	75.00 (16.75)	0.540
Lateral E/A	0.89 (0.32)	1.09 (0.63)	0.111
Lateral MPI	0.56 (0.07)	0.50 (0.18)	0.232
Septal E/A	0.77 (0.25)	0.87 (0.50)	0.087
Septal MPI	0.60±0.21	0.51±0.15	0.094
Anterior E/A	0.98±0.47	0.97±0.60	0.834
Anterior MPI	0.59±0.15	0.51±0.17	0.145
Inferior E/A	0.84±0.23	0.91±0.62	0.264
Inferior MPI	0.61±0.12	0.51±0.15	0.075
Pulmonary arterial pressure (mmHg)	24.00±6.25	28.00±6.75	**0.009**
LV end-diastolic diameter (cm)	4.40± 0.38	4.57± 0.47	0.191
LV end-systolic diameter (cm)	2.50±0.67	2.80±0.70	**0.012***
LV ejection fraction (%)	62.50 (5.00)	60 (0)	**0.001***
Interventricular septum thickness (cm)	0.93±0.15	0.90±0.20	0.825
Left atrium diameter (cm)	3.40±0.35	3.44±0.55	0.537

Data are shown as mean±standard deviation or median (IQR) or n (%). E, peak early diastolic mitral inflow velocity; A, late (atrial) diastolic mitral inflow velocity; MPI, myocardial performance index; LV, left ventricle.

Statistically significant P-values are shown in bold.

TABLE 4. Comparison of Baseline Clinical, Biochemical, and Echocardiographic Characteristics in Ankylosing Spondylitis Patients According to TNF-α Inhibitor use

Parameters	TNF-α blocker– (n=24)	TNF-α blocker+ (n=21)	P-value
Age (years)	43.75±11.64	42.33±11.65	0.686
Gender, male	15 (62.5%)	17 (80.9)	0.151
Duration of disease (years)	3.00 (5.50)	14.00 (20.00)	**0.014**
Hypertension	5 (20.8%)	2 (9.5%)	0.267
Diabetes mellitus	3 (12.5%)	3 (14.2%)	0.600
Body mass index (kg/m²)	27.10±6.08	28.50±6.66	0.873
Systolic blood pressure (mmHg)	110 (10)	120 (10)	0.559
Diastolic blood pressure (mmHg)	70 (10)	70 (5)	0.286
Heart rate at rest (beats/min)	73.00 (19.50)	78.00 (10.00)	0.531
Hemoglobin (g/dL)	12.88±1.93	14.40±1.70	**0.008***
White blood cell count (×10³/µL)	7.38±2.19	8.02±2.63	0.378
Platelet count (×10³/µL)	277.00 (113.00)	251.00 (54.00)	0.139
ESR (mm/h)	15.00 (20.50)	6.00 (23.00)	0.425
CRP (mg/dL)	8.31 (18.81)	4.70 (20.55)	0.600
Urea (mg/dL)	31.20±10.12	28.99±8.61	0.438
Creatinine (mg/dL), mean ± SD	0.70±0.12	0.78±0.16	0.090
Glomerular filtration rate (mL/min)	120.18±23.29	114.92±22.94	0.451
LDL (mg/dL)	109.03±33.87	120.31±24.87	0.215
HDL (mg/dL)	41.18±10.99	43.43±10.41	0.484
Triglyceride (mg/dL)	121.00 (112.00)	146.00 (64.00)	0.488
Total cholesterol (mg/dL)	176.41±39.95	193.71±33.33	0.125
Fasting blood glucose (mg/dL)	97.70±32.94	94.90±27.75	0.764
BASFI	4.36±1.52	4.36±1.87	1.000
BASDAI	4.93±1.89	4.37±1.77	0.312
Lateral MPI	0.54±0.18	0.46±0.22	0.930
Septal MPI	0.51±0.13	0.50±0.25	0.397
Anterior MPI	0.51±0.17	0.55±0.20	0.189
Inferior MPI	0.51±0.17	0.52±0.12	0.528
Pulmonary arterial pressure (mmHg)	28.00 (9.50)	30.00 (8.00)	0.503
LV end-diastolic diameter (cm)	4.61±0.48	4.44±0.38	0.202
LV end-systolic diameter (cm)	2.80±0.70	2.60±1.10	0.392
LV ejection fraction (%)	60.00 (1.00)	60 (0)	0.754
Interventricular septum thickness (cm)	0.90±0.20	0.90±0.20	0.513
Left atrium diameter (cm)	3.48±0.51	3.45±0.51	0.816

Data are shown as mean±standard deviation or median (IQR) or n (%). BASDAI, bath ankylosing spondylitis disease activity index; BASFI, bath ankylosing spondylitis functionality index; CRP, c-reactive protein; ESR, erythrocyte sedimentation rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LV, left ventricle; MPI, myocardial performance index. Statistically significant P-values are shown in bold.

TABLE 5. Comparative Clinical, Biochemical, and Echocardiographic Characteristics of Patients with Ankylosing Spondylitis and Rheumatoid Arthritis

Parameters	Ankylosing spondylitis (n=45)	Rheumatoid arthritis (n=55)	P-value
Age (years)	43.34±11.06	55.16±11.25	**<0.001**
Gender, male	32 (71.1%)	9 (16.4%)	**<0.001**
Hypertension	7 (15.6%)	18 (32.7%)	0.143
Diabetes mellitus	6 (13.3%)	5 (9.1%)	0.191
Body mass index (kg/m²)	27.45±6.02	32.00±6.40	**0.025***
Systolic blood pressure (mmHg)	120 (10)	120 (20)	0.238
Diastolic blood pressure (mmHg)	70 (10)	80 (10)	0.086
Heart rate at rest (beats/min)	75.00 (16.75)	77.00 (16.00)	0.648
Hemoglobin (g/dL)	13.59±1.97	12.59±1.80	**0.010**
White blood cell count (×10³/µL)	7.69±2.40	7.39±2.70	0.564
Platelet count (×10³/µL)	261.00 (80.50)	282.00 (95.00)	0.200
ESR (mm/h)	13.00 (27.00)	21.00 (26.00)	**0.043**
CRP (mg/dL)	5.90 (19.72)	8.72 (16.97)	0.275
Urea (mg/dL)	30.17±9.41	30.69±11.08	0.802
Creatinine (mg/dL)	0.74±0.15	0.73±0.21	0.700
Glomerular filtration rate (mL/min)	117.73±23.02	100.07±23.04	**<0.001**
LDL (mg/dL)	114.29±30.22	115.19±32.04	0.887
HDL (mg/dL)	42.24±10.67	48.49±11.08	0.005
Triglyceride (mg/dL)	122.00 (74.00)	114.00 (59.00)	0.348
Total cholesterol (mg/dL)	184.49±37.62	190.98±39.08	0.401
Fasting blood glucose (mg/dL)	96.40±30.78	100.72±36.66	0.530
Lateral E/A	1.09 (0.63)	0.79 (0.32)	**0.001**
Lateral MPI	0.50±0.18	0.51±0.30	0.724
Septal E/A	0.87 (0.50)	0.70 (0.28)	**0.002**
Septal MPI	0.51±0.15	0.51±0.16	0.629
Anterior E/A	0.97±0.60	0.78±0.44	**0.024**
Anterior MPI	0.51±0.17	0.52±0.21	0.983
Inferior E/A	0.91±0.62	0.71±0.30	**0.001**
Inferior MPI	0.51±0.15	0.53±0.19	0.967
Pulmonary arterial pressure (mmHg)	28.00±6.75	27.00±7.00	0.688
LV end-diastolic diameter (cm)	4.57±0.47	4.55±0.43	0.924
LV end-systolic diameter (cm)	2.80±0.70	2.50±0.70	0.102
LV ejection fraction (%)	60 (0)	60 (0)	0.052
Interventricular septum thickness (cm)	0.90±0.20	1.00±0.20	0.065
Left atrium diameter (cm)	3.44±0.55	3.52±0.49	0.560

Data are shown as mean±standard deviation or median (IQR) or n (%). CRP, c-reactive protein; ESR, erythrocyte sedimentation rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LV, left ventricle; MPI, myocardial performance index.

Statistically significant P-values are shown in bold.

parameters, including all MPI values, did not differ significantly between the two groups. LV dimensions, pulmonary artery pressures, and left atrial diameter were comparable (Table 4).

Comparison Between RA and AS Patients

A detailed comparison of clinical, biochemical, and echocardiographic parameters between patients with AS and RA is presented in Table 5. Ankylosing spondylitis patients were significantly younger than RA patients (P<0.001) and had a higher proportion of male participants (P<0.001). The body mass index was significantly higher in RA patients than with AS (P=0.025). In the evaluation of diastolic function, lateral, septal, anterior, and inferior E/A ratios were significantly higher in AS patients than in RA patients (P=0.001, P=0.002, P=0.024, and P=0.001, respectively), suggesting relatively better diastolic performance. However, MPI values did not differ significantly between the groups. Other echocardiographic and laboratory findings were generally similar (Table 5).

DISCUSSION

This study investigated LV diastolic function in patients with rheumatoid arthritis and ankylosing spondylitis using conventional echocardiographic measures and myocardial performance index derived from tissue Doppler imaging. It also examined whether tumor necrosis factor-alpha inhibitors affected cardiac function. The findings demonstrated that, in RA patients, diastolic dysfunction markers such as the E/A ratio were significantly reduced compared to healthy individuals, while MPI values showed a paradoxical pattern. In AS patients, diastolic parameters did not significantly differ from controls. Additionally, no clear association was found between TNF-α inhibitor use and MPI values in either group.

Cardiovascular disease is one of the most serious extra-articular manifestations of inflammatory rheumatic conditions. It results from a complex interplay of systemic inflammation, endothelial dysfunction, and metabolic abnormalities [18]. Cardiac involvement in RA and AS includes pericardial inflammation, valvular disease, myocarditis, and subclinical myocardial dysfunction, even in asymptomatic patients [19]. Early detection of such complications is crucial, as cardiovascular disease is a leading cause of mortality in these populations [20]. A recent study confirmed significant left ventricular involvement even in RA patients without cardiac symptoms, emphasizing the importance of proactive screening [21].

Several echocardiographic studies have demonstrated increased diastolic dysfunction in RA patients compared to healthy individuals. In one cohort, the prevalence of diastolic dysfunction was found to be higher among RA patients, and it was associated with disease duration and interleukin-6 levels [22]. Similar findings were reported using natriuretic peptide levels to screen for cardiac dysfunction in RA [23]. Akgöl *et al*. [24] also found that RA patients had impaired myocardial relaxation and significantly altered MPI values, highlighting inflammation-driven subclinical myocardial dysfunction. These observations are in line with the present study, which showed significantly reduced E/A ratios in the lateral, anterior, and inferior walls of the left ventricle in RA patients. The consistent decrease in E/A ratios supports the presence of diastolic impairment, as shown in several meta-analyses and systematic reviews [25, 26].

In contrast, MPI values in RA patients showed a slightly unexpected pattern, with lower MPI in some walls than in the controls. Since MPI reflects both systolic and diastolic functions, it may be influenced by changes in systolic performance, preload, and afterload [27]. In this study, LVEF was lower in RA patients, which could have affected the MPI values. Other reports evaluating MPI in RA have shown contradictory results. Some have reported elevated MPI values suggesting global myocardial dysfunction [28-30]. Differences in disease duration, medication use, and sample characteristics may explain these inconsistencies.

Notably, pulmonary artery pressure was significantly elevated in RA patients compared to controls. This may represent an early subclinical consequence of diastolic dysfunction or reflect pulmonary vascular involvement associated with chronic inflammation [31].

The role of TNF-α in cardiovascular pathology has received significant attention, as it contributes to endothelial dysfunction, oxidative stress, and prothrombotic states. The use of TNF-α inhibitors, although clinically effective in controlling inflammation, has generated mixed results in terms of cardiac effects. Some investigations have reported potential risks, particularly in patients with pre-existing heart disease, whereas others have suggested a neutral or even protective effect [32, 33]. In this study, TNF-α inhibitor use was not associated with any significant difference in MPI values or other cardiac parameters. Similar findings were reported in recent observational cohorts [9, 34].

Although less frequent than in RA, cardiac manifestations in AS are not uncommon. Diastolic dysfunction prevalence has been estimated between 9% and 45% [35]. The present analysis found no significant difference between AS patients and healthy controls regarding E/A ratio or MPI values. A potential explanation may be related to demographic differences between groups. The control group was older and included more female participants, factors influencing diastolic function with age, and sex-related remodeling [36]. Notably, previous investigations involving older AS populations with longer disease duration have reported elevated MPI values and impaired diastolic relaxation parameters [37]. In line with our findings, Şahin *et al*. [21] reported that while overt systolic dysfunction may be rare in AS, subtle alterations in myocardial performance could be linked to inflammatory burden.

Nonetheless, pulmonary artery pressure was significantly higher in AS patients, suggesting early vascular involvement or increased ventricular stiffness. Furthermore, in this cohort, MPI values positively correlated with both BASDAI and BASFI scores, suggesting that subclinical myocardial dysfunction may be linked to disease activity, consistent with prior findings [37].

The effect of TNF-α inhibition on myocardial function in AS remains less studied than in RA. Some evidence supports a positive role in improving vascular function and lipid profiles [38]. However, this study did not reveal any significant difference in MPI or other cardiac indices between AS patients receiving or not receiving TNF-α blockers. A recent evaluation of arterial stiffness and intima-media thickness also failed to improve after TNF-α therapy significantly [10].

Strengths and Limitations

This study has several limitations that should be taken into account when interpreting the results. Its cross-sectional design limits the ability to infer causal relationships between echocardiographic findings, disease characteristics, or treatment exposure. The relatively small number of patients, particularly those receiving TNF-α inhibitors, may have reduced the power to detect subtle myocardial alterations. Moreover, information regarding the timing of TNF-α inhibitor initiation and duration of therapy was not available, limiting the ability to assess treatment-related effects on cardiac function. Differences in age and sex distribution between AS patients and controls may have influenced diastolic function parameters, as these variables are known to affect myocardial relaxation. All echocardiographic measurements were conducted by a single operator, without external or blinded reassessment, which may have affected measurement reproducibility. Although tissue Doppler-derived MPI was used as a practical and accessible measure of cardiac function, advanced imaging techniques such as speckle-tracking echocardiography or cardiac MRI were not employed. Additionally, smoking status was not documented, despite its potential role in cardiovascular risk stratification in patients with inflammatory arthritis. Finally, serological markers such as rheumatoid factor and anti-cyclic citrullinated peptide antibodies were not assessed, precluding analysis of their potential association with cardiac involvement.

This study has several notable strengths. First, it simultaneously evaluated two distinct inflammatory rheumatic diseases — RA and AS — under a standardized echocardiographic protocol, enabling direct comparison of subclinical cardiac involvement between the two conditions, which has been rarely reported in the literature. Second, all echocardiographic measurements were performed by a single experienced cardiologist who was blinded to clinical data, enhancing measurement consistency and minimizing observer bias. Third, the systematic application of strict exclusion criteria reduced the likelihood of confounding by pre-existing cardiac conditions. Fourth, the use of tissue Doppler-derived

MPI assessed across multiple left ventricular walls provided a more comprehensive regional evaluation of myocardial performance beyond conventional parameters. Finally, subgroup analyses according to TNF-α inhibitor use offered additional insight into the potential cardiac implications of biologic therapy in both disease populations.

CONCLUSION

This study demonstrates that patients with rheumatoid arthritis exhibit early echocardiographic signs of diastolic dysfunction, as evidenced by reduced E/A ratios and elevated pulmonary artery pressures despite preserved systolic function. In contrast, patients with ankylosing spondylitis showed no significant alterations in conventional or tissue Doppler-derived diastolic indices, although subtle vascular involvement may be present. While offering additional diagnostic information, the myocardial performance index showed variable findings, and its clinical utility in this setting remains uncertain. In subgroup analyses, tumor necrosis factor-alpha inhibitor use was not associated with significant differences in cardiac parameters; however, interpretation of these findings is limited by the absence of data on treatment duration and initiation timing. These findings underscore the importance of routine cardiovascular monitoring in chronic inflammatory diseases and highlight the need for prospective, multi-center studies incorporating advanced imaging modalities to better characterize and predict subclinical myocardial involvement.

Ethics Approval and Consent to Participate

This study was approved by the Necmettin Erbakan University Meram Faculty of Medicine Clinical Research Ethics Boxed (Decision no: 2014/81 and date: 28.05.2014). All procedures were conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from all individual participants included in the study.

Data Availability

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available on request from the corresponding author, upon reasonable request.

Authors' Contribution

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

Conflict of Interest

The author(s) disclosed no conflict of interest during the preparation or publication of this manuscript.

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Generative Artificial Intelligence Statement

The author(s) declare that no artificial intelligence-based tools or applications were used during the preparation process of this manuscript. The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

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The European Research Journal 2026;12(4):501-503 Case Report

DOI: https://doi.org/10.18621/eurj.1832025 *Chest Diseases*

Leukocytoclastic Vasculitis Associated with Nintedanib in Idiopathic Pulmonary Fibrosis: A Case Report

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ABSTRACT

Idiopathic pulmonary fibrosis (IPF) is a progressive and fatal interstitial lung disease. Nintedanib, a tyrosine kinase inhibitor, is one of the approved antifibrotic agents used in IPF treatment. While gastrointestinal side effects are common, cutaneous vasculitic reactions are extremely rare. We report a 70-year-old male with IPF, diagnosed by a radiological usual interstitial pneumonia pattern, who developed leukocytoclastic vasculitis (LCV) ten days after initiation of nintedanib therapy. The patient presented with palpable purpura on both lower extremities, accompanied by abdominal pain and diarrhea. Laboratory tests were unremarkable, and a skin biopsy confirmed LCV. Nintedanib was discontinued, and treatment with oral prednisolone resulted in the resolution of the lesions. Antifibrotic therapy was subsequently switched to pirfenidone. To our knowledge, this is the first reported case of nintedanib-associated LCV. Physicians should be aware of this rare adverse reaction in IPF patients receiving nintedanib.

Keywords: Idiopathic Pulmonary Fibrosis, Nintedanib, Leukocytoclastic Vasculitis, Drug Adverse Reaction

Idiopathic pulmonary fibrosis (IPF) is a progressive and fatal interstitial lung disease characterized by a usual interstitial pneumonia (UIP) pattern. Nintedanib, a tyrosine kinase inhibitor, is one of the approved antifibrotic agents used in IPF treatment. Although gastrointestinal side effects are common, cutaneous vasculitic reactions are extremely rare.

Rare cutaneous adverse effects associated with nintedanib, including skin rashes and inflammatory skin reactions, have been sporadically reported in the literature [1]. We present the first case of leukocytoclastic vasculitis (LCV) associated with nintedanib in a patient with IPF.

CASE PRESENTATION

A 70-year-old male presented with exertional dyspnea for two months. He was an ex-smoker with a 70 pack-year history and no history of tuberculosis, chronic illness, or occupational/environmental exposure. On examination, inspiratory velcro crackles were heard bilaterally in the lower lung zones. Oxygen saturation was 97% on room air.

High-resolution computed tomography (HRCT) revealed interlobular septal thickening, subpleural reticulations, traction bronchiectasis, and honeycombing in both lungs, more prominent in the basal segments (Figure 1). Findings were consistent

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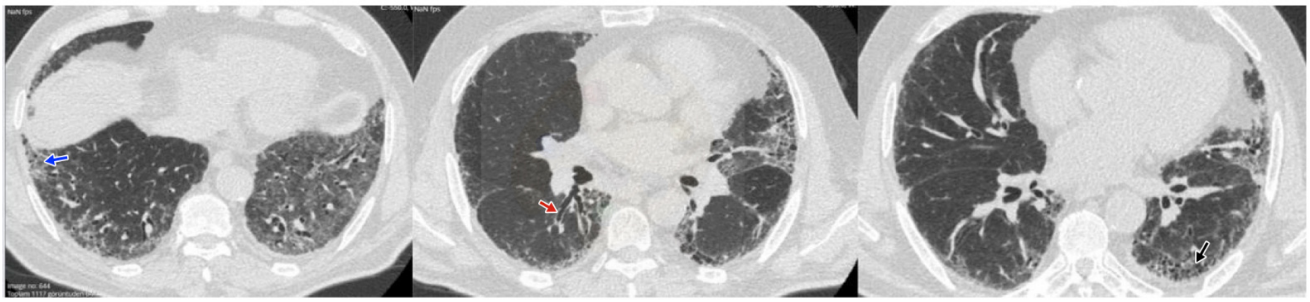

FIGURE 1. High-Resolution Computed Tomography image showing subpleural reticulations (blue arrow), traction bronchiectasis (red arrow), and honeycombing (black arrow) consistent with the usual interstitial pneumonia pattern.

with UIP. Rheumatologic diseases were excluded, and the patient was diagnosed with IPF. After the diagnosis, treatment with nintedanib (150 mg twice daily) was initiated.

Ten days after the initiation of treatment, the patient developed pruritic rashes on the lower extremities (Figure 2). On physical examination, the rashes were identified as palpable purpura. The patient also reported abdominal pain and diarrhea. Laboratory studies, including complete blood count, biochemistry, and peripheral blood smear, were unremarkable. No infectious focus was detected. Due to the presence of palpable purpura and the absence of alternative etiologies, nintedanib was discontinued.

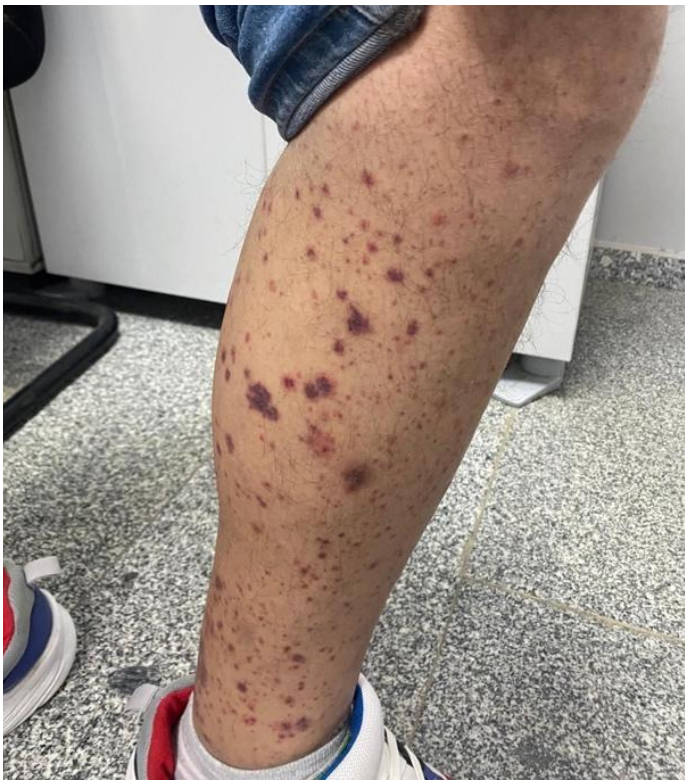

FIGURE 2. Palpable purpura on left lower extremity (Lesions were present on both lower extremities).

During outpatient follow-up, the lesions persisted; therefore, inpatient evaluation was deemed more appropriate. A skin biopsy obtained from the purpuric lesions revealed LCV. Prednisolone (5 mg three times daily) was initiated. During follow-up, the lesions on the lower extremities completely resolved with corticosteroid treatment. After full regression of the lesions, prednisolone was discontinued, and antifibrotic therapy was switched to pirfenidone.

DISCUSSION

LCV is a small-vessel vasculitis that mainly involves the skin. Its pathogenesis involves immune complex deposition in vessel walls, leading to vascular injury [2]. Approximately half of reported LCV cases are idiopathic; however, they may be triggered by infections, medications, systemic diseases, or malignancies. Drug-induced vasculitis usually occurs within 7–21 days after initiation of the responsible agent. Commonly implicated drug groups include antibiotics, nonsteroidal anti-inflammatory drugs, allopurinol, thiazides, and insulin [3].

Nintedanib is a tyrosine kinase inhibitor used in the treatment of IPF and other fibrotic interstitial lung diseases. The most commonly reported adverse effects involve the gastrointestinal system; however, rare adverse events such as thrombocytopenia and thrombotic microangiopathy have also been described

[4]. In our patient, purpuric skin lesions developed 10 days after starting nintedanib, consistent with the typical timeframe of drug-induced vasculitis. Other possible causes, including thrombocytopenia and thrombotic microangiopathy, were excluded before establishing the diagnosis.

Similar vasculitic reactions have been reported with other tyrosine kinase inhibitors and antifibrotic agents, suggesting a possible class-related immune-mediated mechanism [1].

The significance of this case lies in the recognition of LCV as a potential adverse effect of nintedanib. As IPF predominantly affects elderly patients with multiple comorbidities, awareness of this rare reaction is essential to prevent delayed diagnosis and ensure appropriate management.

CONCLUSION

We report the case of leukocytoclastic vasculitis associated with nintedanib in an IPF patient. Early recognition, drug discontinuation, and corticosteroid therapy resulted in clinical improvement. Physicians should remain vigilant for atypical cutaneous adverse effects of antifibrotic therapy, particularly with nintedanib.

Ethics Approval and Consent to Participate

As this is a single-patient case report that does not include identifiable personal information, ethics committee approval was not required. Written informed consent was obtained from the patient for publication of this case and accompanying images.

Data Availability

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available on request from the corresponding author, upon reasonable request.

Authors' Contribution

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

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The European Research Journal 2026;12(4):504-506 Letter to the Editor

DOI: https://doi.org/10.18621/eurj.1812710 *Physical Medicine and Rehabilitation*

Overlooked Source of Shoulder Pain: Pectoralis Minor Myofascial Trigger Points and Dry Needling Efficacy

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Dear Editor,

Shoulder pain is one of the most common causes of musculoskeletal pain. It is most often associated with rotator cuff tendinopathy, subacromial impingement syndrome, or cervical radiculopathy [1]. In some patients, despite persistent shoulder pain, imaging studies revealed no significant pathology. Furthermore, the response to standard treatments (such as analgesic medication and physical therapy) was low. In such cases, the often overlooked clinical presentation of myofascial pain syndrome originating from the Pectoralis Minor (PM) muscle should be considered.

The pectoralis minor muscle is located in proximity to the brachial plexus nerves and the axillary artery and vein; therefore, it is commonly associated with thoracic outlet syndrome and neurovascular compression in the literature. Consequently, related conditions are often described using the term "pectoralis minor syndrome". Some studies have suggested that myofascial trigger points in the PM muscle may play a role in the development of this clinical condition [2]. Nevertheless, only a limited number of studies have addressed myofascial trigger point-related pain in the PM muscle. Lam et al., in their comprehensive review of myofascial pain syndrome and its referred pain patterns, reported that the PM muscle can also cause pain that radiates to the shoulder and anterior chest region (see Figure 1a) [3]. However, awareness of this issue remains low at both the clinical and academic levels. The underlying mechanisms of shoulder pain associated with the pectoralis minor likely involve a combination of neuroanatomical and biomechanical factors. Although isolated lateral pectoral nerve neuropathy or entrapment associated with shoulder and chest pain has been described in a single case report, the current evidence is limited to case observation [4]. In contrast, the biomechanical effects of PM tightness or shortening are better described in the literature. It has been demonstrated that PM tightness or shortening can directly contribute to subacromial impingement (particularly by increasing anterior tilt and internal rotation of the scapula) and that PM tightness can also affect scapular kinematics by restricting upward rotation and external rotation of the scapula [5, 6].

In several observed cases, I identified distinct taut bands and trigger points along the PM muscle upon palpation in some patients with localized, deep, and referred pain in the anterior shoulder region, despite normal imaging findings. In these cases, the pain typically radiated toward the deltoid region or anterior arm. After the dry needling treatment, a significant decrease in the patients visual analog scale scores, an increase in the measured joint range of motion, and

Keywords: Shoulder Pain, Pectoralis Minor Muscle, Myofascial Trigger Points, Dry Needling

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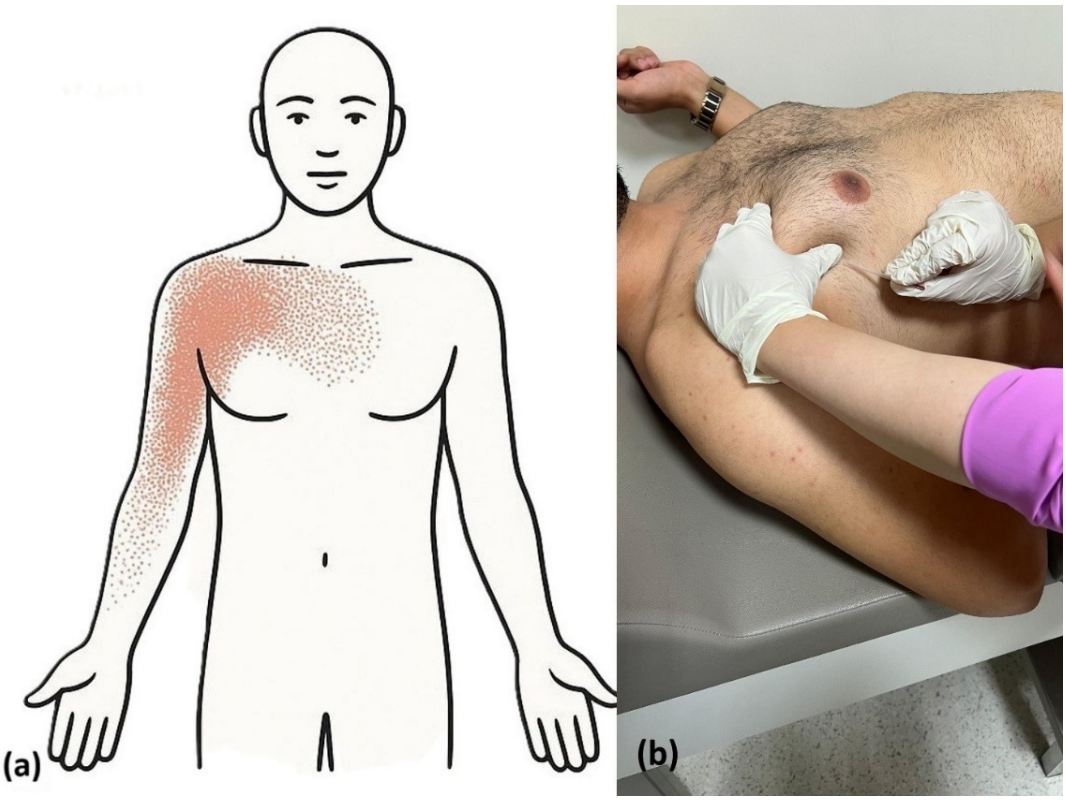

FIGURE 1. (a) Referred pain pattern associated with a pectoralis minor myofascial trigger point. (b) Dry needling technique applied to the pectoralis minor muscle.

functional improvement were observed.

Given the anatomical proximity of the PM muscle to neurovascular and pleural structures, it is clear that the procedure must be performed with great care. Dry needling should be performed with the patient supine and comfortable, with the shoulder in slight abduction and internal rotation. The dry needle should be carefully advanced in a craniomedial direction toward the trigger point identified by palpation with forceps to reach the pectoralis minor muscle. The needle direction and depth must be carefully adjusted anatomically (see Figure 1b) [7]. Following the procedure, light stretching, range of motion exercises for the shoulder joint, and scapular stabilization exercises support the effectiveness of the treatment.

In conclusion, myofascial pain of the PM muscle is a factor that must be evaluated in cases of undiagnosed or treatment-resistant shoulder pain. Including this muscle in the examination may prevent unnecessary testing and interventions and allow for more targeted treatments. Furthermore, controlled studies evaluating the effectiveness of dry needling applied to PM trigger points in shoulder pain are needed to support these findings. Patient consent was obtained for clinical images.

Yours sincerely,

İlknur Akkuş, MD, PhD

Ethics Approval and Consent to Participate

Ethical approval was not required for this article, as it describes clinical observations conducted in accordance with the ethical standards of the Declaration of Helsinki. Written informed consent has been obtained from the patient for the publication of clinical details and images.

Data Availability

Not applicable. This study is based on clinical observations and does not include any datasets.

Authors' Contribution

Study Conception: İA; Study Design: İA; Super-

vision: N/A; Funding: N/A; Materials: İA; Data Collection and/or Processing: N/A; Statistical Analysis and/or Data Interpretation: N/A; Literature Review: İA; Manuscript Preparation: İA; and Critical Review: İA.

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