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Table of Contents

Original Articles

Case Report

The European Research Journal 2026;12(6):606-615 Research Article

DOI: https://doi.org/10.18621/eurj.1789711 *Obstetrics and Gynecology*

In Vitro Analysis of FSH- and LH-Induced Alterations in Progesterone Levels in Human Granulosa-Theca Cells

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Abstract:

Objective: This study aimed to evaluate whether administration of luteinizing hormone (LH) before the ovulation trigger could modulate elevated progesterone levels.

Methods: Human granulosa-theca tissues were obtained from 15 women aged 18–38 years undergoing laparoscopic ovarian cystectomy at the Department of Gynecology and Obstetrics, Hacettepe University. The tissues were cultured in vitro and treated with 12.5 mIU/mL recombinant follicle-stimulating hormone (r-FSH; Group A), 18.75 mIU/mL r-FSH (Group B), or 18.75 mIU/mL r-FSH plus 6.25 mIU/mL recombinant LH (r-LH; Group C). Baseline and post-treatment estradiol (E2) and progesterone concentrations were measured at 0, 24, and 48 hours. Immunofluorescence staining for aromatase, cytochrome P450, and Ki67 was performed to assess steroidogenic activity and cellular proliferation.

Results: A mild trend toward reduced progesterone and increased E2 levels was observed following LH supplementation at both time points; however, these changes did not reach statistically significant. No statistically significante differences were observed in progesterone or E2 levels among the treatment groups at 24 h (P=0.891 and P=0.805, respectively) or 48 h (P=0.878 and P=0.505, respectively). Expression levels of aromatase, cytochrome P450, and Ki67 did not differ significantly between groups at 24 h (P=0.665, P=0.932 and P=0.953, respectively) or 48 h (P=0.911, P=0.674 and P=0.202, respectively).

Conclusion: These findings suggest that although r-LH addition to high-dose r-FSH does not significantly alter progesterone synthesis, it may exert a subtle regulatory effect on steroidogenesis. Larger studies with extended culture durations are needed to further elucidate the biological and clinical significance of these observations.

Keywords: Estradiol (E2), Follicle-Stimulating Hormone, Granulosa Cells, Luteinizing Hormone, Progesterone, Theca Cells

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Premature luteinizing hormone (LH) surges are a major factor reducing in vitro fertilization (IVF) success by impairing endometrial receptivity, lowering oocyte and embryo quality, and consequently decreasing pregnancy rates [1, 2]. While gonadotropin-releasing hormone (GnRH) analogs have reduced premature LH surges to approximately 2% in stimulated IVF cycles [3]. However, subtle elevations in serum progesterone levels during the late follicular phase persist in 35–38% of cycles using GnRH agonist and antagonist protocols. These elevations are closely linked to ovarian response and elevated progesterone levels at the time of human chorionic gonadotropin (hCG) administration [4–6]. The mechanisms underlying these progesterone elevations remain unclear, but evidence suggests that follicle-stimulating hormone (FSH) stimulation alone can increase progesterone production by granulosa cells (GCs) without luteinization [7].

In vitro studies demonstrate that FSH upregulates the expression and activity of 3β-hydroxysteroid dehydrogenase (3β-HSD), converting pregnenolone into progesterone in human GCs [7]. In the absence of LH, progesterone accumulates in GCs, leading to its passage into the systemic circulation and disrupting the hormonal balance essential for endometrial receptivity and implantation. In the presence of LH, however, excess progesterone is transported to theca cells, where it undergoes conversion to androgens by cytochrome P450 enzymes, particularly CYP17A1 (7–9). The activity of these enzymes is not enhanced by FSH stimulation alone in the absence of LH support; progesterone accumulates in GCs as a result and passes into the systemic circulation. The rise of progesterone level before ovulation trigger in IVF cycles is explained molecularly by the direct stimulating impact of recombinant FSH (r-FSH) on 3β-HSD and progesterone synthesis in GCs [10–15]. When LH is introduced, it activates the Δ5 androgen pathway, converting excess progesterone into androstenedione (Δ4 pathway) and testosterone in theca cells. These androgens are subsequently converted into estradiol (E2) in GCs by the aromatase enzyme, effectively reducing excess progesterone in the systemic circulation [16, 17]. The necessity of converting progesterone to androgens lies in maintaining hormonal balance within the ovarian microenvironment. Excessive systemic progesterone levels can disrupt the delicate endocrine regulation required for endometrial receptivity and implantation. Converting progesterone into androgens allows for the subsequent production of E2, which is critical for follicular development and preparing the endometrium for successful implantation. Without this conversion, the large amounts of progesterone produced by GCs during stimulation could enter the bloodstream, leading to premature endometrial maturation. These disruptions ultimately compromise IVF success rates. Without this conversion, the large amounts of progesterone produced by GCs during stimulation could enter the bloodstream, potentially disrupting the hormonal balance critical for endometrial receptivity [7, 16, 17]. This mechanism suggests that the introduction of LH before the ovulation trigger could mitigate the adverse effects of premature progesterone elevation by facilitating its conversion to androgens and, subsequently, estradiol.

These findings underscore the importance of further research into early progesterone elevations and strategies to prevent their occurrence to improve IVF outcomes. Based on this, the present study aims to evaluate the effects of r-LH on progesterone levels in human granulosa-theca tissue culture in response to varying doses of administered r-FSH.

METHODS

Study Design and Participants

Approved by the Hacettepe University Non-Invasive Clinical Research Ethics Committee (14.05.2019, GO19/439), it included 15 patients aged 18–38 with regular menstrual cycles undergoing laparoscopic ovarian cyst surgery. This prospective observational in vitro study cultured healthy stromal and follicular ovarian tissues obtained from patients with benign cysts. Patients with suspected endometriosis were excluded to minimize confounding variables.

Outcomes

The main outcomes were the levels of E2, progesterone, aromatase, cytochrome p450, and Ki67 biomarkers measured at 0, 24, and 48 h. Cytochrome p450 (17α-hydroxylase/17,20-lyase) facilitates the conversion of pregnenolone and progesterone into

dehydroepiandrosterone (DHEA) and androstenedione, acting as a specific marker for theca cells (bs-6695R, bioss, USA). Aromatase (CYP19A1), located in the endoplasmic reticulum, catalyzes the final steps of estrodiol biosynthesis and serves as a granulosa cell marker (bs-1292R, bioss, USA). Ki67, a marker for cell proliferation. Delta values for hormone changes were calculated as ΔE2 (change in E2 levels) and Δprogesterone (change in progesterone levels) for 0–24 hours and 0–48 hours intervals.

Tissue Culture

Healthy stromal and follicular components from cultured tissues were placed in HEPES buffer and to confirmed the presence of appropriate components under a stereomicroscope (Leica M55, Leica CLS 50×). Tissue samples were cut into 1 mm³ pieces and transferred to a basal culture medium containing 5% FBS, 4% L-glutamine, DMEM-F12, and 1% penicillin-streptomycin (Figure 1). After 24 hours of culture, the medium was collected to assess basal hormone levels (0 h). Subsequently, ovarian tissues were cultured with varying hormone combinations: group A (12.5 mIU/mL FSH), group B (18.75 mIU/mL FSH), and group C (18.75 mIU/mL FSH + 6.25 mIU/mL LH) (18). At 24. hours, the medium was collected for basal hormone measurements, and tissues were fixed in paraformaldehyde. The process was repeated at 48 h.

Histological Examination

Fixed tissues were processed using an automatic tissue processor (Leica TP 1020) and embedded in paraffin blocks (Leica EG 1150 H). Serial 5-µm sections were prepared using a microtome (Leica SM2000R). Some sections were stained with hematoxylin and eosin for histopathological scoring, while others were deparaffinized, rehydrated, and treated with 3% hydrogen peroxide to block endogenous peroxidase. Sections were incubated with primary antibodies (Ki-67, cytochrome P450, aromatase; 1:200) for 1 h at room temperature, followed by secondary antibodies (goat anti-rabbit, 1:1000) for 1 h. After washing, sections were stained with DAPI and mounted with antifading medium, and prepared for evaluation. Five randomly selected sections from the mucosa, submucosa, and adventitia layers were imaged using a Leica DM6 B microscope with a Leica DFC 7000T camera (40×). Fluorescence intensities were analyzed with ImageJ software (NIH, USA).

Biochemical Hormone Measurement

The basal and post-FSH/LH culture media were analyzed for progesterone and E2 levels. E2 was measured using a one-step chemiluminescent microparticle immunoassay (Abbott Diagnostics, USA) with a range of 0–1000 pg/mL and a functional sensitivity of 25 pg/mL. Precision for low (5 pg/mL),

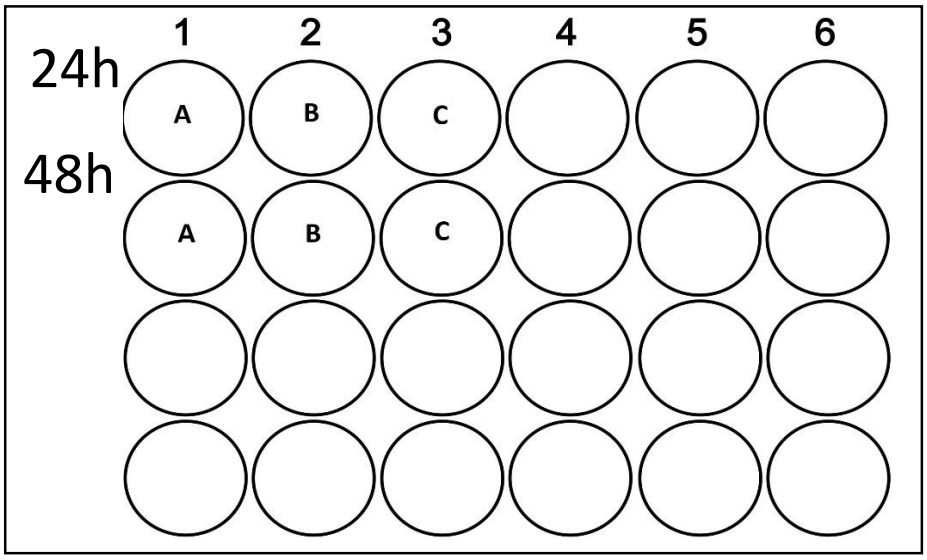

FIGURE 1. Representative view of ovarian tissue placement into the culture dish obtained from each patient before incubation.

moderate (200 pg/mL), and high (800 pg/mL) concentrations of E2 was used for assay calibration. Similarly, for progesterone, control levels were set at low (0.5 ng/mL), moderate (10 ng/mL), and high (35 ng/mL). Hormone analysis results from the cell culture medium were recorded.

Statistical Analysis

Data analysis was performed using SPSS v20.0. Shapiro–Wilk tests assessed data distribution, with results expressed as median (IQR (interquartile range)). A P-value <0.05 was considered significant. Wilcoxon's signed-rank test was used for within-group comparisons of non-normally distributed E2 and progesterone data at 0, 24, and 48 h. The Kruskal–Wallis test compared E2, progesterone, aromatase, and cytochrome p450 between groups A, B, and C. Ki67 marker data, which followed a normal distribution, were analyzed using one-way ANOVA.

RESULTS

Immunofluorescence Labeling Analysis

The tissue samples were fixed and sectioned after 24 and 48 hours of incubation. Immunofluorescence markers were applied, and five random fields were analyzed using the ImageJ v1.53j software.

The GCs were labeled with aromatase antibodies, showing widespread expression along the cell membrane in all groups (Figure 2). Intensity measurements of aromatase labeling revealed no statistically significant differences among the groups at 24 hours (P=0.665) and 48 hours (P=0.911) (Table 1).

Theca cells were labeled with CyP450 antibodies, which showed membrane-localized fluorescent labeling (Figure 3). There were no significant differences in the labeling intensity at 24-h (P=0.932) and 48-h (P=0.674) (Table 1).

Cell proliferation was assessed using Ki67 antibodies, with labeling localized in the nucleus. No statistically significant differences in proliferation were observed between groups at 24 hours (p=0.953) or 48 hours (P=0.202) (Figure 4, Table 1).

Biochemical Analysis

E2 and progesterone concentrations were measured at three time points. Within each group, E2 levels showed no significant differences between 0 and 24 hours (group A, P=0.069; group B, P=0.132; and group C, P=0.414; respectively) or between 0 and 48 hours (P=0.118, P=0.706 and P=0.460, respectively). The median levels within the assay's low-to-moderate range. Similarly, no statistically significant differences in progesterone concentrations were observed within groups between 0 and 24 h (P=0.153, P=0.194 and P=0.673, respectively) or between 0 and 48 h (P=0.929, P=0.965 and P=0.422, respectively). Both progesterone and estradiol levels

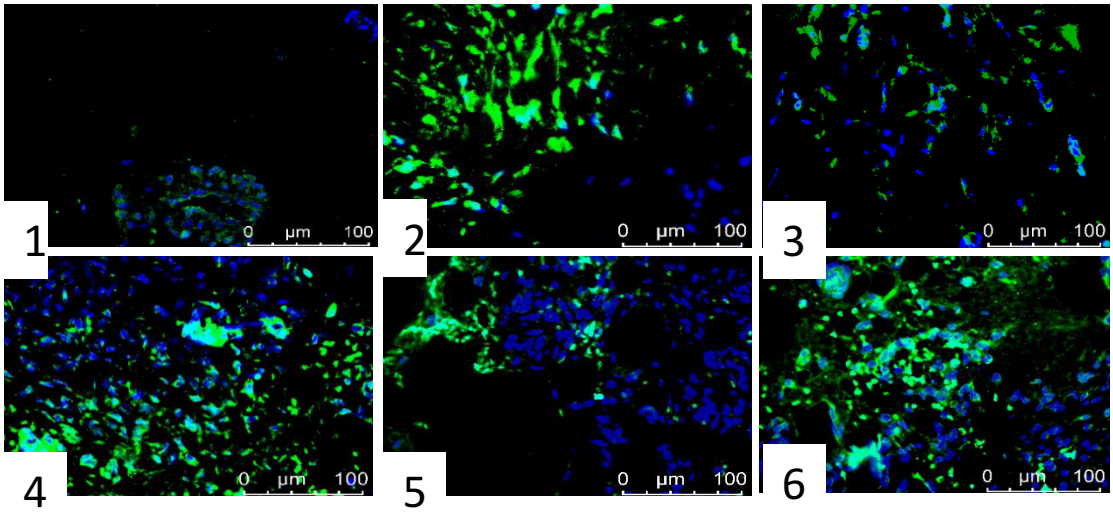

FIGURE 2. Immunofluorescence micrographs showing aromatase protein expression in cultured granulosa–theca tissues. Groups: 1=A24, 2=B24, 3=C24, 4=A48, 5=B48, 6=C48 (Green=aromatase; blue=DAPI. Magnification, ×400).

TABLE 1. Comparison of Median Values (Interquartile Range) Between 0–24 h and 0–48 h for Each Experimental Group.

	24th Hour				48th Hour			
	Group A	Group B	Group C	P-value	Group A	Group B	Group C	P-value
ΔE2	-11 [-279.00;6.00]	-7.0 0 [-471.00;3.00]	-1.00 [-348.00;7.00]	0.805[a]	-6.00 [-255.00;9.00]	0.00 [-54.00;25.00]	1.00 [-168.00;15:00]	0.505[a]
ΔProgesterone	-0.10 [-0.90;0.00]	0.00 [-0.40;0.00]	-0.10 [-0.30;0.60]	0.891[a]	0.00 [-0.40;0.10]	0.00 [-0.90;0.40]	0.00 [-0.40;0.10]	0.878[a]
Aromatase	18.22 [17.81;19.41]	19.07 [17.81;19.41]	18.22 [17.81;20.80]	0.665[a]	18.22 [17.81;19.41]	18.22 [17.81;19.41]	18.89 [17.81;19.41]	0.911[a]
Cytochrome p450	18.19 [17.77;19.33]	17.77 [16.96;18.55]	17.77 [16.96;19.67]	0.932[a]	17.77 [16.75;19.33]	17.77 [15.19;18.55]	17.77 [16.96;19.33]	0.674[a]
Ki67 expression	6.08 [5.56;78]	6.09 [5.05;6.67]	6.08 [5.01; 6.48]	0.953[b]	6.69 [5.37;7.17]	6.82 [6.01;7.75]	6.23 [5.19;6.56]	0.202[b]

Data are shown as median [interquartile range]. ΔE2, change in E2 (estradiol) levels, Δprogesterone, change in progesterone levels. The tissues were cultured *in vitro* and treated with 12.5 mIU/mL recombinant follicle-stimulating hormone (r-FSH; Group A), 18.75 mIU/mL r-FSH (Group B), or 18.75 mIU/mL r-FSH plus 6.25 mIU/mL recombinant luteinizing hormone (r-LH; Group C)

[a]Kruskal-Wallis test, [b]One-Way ANOVA test

remained within the expected low-to-moderate assay range, consistent with the assay's sensitivity. E2 and progesterone values are presented as medians with interquartile ranges (IQR) and are shown in Table 2.

In a follow-up experiment, FSH doses of 25 mIU and 50 mIU (two and four times the 12.5 mIU dose) were administered with LH added to the treatments. Despite the variations in FSH dosage, no significant differences in progesterone levels were observed between groups or across time points (0–24 hours, 0–48 hours) (Table 2). Additionally, no significant differences were observed in the ΔE2 or Δprogesterone between groups (P=0.805 and P=0.503 for ΔE2; P=0.891 and P=0.878 for Δ progesterone) (Table 1).

DISCUSSION

This study aimed to investigate whether recombinant luteinizing hormone (r-LH) administration influences progesterone and E2 secretion in human granulosa–theca cell cultures exposed to varying doses of r-FSH. The results demonstrated that r-LH supplementation did not significantly alter progesterone or E2 concentrations, regardless of FSH dose. Nevertheless, the observed tendencies suggest that r-LH may attenuate non-systemic progesterone accumulation within theca cells by enhancing its conversion to androgens, which are subsequently aromatized to E2 in granulosa cells. This mechanism supports the established "two-cell, two-gonadotropin" model of follicular steroidogenesis, in which LH stimulates androgen production in theca cells and FSH promotes aromatase activity in granulosa cells. Although the differences were not statistically significant, a numerical reduction in progesterone synthesis accompanied by a mild elevation in E2 levels was observed, particularly under high-dose FSH combined with r-LH treatment. These observations provide additional insight into the coordinated regulation of steroidogenic enzyme activity under gonadotropin stimulation and may explain the subtle hormonal balance observed during controlled ovarian stimulation protocols.

The lack of statistical significance observed in this study may be attributed to the small sample size or the limited exposure duration. Future studies incorporating optimized hormone concentrations,

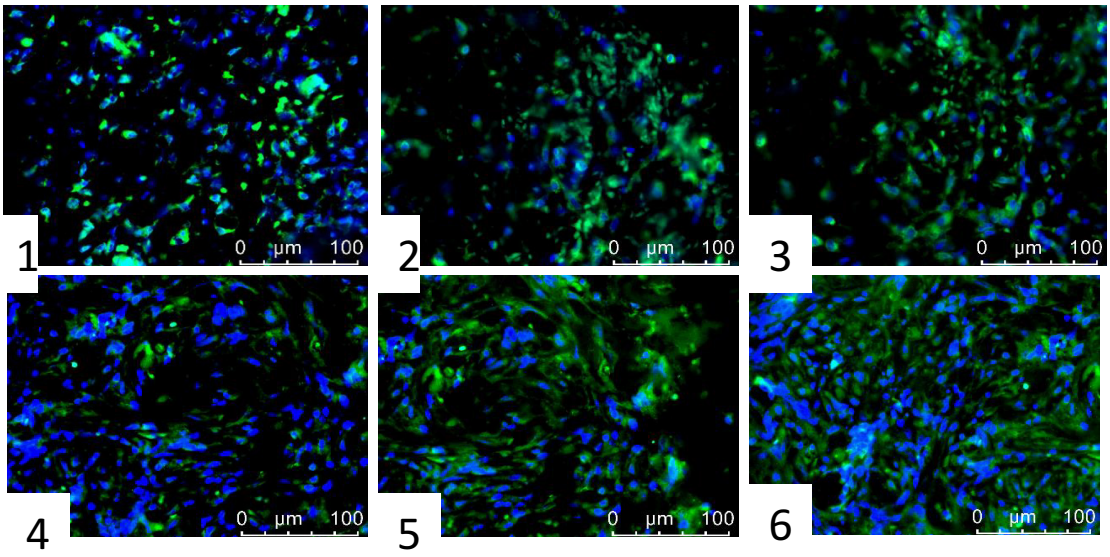

FIGURE 3. Immunofluorescence micrographs showing cytochrome P450 protein expression in cultured granulosa–theca tissues. Groups: 1=A24, 2=B24, 3=C24, 4=A48, 5=B48, 6=C48. (Green=CyP450; blue=DAPI. Magnification, ×400).

extended culture periods, and larger sample cohorts are warranted to confirm these preliminary trends and to better define their potential clinical relevance in IVF settings. This study specifically aimed to determine whether r-LH administration could modulate excessive progesterone accumulation and restore physiological balance in human granulosa–theca cell cultures. The present findings support the hypothesis that LH enhances the conversion of progesterone to androgens within theca cells, subsequently leading to increased E2 synthesis via aromatase activity in granulosa cells. Nevertheless, further research under variable experimental conditions is required to validate this proposed mechanism and elucidate its implications for controlled ovarian stimulation protocols.

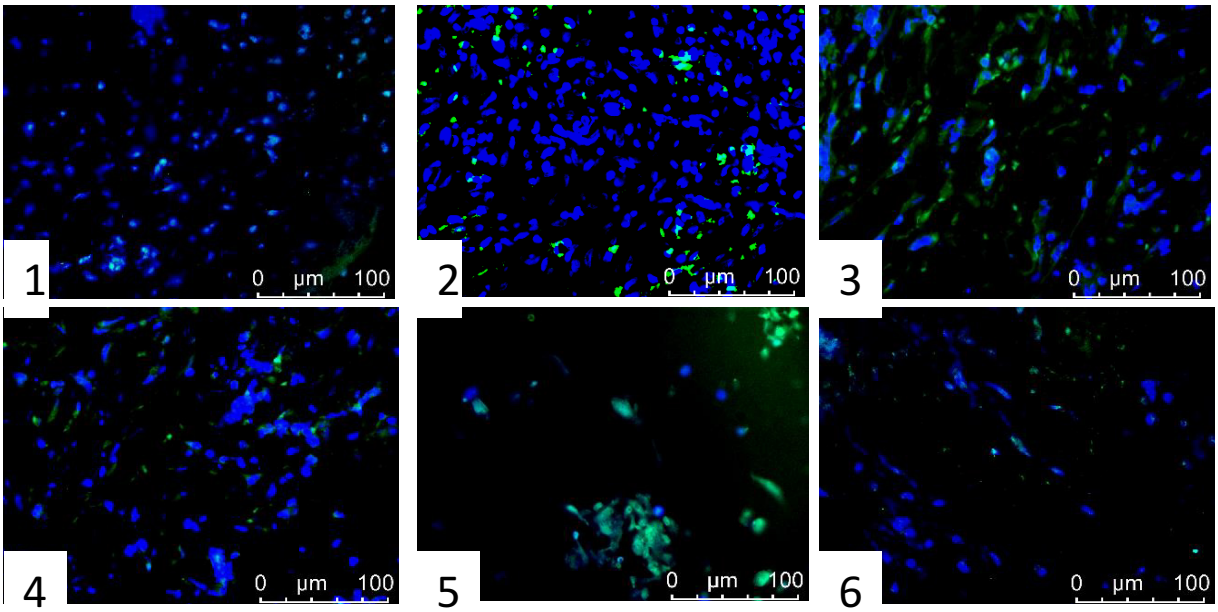

FIGURE 4. Immunofluorescence micrographs showing Ki-67 protein expression in cultured granulosa–theca tissues. Groups: 1=A24, 2=B24, 3=C24, 4=A48, 5=B48, 6=C48. (Green=Ki67; blue=DAPI. Magnification, ×400).

TABLE 2. Median Values (Interquartile Range) for Estradiol and Progesterone Concentrations in Each Experimental Group

	Group A			Group B			Group C		
	0^{th}	24th	P-value	0th	24th	P-value	0th	24th	P-value
Estrodiol	33.0 [24.0;706.0]	42.0 [18.0;331.0]	0.069	41.0 [24.0;801.0]	38.0 [22.0;291.0]	0.132	62.0 [29.0;786.0]	77.0 [29.0;438.0]	0.414
Progesteron	0.6 [0.00;2.00]	0.4 [0.00;0.90]	0.153	0.4 [0.1;1.0]	0.1 [0.00;1.0]	0.192	0.30 [0.10;3.30]	0.20 [0.00;3.90]	0.673
	Group A	**Group B**	**Group C**						
	0^{th}	48^{th}	P-value	0^{th}	48^{th}	P-value	0th	48^{th}	P-value
Estrodiol	65.0 [33;767]	73.0 [32;512]	0.118	88.0 [28.0;682.0]	81.0 [35.0;476.0]	0.706	72.0 [31.0;252.0]	84.0 [22.0;223.0]	0.460
Progesteron	0.4 [0.00;1.40]	0.2 [0.10;1.40]	0.929	1.0 [0.1;2.30]	0.4 [0.1;4.6]	0.965	0.40 [0.10;2.20]	0.30 [0.00;2.60]	0.422

Data are shown as median [interquartile range]. The tissues were cultured *in vitro* and treated with 12.5 mIU/mL recombinant follicle-stimulating hormone (r-FSH; Group A), 18.75 mIU/mL r-FSH (Group B), or 18.75 mIU/mL r-FSH plus 6.25 mIU/mL recombinant luteinizing hormone (r-LH; Group C)
Wilcoxon signed test was uset for all comparsions

The success of IVF cycles often depends on serum progesterone and E2 levels on the day of hCG administration. Studies have shown that elevated progesterone levels on the hCG day, leading to early luteinization, negatively affect endometrial receptivity and implantation success [1, 2]. Under physiological conditions, serum progesterone levels during the follicular phase are typically below 1.5 ng/mL and begin to rise approximately 24 hours after the LH trigger. However, in IVF cycles, supraphysiological hormone levels can lead to elevated progesterone levels during the late follicular phase, occurring either independently or in association with premature LH rises [19-21].

Although the exact cause of progesterone elevation remains unclear, recent research suggests it may be associated with increased FSH stimulation. Elevated peripheral progesterone levels primarily affect the endometrium and the implantation window, potentially causing asynchrony between embryo development and endometrial receptivity [13, 22, 23]. Bosch *et al*. [17] investigated the impact of gonadotropins on follicular-phase serum P concentrations in 112 patients undergoing ovarian stimulation with a GnRH antagonist protocol using 225 IU r-FSH or hp-HMG for oocyte donation. Both groups experienced comparable supraphysiological FSH levels, but patients without LH activity showed significantly higher serum progesterone levels starting from the sixth day of stimulation. This finding suggests that in the absence of LH activity, pregnenolone is predominantly converted to pregnenolone 4 is predominantly converted to P4 without further metabolism without further metabolism. Furthermore, the study highlights that r-FSH is more potent than urinary FSH, emphasizing the importance of FSH selection, particularly in IVF patients prone to elevated pregnenolone 4 levels.

Oktem *et al*. [7] also demonstrated in an in vitro study that r-FSH directly stimulates progesterone production in human granulosa cells. In their study, 15 non-luteinized, FSH-responsive human granulosa cell lines (HGrCl) were stimulated with r-FSH at different time points and concentrations. The results showed no significant changes in 17α-OH expression but revealed upregulation of enzymes and proteins such as 3β-HSD, steroidogenic acute regulatory protein (StAR), aromatase, and 17β-HSD. These findings suggest that

progesterone increases with FSH stimulation in granulosa cells, enters systemic circulation, and persists at high levels in theca cells because the 17α-OH enzyme, which converts progesterone to androgens, remains inactive without luteinization [7-9, 15]. These findings are in line with previous reports suggesting that LH activity contributes to maintaining the physiological balance between androgen and estrogen synthesis during folliculogenesis. Hill *et al*. [24] and Bosch *et al*. [25] demonstrated that excessive progesterone elevation before ovulation triggering may reflect an imbalance in follicular steroidogenesis, often resulting from supraphysiological FSH exposure. The current data support this concept by showing that the addition of r-LH might mitigate such progesterone accumulation, likely through enhanced 17α-hydroxylase/17,20-lyase (CYP17A1) activity within theca cells. Similarly, studies by Venetis *et al*. [26] and Hu *et al*. [11] have highlighted that controlled LH supplementation can optimize follicular maturation and improve hormonal synchrony without significantly increasing systemic progesterone levels. Therefore, even though our results did not reach statistical significance, they point toward a potential modulatory role of r-LH in fine-tuning intrafollicular steroid conversion and preventing premature luteinization.

Hormone evaluations at 24 and 48 hours showed no significant differences in progesterone levels across the groups or between time points (0–24 h: p=0.805; 0–48 h: p=0.878). Similarly, E2 levels showed no statistically significant differences, though a trend toward increased E2 levels was observed in high-dose FSH groups, especially with LH supplementation. After these findings, FSH doses of 25 and 50 Miu - 2 and 4 times the 12.5 mIU identified in the earlier groups - were administered. Six further groups were formed in the same manner by adding LH to these doses, and hormone measurements were carried out once again. In these samples, there was no statistically significant change in the progesterone levels between groups or between 0-24 and 0-48 h. E2 values were also measured in the culture medium, and similar to progesterone, no significant change was observed between the groups. Although statistically significant results were not obtained, a high dose of FSH, administered together with LH at both 24 and 48 h, showed an increase in E2 levels.

Strengths and Limitations

These findings suggest that progesterone in theca cells is converted to testosterone and then to E2 in GCs, with limited systemic circulation of progesterone. Additionally, the decrease in progesterone levels with LH administration supports the hypothesis that LH promotes progesterone conversion in theca cells. However, the absence of significant results may be attributed to the small sample size. Despite these insights, our study has several limitations, including technical challenges in cell isolation and a limited number of tissue samples. Future studies with larger cohorts are warranted to confirm the hormonal dynamics observed and to validate the proposed interactions between FSH, LH, and ovarian steroidogenesis.

CONCLUSION

In summary, our results indicate that varying doses of FSH combined with LH supplementation do not significantly alter overall progesterone production. However, the observed increase in E2 and reduction in progesterone levels suggest the preferential activation of the Δ5 pathway in theca cells and the Δ4 pathway in granulosa cells. While elevated progesterone levels after r-FSH stimulation have been associated with impaired endometrial receptivity and lower implantation rates in IVF cycles, the potential regulatory role of r-LH as a preventive approach remains inconclusive in our study. Further research with optimized LH dosing, prolonged exposure periods, and larger sample sizes is necessary to clarify its impact on steroidogenic balance and clinical outcomes.

Ethics Approval and Consent to Participate

This study was approved by the Hacettepe University Non-Interventional Clinical Research Ethics Committee (Decision No: 2019/13-08-GO 19/439; date: 14.05.2019). 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 obtained from undergone laparoscopic ovarian cystectomy women that participated in this 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: NCA, ACG, SZ, GB, SM, OPA, SFM, LKS; Study Design: NCA, ACG, SZ, GB, SM, OPA, SFM, LKS; Supervision: NCA, ACG, SZ, GB, SM, OPA, SFM, LKS; Funding: Hacettepe BAP; Materials: NCA, ACG, SZ; Data Collection and/or Processing: NCA, ACG, SZ; Statistical Analysis and/or Data Interpretation: SFM, LKS, GB, NCA; Literature Review: GB, LKS; Manuscript Preparation: NCA, SZ, ACG; and Critical Review: NCA, ACG, SZ, GB, SM, OPA, SFM, LKS.

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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Relationship Between Growth Differentiation Factor-15 Levels and Micro-Atrial Fibrillation

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Abstract:

Objective: Micro-atrial fibrillation (micro-AF) represents short, irregular supraventricular tachyarrhythmia episodes that may precede clinical atrial fibrillation (AF). Identifying biomarkers associated with micro-AF could improve early detection and risk stratification. Growth Differentiation Factor-15 (GDF-15), a stress-responsive cytokine related to inflammation and myocardial injury, has been associated with AF, but its relationship with micro-AF remains unclear.

Methods: Ninety patients who underwent 24-hour Holter monitoring due to palpitations were enrolled. Forty-five patients with micro-AF and 45 controls without supraventricular tachyarrhythmia were compared. Serum GDF-15 levels and clinical characteristics were analyzed using standard statistical methods, including logistic regression analysis and Receiver Operating Characteristic (ROC) curve analyses.

Results: The groups were similar in age, sex, and comorbidities. Prior stroke was significantly more frequent in the micro-AF group (28.8% vs. 6.7%, P<0.001). Mean serum GDF-15 levels were significantly higher in patients with micro-AF than in controls (544.2±322.5 vs. 344±138.8 pg/mL, P<0.001). Logistic regression revealed that GDF-15 was associated with stroke risk in univariate analysis but not independently in multivariate analysis.

Conclusion: Elevated GDF-15 levels in individuals with micro-AF suggest an underlying inflammatory or structural atrial substrate, supporting its potential role as a biomarker for early arrhythmia detection and assessment of thromboembolic risk. Larger, prospective studies are warranted to clarify the prognostic significance of GDF-15 in micro-AF.

Keywords: Micro-Atrial Fibrillation, Growth Differentiation Factor-15, Biomarker, Atrial Remodeling, Stroke

Atrial fibrillation (AF) represents one of the most prevalent cardiac arrhythmias observed in clinical settings, substantially increasing the risk of stroke, heart failure, and mortality [1]. With advancements in rhythm monitoring technologies, subclinical atrial tachyarrhythmias that do not fully meet the standard diagnostic thresholds for AF have become more detectable. Among these, micro-atrial fibrillation (micro-AF) is defined by brief, irregular supraventricular tachycardia episodes lasting less than 30 seconds. This entity is considered a potential early marker for the eventual development of clinically manifest AF [2, 3]. Prospective cohort studies have revealed that individuals exhibiting micro-AF are at

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an elevated risk for subsequent AF and ischemic stroke compared to the general population [4, 5]. However, given its subclinical presentation, standardized management strategies for micro-AF remain undefined, and therapeutic decisions are largely guided by expert consensus [6]. Consequently, the identification of biomarkers linked to atrial remodeling, inflammation, and oxidative stress has gained growing attention as a means of refining risk stratification in micro-AF [7, 8]. Growth Differentiation Factor-15 (GDF-15), a member of the transforming growth factor-beta (TGF-β) superfamily, has emerged as a promising biomarker of cardiovascular stress. It is released in response to inflammation, cellular injury, and oxidative stress [9]. Elevated circulating levels of GDF-15 have been associated with AF onset, stroke, and increased mortality across large-scale cohorts [10, 11]. Although its prognostic role is well established in clinical AF, no prior study, to our knowledge, has evaluated GDF-15 levels in patients with subclinical arrhythmias such as micro-AF. Given the overlapping pathophysiological processes - namely inflammation, atrial fibrosis, and prothrombotic activity - it is plausible that GDF-15 is also implicated in micro-AF. Therefore, this study aimed to compare serum GDF-15 levels between patients with and without micro-AF and to assess whether GDF-15 is independently associated with micro-AF presence. Ultimately, we sought to explore the potential role of GDF-15 as a biomarker for early detection and risk assessment in micro-AF.

METHODS

Study Population

This retrospective study included patients who presented to the Cardiology Outpatient Clinic of Tekirdağ Namik Kemal University Hospital had palpitations and underwent 24-hour Holter monitoring following clinical evaluation. Participants were stratified into two groups: Group 1 included patients with micro-AF, and Group 2 served as controls without any supraventricular tachyarrhythmia recorded on Holter. Holter recordings (Schiller MT-101; Schiller AG, Baar, Switzerland) demonstrating tachycardia episodes lasting less than 30 seconds, composed of at least five consecutive supraventricular premature beats with irregular R–R intervals and absent P-waves, were classified as micro-AF. A total of 90 patients were enrolled between January 2024 and July 2024. Eligible participants were over 18 years of age, free from heart failure or valvular heart disease, and diagnosed with micro-AF based on 24-hour Holter analysis. Exclusion criteria included prior diagnosis of paroxysmal AF, prior use of oral anticoagulants (warfarin or direct oral anticoagulants), structural valvular abnormalities, heart failure, thyroid dysfunction, and significant coronary artery disease. Comprehensive medical histories were obtained, documenting age, sex, hypertension, diabetes mellitus, peripheral arterial disease, coronary artery disease, and cerebrovascular events. Physical examination and echocardiographic assessment were performed for all participants, and body mass index (BMI) was calculated using self-reported height and weight. Venous blood samples were collected after a 12-hour fasting period, after a Holter-based diagnosis. Serum GDF-15 levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions. The study protocol was conducted in accordance with the Declaration of Helsinki and approved by the institutional ethics committee.

Statistical Analysis

All analyses were performed using IBM SPSS Statistics version 27.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were assessed for normality using the Kolmogorov–Smirnov test. Data with normal distribution were expressed as mean±standard deviation (SD), while non-normally distributed variables were summarized as medians (interquartile range). Categorical variables were presented as frequencies and percentages. Between-group comparisons were made using the independent samples t-test for normally distributed continuous data and the Mann–Whitney U test for skewed data. Categorical variables were compared using either the Chi-square test or Fisher's exact test when appropriate. Logistic regression analyses (univariate and multivariate) were applied to determine independent predictors of micro-AF. Receiver operating characteristic (ROC) curve analysis was used to evaluate the diagnostic performance of GDF-15 levels

TABLE 1. Baseline Characteristics of the Study Participants

Characteristics	Group 1	Group 2	P-value
Age (years)	64.9±7.8	63.2±6.9	0.302
Male gender, n (%)	22 (48.9)	23 (51.1)	0.833
Diabetes mellitus, n (%)	17 (37.8%)	17 (37.8%)	1.000
Hypertension, n (%)	36 (80%)	31 (68.9%)	0.227
Coronary artery disease, n (%)	12 (26.7%)	13(28.9%)	0.814
Stroke, n (%)	13 (28.8)	3 (6.7)	**<0.001**
Smoking, n (%)	16 (35.6)	21 (46.7)	0.284
Alcohol use, n (%)	10 (22.2)	13 (28.9)	0.468
CHA_2DS_2-VASc score	1 (0-6)	1 (0-6)	1.000
Body mass index	28±2	27±3	0.879
Laboratory parameters			
Creatinine (mg/dL)	0.8±0.2	0.7±0.1	0.227
Hemoglobin (g/dL)	12.3±1.5	12.5± 1.7	0.548
White blood cell (10^3/mm^3)	6.5±1.6	6.4±1.6	0.779
Neutrophil (10^3/mm^3)	3.3±1.2	3.2±1.2	0.670
Platelet (10^3/mm^3)	230 (117-504)	228 (33-425)	0.640
Glucose (mg/dL)	117.9±32.4	102.9±27.2	**0.020**
Lymphocyte($\times10^3$/mm^3)	1.5±0.3	1.3±0.5	0.758
Triglyceride (mmol/L)	183±46	196±48	0.123
C-reactive protein (mg/L)	2.0 (0.2-12)	1.5 (0.1-89)	0.640
Thyroxine (ng/dL)	1.2±0.2	1.2±0.5	0.936
Thyroid-stimulating hormone (µU/mL)	1.5±1.1	1.8±0.9	0.186
GDF-15 (pg/mL)	544.2±322.5	344±138.8	**<0.001**
Medication use			
ACE-I, n (%)	21 (46%)	24 (53.3%)	0.480
ARB, n (%)	11 (24.4%)	13 (28,3%)	0.776
β-Blocker (%)	36 (80.1%)	33 (73%)	0.729
Ca–channel blocker, n (%)	12 (26.6%)	13 (31.2%)	0.796
Diuretic, n (%)	9 (20%)	7 (15%)	0.652
Oral antidiabetic, n %	13 (28.9%)	11 (24.4%)	0.634
Insulin, n %	3 (6%)	2 (4%)	0.730
Statin, n %	25 (34.7%)	21 (30%)	0.327
Echocardiographic variables			
LVEF (%)	62.7±3.81	61.53±4.06	0.189
LVEDD (mm)	47.6±4.1	47.5±3.1	0.889
LA (mm)	35.9±1.2	35.6±1.1	0.247
E wave (cm/s)	0.76±0.16	0.74±0.12	0.683

Data are shown as mean±standard deviation or n (%) or median (interquartile range) where appropriate. Group 1, micro-atrial fibrillation; Group 2, control group; ACE-I, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocker; Pro-BNP, B-type natriuretic peptide precursor; GDF-15, growth differentiation factor 15; LVEF, left ventricular ejection fraction; LVEDV, left ventricular end-diastolic diameter; LA, left atrium; E, Early passive filling of the left ventricle; CHA_2DS_2-VASc Score, congestive heart failure, hypertension, age ≥75 (doubled), diabetes, stroke (doubled), vascular disease, age 65 to 74 and sex category (female).

Statistically significant P-values are shown in bold.

in predicting micro-AF, calculating the area under the curve (AUC), sensitivity, and specificity. A two-tailed P-value < 0.05 was considered statistically significant.

RESULTS

The study cohort consisted of 90 participants, with 45 patients diagnosed with micro-AF and 45 matched controls. No significant differences were observed between the two groups regarding mean age (64.9±7.8 vs. 63.2±6.9 years; P=0.302) or sex distribution (male: 48.9% vs. 51.1%; P=0.833) and body mass index (28±2 vs. 27±3; P=0.879). (Table 1).

The prevalence of comorbid conditions - including diabetes mellitus, hypertension, and coronary artery disease - did not differ significantly between groups (P=1.000, P=0.227, and P=0.814, respectively). Median CHA_2DS_2-VASc scores were similar in both groups. However, a history of prior stroke was significantly more frequent among patients with micro-AF (28.8% vs. 6.7%; P<0.001), suggesting a potential link between micro-AF and subclinical thromboembolic events.

In laboratory analyses, serum GDF-15 concentrations were significantly elevated in the micro-AF group compared with controls (544.2±322.5 vs. 344.0±138.8 pg/mL; P<0.001). Fasting glucose levels were also higher in patients with micro-AF (117.9±32.4 vs. 102.9±27.2 mg/dL; P=0.020), although this appeared to have limited prognostic impact compared with GDF-15. No significant intergroup differences were noted for C-reactive protein (CRP), high-density lipoprotein cholesterol (HDL-C), thyroid-stimulating hormone (TSH), or other hematologic and biochemical parameters. Echocardiographic indices and medication usage were similarly distributed between groups. In the ROC curve analysis to predict micro-AF, micro-AF was predicted with 53.3% sensitivity and 71.1% specificity when the cut-off value for GDF-15 was >373 pg/mL (AUC: 0.708, P<0.001) (Figure 1).

Univariate logistic regression identified GDF-15 levels as significantly associated with stroke incidence (odds ratio [OR]: 1.005; 95% confidence interval [CI]: 1.002–1.009; P=0.014). However, in multivariate analysis, this relationship did not remain statistically significant (OR: 1.029; 95% CI: 0.992–1.067; P=0.123).

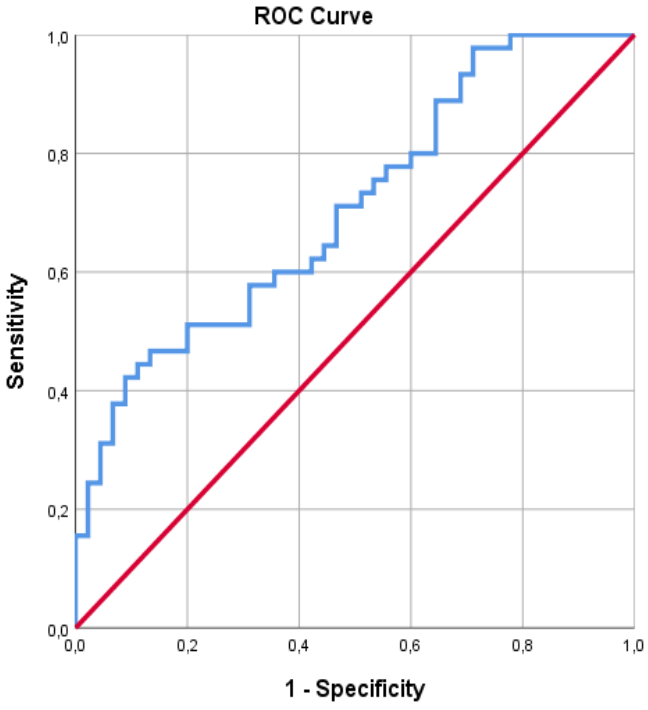

FIGURE. 1. The receiver operating characteristics (ROC) curve analysis for cut-off value of GDF-15 for predicting micro-atrial fibrillation.

DISCUSSION

The present study demonstrated significantly higher GDF-15 levels among individuals with micro-AF, indicating that these subclinical arrhythmias are accompanied by biochemical alterations reflecting myocardial stress and inflammation. Elevated GDF-15 levels may mirror underlying processes of atrial remodeling, fibrosis, and oxidative injury, aligning with previous findings linking this biomarker to AF development and adverse cardiovascular outcomes [12].

GDF-15, secreted primarily by cardiomyocytes, vascular endothelial cells, and macrophages, serves as a stress-responsive cytokine indicative of both inflammation and myocardial damage. Recent cohort data have identified GDF-15 as a robust, independent predictor of new-onset AF, regardless of conventional risk factors. Specifically, analyses from the ARISTOTLE trial highlighted its association with stroke and cardiovascular mortality in AF patients [13]. Furthermore, a 2024 study demonstrated a correlation between elevated GDF-15 levels and structural atrial remodeling, showing increased atrial wall thickness, left atrial enlargement, and enhanced fibrotic burden in individuals with higher biomarker concentrations [14]. These findings support the notion that micro-AF may represent not merely an electrophysiological event but also an early manifestation of structural atrial pathology.

Consistent with prior evidence, our cohort exhibited a significantly greater prevalence of previous stroke among patients with micro-AF. This observation underscores the clinical relevance of detecting such subclinical rhythm abnormalities, as they may signal increased thromboembolic risk even in the absence of sustained AF episodes. Given that GDF-15 reflects mechanisms such as mitochondrial dysfunction, oxidative stress, and apoptosis, it holds promise as a biomarker with broad prognostic utility across cardiovascular disease spectra [15-17].

Strengths and Limitations

To our knowledge, this is the first study to directly evaluate GDF-15 in individuals with micro-AF, thus contributing novel insight into its potential clinical application. A major strength of this study lies in its novel focus on the association between growth differentiation factor-15 and micro-atrial fibrillation, a subclinical arrhythmia entity that remains poorly characterized despite its growing clinical relevance. The use of standardized Holter-based diagnostic criteria for micro-AF enhances reproducibility and clinical applicability. The observed association between micro-AF and prior stroke underscores the clinical importance of early arrhythmia detection and supports the potential utility of GDF-15 as a biomarker reflecting atrial remodeling and thromboembolic risk in this populationElevated GDF-15 levels may signify both existing atrial abnormalities and a predisposition toward overt AF and cerebrovascular events. Regular assessment of GDF-15 could therefore assist in personalized monitoring and risk-adapted therapeutic strategies for patients with micro-AF.

Nonetheless, several limitations should be noted. The model sample size, single-center design, and single-time-point measurement of GDF-15 restrict generalizability. Future large-scale, multicenter prospective studies with serial GDF-15 assessments are warranted to further clarify its temporal and prognostic dynamics.

CONCLUSION

In summary, this study identified significantly elevated serum GDF-15 levels in patients with micro-AF and a higher prevalence of prior stroke compared with controls. These findings suggest that GDF-15 may serve as a valuable biomarker for detecting micro-AF and assessing associated thromboembolic risk. Moreover, elevated GDF-15 could reflect underlying atrial pathology, potentially preceding clinical AF or stroke development. Further multicenter longitudinal studies are essential to establish the prognostic role of GDF-15 in this patient population.

Ethics Approval and Consent to Participate

This study was approved by the Tekirdağ Namık Kemal University Non-Interventional Clinical Research Ethics Committee (Decision No:2025.154.09.02; date: 30.09.2025). 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.

Ethical Statement

One of the authors of this article, CA, is on the editorial board of our journal. While the peer-review process for the author's work is ongoing, their role in our journal has been suspended; thus, their access to the process has been restricted, and the principle of double-blind peer review has been preserved.

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: HO, CA; Study Design: HA, AD, AY; Supervision: HO, CA, AY; Funding: HO, CA, AD; Materials: AD, CA; Data Collection and/or Processing: HO, CA; Statistical Analysis and/or Data Interpretation: AY, CA; Literature Review: HO, AD; Manuscript Preparation: AD, CA; and Critical Review: HO, CA.

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 authors 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 authors in accordance with scientific research methods and academic ethical principles.

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Investigation of Prognostic Biomarkers in Advanced Stage Ovarian Cancer

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Abstract:

Objective: Ovarian cancer is a fatal disease originating from egg cells in the female reproductive system. It is the most lethal gynecological cancer, accounting for 4% of all cancers in women and 25% of female reproductive system cancers. Inflammation plays a significant role in its development. This study aimed to investigate the effects of inflammatory biomarkers on disease monitoring and prognosis in patients with advanced ovarian cancer.

Methods: This study included 29 consecutive patients who underwent surgery for advanced-stage (FIGO stage III–IV) ovarian cancer at Trakya University Gynecology and Obstetrics Clinic between February 2006 and July 2012. Twelve healthy postmenopausal women served as controls. All patients received standard surgical treatment comprising total abdominal hysterectomy, bilateral salpingo-oophorectomy, and omentectomy as cytoreductive surgery to minimize residual tumor.

Results: Mean age of the study group was 52.6±8.8 years (range: 36–77). Twenty-three (79%) patients were stage III and six were stage IV. The control group's mean age was 54.3±8.1 years (range: 49–72), with no significant difference between groups (P=0.467). CA-125, transforming growth factor-beta (TGF-β), and C-reactive protein (CRP) levels were significantly elevated in the patient group (P<0.001 for all). Omental involvement was present in 21 (72%) patients, and subdiaphragmatic peritoneal involvement in 14 (49%). Recurrence was detected in 20 (66%) patients, including all six stage IV patients, within a maximum follow-up of 77 months.

Conclusion: Advanced-stage ovarian cancer appears strongly associated with inflammatory markers. These biomarkers should be considered in patients' routine clinical follow-up and prognostic evaluation.

Keywords: Inflammation, Ovarian Cancer, Prognosis, Surgical Treatment

Ovarian cancer is a fatal disease originating from egg cells in the female reproductive system. It is the most lethal of gynecological cancers, accounting for 4% of all cancers in women and 25% of female reproductive system cancers [1]. Because this cancer manifests late, 70-80% of patients are diagnosed in the advanced stage. Treatment consists of cytoreductive surgery and adjuvant chemotherapy. Chemotherapy is administered to all patient groups except for some early-stage cases. The prognosis of ovarian cancer depends on the stage of the disease, histological features, tumor grade, and the patient's

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general health. Recurrence occurs in approximately 25% of early-stage cases and in more than 80% of advanced-stage cases [2].

Inflammation plays a significant role in the development of ovarian cancer, as in many diseases. Various biomarkers have been investigated to predict the prognosis ovarian cancer [3]. Transforming growth factor-beta (TGF-β), C-reactive protein (CRP), and Cancer Antigen-125 (CA-125) are important biomarkers used in the diagnosis and follow-up of ovarian cancer. The TGF-β family is a large group of extracellular growth factors involved in many processes such as cell proliferation, differentiation, motility, adhesion, and death. While it exerts a tumor-suppressive effect by suppressing epithelial cell division, it also exhibits oncogenic properties in tumoral tissues, mediating events such as uncontrolled proliferation, metaplasia, dysplasia, and aplasia, as well as invasion and metastasis [4, 5]. CA-125 is detected positively in more than half of early-stage cancers and in over 90% of advanced-stage cancers [6]. In addition to its diagnostic value, its relationship with response to chemotherapy and survival during clinical follow-up has been demonstrated [7]. Because the body's response to cancer is through inflammation, high CRP levels can indicate progression. High CRP levels are associated with advanced stages of ovarian cancer and residual tumor size [8].

In this study, we aimed to investigate the effects of biomarkers such as TGF-β, CRP, and CA-125 on disease monitoring and prognosis in patients with advanced ovarian cancer.

METHODS

This study included patients who underwent surgery for advanced-stage ovarian cancer at Trakya University, Gynecology&Obstetrics Clinic between February 2006 and July 2012. Twenty-nine consecutive patients with long-term follow-up were included in the study. Approval for this prospective study was obtained from the Trakya University Faculty of Medicine Ethics Committee (No: 18/11/2009-06). All patients provided informed consent before surgery. Patients with stage III and stage IV cancer according to the International Federation of Gynecology and Obstetrics (FIGO) were included in the study. All patients' ages, follow-up period, recurrence, relaparotomies, and total chemotherapy durations were recorded. Twelve healthy postmenopausal patients who had presented to the Trakya University Faculty of Medicine Gynecology and Obstetrics Clinic were also included as a control group.

During the preoperative and postoperative follow-up of the patients, approximately 4 mL of blood was drawn into tubes to determine serum TGF-β-1 and CRP levels using the Enzyme-Linked Immunosorbent Assay (ELISA) method. Serum CA-125 values were assessed using IMMULITE 2000 (Diagnostic Products Corporation, Los Angeles). TGF β-1 was measured using ASSAYPRO kits, and CRP was measured using the "Enzyme-Linked Immuno Sorbent Assay" method using DRG kits (International Featured as Spotlight Company of 2012 AACC, Los Angeles). All analyzes were performed in the Biochemistry Laboratory of Trakya University Faculty of Medicine. For TGF β, a serum dilution factor of 1/4000, and for CRP, a serum dilution factor of 1/100 was used. Results were finally analyzed using a Biotek µ Quant instrument at 450 nm absorbance.

Of the patients included in the study, 23 (79%) were evaluated as stage III, and 6 patients were evaluated as stage IV. All of these patients underwent surgical treatment. Standard surgical treatment for all ovarian cancer patients included total abdominal hysterectomy, bilateral salpingo-oophorectomy, and omentectomy as cytoreduction surgery, to minimize residual tumor. Of the patients included, no additional medical problems were detected at the time of surgery. Residual tumor size was taken as 0, >2 cm, and <2 cm for each patient. Each patient received 6 cycles of postoperative chemotherapy with paclitaxel and carboplatin, as suggested [1].

Follow-up assessments for these patients were performed at three-month intervals during the first year, and subsequently at intervals determined by disease stage, using CA-125 levels, speculum examination, vaginal smears, transvaginal ultrasonography, computed tomography (CT), chest radiography, and, when necessary, positron emission tomography. Clinical and pathological data included disease stage, tumor grade, ascites, and tumor staging. All chemotherapies received by the patients up to the time their samples were collected were recorded. The

time from the date of primary surgery to the date of disease-related death was considered "overall survival," and the time from the date of surgery to the date of disease development of recurrence or metastasis was considered as “disease free survival” (DFS). Survival and DFS duration were expressed in months. Patients were followed for survival and recurrence for a maximum of 77 months.

Statistical Analysis

In our study, descriptive statistics were used for CA-125, TGF-β, and CRP, including frequency, percentage, arithmetic mean, median, and standard deviation. A time series pilot was used to graphically display bivariate numerical data, which demonstrates how numerical data series change over time. Excel (Serial No: K66FVY7733B8WCK9KTG64BC7D8) was used to calculate numerical values.

RESULTS

Twenty-nine patients with ovarian cancer diagnosed as stage III (according to FIGO) and stage IV (according to FIGO), aged between 36 and 77 (median: 52, mean±SD: 52.6±8.8) years, and 12 postmenopausal healthy controls were included in the study. The ages of the control group ranged from 49 to 72 (median: 53.5, mean: 54.3±8.1) years. Characteristics of the study and control groups are presented in Table 1. The control and study groups were similar in terms of age (P=0.467). CA-125, TGF-β, and CRP values were significantly higher in the patient group (P<0.001, for all variables).

Residual tumor was detected as 0 in 9 (31%) patients, <2 cm in 15 (51%), and >2 cm in 5 (18%) women. When the amount of ascites detected during surgery was evaluated, no ascites was detected in 15 (51%) patients. Ascites was detected as <500 mL in 4 (13%), and >500 mL in 10 (36%) patients. The tumor grade of the patients was as follows: Three (10%) patients were detected as grade 1, seven (24%) patients as grade 2, and 19 (65%) patients as grade 3 (Table 2).

Omental involvement was evaluated as positive in 21 (72%) patients during the operation. Also, subdiaphragmatic peritoneal involvement was detected in 14 (49%) patients. In a mean time period of 15.4+6.6 months, recurrence was detected in 20 (66%) of the 29 patients included in the study. Recurrence was detected in all 6 patients evaluated as stage IV (according to FIGO). Relaparotomy was performed in 6 (20%) of the 29 patients. All were considered tumor-positive before and during the surgery. Six (20%) patients died during a time period of a maximum 77 months of follow-up (Table 2). Survival was calculated as the time from primary surgical treatment to the patients’ death. Mean survival in these 6 patients was 30.5±9.5 months (13-40 months), and mean DFS was 16.2±6.6 months (3-29 months).

In our patient group, due to the insufficient number of patients and the clinical characteristics of the samples collected, we performed individual analyses rather than cumulatively. We present follow-up charts for four of these patients and results from four representative patients are presented as follows:

Preoperative CA-125 level was detected as 797 mIU/mL in one patient. The patient underwent total abdominal hysterectomy, bilateral salpingo-

TABLE 1. Baseline Characteristics

Variables	Patient group (n=29)	Control group (n=12)	P-value
Age (years)	52.6±8.8	54.3±8.1	0.467
CA-125 (mIU/mL)	1111.5±1399.3	5.3±2.75	**<0.001**
TGF-β (mIU/mL)	49.9±29.7	13.6±23.1	**<0.001**
CRP (mg/dL)	10.9±8.9	3.6±3.4	**<0.001**

Data are shown as mean±standard deviation. CA-125, Cancer antigen 125; TGF- β, Transforming growth factor-β; CRP, C reactive protein.

Statistically significant P-values are shown in bold.

TABLE 2. Clinical and Pathological Features of the Patients

Variables		n (%)
Age (years)	≤52	15 (51%)
	>52	14 (49%)
Stage	III	23 (80%)
	IV	6 (20%)
Grade	1	3 (10%)
	2	7 (24%)
	3	19 (65%)
Histological type	Serous	22 (75%)
	Endometrioid	1 (3.5%)
	Clear cell	1 (3.5%)
	Undifferentiated	3 (10%)
	Brenner	2 (7%)
Ascites	None	15 (51%)
	<500 mL	4 (14%)
	>500 mL	10 (35%)
Omental involvement		21 (72%)
Subdiaphragmatic involvement		14 (49%)
Recurrence		20 (66%)
Re-laparotomy		6 (20%)
Residual tumor	none	9 (31%)
	<2cm	15 (51%)
	>2cm	5 (18%)
Mortality		6 (20%)

oophorectomy, pelvic lymph node dissection, omentectomy, and appendectomy. The patient's pathology was evaluated as serous papillary carcinoma, stage 3C, class 3. Pathology revealed omental and diaphragmatic involvement. The patient's postoperative residual tumor burden was >2 cm, and the amount of ascites was >500 mL. After six cycles of postoperative chemotherapy, recurrence occurred at the 22nd month. The patient underwent relaparotomy at the 29th month. Follow-up serum CA-125 values are shown in Figure 1.

In another patient, preoperative CA-125 was 209 mIU/mL. The patient underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, pelvic-paraaortic lymph node dissection, and omental biopsy. There was no residual tumor after the surgery. Pathology revealed stage 3B endometrioid-type adenocarcinoma. After six cycles of adjuvant chemotherapy, no tumor or intra-abdominal ascites, diaphragmatic or omental involvement was detected at the 12th and 36th month follow-up. The tumor was grade 1. A decreasing trend in CA-125 but an increasing trend in TGF-β and CRP were observed at the 12th and 36th month serum samples (Figure 2).

The third patient had a preoperative CA-125 level of 43 mIU/mL. She underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy, pelvic-paraaortic lymph node dissection, and multiple peritoneal biopsies. Pathology revealed serous carcinoma, stage 4, grade 3. Omental and diaphragmatic involvement was observed, but after the surgery no ascites or no residual tumor was present (residual tumor=0). She then received 6 courses of adjuvant chemotherapy. However, the disease regressed in the 16th month of chemotherapy but progressed in the 26th month. When the serum values of the markers are examined, it seems that the values detected for CRP and TGF-β indicate relapse (Figure 3).

The last patient had a preoperative CA-125 level of 3000 mIU/mL. She underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy, and multiple peritoneal biopsies. Pathology revealed serous carcinoma, stage 4, grade 2. Ascites was >500 mL, and residual tumor was >2cm after surgery. No omental or diaphragmatic involvement was observed. The patient's serum samples were examined at the 42nd and 55th months. The patient had recurrences at 24th and 55th months (Figure 4), and she received a total of 30 courses of chemotherapy.

DISCUSSION

We found significantly higher CA-125, TGF-β, and CRP values in women with stage III and IV ovarian epithelial cancer. The mortality rate was 20% in a mean 30.5±9.5 months' time period in these women.

Ovarian cancer is the fifth most common cancer among women and the second most common among

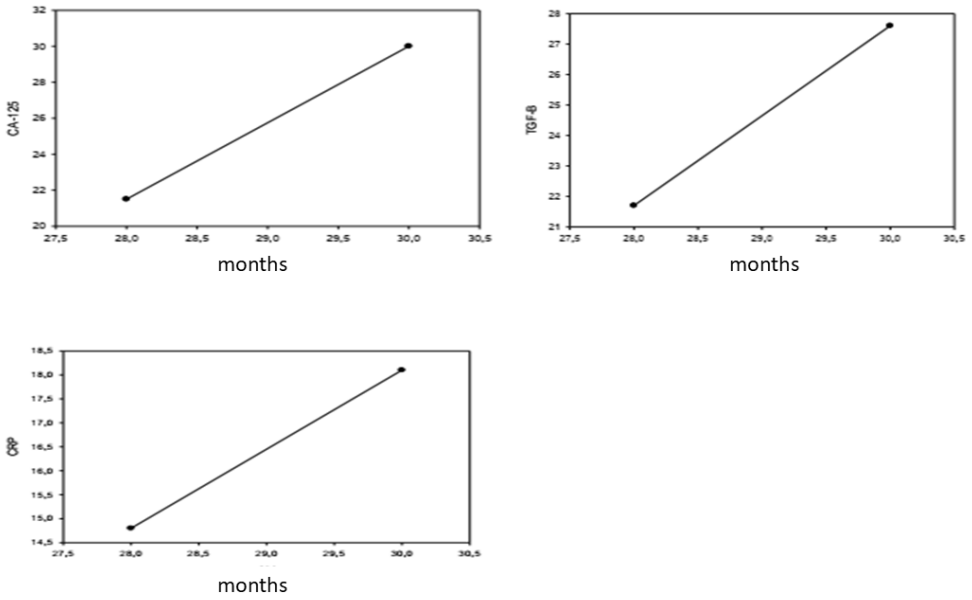

FIGURE 1. Monthly blood levels of biomarkers of a 57-year-old patient who developed recurrence in the 22nd month and underwent re-laparotomy in the 29th month.

gynecological cancers, and it has the highest mortality rate. Because the symptoms of epithelial ovarian cancer (EOC) are nonspecific in the early stages, most patients are in advanced stages at diagnosis. A screening method has not yet been developed for early diagnosis. Further research on biomarkers is important to facilitate prognosis and screening. Studies on EOC have identified cytokines, cancer growth factors, and various genetic mutations that contribute to the development of the disease. Therefore, EOC is thought to result from a multistep carcinogenesis process. For a tumor to manifest clinically, it must overcome many protective mechanisms. These include inhibition of apoptosis, stimulation of angiogenesis, and stromal invasion. Numerous studies support the notion that inflammation increases the risk of EOC [9]. Inflammation of the ovarian surface epithelium is observed in ovulation, endometriosis, and pelvic inflammatory disease. These are known to increase the risk of EOC, particularly by activating cytokines such

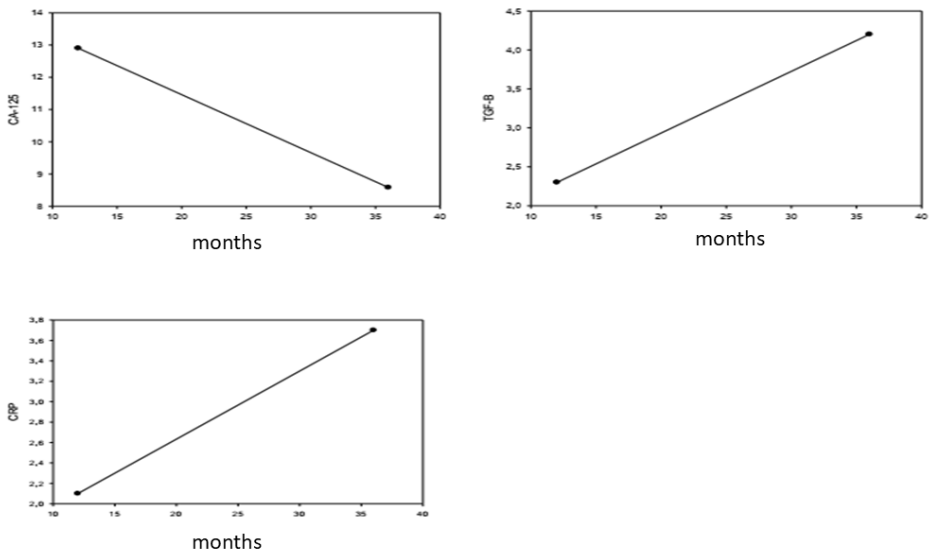

FIGURE 2. Monthly blood levels of biomarkers of a 77-year-old patient with no recurrence detected during follow-up.

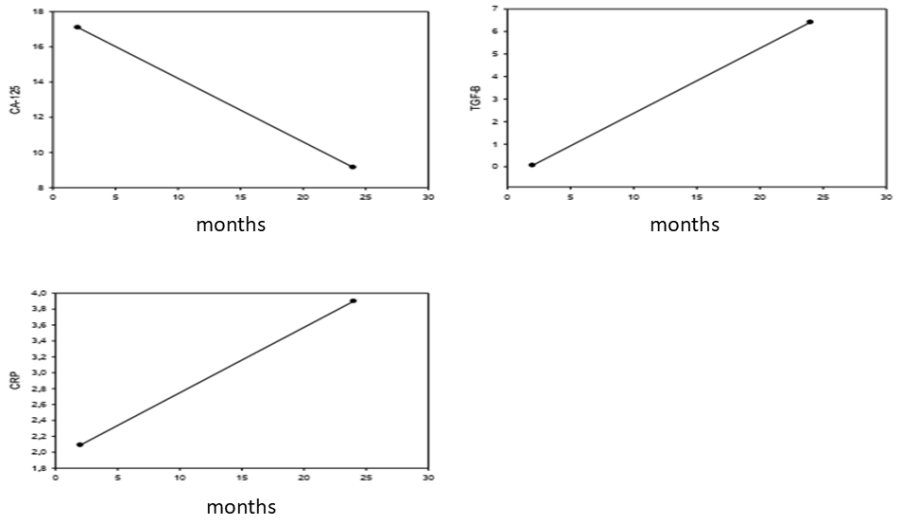

FIGURE 3. **Monthly blood levels of biomarkers of a 50-year-old patient who did not develop recurrence during follow-up.**

as TNFα, interleukin (IL)-1b, and IL-6 [10, 11]. We have found elevated TGF-β and CRP levels in our study. Although our study is a small sample-sized study and the study protocol cannot reveal the relation between inflammation and tumorigenesis, we think that the elevated TGF-β and CRP levels may be a result rather than the cause of the tumoral process.

The majority of epithelial ovarian cancers are serous tumors and are divided into two groups: type 1 and type 2 [12]. Type 1 serous tumors are reported to be low-grade and associated with K-Ras and BRAF gene mutations, while type 2 serous tumors exhibit p53 alterations. Type 2 serous tumors are also thought to arise from tubal intraepithelial carcinoma [12, 13]. Furthermore, the tubal epithelium of women with BRCA1/2 mutations is thought to be associated with high-grade tumor gene expression. In our study, 75% of our patients had serous type and the six women who died also had all serous type. Clinical follow-up of these patients indicated that these women relapsed early and did not respond to chemotherapy, suggesting that they may have type 2 serous tumors.

One of the most studied biomarkers for early diagnosis and screening in EOC is CA-125. It is an important biomarker for disease progression and recurrence [14]. CA-125 is also elevated in benign

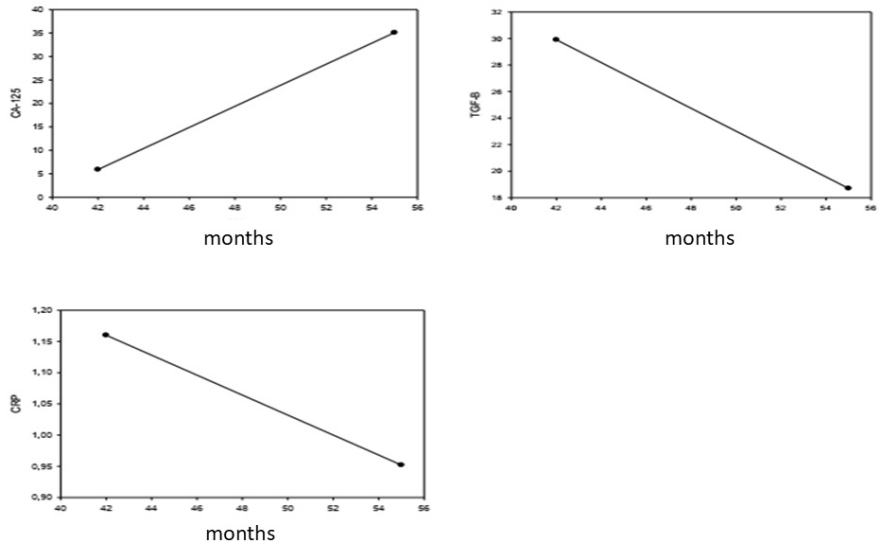

FIGURE 4. **Monthly blood biomarker levels of a 52-year-old patient who developed relapse in the 24th and 55th months.**

conditions such as endometriosis and fibroids. Therefore, it is not a reliable diagnostic marker on its own [15]. Additional biomarkers or molecular biomarker panels should be used for early detection. In our study, all women had advanced-stage ovarian cancer, and thus, as expected, significantly elevated CA-125 levels were found. Actually, CA-125 was suggested not for the diagnosis but for the follow-up in women with ovarian cancer [16].

Peritoneal spread is one of the most common routes of spread in ovarian cancer. Some publications suggest that the amount of intra-abdominal ascites in ovarian cancer may be associated with TGF-β [17]. In our study, no significant difference was found between TGF-β and CRP in terms of survival, but TGF-β and CRP were found to be effective markers for diagnosis when compared with the postmenopausal healthy patient group. When we examined the above mentioned four patients, ascites was present in three, but one woman had no ascites. The woman without ascites received neoadjuvant chemotherapy and had a decreasing trend in TGF-β levels. The TGF-β levels increased in three women who developed ascites, and all had an increasing trend in TGF-β levels. This shows that TGF-β levels can be associated with ascites in women with EOC. Omental metastasis is also a prognostic factor in advanced ovarian cancer [18]. The TGF-β signaling pathway was found to play an active role in omental metastases in ovarian cancer [17]. In our study, when we examined the omental involvement and TGF-β levels in the aforementioned four patients, three had high, but one patient had low TGF-β levels. Like the presence of ascites, a low TGF-β value might be a result of neoadjuvant chemotherapy in that woman who received neoadjuvant chemotherapy. For this reason, we can speculate that preoperative TGF-β levels may be useful for determining the extent of disease, the need for omentectomy, and selecting neoadjuvant chemotherapy.

High preoperative CRP levels can provide information about residual tumors after cytoreductive surgery. This can also help us to select patients who would receive neoadjuvant chemotherapy before cytoreductive surgery. In our patient group, we observed that all four patients had high preoperative CRP values. All four women had residual tumors <2 cm. In line with previous studies, despite the small number of patients, 3 out of 4 showed elevated CRP levels during the follow-up period. Because CRP is an inexpensive and easily obtained method, routine screening can be done. However, this recommendation should be supported by larger population studies for CRP to become a marker that can play a role in ovarian cancer treatment, follow-up, and surgical planning. Because inflammation is known to promote metastasis and increase angiogenesis, high serum CRP levels may reflect increased metastatic potential [8, 19]. On the other hand, we can speculate that elevated serum CRP levels can be an effect of platinum-based chemotherapy.

In our patient group, due to the individual chart evaluations of the four patients, 37% had concordant chart results for TGF-β, CRP, and CA-125. Figure 3 shows an atypical pattern of TGF-β and CRP elevations, suggesting inflammation is negative for tumoral disease but suggests another inflammatory cause. Figure 4 shows an atypical pattern of TGF-β, CRP, and CA-125 patterns for tumoral disease and inflammation. Subsequent CA-125 measurements (CA-125: 153 mIU/mL at the 3rd month follow-up) suggest that the CA-125 elevation indicates tumoral disease, but TGF-β and CRP values seems associated with other inflammatory causes and do not indicate a link between tumoral disease and inflammation. Thus, inflammatory markers can be affected by many factors during treatment. Prospective studies with large patient populations that examine all data individually, including clinical follow-up issues, are needed for more definitive information.

Strengths and Limitations

We found significantly higher CA-125, TGF-β, and CRP values in women with stage III and IV ovarian epithelial cancer. The mortality rate was 20% in a mean 30.5±9.5 months' time period in these women. Also, we demonstrated that advanced-stage ovarian cancer is associated with inflammatory markers. These markers can be considered in patients' clinical follow-up. Although our study is a single-center study, the most important limitation of our study is the small number of patients. Also, blood parameter assessments in our study were performed using ELISA, but the more sensitive RT-PCR method could not be used. Also, the markers are obtained at different time periods which may cause divergent results.

CONCLUSION

In this study, we found that advanced-stage ovarian cancer is associated with inflammatory markers. These markers can be considered in patients' clinical follow-up. Our patient-based analyses demonstrated that TGF-β and CRP levels may be poor prognosticators. Multicenter studies with larger patient populations are needed to obtain more definitive results.

Ethics Approval and Consent to Participate

This study was approved by the Trakya University Faculty of Medicine Clinical Research Ethics Committee (Decision No: 209/06; date: 18.11.2009). 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 patients provided informed consent before surgery.

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

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. 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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Malignancy and Mortality in Idiopathic Inflammatory Myositis: A Retrospective Single-Center Cohort Study

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Abstract:

Objective: Idiopathic inflammatory myositis (IIMs) comprise a heterogeneous group of rare autoimmune muscle diseases characterized by variable clinical presentations and outcomes. While infections are a common cause of early mortality, malignancy has been recognized as the predominant driver of late mortality. This study aimed to evaluate malignancy frequency, mortality, and associated factors in a single-center IIM cohort.

Methods: In this retrospective cohort study, all patients diagnosed with IIM and followed at Pamukkale University Faculty of Medicine between 2010 and 2025 were analyzed. Mortality rates, malignancy prevalence, and potential demographic and clinical determinants were assessed. Multivariable logistic regression analyses were performed, and results were reported as odds ratios with 95% confidence intervals. A two-sided P-value <0.05 was considered statistically significant.

Results: A total of 66 patients were included, nearly three-quarters of whom were female. Concomitant malignancy was identified in 8 (12.1%) patients, and 10 (15.2%) patients died during the follow-up period. Mortality was substantially higher among patients with malignancy compared with those without cancer (50.0% vs. 10.3%). When survivors were compared with deceased patients, the latter exhibited a significantly greater comorbidity burden and a higher prevalence of malignancy. The comorbidity burden did not reach statistical significance in multivariable analyses. Kaplan–Meier survival analysis demonstrated significantly reduced overall survival in patients with concomitant malignancy compared with those without malignancy (log-rank P=0.014).

Conclusion: The prevalence of malignancy in this cohort was consistent with previously published data, supporting the external validity of our findings. Mortality was markedly higher among patients with cancer, and malignancy was disproportionately represented among deceased individuals. Survival analyses further underscored malignancy as a major determinant of poor outcomes in patients with IIMs.

Keywords: Idiopathic Inflammatory Myositis, Mortality, Neoplasms

Idiopathic inflammatory myositis (IIM) represent a rare group of autoimmune muscle disorders that may manifest across all age groups, most characteristically through proximal muscle weakness associated with myositis-specific (MSA) or myositis-associated autoantibodies (MAA). They remain uncommon in the general population, with an estimated prevalence ranging between 0.2 and 2 cases per 100.000 individuals [1]. Accurate diagnosis of IIM relies on an integrated approach, combining autoantibody profiling, advanced imaging such as muscle magnetic resonance imaging (MRI),

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electrophysiological assessment with myopathy electromyography (EMG), and histopathological confirmation by muscle biopsy [2].

Advanced age and diagnostic delay are key determinants of poor long-term prognosis in IIM [3]. Another important determinant was the presence of systemic involvement - particularly when the lungs or heart were affected - which consistently signalled higher mortality. Rapidly progressive interstitial lung disease (ILD) develops in roughly 10% of patients and represents one of the most life-threatening complications of myositis, yet even under optimal management the five-year mortality rate can approach 40% [4]. Patients with IIM are particularly susceptible to infectious complications, with pulmonary infections representing the most common and another clinically significant drivers of mortality [5].

Malignancy represents the other key driver of long-term outcomes, exerting a substantial influence on overall mortality in patients with IIM. The link between IIM and cancer is well established, and neoplastic disease remains one of the principal contributors to death [6]. During the first three years following diagnosis, patients face the highest risk for the development of malignancy [7]. Advanced age, male sex, dysphagia, rapid disease onset, the presence of vasculitis, and cutaneous necrosis have all been associated with an increased risk of malignancy [8]. Moreover, certain subtypes of IIM - most notably dermatomyositis (DM) - along with distinct serological profiles, confer an increased risk of malignancy. Specific autoantibodies, including anti-Transcription Intermediary Factor 1-gamma antibody (anti-TIF1γ) and anti-Nuclear Matrix Protein 2 antibody (anti-NXP2), have emerged as clinically relevant biomarkers, enabling more accurate stratification of cancer risk in affected patients [9]. The newly introduced Epidemiological Useful Clinical-Laboratory-Imaging Development Screening (EUCLIDES) algorithm guides cancer risk assessment in myositis by integrating epidemiological factors, proven screening tools, clinical-laboratory data, and tailored imaging strategies [10]. This algorithm is increasingly being used to stratify cancer risk in patients with myositis [11]. For patients stratified as high risk, current guidelines advise an expanded and more frequent screening approach at diagnosis and throughout the first three years of follow-up [12].

In this field, most available evidence comes from local cohorts with limited geographic and population diversity. This gap is particularly evident in Türkiye, where no up-to-date, standardised screening pathway for myositis-associated malignancy exists and where multicentre data on cancer incidence and mortality in IIM are lacking. In a population-based study from Türkiye conducted by Trakya University between 2004 and 2014, the annual incidence of DM was estimated at 3.7 per million person-years, with an overall prevalence of 32.2 per million. Importantly, this cohort did not report outcomes related to mortality or malignancy [13]. For this reason, we designed the present study to provide locally relevant. We aimed to evaluate our myositis cohort with respect to mortality and malignancy ratios, as well as the associated factors.

METHODS

Study Design and Cohort Definition

This retrospective cohort study included adults diagnosed with IIMs who were evaluated at our tertiary referral center between 2010 and 2025. Only patients aged ≥18 years were eligible. To ensure diagnostic precision, we included exclusively prevalent adult IIM cases fulfilling the 2017 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) [14]. Clinically suspected but unclassified myositis, isolated hyper-CKemia, and non-autoimmune inflammatory myopathies (e.g., toxic, metabolic, infectious) were excluded. Patients lacking essential diagnostic data (clinical, serological, or histopathological) or without sufficient follow-up information were also excluded.

IIM Subtype Classification and Autoantibody Profiling

Each patient was assigned to an IIM subtype using integrated clinical, serological, and histopathological features. In this cohort, subtypes included DM and polymyositis (PM), and myositis overlap syndromes. MSAs and MAAs were evaluated using standardized commercial platforms, primarily line-blot immunoassays (Euroline Myositis Profile, Euroimmun, Lübeck, Germany) and, when applicable, enzyme-linked immunosorbent assay (ELISA) kits from the same manufacturer. The MSA panel

comprised antibodies known to define major clinical phenotypes, including anti-TIF1γ, anti-NXP2, anti–Mi-2, anti–melanoma differentiation–associated protein 5 (anti-MDA5), anti–small ubiquitin-like modifier activating enzyme (anti-SAE1), anti–3-hydroxy-3-methylglutaryl-coenzyme A reductase (anti-HMGCR), anti-histidyl-tRNA synthetase (anti-Jo-1), and other antisynthetase antibodies. The MAA panel included antibodies typically associated with overlap phenotypes, namely anti-Ku, anti-PM-Scl, anti-mitochondrial antibody type M2 (AMA-M2), anti–Sjögren's syndrome–related antigen A (anti-SSA/Ro), anti–Sjögren's syndrome–related antigen B (anti-SSB/La), and extractable nuclear antigen (ENA) antibodies routinely assessed by the commercially available ENA Profile kits [15]. Because autoantibody testing availability varied across the study period, unperformed assays were coded as "not assessed" rather than negative.

Histopathology Review

Muscle biopsy findings were extracted from original pathology reports. For this exploratory analysis, histological features - including necrosis, perifascicular and/or fiber atrophy, inflammation including perivascular infiltrates, major histocompatibility complex class I (MHC-I) upregulation, and complement deposition, vasculitis - were recorded in binary format (present/absent). All specimens had been evaluated by analysing the reports of pathologists at the time of diagnosis. Although no formalized scoring system was used, assessments were performed independently and without knowledge of subsequent clinical outcomes. Centralized re-reading was not feasible due to the retrospective nature of the study.

Ascertainment of Malignancy, Mortality and Follow-up

Malignancies were identified through comprehensive review of electronic medical records, oncology notes, pathology archives, and radiological databases. No external cancer-registry linkage was available for validation. All newly diagnosed oncological disorders were included in the analysis, encompassing both melanoma and non-melanoma skin cancers as well as other malignancies identified during follow-up. Malignancies diagnosed prior to the onset of IIMs were categorized as a history of malignancy; however, for comparative analyses, patients were dichotomized into cancer and non-cancer groups irrespective of timing. Vital status was ascertained using hospital medical records and official death notification systems. Owing to restricted data access and the absence of discharge summaries for some deceased patients, the specific cause of death could not be reliably determined in all cases; limiting analyses to cases with confirmed causes would have substantially reduced the sample size and compromised statistical robustness. Follow-up time was defined as the interval from IIM diagnosis to death or the last documented clinical encounter.

Clinical Variables and Covariates

Baseline data at diagnosis included demographic characteristics, disease duration, clinical manifestations, laboratory parameters such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and creatine kinase (CK), organ involvement (skin, lung, heart), autoantibody profiles, EMG results, and detailed muscle biopsy findings. Information on induction and maintenance therapies was also recorded. ILD was confirmed using high-resolution computed tomography, which was evaluated by experienced thoracic radiologists. Longitudinal data related to malignancy and mortality were systematically documented. All variables were collected from electronic medical records using a standardized data-collection form to ensure completeness and uniformity.

Determining of the Outcome

The mortality and malignancy represent biologically distinct outcomes and should ideally be analyzed separately. However, in the present cohort, the number of events was limited (10 deaths and 8 malignancies), which precluded the construction of statistically robust multivariable models for each outcome independently. Performing separate multivariable analyses under these conditions would have substantially increased the risk of overfitting and yielded unstable estimates with wide confidence intervals. Therefore, to preserve statistical power and model stability, we employed a composite endpoint encompassing death and/or malignancy.

TABLE 1. Baseline Demographic, Clinical, Laboratory, and Treatment Characteristics of the Study Cohort

Variable	Data
Age (years)	59.5 (49-68)
Age at diagnosis (years)	50 (44-63)
Female sex	48 (72.7%)
Smoking status (never / ormer / current)	39 (59.1%) / 17 (25.8%) / 10 (15.1%)
Number of comorbidities	1 (0-2)
Erythrocyte sedimentation rate (mm/h)	22 (13-40)
CRP (mg/dL)	9.2 [4.5-23.4)
CK (U/L)	673 (228-1798)
Skin involvement	33 (50.0%)
Lung involvement	14 (21.2%)
Cardiac involvement	3 (4.5%)
Dysphagia	16 (24.2%)
Positive EMG finding	19 (28.8%)
Muscle biopsy performed	47 (71.2%)
Skin biopsy performed	25 (37.9%)
Muscle biopsy – inflammation	39 (59.1%)
Muscle biopsy – atrophy	28 (42.4%)
Muscle biopsy – vasculitis	16 (24.2%)
Muscle biopsy – necrosis	7 (10.6%)
Overlap syndrome present	21 (31.8%)
Most common overlap type	Sjögren's syndrome
Malignancy development	8 (12.1%)
Types of malignancy	Breast (n=2), thymoma (n=1), brain (n=1), ovarian (n=1), colon (n=1), nasopharynx (n=1), lung (n=1)
Timing of malignancy to diagnosis	Before: n=2; After: n=6
Mortality	10 (15.2%)
Pulse steroid ≥3 g	17 (25.8%)
IVIG use	16 (24.2%)
Induction DMARD	MTX: 29 (43%), AZA: 11(17%), CyP: 10 (15%), others: 16 (24%)
Maintenance DMARD	MTX: 25 (38%), RTX: 13 (20%), AZA: 10 (15%), others: 18 (27%)
Avascular necrosis	4 (6.1%)
Total follow-up duration (months)	48 (30-84)
Diagnosis–cancer interval (months)	Median 18 (excluding pre-diagnosis cases)

Data are shown as median (interquartile range) or n (%) where appropriate. CRP, C-reactive protein; CK, creatine kinase; EMG, electromyography; DMARD, disease-modifying antirheumatic drug; ANA, antinuclear antibody; SCC, squamous cell carcinoma; MTX, methotrexate; AZA, azathioprine; CYP, cyclophosphamide; MMF, mycophenolate mofetil; PLQ, hydroxychloroquine; RTX, rituximab; IVIG, intravenous immunoglobulin; AVN, avascular necrosis.

Ethical Considerations

Ethical approval for the study was granted by the Local Ethics Committee (Approval No. 15; Date: 12/08/2025). In accordance with national regulations governing retrospective studies without direct patient contact or identifiable intervention, the requirement for informed consent was formally waived by the ethics committee.

Statistical Analysis

All statistical analyses were performed using SPSS Statistics software (IBM Corp., Armonk, NY, USA). The normality of continuous variables was assessed using the Shapiro–Wilk test. Continuous variables not following a normal distribution were summarized as median and interquartile range (IQR), and comparisons between groups were performed using the Mann–Whitney U test. Categorical variables were presented as counts and percentages (%), and comparisons between two groups were conducted using Pearson's chi-square test or Fisher's exact test, as appropriate. Variables potentially associated with death and/or cancer development were first examined using univariate analysis. Variables with a p-value <0.10 in the univariate analysis or considered clinically relevant were included in a multivariate logistic regression model. Results of the multivariate analysis were reported as odds ratios (OR) with 95% confidence intervals (CI). For all statistical tests, P-value <0.05 was considered statistically significant.

RESULTS

This study included 66 patients, of whom 48 (72.7%) were female. The median current age was 59.5 years (IQR: 49–68), and the median age at diagnosis was 50 years (IQR: 44–63). At diagnosis, the median ESR was 22 mm/h (IQR: 13–40), CRP: 9.2 mg/dL (IQR: 4.5–23.4), and CK: 673 U/L (IQR: 228–1798). Cutaneous involvement was observed in 33 (50.0%) patients, ILD in 14 (21.2%), and cardiac involvement in 3 (4.5%). Abnormal EMG findings were detected in 19 (28.8%) patients. Muscle biopsy was performed in 47 (71.2%) patients and skin biopsy in 25 (37.9%) (Table 1).

Histopathology most commonly showed inflammation (59.1%), followed by atrophy (42.4%), vasculitis (24.2%), and necrosis (10.6%). Overlap syndromes were present in 21 patients (31.8%), most frequently Sjögren's syndrome (SjS), with less frequent associations with Systemic Lupus Erythematosus (SLE) and Systemic sclerosis (SSc). Within the serological profile of IIM, anti-Jo-1 is the most frequently detected MSA, whereas anti-SSA predominates among MAA. Notably, Antinuclear Antibody (ANA) positivity is strikingly high, observed in approximately 77% of patients (Table 1). In this cohort, malignancy was identified in 8 (12.1%) patients. Of these, 2 patients had a documented history of malignancy prior to the diagnosis of IIMs, while malignancy developed after IIM diagnosis in the remaining 6 patients. Ten (15.2%) patients died during follow-up. Pulse steroid therapy ≥3 g was administered to 17 (25.8%) patients, and Intravenous immunoglobulin (IVIG) to 16 (24.2%). Methotrexate (MTX), was the most frequently used induction and maintenance Disease-Modifying Antirheumatic Drug (DMARD), followed by Azathioprine (AZA), Cyclophosphamide (CyP) for induction regimen. Avascular necrosis occurred in 4 (6.1%) patients. The median follow-up duration was 48 months (IQR: 30–84), and among patients with malignancy diagnosed after myositis, the median time to cancer was 18 months (Table 1).

In the comparison between patients with and without malignancy (Table 2), those in the cancer group were significantly older at both the time of evaluation (median 67.0 vs. 57.5 years, P=0.012) and at diagnosis (median 62.5 vs. 48.0 years, P=0.010). The cancer group also exhibited higher median ESR values (41.5 vs. 19.5 mm/h, P=0.016). Mortality was markedly higher among patients with malignancy (50.0% vs. 10.3%, P=0.009), suggesting that malignancy may be a major contributor to increased mortality in myositis.

In this cohort of 66 patients, 10 individuals (15.2%) died during follow-up (Table 3). Patients dead in the follow up had significantly higher median age and age at diagnosis compared with survivors (P=0.010 and P=0.023, respectively). The number of comorbidities and presence of cancer were also significantly greater among deceased patients (median=2, IQR 1–2; P=0.001), suggesting a substantial impact of coexisting diseases on survival.

TABLE 2. Comparison of Clinical and Laboratory Characteristics Between Patients with and Without Malignancy

Variable	Malignancy (+) (n=8)	Malignancy (–) (n=58)	P-value
Age (years)	67.0 (63.8–72.0)	57.5 (44.2–65.8)	**0.012**
Age at diagnosis (years)	62.5 (56.5–64.2)	48.0 (39.2–57.8)	**0.010**
CK (U/L)	278.0 (154.2–426.2)	960.0 (286.2–1978.0)	0.102
CRP (mg/dL)	4.5 (4.5–4.5)	5.0 (3.0–15.0)	1.000
ESR (mm/h)	41.5 (28.5–45.8)	19.5 (14.0–34.8)	**0.016**
Comorbidity count	1 (0–2)	0 (0–1)	0.292
Follow-up (months)	84 (24–84)	48 (24–96)	0.635
Female sex	6 (75.0%)	42 (72.4%)	1.000
Smoking status			0.879
Never	4 (50.0%)	35 (60.3%)	
Former	3 (37.5%)	14 (24.1%)	
Current	1 (12.5%)	9 (15.5%)	
Skin involvement	3 (37.5%)	30 (51.7%)	0.490
Lung involvement	2 (25.0%)	12 (20.7%)	1.000
Cardiac involvement	1 (12.5%)	2 (3.4%)	0.338
Dysphagia	3 (37.5%)	13 (22.4%)	0.386
Overlap syndrome	4 (50.0%)	17 (29.3%)	0.244
Muscle biopsy performed	7 (87.5%)	40 (69.0%)	0.420
Inflammation	5 (62.5%)	34 (58.6%)	1.000
Atrophy	4 (50.0%)	24 (41.4%)	0.709
Vasculitis	3 (37.5%)	13 (22.4%)	0.386
Necrosis	2 (25.0%)	5 (8.6%)	0.193
Pulse steroid ≥ 3 g	3 (37.5%)	14 (24.1%)	0.398
IVIG use	3 (37.5%)	13 (22.4%)	0.386
Mortality	4 (50.0%)	6 (10.3%)	**0.009**

Data are shown as median (interquartile range) or n (%) where appropriate. CK, creatine kinase; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IVIG, intravenous immunoglobulin.

Statistically significant P-values are shown in bold.

In this study, death and/or cancer occurrence was defined as a "composite endpoint," and all potential demographic, clinical, laboratory, and histopathological variables were evaluated using logistic regression analysis. In the univariable analysis, a higher number of comorbidities was associated with increased risk (OR=8.61, P=0.012). In the multivariable logistic regression model (Table 4), neither older age nor a higher comorbidity burden reached statistical significance. Age was associated with an odds ratio (OR) of 1.056 (P=0.081), while the cumulative number of comorbidities showed an OR of 2.082 (P=0.074). No significant associations were found between organ involvement, muscle involvement patterns, specific autoantibody profiles, or therapeutic regimens, including pulse corticosteroids, IVIG, MTX, AZA, CyP, rituximab (RTX), mycophenolate mofetil (MMF), and the occurrence of cancer or death.

Patients with malignancy exhibited a markedly higher mortality rate compared with those without

TABLE 3. Comparison of Clinical Variables by Mortality Status

Variable	Death (+)	Death (–)	P-value
Age (years)	70.0 (65.0–73.8)	58.0 (44.8–65.0)	**0.010**
Age at diagnosis (years)	62.0 (55.5–64.8)	48.0 (39.8–57.2)	**0.023**
CK (U/L)	796.0 (195.2–1822.5)	673.0 (173.5–1964.0)	0.950
CRP (mg/dL)	4.5 (4.5–4.5)	5.0 (3.0–15.0)	1.000
ESR (mm/h)	29.5 (17.5–47.0)	22.5 (14.0–36.8)	0.220
Number of comorbidities	2.0 (1.0–2.0)	0.0 (0.0–1.0)	**0.001**
Presence of cancer	4 (40%)	4 (6.9%)	**0.015**
Follow-up duration (months)	54.0 (27.0–81.0)	48.0 (24.0–108.0)	0.726

Data are shown as median (interquartile range) or n (%) where appropriate. CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; CK, creatine kinase.

Statistically significant P-values are shown in bold.

malignancy. Owing to the small number of events, Fisher's exact test was applied to assess between-group differences (P=0.014) (Table 5).

Kaplan–Meier survival curves comparing overall survival between patients with and without malignancy. Patients with malignancy (red line, n=8) exhibited a markedly lower cumulative survival probability over the follow-up period compared with patients without malignancy (blue line, n=58). Survival probability declined more rapidly in the malignancy group, whereas survival remained relatively stable among patients without cancer. The between-group difference was statistically significant (P=0.014, log-rank test) (Figure 1).

DISCUSSION

In this study, we examined a wide spectrum of clinical, serological, histopathological, and treatment-related variables to assess their potential relationships with malignancy and mortality in IIMs. Mortality was substantially higher in patients with cancer, and those who died exhibited a greater comorbidity burden together with a higher prevalence of malignancy than survivors. Notably, our work contributes one of the few datasets from Türkiye to address malignancy- and mortality-related patterns in IIMs, providing region-specific evidence in an area where the literature remains limited. In a large multicenter retrospective cohort from Spain, Nuño-Nuño *et al*. [16], likewise reported considerable mortality among patients with IIM. They identified advanced age at diagnosis, the presence of cancer, and elevated disease activity at onset as independent predictors of poor prognosis. Over a median follow-up of 9.7 years, the mortality rate was markedly higher than in our cohort, reaching 24%. Consistent with our findings, cancer was among the leading causes of death [16]. Similarly, in our cohort, older age was significantly more common both among patients who died compared with survivors and among those with malignancy compared with those

TABLE 4. Multivariable Logistic Regression Analysis for Predictors of Death and/or Cancer in Patients with Myositis

Variable	β Coefficient	OR	95% CI (Lower–Upper)	P-value
Older age (years)	0.055	1.056	0.993 – 1.123	0.081
Number of comorbidities	0.734	2.082	0.928 – 4.668	0.074
Muscle biopsy – inflammation	1.063	2.893	0.619 – 13.527	0.182

OR, odds ratio; CI, confidence interval.

TABLE 5. Mortality According to Malignancy Status in Patients with Idiopathic Inflammatory Myopathies

Malignancy status	Total patients (n)	Deaths (n)	Mortality rate (%)	P-value
No malignancy	58	6	10.3	**0.014***
Malignancy present	8	4	50.0	

*Fisher's exact test. Statistically significant P-value is shown in bold.

without. Several studies have published registry-based data describing the clinical features of patients with IIM, including the frequency and distribution of associated malignancies. For instance, a study from South Australia found a modestly increased cancer risk in IIM patients compared with the general population (standardized incidence ratio = 1.39; P=0.047). Lung and prostate cancers were the most frequently observed malignancies, with 28% diagnosed within the first year after IIM onset [17]. In our cohort, the overall malignancy rate was 12.1%, with breast cancer representing the most frequent subtype. In a similar fashion, a Colombian cohort of 112 patients with IIM reported a malignancy rate comparable to ours at 11.6%. In that study, thyroid cancer was the most frequently observed type, with a mean interval of 11 months to cancer development [18]. In another DM cohort, although the overall prevalence of malignancy was comparatively low, breast cancer again emerged as the most commonly reported type, mirroring our findings [19]. This observation may, at least in part, be attributable to the female predominance within our cohort. Similarly, elevated ESR levels at the time of diagnosis, in addition to increased age, have been identified in several studies as factors associated with malignancy development [20].

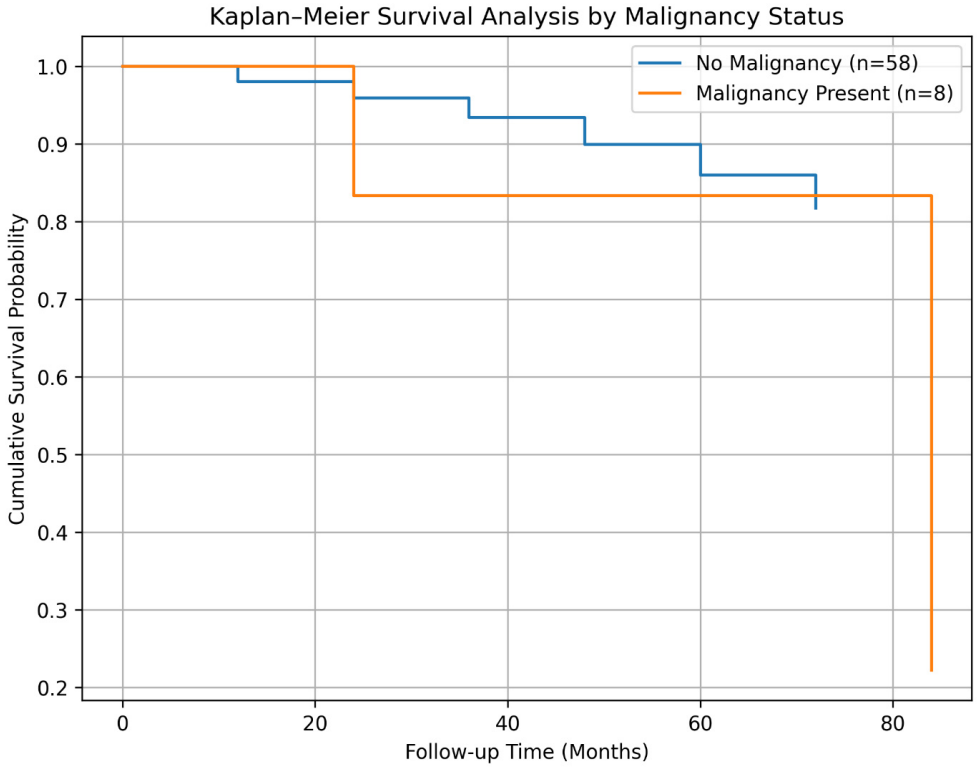

FIGURE 1. Kaplan–Meier survival curves illustrating significantly reduced overall survival in patients with idiopathic inflammatory myopathies and concomitant malignancy compared with those without malignancy.

In our cohort, malignancy developed after a median of 18 months from the diagnosis of myositis. A population-based cohort study from Sweden, published in 2021, examined 1.419 patients with IIM and found a markedly elevated risk of cancer both before and after the diagnosis of myositis. The risk was already increased prior to disease onset (OR 1.5; 95% CI 1.3–1.8), and remained high following diagnosis, with the spectrum of cancer types varying depending on whether they developed before or after myositis [21]. Interestingly, two patients were diagnosed with malignancy prior to the diagnosis of myositis in our cohort.

In a 2024 review, El Hasbani *et al*. [22], reported that smoking aggravates the development of ILD and accelerates atherosclerosis in patients with IIM. In another study, anti-TIF1γ positivity was shown to be associated with lung cancer in patients with elderly onset DM, whereas the presence of ILD appeared to play a protective role. In that study, smoking history was also found to increase the risk of lung cancer [23].There was no difference in smoking status between the groups with and without cancer in our study.

In a biopsy-confirmed IIM, distinct muscle biopsy patterns align with specific myositis autoantibodies [24]. In addition, in a juvenile DM cohort, a higher total histopathology score at baseline was found to be predictive of a long-term treatment necessity and prognosis [25]. According to recent studies, no appreciable differences have been identified in the muscle pathology of patients with PM, irrespective of malignancy status. In contrast, among individuals with DM, rare infiltrative pathological patterns occur more frequently in those with concomitant cancer, suggesting a potentially meaningful association [26]. However, the biopsy features were comparable between patients with and without malignancy in our cohort.

Strengths and Limitations

The principal limitations of this study arise from its single-center and retrospective design, which may constrain the generalizability of the findings. The modest sample size and low number of outcome events also limit the statistical power to detect subtle or clinically meaningful associations. Furthermore, the extended inclusion period (2010–2025) introduces potential era-related variability, as antibody assays, imaging techniques, malignancy screening protocols, and therapeutic strategies have evolved considerably over time. These changes may have contributed to surveillance or ascertainment bias. Additional methodological constraints include the lack of standardized scoring for pathological assessments. As with all observational studies, causal relationships cannot be inferred. A larger, multicenter cohorts will be required to disentangle predictors of mortality and malignancy as separate endpoints.

Despite these limitations, the study has notable strengths. It represents one of the earlier efforts from Türkiye to investigate malignancy and mortality in IIMs and integrates comprehensive histopathological evaluations alongside clinical and serological data. As a tertiary referral center, our institution manages complex cases from multiple surrounding provinces, a factor that likely enhances the representativeness of our cohort and provides a more accurate reflection of the regional patient population.

CONCLUSION

The prevalence of malignancy in our cohort was comparable to previously reported rates, indicating broad agreement with the existing literature. Mortality was higher among patients with cancer, and those who died tended to have both malignancy and a greater burden of comorbid conditions. Although older age and a higher comorbidity burden showed a tendency toward poorer outcomes, these associations did not reach statistical significance and should be interpreted cautiously. Moving forward, larger multicenter studies incorporating time-to-event analyses will be crucial to determine whether these variables act as independent predictors and to enhance risk stratification strategies for patients with IIMs.

Ethics Approval and Consent to Participate

This study was approved by the Pamukkale University Non-Interventional Clinical Research Ethics Committee (Decision No: 2025/15; date: 12.08.2025). 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: FU; Study Design: FU; Supervision: VÇ; Funding: N/A; Materials: FU; Data Collection and/or Processing: FU; Statistical Analysis and/or Data Interpretation: FU; Literature Review: FU, VÇ; Manuscript Preparation: FU; and Critical Review: FU, VÇ.

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ChatGPT (version 5.1) was used solely for English language editing, including grammar, wording, coherence, and clarity. No artificial intelligence tools were involved in the study design, data collection, analysis, statistical evaluation, interpretation of results, or literature search. All scientific content and conclusions are the full responsibility of the authors and adhere to established academic and ethical standards.

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Effects of Emergency Department Analgesia on the Management Timeline and Clinical Outcomes of Acute Appendicitis

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Abstract:

Objective: This study aimed to evaluate the association between the administration of analgesics (Opioids or Nonsteroidal Anti-Inflammatory Drugs [NSAID]) after the initial examination in the emergency department and the diagnostic process of acute appendicitis, the timing of surgery, and postoperative complications in patients presenting with abdominal pain and suspected acute appendicitis.

Methods: A total of 925 patients aged 18–65 years who underwent surgery for acute appendicitis between January 2020 and May 2025 were retrospectively reviewed. Two age- and sex-matched groups were formed: 98 patients who experienced a delay in surgical treatment (case group) and 98 patients who underwent surgery within 24 hours following diagnosis (control group). Delay in surgical treatment was defined as an interval exceeding 24 hours from the initial examination to surgery. Early analgesia was defined as the administration of parenteral analgesics after the initial examination. Group comparisons used Fisher's exact test for categorical variables and t-test or Mann–Whitney U test as appropriate for continuous variables, with two-sided α=0.05.

Results: The groups were similar with respect to demographic and clinical characteristics. The rates of early opioid use were 24.5% in the case group and 26.5% in the control group, with no significant difference between them (P=0.742). In contrast, early NSAID use was significantly higher in the case group (32.7% vs. 18.4%, P=0.021). The delayed-treatment group had markedly higher rates of perforation (28.6% vs. 12.2%, P=0.004) and postoperative abscess (14.3% vs. 4.1%, P=0.013).

Conclusion: Early opioid analgesia was not associated with diagnostic delay or complications, suggesting it may be used safely in patients with acute appendicitis. In contrast, NSAID administration was associated with diagnostic delays, leading to postponed surgical intervention and higher complication rates. It is therefore advisable to avoid NSAID use, particularly in patients presenting with atypical abdominal pain.

Keywords: Acute Appendicitis, Analgesia, Opioids, Nonsteroidal Anti-Inflammatory Drugs

Acute appendicitis is one of the most common surgical causes of acute abdominal pain in patients presenting to the emergency department [1]. The optimal treatment for patients diagnosed with acute appendicitis is early appendectomy, which helps prevent complications [2]. However, the diagnostic

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process can sometimes be challenging, and delays in diagnosis and treatment may result in undesirable outcomes such as perforation, intra-abdominal abscess, and increased morbidity [3]. Pain control is a fundamental component of patient care in the emergency department. However, the traditional surgical perspective has opposed the use of analgesics before a definitive diagnosis, due to concerns that analgesia might obscure clinical findings [4]. More recent studies, however, have demonstrated that early administration of opioid analgesics does not negatively affect diagnostic accuracy [5, 6]. International guidelines likewise recommend adequate management of pain [7]. Nonetheless, the majority of studies in the literature have primarily focused on opioid analgesics. The role of nonsteroidal anti-inflammatory drugs (NSAIDs) in acute appendicitis has been less extensively investigated [8]. By suppressing inflammation and peritoneal irritation, NSAIDs may mask the clinical presentation, potentially increase the risk of misdiagnosis and adversely affect the timing of treatment [9, 10]. Evidence supporting the suppressive effect of NSAIDs on peritoneal inflammation has been reported in animal experiments and case series [11, 12]. This study aimed to evaluate the impact of early administration of opioid and NSAID analgesia on the diagnostic process and surgical management in patients presenting with suspected acute appendicitis.

METHODS

Our study included patients who presented with abdominal pain to the Emergency Department of Amasya University Şerefeddin Sabuncuoğlu Training and Research Hospital between January 2020 and May 2025 and were diagnosed with acute appendicitis. The study protocol was approved by the Amasya University Non-Interventional Research Ethics Committee (Protocol No: 2025/145).

The case group comprised patients with a treatment delay, defined a priori as >24 hours from the first ED assessment to the start of surgery. Controls were drawn from the same source population and time window and underwent surgery within 24 hours. For each case, one control was randomly selected from all eligible candidates matched on age (±5 years) and sex to minimize confounding by these strong determinants of presentation and outcomes. Importantly, analgesic exposure (opioid or NSAID) was not used as a matching factor to avoid over-matching. This design reduces the risk of selection bias because cases and controls arise from the same underlying population under identical ascertainment procedures.

For each patient in the case group, a control patient was randomly selected from the control group matched for age (±5 years) and sex. In total, 98 matched controls were identified for 98 cases. Data were obtained from the hospital information management system (HBYS) and patient medical records.

Age, sex, and body mass index (BMI); The time interval from symptom onset to emergency department presentation. vital signs including temperature, heart rate, and blood pressure, Leukocyte counts and C reactive protein (CRP) level, Diagnostic imaging modalities (Ultrasonography, Computed Tomography) and radiology reports; Type of analgesics administered (opioids: morphine, pethidine, fentanyl; NSAIDs: ketorolac, diclofenac), along with dosage and timing of administration; Time of surgery, surgical approach (open or laparoscopic); Complications documented in the operative notes (perforation, intra-abdominal abscess) and length of hospital stay was evaluated.

Statistical Analysis

The statistical evaluation was performed with IBM SPSS Statistics 27.0 (Windows). Normality of distribution for continuous data was assessed by the Shapiro–Wilk test. Variables showing normal distribution were summarized as mean±standard deviation; otherwise, medians were reported. Intergroup differences were analyzed using the Independent Samples t-test for normally distributed data and the Mann–Whitney U test for data without normal distribution. Categorical variables were expressed as frequency and percentage, and Fisher's exact test was applied for comparisons. Results with P-values below 0.05 were considered significant.

RESULTS

Between January 2020 and May 2025, a total of 925 patients underwent surgery for acute appendicitis.

TABLE 1. Baseline Demographic and Clinical Profiles of the Case and Control Groups

Variable	Case group (n=98)	Control group (n=98)	P-value
Age (years)	36.4±12.8	35.9±13.1	0.785
Sex (female/male)	53 / 45	54 / 44	0.886
Body mass index (kg/m²)	26.1±4.3	25.7±3.9	0.482
Symptom duration (hours)	28.5 (18.0–42.3)	26.0 (16.0–38.0)	0.224
Admission temperature (°C)	37.6±0.8	37.5±0.7	0.341
Admission heart rate (beats/min)	92±14	90±13	0.278
Leukocyte count (×10³/µL)	14.8±3.9	14.3±3.5	0.321
CRP (mg/L)	48.5 (22.0–95.8)	42.0 (18.3–88.0)	0.189

Data are shown as mean±standard deviation or frequency (n) or median (minimum-maximum). CRP, C-reactive protein

Among them, 98 (10.6%) patients met the criterion of-diagnostic delay exceeding 24 hours and were assigned to the case group. For each case, one control patient matched by age and sex was selected, resulting in a control group of 98 patients. The demographic and baseline clinical characteristics of both groups are compared in Table 1. No statistically significant differences were observed between the groups regarding body mass index, the interval from symptom onset to emergency department admission (hours), body temperature (°C), heart rate, leukocyte count, or C-reactive protein (CRP) level (P>0.05).

The distribution and types of analgesic use in the case and control groups are presented in Table 2. The proportion of patients who received early analgesia (opioid or NSAID) was higher in the case group (57.1%) compared to the control group (44.9%); although this difference did not achieve statistical significance, it was close to the borderline level (P=0.087). The frequency of opioid administration was comparable between the groups (24.5% in the case group vs. 26.5% in the control group; P=0.742). By contrast, NSAID use was significantly more frequent in the case group (32.7% vs. 18.4%; P=0.021). The significant association found between NSAID use and a delay in surgical treatment suggests that NSAIDs may represent a potential risk factor.

Postoperative complication rates for the case and control groups are detailed in Table 3. The incidence of perforation was 28.6% in the case group compared with 12.2% in the control group (P=0.004). The occurrence of postoperative abscess was also significantly higher among case group (14.3% vs. 4.1%; P=0.013). Although gangrenous appendicitis was more frequent in the case group (16.3% vs. 9.2%), this difference did not reach statistical significance (P=0.134). The total major complication rate (including perforation, abscess formation, and gangrene) was significantly higher in the case group (48.0%) compared to the control group (24.5%; P=0.001)

Table 4 summarizes the imaging modalities and surgical techniques employed in the case and control groups. No statistically significant difference was observed between the groups regarding the use of ultra-

TABLE 2. Early Analgesic Utilization Across Case and Control Groups

Type of analgesic	Case group (n=98)	Control group (n=98)	P-value
Any early analgesic	56 (57.1%)	44 (44.9%)	0.087
Opioid	24 (24.5%)	26 (26.5%)	0.742
NSAID	32 (32.7%)	18 (18.4%)	0.021

Data are shown as frequency (%). NSAID, nonsteroidal anti-inflammatory drugs

TABLE 3. Distribution of Postoperative Complications Across Case and Control Groups

Complication	Case group (n=98)	Control group (n=98)	P-value
Perforation	28 (28.6%)	12 (12.2%)	**0.004**
Gangrenous appendicitis	16 (16.3%)	9 (9.2%)	0.134
Postoperative abscess	14 (14.3%)	4 (4.1%)	**0.013**
Surgical site infection	10 (10.2%)	7 (7.1%)	0.445
Total major complications	47 (48.0%)	24 (24.5%)	**0.001**

Data are shown as frequency (%).
Values with P<0.05 are presented in bold.

sonography or computed tomography, and the diagnostic yield for appendicitis was comparable in both modalities. Similarly, the frequency of laparoscopic procedures did not differ significantly between the two groups. In contrast, the length of hospital stay was significantly prolonged in the case group compared to the controls (3 vs. 1 days; P<0.001). This outcome highlights the adverse impact of delayed surgical intervention on hospital resource utilization.

While our age- and sex-matched design with random control selection from the same source population reduces the risk of selection bias, unmeasured confounding cannot be excluded in this retrospective, single-center study. Therefore, our findings should be interpreted as associations rather than causal effects.

DISCUSSION

This study demonstrated that the early administration of NSAIDs in patients with acute appendicitis was associated with a risk of delayed surgical treatment initiation compared to opioid use. Furthermore, the findings substantiated that this delay was correlated with more severe clinical outcomes, including higher rates of perforation and intra-abdominal abscess formation. Early use of opioids was not found to be associated with treatment delay or postoperative complications. Our findings are consistent with the existing literature regarding pain management. Several randomized controlled trials and meta-analyses have demonstrated that the administration of opioid analgesics does not significantly alter physical examination findings, does not increase the rate of misdiagnosis, and substantially improves patient comfort [5, 6, 13]. In a study conducted by Thomas *et al*. [14], the use of morphine was shown not to impair diagnostic accuracy and was even associated with improved patient satisfaction. Our study reinforces these findings, confirming that opioids can be used safely

TABLE 4. Distribution of Imaging Techniques and Operative Approaches in the Case and Control Groups

Variable	Case group (n=98)	Control group (n=98)	P-value
Ultrasonography performed	78 (79.6%)	82 (83.7%)	0.458
CT performed	65 (66.3%)	58 (59.2%)	0.295
Appendicitis detected on USG	45/78 (57.7%)	58/82 (70.7%)	0.082
Appendicitis detected on CT	52/65 (80.0%)	52/58 (89.7%)	0.138
Laparoscopic surgery	60 (61.2%)	68 (69.4%)	0.226
Length of hospital stay (days)	3.0 (2.0–6.0)	1.2 (1.0–1.8)	**<0.001**

Data are shown as frequency (%) or median (minimum-maximum). CT, computed tomography; USG, ultrasonography.
Values with P<0.05 are presented in bold.

by demonstrating this effect through a tangible clinical outcome, namely the absence of surgical treatment delay. Nevertheless, the most striking finding of our study was the indication of a potential risk associated with NSAID use. Our results support the retrospective study by Frei *et al*., which suggested that the administration of NSAIDs might be linked to delays in the surgical management of appendicitis [15]. The exact mechanism underlying this adverse effect of NSAIDs has not been fully elucidated; however, it is hypothesized that by inhibiting prostaglandin synthesis, these drugs may suppress peritoneal inflammation and thereby diminish rebound tenderness, guarding, and pain [9, 11]. Such an effect may mislead clinicians, particularly in patients who do not initially present with clear signs of peritonitis or who exhibit atypical symptoms, potentially resulting in premature discharge or postponement of diagnostic imaging [10, 16]. The observation that NSAID use is associated with diagnostic and surgical delays should be considered clinically relevant and taken into account when determining treatment strategies.

One of the most important findings of our study is that delayed surgical intervention was associated with higher complication rates. In the case group, the incidences of perforation and intra-abdominal abscess were significantly increased. These results are entirely consistent with the literature emphasizing the importance of early surgical management in appendicitis [2, 17, 18]. Consistent with the existing literature, a delay in surgical intervention was found to elevate the risk of complications by facilitating the progression of uncomplicated appendicitis to gangrenous or perforated appendicitis [19, 24].

Strengths and Limitations

This study has certain limitations. Its retrospective design necessitated reliance on previously recorded data, which may be influenced by incomplete documentation or inconsistencies in medical records. Furthermore, as the investigation was conducted in a single center, the generalizability of our findings to broader patient populations is limited. In addition, factors that could influence the choice of analgesics (such as clinician experience and variations in patients' perception of pain) could not be fully controlled. Although our results highlight a potentially important association between NSAID use and delays in surgical management, these observations alone are insufficient to establish a causal relationship.

CONCLUSION

In patients presenting to the emergency department with suspected acute appendicitis, the use of opioid analgesics was not associated with diagnostic errors, surgical delays, or an increased risk of complications, indicating that opioids can be administered safely. In contrast, early use of NSAIDs was found to be related to delays in both diagnosis and surgical intervention. Such delays were significantly associated with higher rates of perforation and intra-abdominal abscess. Emergency physicians should be aware of the potential risks of NSAID administration, particularly in patients with atypical clinical presentations, and should tailor their pain management strategies accordingly.

Ethics Approval and Consent to Participate

This study was approved by the Amasya University Non-Interventional Research Ethics Committee (Decision No: 2025/145; date: 02.10.2025) 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

I confirm; the data supporting the findings of this study can be obtained from the corresponding author upon reasonable request.

Authors' Contribution

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

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Generative Artificial Intelligence Statement

No generative artificial intelligence tool or service was used in the preparation of this manuscript. All content was created, reviewed, and finalized by the authors in accordance with scientific research methods and academic ethical principles.

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Pediatric Vitamin D Status: Age, Gender, and Seasonal Determinants in a Comprehensive Retrospective Cohort

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Abstract:

Objective: Vitamin D plays a critical role in bone health, immune function, and overall growth and development in children. So, the study aimed to determine serum vitamin D levels in a large sample, examine their relationship to age groups, gender, seasonal and monthly, and identify risk groups by demonstrating the prevalence of vitamin D deficiency in the pediatric age group.

Methods: A retrospective study was conducted using serum vitamin D level data from pediatric patients presenting to the Pediatrics Outpatient Clinic. Children were divided into four age groups based on developmental stages: 1-4, 5-8, 9-12, and 13-17 years. Vitamin D levels were categorized as severe deficiency, deficiency, insufficiency, and normal. The data were analyzed for age, gender, seasonality, and monthly distribution, and the relationships between these variables were evaluated using comprehensive statistical methods.

Results: Only 6.5% of the average vitamin D levels were found to be normal. Deficiency, insufficiency, or severe deficiency was detected in 93.5%. A weak but significant negative correlation was observed between age and vitamin D levels. While levels were similar between genders, severe deficiency was higher in females. The highest values were observed in summer and the lowest in winter, with July-September being the peak and January-February the trough.

Conclusion: Our study revealed that vitamin D deficiency is common in children and a critical public health problem. Decreasing levels with age, seasonal cycles, and gender differences indicate that the risk becomes more pronounced. These findings highlight the need for supplementation plans and awareness-raising strategies, particularly during winter and spring.

Keywords: Vitamin D Status, Pediatric Population, Deficiency, Analytical Determinants

Vitamin D (Vit D) plays a vital role not only in bone health but also in immune and metabolic functions during childhood. As an illustration, rickets, one of the classic complications of Vit D deficiency in childhood, is characterized by the softening of developing bone resulting from impaired bone mineralization. It can cause serious health problems in children, such as growth retardation, motor delays, and bone deformities [1]. Furthermore, Vit D exerts regulatory effects on innate components of the immune system. The presence of Vit D receptors, particularly in immune cells such as monocytes, macrophages, and

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dendritic cells, suggests that this Vit D inhibits antimicrobial peptide synthesis and weakens the immune response when serum levels are low [2]. Vit D in steroid form is found in the human body in the forms of D_2 (ergocalciferol) and D_3 (cholecalciferol) [3]. More than 90% of Vit D is converted to previtamin D3 from 7-dehydrocholesterol in the skin during exposure to sunlight, which is thermally converted to Vit D3. Therefore, Vit D, D2, or D3, is hydroxylated first to 25(OH)D in the liver and then to 1,25(OH)2D in the kidneys. 25(OH)D is the main circulating form of Vit D and has a half-life of 2–3 weeks [4]. Since the 25(OH)D level in serum is considered the most reliable biological marker of Vit D status in the body, they have identified it as a strong and reliable indicator for monitoring Vit D status in infants and children [5]. Studies conducted in various geographic regions reveal that differences in sunlight exposure, lifestyle, dietary habits, and latitude also influence serum 25(OH)D levels. And age, gender, season have each been reported to have significant effects on Vit D levels, and the interaction effect between age and season is also significant [4]. It has also been noted that the high prevalence of Vit D deficiency in children is particularly related to nutritional status and seasonal factors [6]. Vit D levels have been reported to be low across age groups, particularly in the 7-18 age group. In gender-based assessments, males are frequently reported to have higher Vit D levels than females. It has also been reported that levels rise in the summer and drop significantly in the winter [7]. Beyond bone metabolism, emerging evidence indicates that Vit D also plays an important role in immune regulation. Accordingly, our study aims to assess vitamin D levels in children aged 0-17 based on age, gender, season, and month, expecting that these factors influence vitamin D status and reveal subgroups at increased risk for deficiency.

METHODS

Study Design and Data Collection

Our study included a total of 15.981 pediatric patients aged 1 to 17 who presented to the Pediatrics Outpatient Clinic of Mardin Training and Research Hospital between 01.01.2018-31.12.2018. The study was approved by the Mardin Artuklu University Non-Interventional Clinical Research Ethics Committee (number: 2025/1-4, date: 07.01.2025).

The study included retrospective and cross-sectional data on Vit D levels in children. Demographic information such as age and gender of the patients, month and season information regarding the application period, and laboratory results of Vit D (25-OH D) levels were obtained from the hospital automation system.

As part of the preliminary analysis, participants were divided into four age groups based on their developmental stages: 1-4, 5-8, 9-12, and 13-17 years. Vit D levels were classified into four categories: <10 ng/mL (severe deficiency), 10-19.99 ng/mL (deficiency), 20-29.99 ng/mL (insufficiency), and ≥30 ng/mL (normal). Additionally, seasonal and monthly data in text format were converted to numerical codes to facilitate statistical analyses (e.g., Winter = 1, Spring = 2...; January = 1, February = 2...). Vit D categories were based on established clinical cut-offs recommended by the Endocrine Society and other pediatric guidelines. Seasonal and monthly information was numerically coded solely to facilitate statistical analyses.

Study inclusion criteria: Pediatric age group between 1 and 17 years old were included in the study.

Study exclusion criteria: Conditions such as diabetes mellitus, pregnancy, cancer, rheumatic diseases, febrile illnesses, inflammatory diseases, renal dysfunction, vitamin D supplementation, autoimmune disease, liver and thyroid dysfunction, and acute and chronic infections were identified as exclusion criteria.

Statistical Analysis

Statistical analysis of the data was performed using SPSS 27.0. Descriptive statistics are given as mean±standard deviation (Mean±SD) for continuous variables and as number (n) and percentage (%). The Kolmogorov-Smirnov test was used to assess the data's conformity to a normal distribution, and since it was determined that the data were not normally distributed ($P < .001$), non-parametric tests were used for comparisons between groups. In group comparisons, the Mann-Whitney U test was used for two independent groups, and the Kruskal-Wallis H test was used for comparisons of more than two independent groups (seasons, months, age groups). If the Kruskal-Wallis H test was significant, post-hoc analysis was performed using the Mann-Whitney U test with Bon-

ferroni correction to determine the source of the difference. Spearman correlation analysis was used to examine the relationship between variables. Statistical significance was set at P<0.05 in all analyses.

RESULTS

Demographic Characteristics and General Vitamin D Assessments

The mean age of the participants was 7.59±5.28 years. Females constituted 51.6% of the sample (n=8,251), while males constituted 48.4% (n=7,730). When the distribution by age group was examined, the largest group was children aged 1-4 (37.5%), followed by children aged 13-17 (23.7%) (n=3,786), those aged 5-8 (21.2%) (n=3,388), and those aged 9-12 (17.6%) (n=2,809) (Table 1).

The overall mean Vit D level in the study group was found to be 16.24±13.06 ng/mL. Classification based on Vit D sufficiency revealed that only 6.5% (n=1.035) of participants had normal Vit D levels (>30 ng/ml). 44.1% (n=7.047) of the individuals were classified as "Deficiency," 30.5% (n=4.872) as "Severe deficiency," and 18.9% (n=3.027) as "Insufficiency". In total, 93.5% of the individuals included in the study were found to have Vit D levels below the ideal level (Table 1).

Evaluation of Vitamin D Levels by Gender and Age Group

The relationship between Vit D status categories and gender was examined using the Chi-Square test. The analysis revealed a statistically significant difference in the distribution of Vit D status by gender ($\chi^2(3, N=15981) = 297.883$, P<0.001). A detailed examination revealed that the rate of 'Severe Deficiency' was significantly higher in females (36.2%) than in males (24.4%). Conversely, the rates of 'Insufficient' (22.2% vs. 15.9%) and 'Normal' (7.4% vs. 5.6%) Vit D levels were higher in males than in females. General Vit D deficiency (<20 ng/mL) was detected in 78.6% of girls and 70.3% of males (Table 2).

Vitamin D Assessment by Age Groups

Chi-Square test analysis revealed a highly statistically significant association between Vit D status and age groups ($\chi^2(9, N=15981) = 1465.163$, P<0.001). The findings show a strong trend towards an increase in both the prevalence and severity of Vit D deficiency with increasing age. While the rate of 'Severe Deficiency' (<10 ng/mL) was 18.8% in the 1-4 age group, this rate gradually increases with age, reaching 46.5% in the 13-17 age group (adolescence). In contrast, the rate of having 'Normal' Vit D status (>30 ng/mL) was highest at 12.0% in the 1-4 age group, while this rate

TABLE 1. Descriptive Statistics Regarding Demographics and Vitamin D Levels of Participants

Variable	Category	Value
Age (year)		7.59±5.28
Gender	Female	8,251 (51.6)
	Male	7,730 (48.4)
Age groups (years)	1-4	5,998 (37.5)
	5-8	3,388 (21.2)
	9-12	2,809 (17.6)
	13-17	3,786 (23.7)
Vitamin D (ng/mL)		16.24±13.06
Vitamin D (ng/mL)	Normal (≥30)	1,035 (6.5)
	Insufficiency (20-29.99)	3,027 (18.9)
	Deficiency (10-19.99)	7,047 44.1
	Severe deficiency (<10)	4,872 (30.5)
Total		15,981 (100.0)

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

TABLE 2. Distribution of Vitamin D Status by Gender

Vitamin D status	Male (n=7,730)	Female (n=8,251)	Total (n=15,981)
Severe deficiency	1,889 (24.4%)	2,983 (36.2%)	4,872 (30.5%)
Deficiency	3,547 (45.9%)	3,500 (42.4%)	7,047 (44.1%)
Insufficiency	1,719 (22.2%)	1,308 (15.9%)	3,027 (18.9%)
Normal	575 (7.4%)	460 (5.6%)	1,035 (6.5%)

Data are shown as n (%). Chi-square test result: P<0.001

decreases to 2.9% during adolescence. This trend towards worsening Vit D status with increasing age was also statistically supported by the linear association test (Linear-by-Linear Association, P<0.001), which was found to be significant (Table 3).

Changes in the Relationship Between Age and Vitamin D Levels According to Gender

The linear relationship between age and Vit D levels was examined using Spearman correlation analysis. The analysis revealed a statistically significant, weak, and negative correlation between age and serum Vit D levels (rho = -0.330, P<0.001). This negative relationship between age and Vit D was also visually confirmed in scatter plots stratified by gender. A similar trend toward a decrease in Vit D levels with increasing age was observed in both male and female participants (Figure 1).

Evaluation of Changes in Vitamin D Levels by Season

The Kruskal-Wallis H test, which was conducted to determine whether Vit D levels differed according to seasons, showed that there was a highly statistically significant difference between seasons (H(3)= 2542.287, P<0.001). In order to determine the seasons between which this general difference occurred, pairwise comparisons were made using Mann-Whitney U tests with Bonferroni correction (significance level was set at P<0.008). According to the mean rank values, Vit D levels are ranked as Summer > Autumn > Spring > Winter. The highest Vit D levels were detected in Summer (Mean Rank: 10122.90), while the lowest levels were observed in Winter (Mean Rank: 5386.13). Additionally, it was found that Autumn (Avg. Rank: 9366.04) levels were significantly higher than Spring (Avg. Rank: 7692.56) levels due to the effect of Vit D stored during the summer (Table 4).

Prevalence of Vitamin D Deficiency by Season

A chi-square test was performed to further examine the effect of seasonal variation on Vit D sufficiency status. The analysis confirmed a highly statistically significant relationship between the distribution of Vit D status categories and the seasons ($\chi^2(9, N=15981) = 2521.187$, P<0.001). Cross-tabulation analyses revealed a dramatic seasonal fluctuation in the prevalence of Vit D deficiency. While severe deficiency (<10 ng/mL) was detected in more than half of the participants (56.4%) in winter, this rate decreased to

TABLE 3. Distribution of Vitamin D Status by Age Groups

Vitamin D status	1-4 years (n=5,998)	5-8 years (n=3,388)	9-12 years (n=2,809)	13-17 years (n=3,786)
Severe deficiency	1,125 (18.8%)	991 (29.2%)	995 (35.4%)	1,761 (46.5%)
Deficiency	2,567 (42.8%)	1,620 (47.8%)	1,353 (48.2%)	1,507 (39.8%)
Insufficiency	1,584 (26.4%)	629 (18.6%)	405 (14.4%)	409 (10.8%)
Normal	722 (12.0%)	148 (4.4%)	56 (2.0%)	109 (2.9%)

Data are shown as n (%). Chi-Square: P<0.001

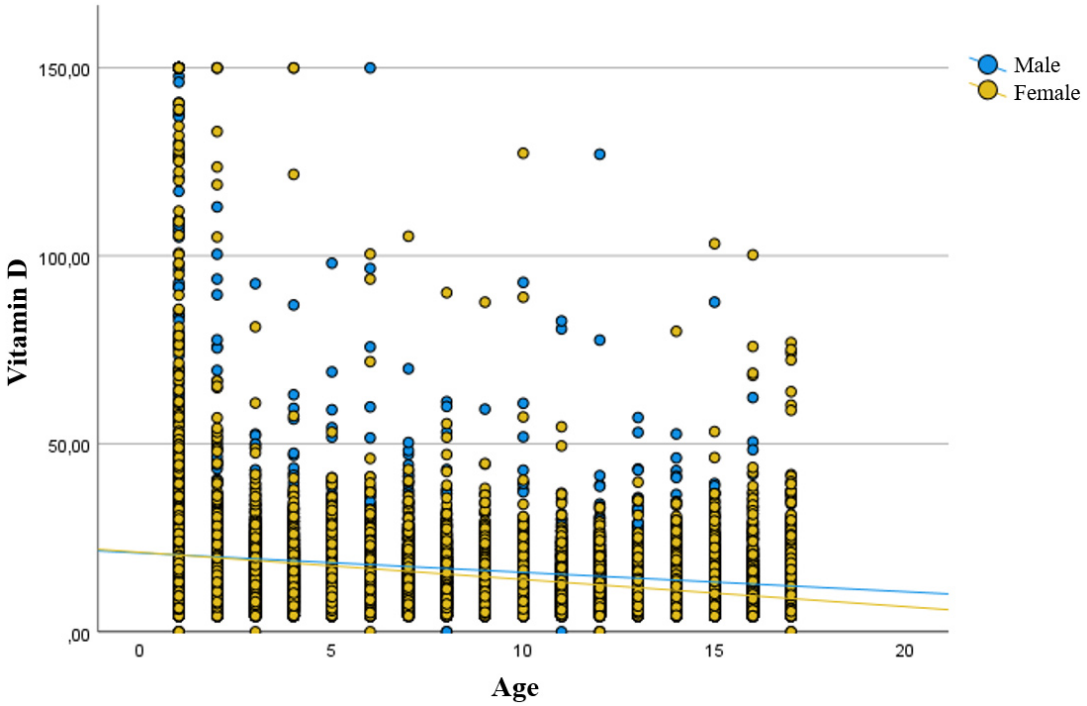

FIGURE 1. Scatter plot of the relationship between age and vitamin D levels by gender.

12.4% in summer. Vit D deficiency (<20 ng/mL) (sum of severe deficiency and deficiency groups) was observed to reach a significantly high rate of 87.3% in winter. This rate decreased to 61.3% in summer. Conversely, the proportion of individuals with 'Normal' Vit D levels (>30 ng/mL) is only 4.4% in winter, reaching its highest value in summer (8.6%) and autumn (8.5%) (Table 5).

Differences in Vitamin D Levels by Months

To conduct a more detailed analysis of seasonal effects, Vit D levels were compared over 12 months. The Kruskal-Wallis H test revealed a highly statistically significant difference in Vit D levels between months ($H(11)= 3044.525$, $P<0.001$). The monthly distribution of mean rank values indicates that Vit D levels exhibit a significant cycle throughout the year. Vit D levels were observed to reach their lowest levels in January (mean rank: 4828.42) and February (mean rank: 4939.42), to increase from March onward, and to reach their highest values in July (mean rank: 10454.35) and September (mean rank: 10369.80). A significant decrease was observed again from October onward (Table 6).

TABLE 4. Comparison of Vitamin D Levels by Season

Seasons	N	Mean Rank
Winter	4,386	5386.13[a]
Spring	4,492	7692.56[b]
Summer	3,962	10122.90[c]
Autumn	3,141	9366.04[d]

Kruskal-Wallis H Test result. Test statistic: $H(3)= 2542.287$, $P<0.001$. Different superscript letters (a, b, c, d) indicate that all groups are statistically significantly different from each other as a result of Bonferroni-corrected post-hoc tests (all $P<0.008$).

Prevalence of Vitamin D Deficiency by Months

The Kruskal-Wallis H test, performed to compare Vit D levels on a 12-month basis, revealed a highly statistically significant difference between months ($H(11)= 3044.525$, $P<0.001$). Chi-Square analysis, performed to evaluate the clinical reflection of this cycle, also confirmed a very strong relationship between the distribution of Vit D status and months ($\chi^2(33) = 3155.455$, $P<0.001$). According to monthly deficiency prevalences (Table 7):

•The 'Serious Deficiency' rate peaks in January

TABLE 5. Distribution of Vitamin D Status categories by Season

Vitamin D status	Winter (n=4,386)	Spring (n=4,492)	Summer (n=3,962)	Autum (n=3,141)
Severe deficiency	2,473 (56.4%)	1,384 (30.8%)	490 (12.4%)	525 (16.7%)
Deficiency	1,354 (30.9%)	2,181 (48.6%)	1,938 (48.9%)	1,574 (50.1%)
Insufficiency	366 (8.3%)	692 (15.4%)	1,194 (30.1%)	775 (24.7%)
Normal	193 (4.4%)	235 (5.2%)	340 (8.6%)	267 (8.5%)

Data are shown as n (%). Chi-square test result: $\chi^2(9)=2521.187$, $P<0.001$

(65.5%) and February (59.7%), falling to its lowest level of 9.3% in July.

•The prevalence of overall Vit D deficiency is most critical in late winter and early spring. This rate reached its highest point of the year in February, affecting 90.2% of participants.

•The lowest rate of 'normal' Vit D levels was observed in February with 3.2%, while the highest was observed in September with 11.9%.

DISCUSSION

In this large-scale study of 15,981 pediatric participants, we found that Vit D deficiency is highly prevalent across all age groups, with only a small proportion of children achieving normal serum levels. Our findings demonstrate the extent of the problem within a large hospital-based population, even if some of this pattern - especially the greater rates of deficiency in older children - is consistent with earlier reports. Despite regular supplementation regulations in many areas, the noticeably high early childhood insufficiency rate raises the possibility that present preventive measures are either inadequate or inconsistently applied. Studies involving children aged 0-18 have reported higher Vit D levels in the infant group, with levels tending to decline with age [8, 9].

TABLE 6. Comparison of Vitamin D Levels by Months

Months	N	Mean Rank
January	1,706	4828.42
February	1,453	4939.42
March	1,562	6588.70
April	1,761	7754.04
May	1,169	9074.88
June	1,088	9814.48
July	1,660	10454.35
August	1,214	9946.11
September	1,407	10369.80
October	1,390	8877.71
November	344	7233.69
December	1,227	6690.53

Kruskal-Wallis H Test results. Test statistic: $H(11) = 3044.525$, $P<0.001$

The average Vit D levels were found to be 16.24±13.06 ng/mL, with only 6.5% at normal levels and approximately 93.5% below the ideal level. This rate is quite high and demonstrates the prevalence of Vit D deficiency. One study reported that Vit D levels decrease with age, and the rate of deficiency increases [10]. It is noteworthy that the mean level in our data (approximately 16-17 ng/mL) was 30.5% in the severe deficiency group. While better levels were observed in areas where Vit D supplementation was administered, particularly during infancy (<1 year of age), a decline was observed during the school-age period and in adolescents due to factors such as supplementation, nutritional deficiencies, and lack of sun exposure. Furthermore, the gender gap is a frequently reported variable in the literature, with many studies [8, 11] showing that deficiency is more prevalent in females. In our study, overall Vit D levels and the rate of "Severe Deficiency" were found to be higher in girls. Contrary to our data, some reported studies have observed higher Vit D levels in males [8, 12].

Spearman correlation analysis showed a statistically significant, weak, but negative correlation between age and serum Vit D levels. This suggests a general trend toward a decrease in Vit D levels with increasing age. Furthermore, the similar decrease observed in both genders in scatterplots grouped by sex

TABLE 7. Distribution of Vitamin D Status Categories by Months (%)

Vitamin D status	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Severe deficiency	65.5	59.7	41.4	30.7	18.2	16.4	9.3	14.7	11.1	17.7	36.9	39.9
Deficiency	22.0	30.6	43.6	49.8	53.2	49.9	50.2	47.5	46.2	55.7	43.6	43.6
Insufficiency	7.7	6.6	11.4	15.3	21.0	28.3	31.5	30.0	20.9	13.3	14.0	11.5
Normal	4.9	3.2	3.6	4.2	7.6	7.3	9.9	7.9	11.9	3.3	5.5	5.0

Data are shown as percentage (%). Chi-square test result: $\chi^2(33) = 3155.455$, $P<0.001$

suggests that this age-related relationship is independent of gender. A study of 1,510 children found a significant negative correlation between Vit D levels and age [13]. According to our findings, the highest Vit D levels were seen in younger age groups, with levels decreasing significantly with age. One study reported that age, gender, and season all had significant main effects on Vit D levels, and that the combined effects of age and season were also significant [4]. Our findings of higher severe deficiency rates in girls may indicate behavioral, cultural, or physiological variables influencing sun exposure or supplementing adherence. Finding these sex-specific trends is therapeutically significant since it could direct more specialized public health initiatives.

Our findings demonstrate a highly statistically significant difference in Vit D levels across seasons. Pairwise comparisons using Mann-Whitney U tests with Bonferroni correction yielded a ranking of Summer > Autumn > Spring > Winter, with the highest levels observed in summer and the lowest in winter. One study observed seasonal variation in 25(OH)D concentrations in children and adolescents (aged 3-18), with the deficiency rate reaching as high as 80.4% in winter [14]. Indeed, this finding closely parallels ours (lowest in winter, highest in summer). A similar study noted that Vit D deficiency is quite common in children, with a particularly strong seasonal pattern among girls (aged 13-18). Levels increased significantly after summer, reflecting differences in sun exposure and time spent outdoors across countries, while levels decreased in winter and spring [15]. Therefore, our findings suggest that the low levels between winter and spring may be due to a lack of sunlight and differences in supplementation/outdoor time. In this context, public health policies should take seasonal changes into account, and supplementation and awareness programs should be developed for at-risk groups, particularly during the winter and spring periods. Overall, our findings not only confirm established patterns but also contribute population-specific data that highlight critical periods (Winter-Spring), vulnerable subgroups (adolescents, girls), and gaps in current public health practices. These insights may support the development of more effective, regionally adapted supplementation and screening policies.

Our findings indicate highly significant differences in Vit D levels across months. The mean rank values follow a distinct cycle throughout the year, with lowest levels occurring in January and February and highest in July and September. This pattern suggests that Vit D synthesis is closely linked to seasonal and monthly variations in sunlight exposure. One study found that Vit D levels were lowest in March and highest in August and September in a group of children not receiving supplements [16]. Another study reported that Vit D concentrations were lowest in March-April and highest in August, and this seasonal difference was more pronounced, especially in individuals not using supplements [17]. These findings are consistent with the observed rise-and-fall data between the January-February lows and the July-September highs in our study. In summary, our findings are generally consistent with national and international literature and clearly demonstrate the existence of a monthly cycle. This points to the need for monthly public health monitoring and/or precautions for months with a high percentage risk (e.g., an intensive information and reinforcement strategy during periods such as January-February). Furthermore, future analyses that incorporate additional variables such as sunlight intensity, UVB radiation levels, cloudiness, and time spent outdoors into this period would be beneficial.

Strengths and Limitations

The strengths of this study include its large sample

size and detailed month-by-month analysis, which allowed us to visualize the cyclical nature of Vit D levels across the year. However, several limitations must be acknowledged. First, the retrospective, hospital-based design may introduce selection bias. Children presenting to healthcare facilities may differ from the general population in health status. As a result, the prevalence of deficiency observed here may not fully reflect community-level data. Second, potential confounders such as dietary intake, sunlight exposure duration, clothing habits, and supplementation history were not recorded and may have affected Vit D levels. Future prospective studies incorporating lifestyle and environmental variables would provide a more comprehensive understanding of the determinants of Vit D status.

CONCLUSION

This study, using a large sample, thoroughly evaluated the distribution of Vit D levels in children, considering age, gender, and seasonal variables. Our findings revealed that Vit D levels were below the ideal range in the majority of children, and that rates of severe deficiency were higher than those reported in many studies in the literature. A significant decreasing trend in serum Vit D levels was observed with age. Furthermore, factors such as seasonal and monthly cycles, sunlight exposure, and lifestyle are among the determinants of Vit D status in children. These data strongly support the notion that Vit D deficiency is a significant health problem at the population level, demonstrating that the risk is particularly pronounced in adolescents and girls during winter and spring. Therefore, national and international health policies should take seasonal variations into account and develop preventive strategies during risk periods, along with regular Vit D supplementation and widespread awareness programs in children.

Ethics Approval and Consent to Participate

This study was approved by the he Mardin Artuklu University Non-Interventional Clinical Research Ethics Committee (Decision No: 2025/1-4; date: 07.01.2025). 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. 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: AD, SCY; Study Design: AD, SCY, HI; Supervision: AD, SCY, HI; Funding: N/A; Materials: AD, SCY, HI; Data Collection and/or Processing: AD; Statistical Analysis and/or Data Interpretation: HI, AD, SCY; Literature Review: AD, SCY, HI; Manuscript Preparation: AD, SCY, HI; and Critical Review: AD, SCY, HI.

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 used artificial intelligence tools (ChatGPT, OpenAI) only for language editing and reference formatting. 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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Relationship Between Orthorexia Nervosa, Eating Attitudes and Obsessive-Compulsive Disorder Among Nursing Students: A Cross-Sectional Study

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Abstract:

Objective: Objectives: This study aims to determine the levels of orthorexic tendencies, eating attitudes, and obsessive-compulsive symptoms in nursing students, examine the relationships among these variables, and investigate whether orthorexic tendencies and eating attitudes predict obsessive-compulsive symptoms.

Methods: The study was designed as a descriptive, cross-sectional study. Participants were selected using purposive sampling. Descriptive Characteristics Information Form, Orthorexia-11 Scale, Eating Attitudes Test (EAT) and Padua Inventory-Washington State University Revision (PI-WSUR) scales were used. This study was reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology checklist for cross-sectional studies.

Results: The mean score was 19.11±11.53 in the EAT participants, 28.30±4.16 in the Orthorexia-11 Scale and 56.35±23.85 in the PI-WSUR. A positive correlation was found between the participants' orthorexia nervosa tendencies and obsessive-compulsive symptoms. It was observed that as the participants' EAT mean scores increased, their obsessive-compulsive symptoms also increased. In the regression analysis, it was seen that orthorexia nervosa tendencies and eating attitudes were significant predictors of obsessive-compulsive symptoms.

Conclusion: The findings obtained from the study are important in terms of shedding light on the relationship between orthorexia nervosa, eating attitudes and obsessive-compulsive symptoms in nursing students. Given that health sciences students are a high-risk group for eating disorders, it is essential to include courses on eating disorders in the curriculum and implement regular psychological screenings in universities.

Keywords: Orthorexia Nervosa, Eating Attitudes Test, Obsessive-Compulsive Disorder, Nursing Students

Orthorexia nervosa (ON) is a proposed eating disorder defined by an obsessive focus on healthy eating, which can result in significant psychological distress and social impairment. The term was introduced by Steven Bratman in the late 1990s, highlighting the extreme preoccupation with food quality rather than quantity, thereby distinguishing it from other eating disorders like anorexia nervosa and bulimia nervosa [1, 2]. Individuals with ON frequently classify foods as

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"healthy" or "unhealthy," leading to rigid dietary restrictions that can negatively affect their physical and mental well-being [3, 4]. ON is considered a behavioral and personality disorder due to excessive attention paid to consuming healthy foods, excessive time spent on this pursuit, and the disruption of daily functioning [5]. Although ON is not yet included in the DSM-5 criteria, it is a growing concern, particularly among health-conscious populations and certain professional groups.

The clinical features of ON include perfectionism, anxiety, and a desire for control, which are also common in other eating disorders [6, 7]. Unlike anorexia nervosa, which is primarily concerned with weight loss and body image, orthorexia emphasizes the perceived healthiness of food choices and often results in social withdrawal to avoid exposure to "unhealthy" foods [1, 8]. Research suggests that ON may serve as a maladaptive coping mechanism, especially for individuals experiencing health anxiety, as strict adherence to dietary rules is believed to prevent illness [9, 10]. Moreover, studies have explored the relationship between ON and Obsessive-Compulsive Disorder (OCD), identifying overlapping traits such as ritualistic behaviors, compulsions, and food-related anxiety [11, 12].

OCD is a chronic mental health condition marked by obsessions, defined as intrusive, unwanted thoughts or images, and compulsions, defined as repetitive behaviors aimed at reducing distress or preventing a feared event [13, 14]. Both OCD and ON significantly impair daily functioning and social interactions [15]. Studies have shown that individuals with orthorexic tendencies may present elevated obsessive-compulsive traits, such as strict food preparation rituals, heightened food-related anxiety, and significant discomfort when deviating from dietary norms [16-18]. These findings suggest that ON may reflect a food-related manifestation of compulsive symptomatology [19, 20].

Eating attitudes (defined as a person's beliefs, behaviors, and feelings about food and eating) are central to understanding ON, particularly among adolescents and young adults. Positive eating attitudes are associated with balanced food intake and psychological well-being, while negative attitudes are linked to disordered eating patterns and dietary rigidity. A growing body of research indicates that a substantial proportion of young adults, particularly those in health-related fields, exhibit symptoms of orthorexia due to internalized ideals about health and nutrition [6, 21]. These individuals may devote considerable time to food selection and preparation, often at the expense of social interaction and mental health [22]. Cultural norms, body image dissatisfaction, and media influence further contribute to maladaptive eating behaviors [23, 24]. Over time, this preoccupation with healthy eating can evolve into an obsession that mimics compulsive behavior, underscoring the need for multidimensional assessment and intervention.

Gender is a critical yet understudied variable in this context. Epidemiological data suggest that women are more likely than men to experience disordered eating attitudes, obsessive-compulsive symptoms, and ON tendencies [23, 25]. Women are disproportionately subjected to sociocultural pressure regarding appearance, diet, and health, which may explain their greater vulnerability to these conditions [8, 24]. Nevertheless, findings are inconsistent, with some studies reporting no significant gender differences in orthorexic or obsessive-compulsive behaviors [19, 26]. These mixed results reveal the necessity of further research that directly addresses the role of gender in the interaction between ON, eating attitudes, and OCD symptoms, especially in young adults preparing for health-related careers.

While numerous studies have independently explored ON, eating attitudes, and OCD symptoms, few have examined their intersection, and even fewer have investigated how these relationships may vary by gender. This study aims to address this gap by assessing the relationships among orthorexia nervosa, eating attitudes, and obsessive-compulsive symptoms in nursing students, a population especially vulnerable due to their health education and internalized health ideals. Furthermore, the study explores whether these variables differ by gender, offering a novel contribution to the existing literature. By illuminating these associations, this research seeks to support early detection and targeted interventions for disordered eating and obsessive traits in future healthcare professionals.

Research questions are the following: (1) What is

the level of ON tendencies, eating attitudes and OCD symptoms in nursing students?; (2) Is there a relationship between ON, eating attitudes and OCD in nursing students? and (3) Are ON and eating attitudes predictive of obsessive-compulsive symptoms in nursing students?

METHODS

Study Design

The study was conducted using descriptive, cross-sectional research design. This study was reported in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) checklist for cross-sectional studies

Participants and Sampling Methods

The population of the study consisted of 456 undergraduate nursing students enrolled in the Department of Nursing at Van Yüzüncü Yıl University, Faculty of Health Sciences, during the fall semester of the 2023-2024 academic year. Participants were recruited using a convenience sampling method and participation was based on voluntary consent. All eligible undergraduate nursing students within the defined population were invited to participate in the study.

A total of 249 students who met the inclusion criteria and voluntarily agreed to participate were included in the final sample. The remaining 207 students were not included for the following reasons: 133 students declined to participate, 42 students were absent or could not be reached during the data collection period, and 32 students submitted incomplete or invalid questionnaires.

The inclusion criteria were: being 18 years of age or older, being currently enrolled as a nursing student at Van Yüzüncü Yıl University, having no visual or hearing impairment, and voluntarily agreeing to participate in the study. The exclusion criterion was the presence of a diagnosed psychological disorder.

Data Collection

Data were collected using face-to-face, self-administered questionnaires between October and November 2023. Prior to data collection, permission was obtained from the relevant course instructors to administer the questionnaires during scheduled class hours. The researchers visited the classrooms in person, provided a brief explanation of the study objectives, and informed students about the voluntary nature of participation, confidentiality, and their right to withdraw at any time.

Students who agreed to participate were provided with a written informed consent form along with the data collection instruments. Data collection took place in quiet, distraction-free classroom environments, where participants completed the questionnaires individually based on self-report. The researchers remained present throughout the data collection process to ensure standardized administration and to address any questions raised by the participants. Completion of the questionnaires required approximately 15-20 minutes, after which the completed forms were collected directly by the researchers.

Data Collection Tools

Descriptive Characteristics Information Form

The form, created by the researchers in line with the literature, consists of a total of 15 questions to determine the students' sociodemographic characteristics (age, gender, marital status, department, class, family structure, income status) and biopsychosocial perceptions (relationship with family, relationship with friends, effective coping, physical health) [3, 20, 26-28].

Orthorexia 11 Scale (ORTHO-11)

The ORTO-15 was developed by Donini *et al*. [5]. In its original form, the scale is a 15-item with 4-point Likert-type responses (Always, Often, Sometimes, Never) self-assessment instrument. The Turkish adaptation of the scale was conducted by Arusoğlu *et al*. [29] and four items with low factor loadings were removed, resulting in an 11-item Turkish version of the scale. The items investigate individuals' behaviours in choosing, purchasing, preparing, and consuming foods that they consider healthy. Answers that are distinctive for orthorexia are given a score of "1", and answers that indicate a tendency towards normal eating behavior are given a score of "4". Low

scores indicate an orthorexic tendency. The scale is evaluated based on the total score. The cut-off score of the scale is 27. Internal consistency calculated with Cronbach alpha was found to be 0.62 [29]. In this study Cronbach alpha was found to be 0.75.

Eating Attitudes Test (EAT-40)

It was developed by Garner & Garfinkel [30] to identify adolescents with eating disorders and to measure symptoms of anorexia nervosa, and can be applied to individuals over the age of eleven. It was adapted into Turkish by Savaşır and Erol [31]. It is a self-report scale consisting of 40 items with 6-point Likert-type responses (Always, very often, Often, Sometimes, Rarely, Never). The cut-off point is 30 points. For items 1, 18, 19, 23, 27, 39, sometimes 1 point, rarely 2 points, never 3 points and other options are evaluated as 0 points. For the other items of the scale, always is calculated as 3 points, very often as 2 points, frequently as 1 point and other options as 0 points. The scale is evaluated based on the total score. Internal consistency calculated with Cronbach alpha was found to be 0.70 [31]. In this study Cronbach alpha was found to be 0.83.

Padua Inventory-Washington State University Revision (PI-WSUR)

Participants' obsessive-compulsive symptom levels were assessed using PI-WSUR. It is a 39-item self-report scale that assesses obsessions and compulsions. The participant is asked to indicate how much he/she is bothered by each statement using a five-point Likert scale (ranging from 0 = Not at all to 4 = Very much). Burns *et al*. [32] revised the original Padua Inventory to address the differentiation between worry and obsession and created this version by removing items that caused confusion [32, 33]. In their study, they reported that this version has a 5-factor structure and that this version of the inventory is also valid and reliable. The Turkish adaptation was made by Yorulmaz *et al*. [34]. The scale consists of 5 sub-dimensions: checking compulsions, contamination obsessions and washing compulsions, obsessional impulses to harm self/others, dressing/grooming compulsions and obsessional thoughts of harm to self/others. There are five options in each item. These are; not at all (0), very little (1), a lot (2), quite a lot (3) and extremely (4), respectively. A score of zero for each item indicates that the symptom in the question is not experienced at all, while a score of four indicates that the symptom is experienced severely. The total score that can be obtained from the inventory is between 0 and 156. In the Turkish adaptation study of the inventory, the Cronbach alpha value was stated as 0.93 [34]. In this study Cronbach alpha was found to be 0.92.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics version 26.0. Normality of the continuous variables was assessed prior to inferential analyses using visual inspection of histograms and Q-Q plots, as well as examination of skewness and kurtosis values. Skewness and kurtosis values within ±1.5 were considered indicative of an approximately normal distribution. The distributions were considered approximately normal based on these criteria, and therefore parametric statistical analyses were applied. Descriptive statistics, including frequencies, percentages, means, and standard deviations, were calculated for sociodemographic characteristics and scale scores. In addition to descriptive statistics, independent samples t-tests were performed to compare mean scale scores between female and male participants. Chi-square tests of independence were used to examine associations between gender and categorical variables related to eating attitudes. Assumptions for each analysis were evaluated prior to testing, and appropriate statistical procedures were applied accordingly. Before conducting regression analysis, Pearson correlation coefficients were computed to assess bivariate relationships among ORTHO-11, EAT-40, and PI-WSUR scores. Correlation coefficients of ≥ 0.30 were considered adequate for inclusion in the regression model. The strength of correlations was interpreted as follows: poor (0.00-0.20), fair (0.21-0.40), good (0.41-0.60), very good (0.61-0.80), and excellent (0.81-1.00) [35]. To examine the predictive effects of orthorexia nervosa and eating attitudes on obsessive–compulsive symptoms, a multiple linear regression analysis (enter method) was performed. Regression assumptions,

TABLE 1. Socio-Demographic Characteristics of Participants

Variables	Data
Age (years)	21.04±2.2 (17-36)
Gender	
Female	180 (72.3%)
Male	69 (27.7%)
Class	
First Class	91 (36.5%)
Second Class	6 (2.4%)
Third Class	81 (32.5)
Fourth Class	70 (28.1)
The relation of parents	
Married	231 (92.8%)
Divorced	2 (8%)
Living separately	3 (1.2%)
Mother deceased	1 (0.4%)
Father deceased	11 (4.4%)
Other	1 (0.4%)
Educational status of mother	
Illiterate	90 (36.1%)
Primary school graduate	95 (38.2%)
Secondary school graduate	38 (15.3%)
High school graduate	17 (6.8%)
University graduate	5 (2%)
Educational status of father	
Illiterate	17 (6.8%)
Primary school graduate	82 (32.9%)
Secondary school graduate	70 (28.1%)
High school graduate	49 (19.7%)
University graduate	29 (11.6%)
Marital status	
Married	11 (4.4%)
Single	220 (88.4%)
I have a partner	18 (7.2%)
Employee status of father	
Has a permanent job	118 (47.4%)
Works part time	66 (26.5%)
Does not work	61 (24.5%)

including linearity, normality of residuals, multicollinearity, and homoscedasticity, were tested and met. A P-value of <0.05 was considered statistically significant for all analyses.

RESULTS

The sociodemographic characteristics of the participants are given in Table 1. Most of the study participants were female (72.3%). 60.6% of the participants are sophomores and above. It was found that 92.8% of the participants have married parents and 88.4% are single. The mothers' educational status of 98% of the participants and the fathers' educational status of 87.5% were high school graduates or below. More than half of the participants had a nuclear family type, and 69.9% of them stated that they had a democratic family type. More than half of the participants described their family and friend relationships as good. 94.8% of the participants did not have any physical illness.

Descriptive statistics of the participants' scores on ORTHO-11, EAT-40, PI-WSUR, and its subdimensions by gender are presented in Table 2.

The mean EAT-40 score was 18.26 ± 10.39 for female participants and 23.83 ± 15.87 for male participants. Considering that the cut-off score of the EAT-40 is 30, the mean scores of both female and male participants were below the clinical threshold, indicating no deterioration in eating attitudes within the study sample.

The mean ORTHO-11 score was 28.18 ± 3.93 among female participants and 29.92 ± 4.39 among male participants. Given that ORTHO-11 scores range from 11 to 44, with higher scores indicating a lower tendency toward orthorexia nervosa, the findings suggest that orthorexic tendencies were relatively low among both female and male participants.

Obsessive–compulsive symptoms were assessed using the PI-WSUR. The mean PI-WSUR total score was 57.00±23.46 for female participants and 54.58±24.98 for male participants. With respect to the PI-WSUR subscales, higher mean scores were observed in the contamination obsessions and washing compulsions subscale (20.20±8.52), followed by

TABLE 1 Continued. Socio-Demographic Characteristics of Participants

Variables	Data
Employee status of mother	
Has a permanent job	4 (1.6%)
Works part time	8 (3.2%)
Does not work	235 (94.4%)
Perceived family economic situation	
(5000 TL under)	14 (5.6%)
(5000-8000 TL)	31 (12.4%)
(8000-1500 TL)	135 (54.2%)
(15000-20000 TL)	48 (19.3%)
(Above 200000 TL)	19 (7.6%)
Family structure	
Democratic	174 (69.9%)
Strongly regulatory	52 (20.9%)
Extremely repressive	22 (8.8%)
Family type	
Nuclear	181 (72.7%)
Extended	67 (26.9%)
The relationship with family	
Good	175 (70.3%)
Average	65 (26.1%)
Bad	8 (3.2%)
The relationship with friends	
Good	154 (61.8%)
Average	86 (34.5%)
Bad	9 (3.6%)
Coping with problems with family or friends	
I can produce solutions with composure	206 (82.7%)
I act reserved and wait for a move from the other party	23 (9.2%)
I think I am inadequate in solving problems	17 (6.9%)
Having a physical illness	
Yes	12 (4.8%)
No	236 (94.8%)
Total	249 (100%)

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

checking compulsions (16.56±9.17) across the total sample.

Descriptively, female participants showed higher mean scores on the contamination obsessions and washing compulsions and obsessional thoughts of harm to self/others subscales, whereas male participants demonstrated higher mean scores on the obsessional impulses to harm self/others subscale. These differences are presented descriptively, and inferential statistical comparisons are reported separately.

Independent samples t-tests were conducted to examine gender differences in EAT-40, ORTHO-11, and PI-WSUR total and subscale scores. No statistically significant gender differences were found in EAT-40 ($t = 1.85$, $P=0.073$) or ORTHO-11 total scores ($t = 0.69$, $P=0.490$). Similarly, no significant gender difference was observed in the PI-WSUR total score ($t = -0.68$, $P=0.495$).

Regarding PI-WSUR subscales, a statistically significant gender difference was identified only in the obsessional impulses to harm self/others subscale, with male participants scoring higher than female participants ($t = 2.29$, $P=0.024$). No statistically significant gender differences were observed for checking compulsions, contamination obsessions and washing compulsions, dressing/grooming compulsions, or obsessional thoughts of harm to self/others (all $P>0.05$).

The frequencies of ON and eating attitude deterioration among the participants are presented in Table 3. Eating attitude deterioration was observed in 8.3% of female participants and 10.1% of male participants. Pearson's chi-square analysis indicated no statistically significant association between gender and eating attitude deterioration, $\chi^2(1, N = 249) = 0.20$, $P=0.652$.

Regarding ON tendencies, 35.6% of female participants and 34.8% of male participants were classified as having a tendency toward ON. Similarly, no significant association was found between gender and ON tendency, $\chi^2(1, N = 249) = 0.01$, $P=0.909$.

The relations between ORTHO-11, EAT-40 and PI-WSUR were given in the Table 4. It was determined that there was a low level negative significant relationship between the mean score of the ORTHO-11 and PI-WSUR ($r=-.159$). In other words, as the participants' orthorexia nervosa symptoms increased,

TABLE 2. Mean Scores of Participants in ORTHO-11, EAT-40, PI-WSUR and Sub-dimensions by Gender

Scale	Gender	Mean±SD	(Min-Max)	P-value*
ORTHO-11	Female	28.18±3.93	15-38	0.49
	Male	29.92±4.39	21-41	
	Total	28.30±4.16	15-39	
EAT-40	Female	18,26±10,39	3-81	0.07
	Male	23.83±15.87	7-79	
	Total	19.11±11.53	3-81	
PI-WSUR Total	Female	57.00±23.46	7-124	0.49
	Male	54.58±24.98	9-120	
	Total	56.35±23.85	7-124	
Checking compulsions	Female	16.48±9.05	0-37	0.82
	Male	16.77±9.55	0-40	
	Total	16.56±9.17	0-40	
Contamination obsessions and washing compulsions	Female	20.52±8.47	0-40	0.33
	Male	19.34±8.64	2-40	
	Total	20.20±8.52	0-40	
Obsessional impulses to harm elf/others	Female	3.93±5.56	0-36	**0.02**
	Male	6.17±7.07	0-34	
	Total	4.53±6.07	0-36	
Dressing/grooming compulsions	Female	4.92±3.17	0-12	0.49
	Male	4.61±3.03	0-12	
	Total	4.83±3.13	0-12	
Obsessional thoughts of harm to self/others	Female	10.68±6.08	1-28	0.06
	Male	9.11±5.65	0-28	
	Total	10.25±5.99	0-28	

ORTHO-11, orthorexia 11 scale; EAT-40, eating attitudes-40 test; PI-WSUR, Padua Inventory Washington State University revision.

*independent sample T test. Statistically significant P-value is shown in bold.

their obsessive-compulsive symptoms increased. In addition, it was seen that there was a positive significant relationship between the participants' means score of the EAT-40 and PI-WSUR (r=.286).

One of the assumptions for performing regression analyses is that there are significant correlation relationships between variables. Accordingly, a prospective model was created in which eating attitudes and ON predict OCD. As a result of the multiple linear regression analysis, it is seen that the model created is statistically significant (F: 11.005, P<0.001). Accordingly, it is seen that the mean score of the ORTHO-11 and EAT-40 together explain approximately 10% of the variance on PI-WSUR. Accordingly, it is seen that ON (β=-.173, P<0.05) and eating attitudes (β=.290, P<0.05) are significant predictors of obsessive-compulsive symptoms (Table 5).

DISCUSSION

This descriptive, cross-sectional study was conducted with 249 nursing students to examine the relationships between orthorexia nervosa tendencies, eating

TABLE 3. Prevalence of Orthorexia Nervosa Tendencies and Eating Attitudes According to Gender

		n	%	P-Value**
ORTHO-11				
No tendency to ON	Female	116	64.4	χ^2:0.20, P=0.65
	Male	45	65.2	
	Total	161	64.7	
There is a tendency towards ON	Female	64	35.6	
	Male	24	34.8	
	Total	88	35.3	
EAT-40				
Eating Attitude Normal	Female	165	91.7	χ^2= 0.01, P=0.90
	Male	62	89.9	
	Total	227	91.2	
Deterioration in Eating Attitude	Female	15	8.3	
	Male	7	10.1	
	Total	22	8.8	

ORTHO-11, orthorexia 11 scale; EAT-40, eating attitudes test-40; ON, orthorexia nervosa.
**Ki-square test.

attitudes, and obsessive-compulsive symptoms. Considering that nursing students are a risk group for disordered eating behaviors due to their health-related education and exposure to health norms, identifying these psychological tendencies is important. The findings of the study are expected to contribute to the development of educational and preventive interventions tailored for nursing students by clarifying how ON tendencies, eating attitudes, and obsessive-compulsive symptoms interact in this population. Previous studies have suggested that individuals in health-related fields may internalize rigid health and dietary norms, which may increase their vulnerability to orthorexic and obsessive behaviors [6, 23]. In this context, nursing students represent a particularly relevant group for investigating the interplay between eating-related attitudes and psychological symptoms. This study builds upon this body of literature by exploring how ON and eating attitudes relate to obsessive-compulsive

TABLE 4. The Correlation Between ORTHO-11, EAT-40 and PI-WSUR

		1	2	3
1-ORTHO-11	r	1		
	P-value			
2-EAT-40	r	0.020	1	
	P-value	0.783		
3-PI-WSUR	r	-0.159*	0.286**	1
	P-value	**0.016**	**<0.001**	

ORTHO-11, orthorexia 11 scale; EAT-40, eating attitudes test-40; PI-WSUR, Padua Inventory Washington State University revision.
*Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed). Statistically significant P-values shown are in bold.

TABLE 5. The Results of Multiple Linear Regression Analysis of ORTHO-11 and EAT-40 Predicting PI-WSUR

Variables	B	SE	β	t	P-value	F	P-value
Constant	73.073	11.563		6.320	**<0.001**	11.005	**<0.001**
ORTHO-11	-0.971	0.398	-0.173	-2.440	**0.016**		
EAT-40	0.596	0.145	0.290	4.104	**<0.001**		

ORTHO-11, orthorexia 11 scale; EAT-40, eating attitudes test-40; PI-WSUR, Padua Inventory Washington State University revision; SE, standard error.

$R=0.33$, $R^2=0.11$, $R^{2adj}=0.10$

Statistically significant P-values are shown in bold.

symptoms within this specific academic and developmental context.

The participants' orthorexia nervosa tendencies were assessed using the ORTHO-11 scale, in which lower scores indicate higher ON tendencies [5, 29]. Participants scoring 27 or below were classified as having a high tendency toward orthorexia nervosa. The mean ORTHO-11 score was 28.18±3.93 for female participants and 29.92±4.39 for male participants, indicating relatively similar orthorexic tendencies across genders. Consistent with this finding, independent samples t-test results showed no statistically significant difference between female and male participants in ORTHO-11 total scores, suggesting that orthorexic tendencies did not differ meaningfully by gender in the present sample. According to the 27-point cut-off, 35.6% of female students and 34.8% of male students in this study were classified as having orthorexic tendencies. In line with the descriptive findings, chi-square analysis revealed no significant association between gender and orthorexia nervosa tendency, further supporting the absence of gender-based differences in ON classification. These prevalence rates are lower than those reported in previous studies using ORTHO-11 or ORTHO-15. For instance, Arslantaş *et al*. [36] reported an average score of 27.34±4.53 among nursing students, while Brytek-Matera *et al*. [23] found ON risk in 68.55% of female and 43.18% of male university students using the ORTHO-15 scale. Studies conducted with Turkish healthcare professionals have shown ON prevalence ranging from 43.6% to 60.1% [25, 37, 38], and in international samples, the risk varies widely from 57.6% to 81.9% [39, 40]. It has been argued that ON may be more common in health science students due to their exposure to dietary and health-related content during their training [41]. While many studies report a higher prevalence in women [23, 42], other findings suggest that men may also be at risk [38, 43, 44]. The lack of significant gender differences observed in the present study may be attributable to sample characteristics, shared educational exposure, or sociocultural factors, including gender distribution within health education programs and varying norms related to body surveillance and health behaviors. Such inconsistencies across studies underscore the influence of cultural, psychosocial, and educational contexts, as well as methodological differences in sampling and measurement.

The total mean score of the participants on the EAT-40 was 19.11±11.53, which is below the clinical cutoff score of 30, indicating generally healthy eating attitudes among most of the sample [31]. The EAT-40 scale is used to assess problematic eating behaviors, and scores above 30 are considered indicative of a possible eating disorder or significant deterioration in eating attitudes [30]. Based on this cutoff, 8.8% of the participants were identified as having disordered eating attitudes. When examined by gender, the prevalence of disordered eating attitudes was 8.3% among female participants and 10.1% among male participants. Although this distribution suggests a slightly higher proportion among male students, chi-square analysis revealed no statistically significant association between gender and eating attitude deterioration, suggesting that the observed difference lacks statistical significance. Consistently, independent samples t-test results showed no significant gender difference in EAT-40 mean scores, further supporting the absence of gender-based differences in eating attitudes within the present sample.

This finding is noteworthy considering that previous studies have generally reported higher levels of disordered eating among female university students. Studies conducted in Turkey found EAT-40 mean scores ranging from 15.66±8.50 to 20.9±9.3, which is consistent with our findings [36, 45]. However, Arslantaş *et al*. [36] found a much higher prevalence (84.5%) of disordered eating attitudes in a university sample, where 77.9% of participants were female, indicating a potentially stronger gender-related vulnerability.

The relatively low prevalence of disordered eating attitudes observed in the present study may be partially attributable to regional sociocultural factors. Eastern Turkey is characterized by a more conservative social structure and traditional gender role expectations compared to more urbanized western regions. Previous research has demonstrated that sociocultural influences, including body image ideals, social pressures, and media exposure, play a significant role in the development of eating disorders [46, 47]. In more conservative contexts, where body-centered social comparison may be less prominent, disordered eating attitudes may emerge less frequently or present differently. Nevertheless, further research is needed to clarify how regional sociocultural contexts shape eating attitudes among university students across Turkey.

The mean total score of female participants on the PI-WSUR was 57.00±23.46, while that of male participants was 54.58±24.98. Given that total PI-WSUR scores range from 0 to 156, these values indicate a moderate level of obsessive–compulsive symptoms in the present sample [33, 34]. Although there is no universally accepted clinical cutoff score for the PI-WSUR, previous studies conducted in non-clinical populations have reported mean scores ranging between 48 and 75 [15, 16, 26, 48]. In this context, the scores observed in our study are consistent with the expected range for university students. Accordingly, the scores observed in this study fall within the expected range for university student samples. Moreover, independent samples t-test analysis revealed no statistically significant gender difference in PI-WSUR total scores, suggesting comparable overall obsessive-compulsive symptom severity between female and male participants.

With respect to symptom dimensions, descriptive differences were observed across genders. Female participants reported higher mean scores on the contamination obsessions and washing compulsions subscale, whereas male participants showed higher mean scores on obsessional impulses to harm self/others. However, inferential analyses indicated that a statistically significant gender difference emerged only for obsessional impulses to harm self/others, with male participants scoring higher on this subscale. No significant gender differences were found for checking compulsions, contamination obsessions and washing compulsions, dressing/grooming compulsions, or obsessional thoughts of harm to self/others.

These findings are partially consistent with prior research suggesting that contamination and cleaning symptoms tend to be more prevalent among women, whereas checking and harm-related symptoms are more commonly reported among men [49-51]. Such patterns are often interpreted within the framework of traditional gender roles, which emphasize cleanliness, caregiving, and responsibility among women. Nevertheless, other studies have reported reversed patterns or no significant gender differences, highlighting the role of cultural and psychosocial context in shaping the expression of obsessive-compulsive symptoms [52]. In the present study, the regional sociocultural setting, characterized by more traditional gender roles and conservative norms, may have influenced symptom expression, particularly among female participants. Additionally, educational stressors and developmental factors associated with the university period may contribute to the predominance of contamination- and checking-related symptoms, which are frequently linked to anxiety regulation and perceived control [50, 53].

The positive correlation between EAT-40 and PI-WSUR scores indicates that higher disordered eating attitudes are associated with greater obsessive-compulsive symptom severity. Similar findings were reported by studies showing higher obsession scores among students with high eating disorder risk [54, 55]. The comorbidity of OCD and eating disorders is well established; shared features include perfectionism, anxiety, and cognitive rigidity. Furthermore, a significant negative relationship between ORTHO-11 and PI-WSUR scores supports previous findings linking ON and obsessive-compulsive behaviors [56]. ON, characterized by obsession with healthy eating,

often overlaps with obsessive symptoms.

This correlation may stem from shared mechanisms such as rigid thinking, heightened anxiety, and compulsive coping patterns. Among nursing students, exposure to health norms, body-related expectations, and academic pressure may foster restrictive eating and ritualistic control behaviors. Perfectionism, common in both OCD and eating disorders, can lead to excessive dietary monitoring and ritualization to reduce perceived risks or dissatisfaction. Cognitive features such as intolerance of uncertainty and fear of mistakes may further reinforce these behaviors. Moreover, in traditional societies emphasizing discipline, appearance, and morality, these traits may be culturally reinforced, particularly among women [46, 47].

Multiple regression analysis showed that both ON tendencies and eating attitudes were significant predictors of obsessive-compulsive symptoms, explaining about 10% of the variance in PI-WSUR scores. ON (β = –.173) was a negative predictor due to ORTHO-11 scoring, while disordered eating attitudes (β = .290) positively predicted obsessive-compulsive symptoms. These results support previous findings highlighting overlapping characteristics, including perfectionism, cognitive rigidity, anxiety, and compulsive rituals, between eating disorders and OCD [7, 8, 26, 48, 57]. Individuals with ON often display obsessive behaviors related to food selection and control [16, 48, 58], while disordered eating attitudes involve intrusive thoughts and ritualistic eating patterns [8].

Other factors such as intolerance of uncertainty, trait anxiety, and distress intolerance may also contribute [22, 23]. Considering the multiple academic, social, and gender-related stressors faced by nursing students, OCD symptoms likely arise from a combination of cognitive, emotional, and environmental influences. The modest variance explained by the model underscores the multifactorial nature of obsessive-compulsive symptomatology and highlights the need for future studies addressing broader psychological and contextual variables.

Strengths and Limitations

A key strength of this study is its focus on nursing students, a clinically relevant high-risk group for disordered eating behaviors and obsessive-compulsive symptoms. The simultaneous assessment of orthorexic tendencies, eating attitudes, and obsessive-compulsive symptoms offers a clinically meaningful perspective on the overlap between eating-related behaviors and compulsive psychopathology. The use of validated and widely accepted assessment tools enhances the clinical reliability of the findings. Furthermore, identifying orthorexic tendencies and eating attitudes as significant predictors of obsessive-compulsive symptoms provides important implications for early screening and preventive mental health interventions in university and clinical settings.

This study has several limitations. First, its cross-sectional design precludes causal inferences and limits the generalizability of the findings. The use of self-reported measures may have introduced social desirability bias. Additionally, the study was conducted at a single center with a predominantly female sample, which may further affect representativeness.

While gender-based comparisons were conducted, other sociodemographic variables (e.g., age, academic year, and income level) were collected to describe the sample characteristics but were not included in inferential analyses. Accordingly, the absence of inferential analyses across additional sociodemographic subgroups should be considered a limitation. Future longitudinal and multi-center studies with larger and more diverse samples are recommended to examine the potential influence of these factors using multivariate analytical approaches. In addition, although individuals with a diagnosed psychiatric disorder were defined as an exclusion criterion, the presence of psychiatric diagnoses among participants was based on self-report and was not verified through a structured clinical interview. Therefore, it is possible that some participants with undiagnosed or unreported psychiatric conditions were included in the sample, which may have influenced the findings.

CONCLUSION

In conclusion, the findings of this study indicate generally healthy eating attitudes, relatively low orthorexia nervosa tendencies, and moderate levels of

obsessive–compulsive symptoms among nursing students. Although no significant gender differences were observed in most variables, the presence of disordered eating attitudes and orthorexic tendencies in a notable proportion of students highlights the importance of preventive and supportive approaches in university settings. Based on these findings, providing training and counseling services addressing eating disorders, orthorexia nervosa, and obsessive-compulsive symptoms may be beneficial for nursing students. Such interventions could be incorporated into existing student counseling and health promotion services to support early identification and promote healthier coping strategies. Future research should examine the effectiveness of these supportive interventions in improving students' psychological well-being.

Ethics Approval and Consent to Participate

Ethical approval for this study was obtained from the Van Yüzüncü Yıl University Non-Interventional Clinical Research Ethics Committee (Decision no: 2023/09-07; Date: 18.09.2023). Institutional permission was also obtained from the institution where the study was conducted prior to data collection. Permission to use the measurement instruments was obtained from the authors who conducted the Turkish validity and reliability studies of the scales. All participants were informed about the purpose and procedures of the study, and written informed consent was obtained prior to participation. Participation was voluntary, and participants were informed of their right to withdraw from the study at any time without any consequences. The study was conducted in accordance with the principles of the Declaration of Helsinki. Data confidentiality was ensured by anonymizing all participant information, assigning identification codes instead of personal identifiers, and restricting data access to the research team only. The collected data were stored securely 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: NSKD, CHA, KA; Study Design: NSKD, CHA, KA; Supervision: NSKD, CHA; Funding: NSKD, CHA, KA; Materials: NSKD, CHA, KA; Data Collection and/or Processing: NSKD, CHA, KA; Statistical Analysis and/or Data Interpretation: NSKD, CHA; Literature Review: NSKD, CHA, KA; Manuscript Preparation: NSKD, CHA, KA; and Critical Review: NSKD, CHA.

Conflict of Interest

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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 Mediating Role of Health Perception in the Effect of Trust in Physicians on Treatment Compliance: The Case of Sakarya Province

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Abstract:

Objective: Trust is a key element of healthcare services, as individuals prefer to receive treatment from physicians they trust in both knowledge and practice. Based on this, the study examines the mediating role of health perceptions in the effect of trust in physicians on treatment adherence.

Methods: The research was conducted in Sakarya Province between 25 October and 30 December 2024, using face-to-face and online surveys. The population included residents aged 18 and over, and the required sample size was 384 at a 95% confidence level. Using convenience sampling, data were obtained from 471 individuals.

Results: The results show that trust in physicians increases treatment compliance while decreasing health perception, with health perception partially mediating this relationship. Treatment compliance depends on trust levels and individuals' health evaluations. The positive relationship between trust in physicians and treatment adherence ($r = 0.527$) and its negative relationship with health perception ($r = -0.442$) confirm both directional effects. This pattern demonstrates the multidimensional nature of patient behaviour. Therefore, effective health communication and strong physician–patient interaction are critical for improving treatment compliance.

Conclusion: Trust in physicians and health perception are key determinants of treatment compliance. The mediating role of health perception indicates its strong effect on transforming trust in physicians into treatment adherence. The study highlights the importance of strengthening trust in physicians and improving individuals' health perceptions through effective communication strategies to enhance treatment compliance.

Keywords: Trust in Physicians, Health Perception, Treatment Compliance

As the most fundamental component of hospital healthcare services, physicians are in constant interaction with patients and their families while providing medical care. In this interaction, trust is paramount because it plays a critical role in informing patients and alleviating their anxiety. Trust is a fundamental factor in the physician-patient relationship [1]. In the context of transformations in healthcare systems, however, the concept of trust has become more fragile. An increase in the number of patients and shorter examination times directly affect treatment outcomes. Therefore, the trust that patients place in their physicians has become even more important. While trust and patient satisfaction

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contribute to a healthier treatment process, a lack of trust can lead patients to seek different doctors and result in unnecessary diagnostic tests being repeated [2].

Trust between individuals is shaped through repeated interactions. In the context of trust in physicians, patients' beliefs and expectations about how they will be treated by the physician are extremely influential [3]. At the same time, healthcare professionals' trust in their institutions from a human resources perspective will strengthen the general sense of trust among stakeholders [4].

Various factors influence a patient's trust in their physician. The most important of these are the physician's ability to devote sufficient time to the patient, address their concerns, and provide satisfactory explanations. It is also important to give patients the opportunity to ask questions, and to respond to them politely and patiently. Approaching patients with empathy and being sensitive to privacy issues is central to building trust. Furthermore, a physician's gender, education, experience, reputation and social media presence also influence patients' trust in them [5, 6].

From the moment they enter the hospital, patients expect an environment where they can feel safe and comfortable. Patients are often anxious, worried and impatient. A hospital visit is one of the most sensitive experiences for patients and their relatives. Negative experiences with healthcare professionals or the system can undermine patients' confidence in treatment and even cause them to abandon it. Such experiences can also influence preferences regarding healthcare facilities [7]. Medicine has traditionally been regarded as a sacred and honourable profession, and physicians are expected to prioritise their patients' well-being under all circumstances. In this regard, patients' trust in their physicians can prevent unnecessary use of healthcare services. Trust in the healthcare system is not one-sided and is not directed solely at physicians. When healthcare professionals work together as a team, their competence is strengthened, as is patients' trust in medical expertise. It should be emphasised that healthcare delivery requires a multidisciplinary team approach rather than individual effort [8].

In today's society, many diseases are treatable. However, individuals are also expected to adopt preventive health measures and make behavioural changes in their social lives [9]. Health perception encompasses individuals' thoughts, beliefs and expectations regarding their health status. This perception can be positive, reflecting good health, or negative, reflecting poor health [10]. Various factors contribute to an individual developing a positive or negative perception of their health, including their personal medical history, how they perceive their ability to cope with problems, their personal preferences, their cultural values, their education and other individual characteristics [11].

An individual's perception of health is closely related to their understanding of its importance. As health literacy increases, the perception of health becomes more accurate and comprehensive. Trust in physicians and health professionals also affects individuals' compliance with treatment processes within the framework of their health perception. In this regard, regular exercise and avoiding a sedentary lifestyle are important factors. Conversely, harmful habits such as alcohol consumption, smoking and substance use indicate a poor perception of health. Conversely, when individuals prioritise their health and incorporate health services into their daily lives, they are expected to exhibit positive health behaviours [12, 13].

Health perception also reflects individuals' responsibility for their own health. Quality of life is closely related to transforming health perception into a sustainable lifestyle behaviour [14]. Attitudes play an important role in shaping health behaviours as they directly influence beliefs about health-related behaviours [15]. For healthy individuals, maintaining a positive perception of health depends on social, environmental and personal factors, which are all interrelated in preserving health. The preservation and management of health perception encompasses all interventions aimed at reducing life risk factors and preventing possible complications [16].

Improving public health relies on individuals' beliefs and perceptions regarding health, as these motivate them to take action. Receiving health education at an early age increases individuals' health perception levels, supporting the resilience of health systems [17]. An individual's educational level and life experiences influence their health-related decisions [18]. Furthermore, health perception serves as a reference point for health economists and

policymakers. It plays an important role in shaping health expenditure, marketing strategies and promotional activities among all stakeholders [19].

Adherence to treatment refers to patients voluntarily cooperating in taking prescribed medications and actively participating in the treatment process to overcome their illness in a healthy manner [20]. This includes following medical instructions, adhering to planned treatments and heeding the recommendations of healthcare professionals. [21] Alignment between the values of providers and patients facilitates adherence, whereas non-adherence may result from patients not taking their illness seriously enough. Many patients who do not adhere to treatment discontinue it of their own accord [22, 23]. In this context, adherence also depends on patients' compliance with lifestyle recommendations and the quality of communication they establish with their physician.

Compliance and non-compliance can be defined as conscious or unconscious behaviours towards prescribed medications. Conscious compliance occurs when the patient chooses to follow the treatment despite being aware of possible side effects or adverse outcomes (provided they have sufficient health literacy). Conversely, non-compliance can be influenced by various factors. For instance, even if a patient intends to comply, factors such as forgetfulness, difficulty accessing medication, or cost may hinder compliance [24].

Another aspect of non-compliance is when a patient initially takes the prescribed medication and complies with the treatment, but is unable to continue doing so, particularly due to communication problems with their physician. Difficulties in accessing healthcare facilities also play an important role. These factors can unintentionally have a negative impact on the treatment process. It has been observed that individuals with chronic diseases eventually stop seeking support from healthcare professionals, experiencing a decline in treatment adherence approximately six months later [25, 26].

Patient non-compliance with treatment is influenced by cultural background, economic status, the country's healthcare system, the nature of the disease and the treatment methods used. Personal factors such as level of knowledge, expectations, stress levels, motivation to cope with the disease, trust in the physician, perception of health and feelings of fatigue or weariness are particularly important [27]. Non-compliance with treatment affects not only the individual, but also their relatives and the general health of the community.

A trust-based relationship is healthy and has a positive impact on patients' treatment processes [28]. If there is mistrust between the physician and the patient, the likelihood of treatment decreases. In this case, patients may discontinue their treatment. Clinical treatment processes are faster for patients who trust their physicians [29].

Patient-centered communication strengthens patients' trust in their physician. This positively impacts treatment compliance processes. Research shows that effective communication with patients has been proven to increase their level of trust in their physician, and this result also increases treatment success [30].

Trust in the physician is fundamental to patient–physician relationships. In this context, it directly affects patients' compliance with the treatment process. This research was conducted within the framework of the Health Belief Model and Social Change Theory [31, 32]. The Health Belief Model posits that individuals shape their health-related behaviours based on their perceived disease risks, benefits, and barriers. Social Change Theory emphasises the need to evaluate trust in relationships. In light of these theoretical frameworks, the effects of physician trust and individuals' perceptions of health on treatment adherence can be better understood. Furthermore, individuals' perceptions of health are closely related to both their physical health status and their lifestyles. This study aims to examine the mediating role of health perceptions in the effect of physician trust on treatment adherence. The research question is defined as follows: 'How does individuals' trust in their physicians affect their treatment adherence through their perceptions of health?' The Health Belief Model and Social Exchange Theory offer a conceptual basis for explaining the interaction between trust in physicians and health perception. This theoretical framework seeks to illuminate how trust influences individuals' health perceptions and through which pathways this effect supports treatment adherence.

This study differs from previous research in that it considers the relationship between trust in

physicians, health perception and treatment adherence within a different model scope. Similar studies in the literature have examined the variables used in this study separately. This study examines the three research variables simultaneously and takes their effects on patient behaviour into account for evaluation. Additionally, the study emphasises the mediating role of these relationships by examining the consistency of health perceptions and trust in physicians. These are among the most important components of the service sector in terms of patient compliance with treatment. Very few studies in the relevant literature examine the interaction between these variables. In this context, the research is unique and offers a fresh perspective on the existing literature. The study addresses the concepts of trust in physicians, health perception and treatment compliance, examining the effects of these variables on each other. The hypotheses summarised below were developed, the data were analysed and evaluated, and the results are presented.

Research Hypotheses and Model

Trust in physicians enables patients to adopt a more positive approach to the treatment process. This trust increases patients' compliance with recommended treatments and accelerates their recovery. Trust in physicians positively affects treatment outcomes and improves patients' health status by encouraging better compliance with treatment procedures [33].

A review of the relevant literature reveals that Hall *et al*.'s study [34] determined that the trust relationship between physician and patient positively affects treatment compliance. Similarly, another study found that patients who established a trust-based relationship with their physician showed a higher level of compliance with treatment. Atıcı's study [35] also emphasised that strengthening physician trust increases compliance with treatment processes. In line with these findings, it can be concluded that physician trust increases treatment compliance.

Therefore, the following research hypothesis addressing the relationship between physician trust and treatment compliance was formulated:

H1: Trust in physicians positively affects treatment compliance.

Trust in physicians is an important factor that positively influences individuals' perception of health. However, some studies show that when the trust relationship with the physician is weak, individuals' perception of health may be negatively affected. A lack of trust can reduce patients' participation in treatment processes and cause them to develop negative attitudes towards health services. This can also have a negative effect on health outcomes.

For instance, Hall *et al*.'s [34] study revealed that patients with low trust in their physicians paid less attention to health information and exhibited poor treatment compliance. Çakmak and Uğurluoğlu's [32] research also indicated that a weak physician–patient trust relationship could negatively affect patients' perception of their health.

In light of this information, the following hypothesis was developed:

H2: Trust in physicians negatively affects health perception.

Individuals' perceptions of their health status may negatively impact their participation in treatment processes and adherence to treatment. For instance, when an individual perceives their health status as poor, they may be reluctant to participate in treatment or have difficulty adhering to medical recommendations [36].

Güven's [37] study showed that trust in physicians can negatively affect health behaviours and overall health perception. A study by Özdemir *et al*. [12] found that trust in physicians can negatively affect health perception, thereby weakening belief in the treatment process.

Based on these findings, the following hypotheses are proposed:

H3: Health perception negatively affects treatment compliance.

H4: Health perception acts as a mediating variable in the relationship between trust in physicians and treatment compliance (Figure 1).

METHODS

Study Procedure

The study was conducted in Sakarya, Turkey, between 25 October and 30 December 2024. Data

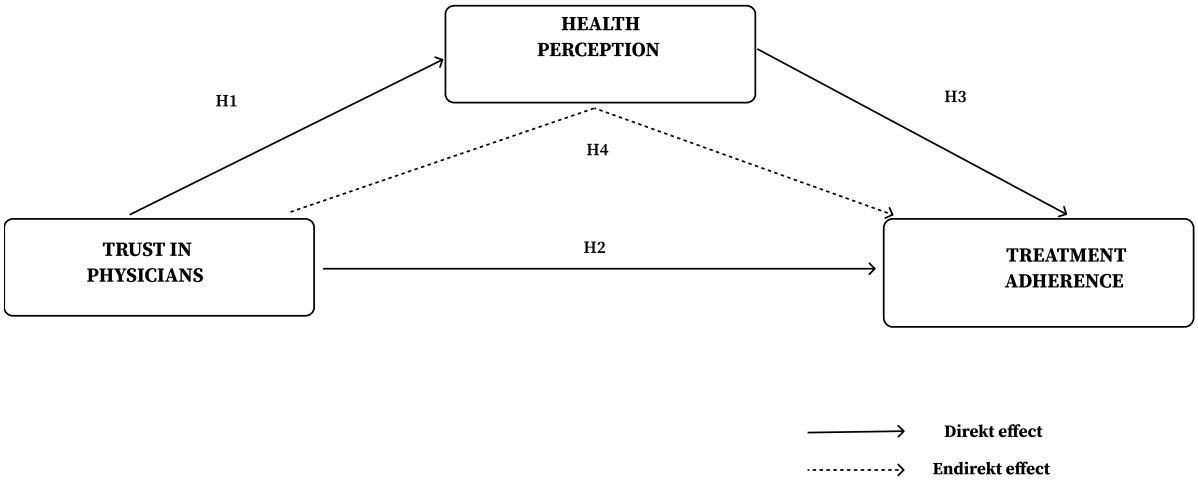

FIGURE 1. **Research model.**

were collected from 471 participants aged 18 years and over via convenience sampling. Based on a 95% confidence level, the minimum sample size was calculated as 384 individuals [40]. Participants' demographic characteristics, such as age, gender, and education level, were recorded to provide detailed profiles. Data were collected through a four-part questionnaire and measured using scales for trust in physicians (α=0.758), health perception (α=0.629) and treatment compliance (α=0.730). All scales showed acceptable reliability. Ethical considerations were observed and informed consent was obtained from all participants prior to the commencement of the study.

Participants

Data were collected from a total of 471 individuals. Of these, 35.9% (n=169) were male and 64.1% (n=302) were female. The mean age of participants was 31.46±8.73 years. In terms of age distribution, 56.6% (n=267) of participants were aged 25 or under, 12.9% (n=61) were aged 26–35, 14.0% (n=66) were aged 36–45, and 16.3% (n=77) were aged 46 or over. In terms of educational level, 21.6% (n=102) of participants had completed high school or less, 42.8% (n=202) had an associate's degree, 21.4% (n=101) had a bachelor's degree and 14.0% (n=66) had completed postgraduate studies.

Data Collection Instruments

A questionnaire consisting of four sections was used as the data collection instrument in this study. The first section included seven questions aimed at identifying the participants' demographic characteristics.

The second section utilised the scale developed by Deniz and Çimen in their study [38] 'A Research on Determining the Level of Trust in Physicians'. This scale contained 11 items, each rated on a five-point Likert scale (1 = strongly disagree, 5 = strongly agree). The reliability of the scale was confirmed with a Cronbach's alpha coefficient of 0.758 for the Trust in Physicians Scale.

The third section included the Health Perception Scale, adapted from Kadıoğlu and Yıldız's [39] study, 'Validity and Reliability of the Turkish Version of the Health Perception Scale'. Reliability analysis showed a Cronbach's alpha coefficient of 0.629 for the Health Perception Scale.

The fourth section comprised the Treatment Adherence Scale, derived from the study by Deniz *et al*., [40] 'The Mediating Role of Shared Decision-Making in the Effect of the Patient–Physician Relationship on Compliance with Treatment'. The reliability analysis yielded a Cronbach's alpha coefficient of 0.730 for the treatment adherence scale. In light of these results, it was determined that all three scales had acceptable reliability.

Statistical Analysis

The data obtained from the study were analysed using the SPSS 22.0 program. First, descriptive statistics were used to demonstrate the characteristics

of the sample and the general distribution of the variables. Counts and percentages were reported for categorical variables and mean, standard deviation, minimum and maximum values were reported for scale-type variables. To assess the reliability of the scales, the Cronbach's alpha coefficient was calculated for each one.

Pearson correlation analysis was performed to examine the relationships between the variables in the research model. This analysis showed the direction and significance of the relationships between trust in physicians, health perception and treatment compliance, and tested the preconditions for mediation analysis.

Multiple linear regression analyses were then applied to test the predicted effects in the model. The following were tested in order: (i) the effect of trust in physicians on health perception; (ii) the direct effect of trust in physicians on treatment compliance; and (iii) the effect of health perception on treatment compliance.

To examine the mediating role of health perception in the relationship between trust in physicians and treatment adherence, the steps proposed by Baron and Kenny were followed. In the final step, the independent and mediating variables were included in the same model and the decrease in the coefficient of the independent variable was evaluated. To confirm the significance of the mediating effect, bootstrap-based confidence intervals for the indirect effects were calculated. A significance level of $P<0.05$ was accepted for all analyses.

The statistical analyses were conducted using SPSS version 22.0. Descriptive statistics were calculated to determine participants' levels of trust in physicians, health perception and treatment adherence. All findings obtained during data analysis were evaluated at a 95% confidence interval and 5% significance level.

RESULTS

The findings obtained from this research are presented below. As shown in Table 1, the descriptive statistics revealed the following mean scores: trust in physicians (3.39±0.60); health perception (2.88±0.52); and treatment adherence (3.54±0.81). These values demonstrate that, while their health perception remained at a moderate level, participants reported a relatively high level of trust in physicians and treatment adherence (Table 1).

According to the correlation analyses, presented in Table 2, there was a positive and statistically significant relationship between trust in physicians and treatment adherence ($r = 0.527$), indicating that individuals who trusted their physicians more tended to report higher adherence to treatment. In the same table, trust in physicians was found to be negatively and significantly related to health perception ($r = –0.442$), suggesting that as trust in physicians increased, participants' perception of their own health decreased. In addition, health perception was negatively associated with both trust in physicians and treatment adherence, showing that individuals who perceived themselves as healthier were less likely to adhere to treatment recommendations (Table 2). The reliability analyses also demonstrated that the scales used in the study had acceptable to good internal consistency: Trust in Physicians Scale $\alpha = 0.758$, Treatment Adherence Scale $\alpha=0.730$, and Health Perception Scale $\alpha = 0.629$.

Regression analyses, the results of which are likewise summarized in Table 2, confirmed these relationships at the predictive level. First, trust in physicians significantly and negatively predicted health perception ($\beta = –0.377$, $P<0.001$), meaning that a one-unit increase in trust in physicians reduced health perception by 0.377 units. Second, trust in physicians significantly and positively predicted

TABLE 1. Descriptive Statistics and Correlations

Variables	Average	Standard deviation	Cronbach alfa	1	2	3
Trust in physicians	3.39	0.60	0.758	—		
Health perception	2.88	0.52	0.629	-0.442*	—	
Treatment adherence	3.54	0.81	0.730	0.527*	-0.443*	—

*Indicates statistically significant correlations ($P<0.05$).

TABLE 2. Impact Analyses

Effect	β	S.H.	t	P-value	LLCI	ULCI
Model 1: TP→ HP						
Constant	4.156	0.1218	34.1	**<0.001**	3.917	4.396
HG → SA	-0.377	0.0354	-10.7	**<0.001**	-0.447	-0.307
Model 2: TP, HP → TA						
Constant	2.864	0.3288	8.71	**<0.001**	2.218	3.510
TP→ TA	0.555	0.0570	9.74	**<0.001**	0.443	0.667
HP→ TA	-0.415	0.0668	-6.21	**<0.001**	-0.546	-0.283

TP, trust in physicians; HP, health perception; TA, treatment adherence; HG, trust in physicians; SA, health perception; SH, standard error; LLCI, lower limit of the confidence interval; ULCI, upper limit of the confidence interval.
Statistically significant P-values are shown in bold.

treatment adherence (β = 0.555, P<0.001); therefore, higher trust in physicians was associated with greater adherence to treatment. Third, health perception significantly and negatively predicted treatment adherence (β = –0.415, P<0.001), indicating that as individuals' perception of being healthy increased, their motivation to follow treatment decreased. This pattern may be interpreted as an indication that feeling healthy can reduce the perceived necessity for treatment (Table 2).

Finally, the mediation analysis results, displayed in Table 3, showed that health perception played a mediating role in the relationship between trust in physicians and treatment adherence. The indirect effect of trust in physicians on treatment adherence through health perception was significant (β=0.156), while the total effect of trust in physicians on treatment adherence remained strong and significant (β = 0.712, P<0.001). These findings indicate that part of the positive effect of trust in physicians on treatment adherence operates through individuals' perception of their health status, and that the inclusion of the mediator improves the explanatory power of the model (Table 3). In light of all these analyses, all of the hypotheses proposed in the study were supported and accepted.

These results demonstrate that a perception of good health strengthens the relationship between trust in physicians and compliance with treatment. All of the study's proposed hypotheses have been supported and accepted (Figure 2).

DISCUSSION

This study shows that patients' trust in their doctors is related to their perception of the treatment process. However, it also reveals that individuals who are ill

TABLE 3. Analysis of the Mediation Effect

Effect	β	S.E.	t	P-value	LLCI	ULCI
Direct effect: TP → HP	-0.377	0.0353	-10.68	**<0.001**	-0.446	-0.308
Direct effect: HP → TA	-0.415	0.0666	-6.23	**<0.001**	-0.545	-0.284
Direct effect: TP → TA	0.555	0.0568	9.77	**<0.001**	0.444	0.667
Indirect effect: TP → HP → TA	0.156	0.0291	5.38	**<0.001**	0.099	0.213
Total effect: TP → TA	0.712	0.0530	13.43	**<0.001**	0.608	0.816

TP, trust in physicians; HP, health perception; TA, treatment adherence; HG, trust in physicians; SA, health perception; SE, standard error; LLCI, lower limit of the confidence interval; ULCI, upper limit of the confidence interval.
Statistically significant P-values are shown in bold.

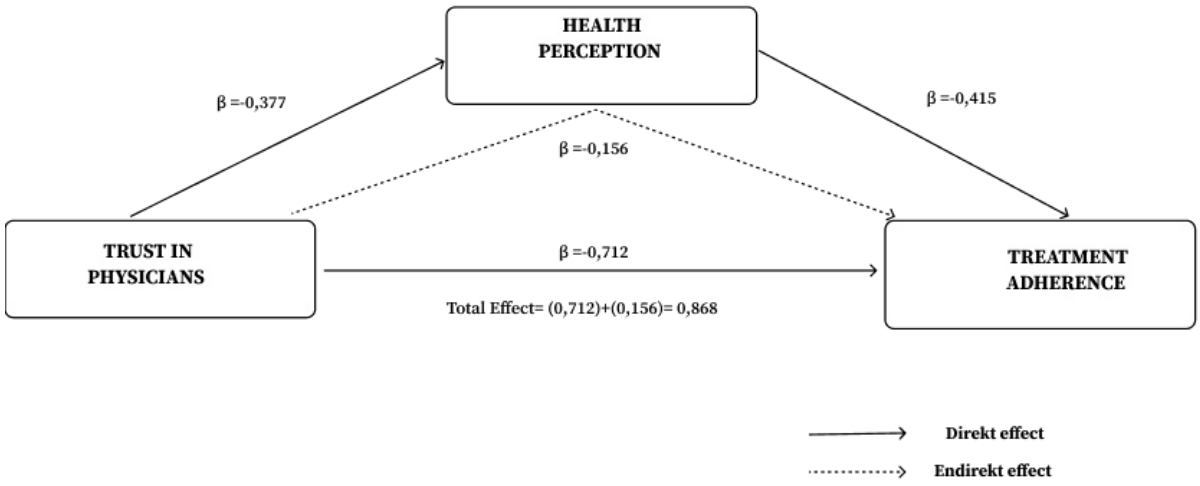

FIGURE 2. Mediating effect. (Illustrates the mediating role of health perception in the relationship between trust in physicians and treatment adherence.

have a low perception of their own health. Analysis of the study data shows that there is a mechanism which partially explains how patients' feelings about their health status affect their trust in doctors and compliance with treatment. In this context, perceptions of health and behaviours exhibited in terms of adherence to treatment protocols and durations, along with trust in doctors, stand out as key elements. The results obtained in this study are consistent with those of other studies in the literature, which show that trust in doctors influences patients' health-related decisions and habits. When evaluated holistically, the results reveal a new perspective by attempting to clarify patients' perceptions of trust and their desire to comply with treatment. Similar studies in the literature show that the sense of trust gained in the doctor–patient relationship facilitates compliance with treatment [34]. Another study by Thom [41] concluded that trust in physicians due to information asymmetry positively affects patient health outcomes. A meta-analysis by Birkhauer *et al*. [42] concluded that patients' trust in their physicians strengthens both their treatment satisfaction and psychological well-being. Another relevant study, by Safran *et al*. [43], found that patients with high levels of trust were more likely to comply with recommended treatments. Together, these studies show that patients' trust in their physicians is a critical determinant of treatment compliance.

This study's finding that health perception plays a mediating role in physician trust and treatment compliance is consistent with other studies showing the effect of health perception on individuals' health behaviours. Jylha [44] demonstrated that health perception predicts future health behaviours in patients. Another study by Idler and Idler [45] concluded that subjective health perception creates differences among patients in terms of disease prevalence and contagion. Furthermore, Hittner and Swickert [46] concluded that individuals with low health perception find it difficult to understand advice from physicians and comply with treatment. Additionally, Bailis *et al*.'s [47] research results show that individuals' health perception significantly affects behavioural health outcomes. Considering this study and others in the literature, it is evident that health perception is significant in the relationship between physician trust and treatment compliance. It is also thought that the results emerging from these studies will provide a comprehensive overview of the literature.

The negative effect of health perception on treatment adherence may be linked to individuals who feel healthy perceiving less need for medical intervention and therefore not fully following treatment recommendations. Despite high trust in physicians, lower health perception may reflect patients' more serious evaluations of their condition, increasing their reliance on a trusted physician. Taken together, these findings demonstrate that patients' perceptions of their health play a critical psychological role in shaping their treatment-related behaviours.

The research findings reveal that physician trust

increases treatment adherence while decreasing health perception, and that health perception acts as a mediator in the relationship between these two variables. However, these results may vary depending on cultural structures and health systems. For instance, in certain cultures, a high level of respect for medical authority may impact individuals' perceptions of health responsibility. Hofstede [48]. In this context, the tendency of healthy participants to be non-compliant with treatment can be linked to health beliefs and levels of authority that vary from culture to culture. Consequently, interventions that enhance health awareness and improve communication between physicians and patients at both the national and cross-cultural levels are critical for increasing treatment compliance.

This research examines the impact of trust in healthcare service delivery and health perception on treatment processes within a comprehensive model. This model is increasingly important today and the research makes recommendations based on its findings. The results of the research indicate that healthcare managers and policymakers need to strengthen communication between physicians and patients, promote trust-building practices and support health literacy interventions. Increasing trust in physicians will positively affect treatment compliance. Furthermore, it should be noted that variability in health perception may affect motivation for treatment. Therefore, future studies should expand to include different samples and models that incorporate various psychosocial variables, as this will contribute to improving the quality of healthcare services.

Strengths and Limitations

Among the strengths of this study is its focus on a mechanism that has been limitedly examined in the literature by investigating the mediating role of health perception in the relationship between trust in physicians and treatment compliance. Conducting the study specifically in Sakarya Province enabled a detailed examination of regional patient–physician relationships and individuals' health perceptions. The use of measurement instruments with established validity and reliability enhanced the scientific rigor and consistency of the findings. Moreover, the proposed research model provides a theoretical contribution by offering a comprehensive framework for explaining individuals' treatment compliance behaviors.

Among the limitations of the study, the fact that it was conducted solely in Sakarya Province restricts the generalisability of the findings to other regions. Differences in participants' demographic characteristics and individual variations in health perceptions further complicate the universal interpretation of the results. The use of self-report measures constitutes another limitation, as it relies on the assumption that participants provided accurate and conscious responses. In addition, the cross-sectional research design limits the ability to draw causal inferences regarding the relationships among the variables.

CONCLUSION

This study has revealed that trust in physicians has a positive effect on treatment compliance and a negative effect on health perception. Furthermore, it was determined that health perception plays a mediating role in the effect of trust in physicians on treatment compliance. These findings suggest that trust in physicians and health perception are key factors in treatment compliance.

The study offers various recommendations for healthcare administrators and relevant stakeholders. Strategies that could increase treatment adherence include organising training and seminars aimed at strengthening physician-patient communication, adopting trust-based approaches and regularly evaluating patient feedback. Understanding the effects of trust in physicians and health perception on treatment adherence is important for improving health expectations at an individual and societal level.

The aim of this research is to contribute to the existing literature by revealing the relationships between trust in physicians, health perception and treatment adherence. Future studies involving larger sample sizes and different variables could enhance the quality of healthcare services.

Ethics Approval and Consent to Participate

This study was approved by the Sakarya University Social and Human Sciences Ethics

Committee (Decision No: 2024/75-23; Date: 16.10.2024). All procedures were conducted in accordance with the ethical standards of the institutional and national research committees and with the 1964 Helsinki Declaration and its subsequent amendments. Written informed consent was obtained from all individual participants included in the study.

Data Availability

The authors confirm that all data generated or analyzed during this study are included in this published article. The data supporting the findings of the study are available from the corresponding author upon reasonable request.

Authors' Contribution

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

Conflict of Interest

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Generative Artificial Intelligence Statement

The author used artificial intelligence tools (ChatGPT, OpenAI) only for language editing and reference formatting. 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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Effects of COVID-19 Lockdown on Time in Therapeutic Range (TTR) for Warfarin Users

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Abstract:

Objective: This study investigated the impact of COVID-19 lockdown measures on Time in Therapeutic Range (TTR) for patients receiving warfarin therapy.

Methods: warfarin for at least one year before and after March 11th, 2020 (the start of pandemic) were included (n=112). Demographic data, comorbidities, medications, and International Normalized Ratio (INR) results were collected. TTR was calculated using the Rosendaal method.

Results: The mean TTR before the pandemic was 56.91%, significantly higher than the 40.23% observed during the pandemic (P<0.001). INR measurement intervals also increased significantly during the pandemic (34.2 days pre-pandemic vs. 50.9 days during the pandemic, P<0.001). This effect was most pronounced in patients over 65 years old (P<0.001).

Conclusion: COVID-19 lockdown measures significantly decreased TTR and extended INR monitoring intervals for warfarin users. These findings were particularly concerning in the elderly population. Strategies to ensure optimal warfarin monitoring during pandemics or disasters are crucial to prevent complications.

Keywords: COVID Lockdown, Warfarin, Time in Therapeutic Range, INR Monitoring

Oral anticoagulant therapy, particularly with the anti-vitamin K agent warfarin, plays a crucial role in both primary and secondary antithrombotic prophylaxis for patients with venous thromboembolism, atrial fibrillation (AF), and cardiac mechanical valves [1]. Careful monitoring of coagulation status is essential to prevent potentially life-threatening major haemorrhagic complications. Warfarin therapy presents a unique challenge due to its narrow therapeutic window. To mitigate this challenge and ensure optimal outcomes, patients on warfarin therapy require close follow-up and meticulous dose adjustments. This vigilance is crucial to minimise the risk of both bleeding and thrombotic complications [2]. An International Normalized Ratio (INR) below 2.0 is associated with an increased risk of stroke, while an INR exceeding 4.0 is linked to a higher risk of major bleeding events [3]. For stable oral anticoagulant therapy patients, the interval between INR measurements should not exceed four weeks [4]. Time in therapeutic range (TTR) has become valuable for assessing warfarin therapy quality. Numerous studies have consistently demonstrated a strong correlation between TTR and

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adverse event rates, solidifying TTR's role as a valid and practical tool for quality control evaluation.

The COVID-19 pandemic, declared in March 2020, prompted widespread lockdowns and social distancing measures that substantially transformed healthcare delivery [5]. Fear of infection and mobility restrictions, particularly among individuals over 65 years of age, led to a decline in outpatient visits.

This study examined how the COVID-19 pandemic influenced healthcare utilization patterns among warfarin users, specifically focusing on the impact of public apprehension towards hospital visits and the restrictions imposed by lockdowns.

METHODS

Study Population

We completed this study as a retrospective cohort study of patients receiving warfarin in the outpatient clinics of Malatya Training and Research Hospital, one of the region's leading tertiary care hospitals. We included patients who continued consecutive warfarin treatment for at least one year, six months before and six months after 11/03/2020, the turning point of the pandemic for our country.

This study investigated the impact of the COVID-19 pandemic on warfarin management among patients receiving follow-up care at our hospital. We included patients who utilized warfarin for various indications and were actively followed up from January 1st, 2019, to December 31st, 2020. Patients who did not have a pre-pandemic follow-up history at our institution or who discontinued follow-up after the pandemic began were excluded. The COVID-19 pandemic's official start in Turkiye, marked by the first reported case on March 11th, 2020, served as the demarcation point for this study. The preceding six months, from January 1st, 2020, to March 10th, 2020, were designated as the pre-pandemic period. Conversely, the subsequent six months, from March 11th, 2020, to December 31st, 2020, were defined as the pandemic period. This study retrospectively analyzed the hospital admissions of warfarin users within our institution for one year, encompassing the six months before and after March 11th, 2020 (the date of the first reported COVID-19 case and subsequent restrictions in Turkiye). Demographic characteristics, co-morbidities, and medication use were extracted from patients' electronic medical records. Additionally, the CHA2DS2-VASc score was calculated for each participant [6]. We evaluated the frequency of INR monitoring and the distribution of INR results during the pre-pandemic and pandemic periods, for patients with isolated mitral valve replacements (MVR) or combined aortic and mitral valve replacements (AVR+MVR), a therapeutic INR was defined as a value between 2.5 and 3.5. In contrast, for patients with AF or isolated aortic valve replacements (AVR), the target INR range was set between 2.0 and 3.0 [7]. TTR was calculated using the Rosendaal linear interpolation method, which assumes a linear change between consecutive INR measurements [TTR = (Days in range / Total days observed) × 100]. Employing the Rosendaal method, we calculated the TTR for patients before and after the pandemic period [8]. The National Institute for Health and Care Excellence (NICE) criteria were used to assess anticoagulation status, with TTR below 65% signifying poor control [9].

The local clinic's ethics committee approved all procedures used in the study, which was conducted in accordance with the Declaration of Helsinki. Written and signed consent was obtained from each participant.

Statistical Analysis

Statistical analyses were conducted using SPSS software (IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY, USA, IBM Corp.). Prior to conducting parametric statistical tests, the normality of the data distribution was evaluated. The Kolmogorov-Smirnov test, appropriate for sample sizes exceeding 50, was employed to assess the normality of TTR values. The test results indicated no significant deviations from normality (P=0.16), suggesting that the data distribution closely approximates a normal distribution. This finding supported the utilization of parametric statistical tests for further analysis. Skewness and kurtosis values were also examined to substantiate the normality assumption further. Both skewness and kurtosis fell within the acceptable range of ±1, further confirming the normality of the data distribution. A paired t-test was employed to compare TTR values between the

pre-pandemic and pandemic periods to investigate the impact of the pandemic on TTR values. Independent t-tests were utilized to examine the relationship between TTR and maximum days without INR values across different age groups. Cohen's d values were calculated to measure the effect size of the observed differences. Cohen's d values of 0.02, 0.05, and 0.08 were interpreted as small, medium, and large effects, respectively (Cohen, 1988). The significance level for all statistical tests was set at α=0.05.

RESULTS

This study included 112 patients receiving warfarin therapy, 61 (54.5%) women. The mean age was 61±14 years. Notably, 52 (46.4%) patients fell within the 65+ age group, where stricter restrictions were implemented. Warfarin use was primarily for mechanical heart valves in 92 (82.1%) patients, while 20 (17.9%) used it solely for AF. Among the AF patients, eleven lacked valve disease that would preclude direct oral anticoagulants (DOAC). The average CHA2DS2-VASc score was 2.48±1.59. Comorbidities included diabetes mellitus (33 patients, 29.5%), hypertension (56 patients, 50%), coronary artery disease (24 patients, 21.4%), heart failure (32 patients, 28.6%), and atrial fibrillation (40 patients, 35.7%). Additionally, 14 patients (12.5%) had a history of cerebrovascular events, and 15 (13.4%) had a glomerular filtration rate below 50 mL/min. Lastly, 23 patients (20.5%) used statins, proton pump inhibitors, amiodarone, and carbamazepine, which can interact with warfarin. A detailed breakdown of baseline patient characteristics is provided in Table 1. Analysis revealed a significant decrease in the frequency of INR monitoring during the pandemic period compared to the Pre- pandemic period. On average, patients underwent blood tests for INR measurement every 34.2±0.95 days in the Pre-pandemic period, whereas this interval increased to 50.9±0.99 days in the pandemic period (P<0.001). This translates to a decrease in the average number of blood tests from 5.45±0.95 in the Pre-pandemic period to 3.79±0.99 in the pandemic period. Stringent restrictions were implemented in our country during the COVID-19 pandemic due to the increased mortality risk observed in the over-65 age group. Table 2 details the pre-pandemic and pandemic INR monitoring frequencies stratified by patient age categories. The analysis revealed statistically significant longer INR measurement intervals in the over-65 age group (P<0.001).

Before March 11th, 2020 (considered the start of the COVID-19 pandemic in Turkiye), 42.8% of patients achieved their target INR range at their last measurement. However, following the pandemic's onset, the first INR measurement obtained for these patients fell outside the target range. The analysis revealed a significant increase in the time interval between INR measurements during the pandemic. On average, patients waited 80.8 days between their last pre-pandemic INR and their first INR measurements

TABLE 1. Baseline Characteristics of the Patients Using Warfarin

Age (years)	61±14
Female gender	61 (54.5 %)
Diabetes mellitus	33 (29.5 %)
Hypertension	56 (50 %)
Heart failure	32 (28.6 %)
Stroke	14 (12.5 %)
Coronary artery disease	24 (21.4 %)
Hemoglobin (g/dL)	12.2±1.4
Platelets, ($\times 10^9$/)L	228±56
AST (U/L)	32±24
ALT (U/L)	28±10
Creatinine (mg/dL)	1.03±0.4
GFR<50 (mL/min/1.73 m^2)	15 (13.4 %)
CHA_2DS_2-VASc score	2.48±1.59
Atrial fibrillation	40 (35.7 %)
Drug interactions	23 (20.5 %)
Indication of Vitamin K antagonist	
Mechanical valve	92 (82 %)
Non-valvular AF	11 (10 %)
Valvular AF	9 (8 %)

Data are shown as mean±standard deviation or n (%) where appropriate. AF, atrial fibrillation; ALT, alanine amino transferase; AST, aspartate amino transferase; CHA2DS2-VAScscore, congestive heart failure, hypertension, age (>65:1point, >75:2points), diabetes, previous stroke/transient ischemic attack (2 points), vascular disease and female gender; GFR, glomerular filtration rate.

TABLE 2. Average Days Between INR Measurements by Age Group

Period	<65-year-olds (n=60)		>65-year-olds (n=52)		t(110)	Cohen's d
	M	SD	M	SD		
Pre-pandemic period	32.91	6.87	35.75	7.66	-2.07*	-0.39
Pandemic period	45.60	11.98	57.06	14.26	-4.62***	-0.88
All period	37.45	6.13	43.28	7.76	-4.44***	-0.84

INR, international normalized ratio; M, mean; SD, standard deviation.
*P<0.01, ***P<0.001.

while the pandemic was ongoing. This interval was even longer in the over-65 age group, averaging 102.8 days (P<0.001), compared to younger patients. Analysis of time in TTR revealed a significant decrease during the pandemic period compared to the Pre-pandemic period. The average TTR in the Pre-pandemic period was 56.91%, whereas it dropped to 40.23% in the pandemic period (Table 3). Further evaluation stratified by age categories (Figure 1) demonstrated that patients over 65, a group with stricter pandemic restrictions, exhibited lower TTR values than younger patients in both Pre- pandemic period and pandemic period.

DISCUSSION

Warfarin remains the cornerstone of stroke prevention strategies. However, its narrow therapeutic window presents a significant challenge in achieving optimal clinical use. Multiple factors influence the quality of anticoagulation with warfarin, including patient characteristics (diet, age), comorbidities, concomitant medications, and the drug's pharmacokinetic and pharmacodynamic properties. TTR serves as a critical metric for assessing anticoagulation quality and has been demonstrably linked to adverse outcomes such as stroke, haemorrhage, and mortality. Randomized controlled trials reveal that only approximately 60% of patients achieve the desired TTR threshold with warfarin treatment [10]. This study investigated the impact of the COVID-19 pandemic on TTR for warfarin users. We observed a significant decrease in average TTR during the pandemic period compared to the pre-pandemic period (P<0.001). This decline can be attributed, in part, to a reduction in outpatient clinic visits for INR monitoring during the pandemic's initial stages. Hospitals prioritized COVID-19 diagnosis and treatment, leading to fewer patients seeking clinic INR monitoring. Additionally, concerns about nosocomial transmission likely discouraged patients from frequent hospital visits, particularly those worried about COVID-19 infection.

Consistent with previous findings, our study demonstrated lower TTR values in the over-65 age group, both pre-pandemic and during the pandemic (P< .001). The VARIA study reported lower TTR in warfarin users exceeding 55 years of age, and similar age-related TTR reductions have been observed in patients with atrial fibrillation [11,12]. Potential explanations for this negative correlation between age and TTR include age-related changes in drug

TABLE 3. Age-Stratified Comparison of TTR

Age	Pre-pandemic TTR		Pandemic TTR		t	Cohen's d
	M	SD	M	SD		
<65-year-olds (n=60)	59.74	19.66	47.49	22.33	3.80***	0.49
>65-year-olds (n=52)	53.65	22.93	31.85	21.13	5.57***	0.77
Full group (n=112)	56.91	21.36	40.23	23.06	6.57***	0.62

M, mean; SD, standard deviation; TTR, time in therapeutic range.
***P<0.001.

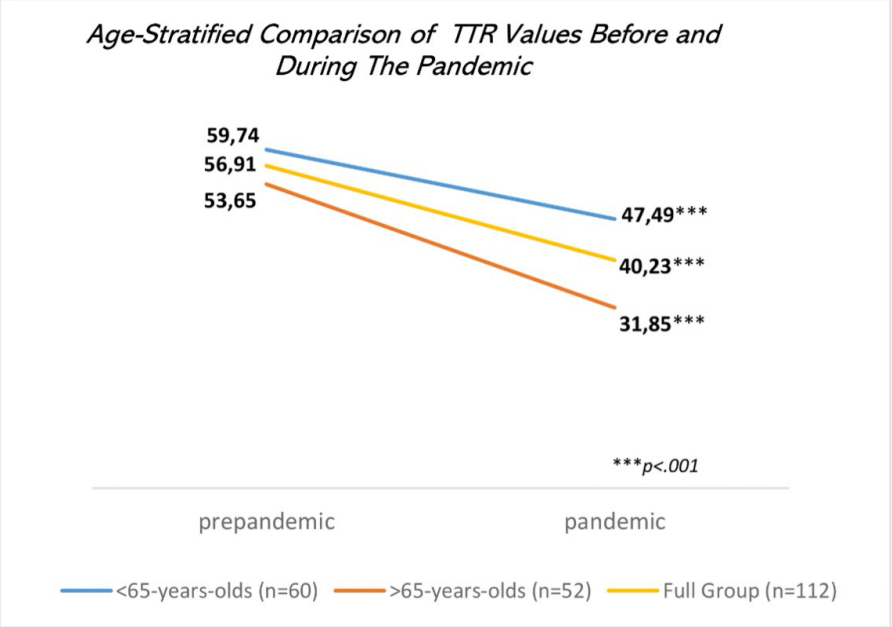

FIGURE 1. Target TTR values before (6 months) and during (6 months) the pandemic.

metabolism, increased comorbidities in elderly patients, and cognitive decline.

However, our study not only confirms lower TTR in the over-65 population pre-pandemic and pandemic but also reveals that this age group experienced the most significant TTR decline during the pandemic. This finding can likely be attributed to two key factors: heightened reluctance among elderly patients to visit crowded environments like hospitals due to their increased vulnerability to COVID-19 mortality, and stricter enforcement of pandemic restrictions for individuals over 65 in our country. A prospective study by Turk *et al*. [13] investigating TTR in Turkish AF patients reported an average TTR of 42%. Our analysis revealed a pre-pandemic period TTR of 56.91% for all warfarin-treated patients, significantly decreasing to 40.23% during the pandemic period.

Current guidelines recommend INR testing intervals no longer than 4-6 weeks for stable patients on warfarin [14]. Our study revealed a significant increase in INR monitoring intervals during the pandemic compared to the pre-pandemic period (34.2 days vs. 50.9 days, $P<0.05$). Notably, the longest intervals were observed in the over-65 age group, where stricter pandemic restrictions were implemented. In this group, INR measurements were performed every 57 days on average during the pandemic. These extended intervals significantly exceeded the recommended guidelines. Subtherapeutic INR levels are known to elevate the risk of both thromboembolic events and bleeding complications [15]. A study by Shalev *et al*. [16] involving 4408 chronic AF patients demonstrated superior TTR with frequent, regular INR monitoring (intervals ≤ 3 weeks with 7-day multiples) compared to less frequent or irregular schedules.

Our analysis identified a concerning gap of 80.8 days between the last pre-pandemic INR test and the first INR test performed after the pandemic's onset. This interval further extended to 102.8 days in the over-65 age group. These extended periods significantly exceed the recommended 1-month interval and pose a potential risk of severe complications.

Furthermore, only 33% of the first INR measurements obtained following the pandemic fell within the target range. Additionally, while patients were expected to undergo an average of 5-6 INR tests during the first six months of the pandemic, the average number of examinations during this period was only 3.79.

Our findings highlight a concerning trend: pre-existing suboptimal TTR levels in the Turkish

population are further declining during the COVID-19 pandemic to a degree potentially compromising patient safety. As previously established, inadequate TTR is associated with increased risks of both thromboembolic and bleeding complications [15]. While this study, due to its observational design, did not capture the incidence of adverse events associated with suboptimal TTR, other studies have documented the significant morbidity and mortality associated with poor TTR control. Notably, our data reveal a disproportionate impact on the over-65 age group, a population subject to stricter pandemic restrictions. This vulnerability emphasizes the potential dangers posed by disruptions to healthcare access during pandemics or other public health emergencies.

The results of this study underscore the urgent need for healthcare providers to develop strategies to mitigate the risks identified. Educational initiatives aimed at patients, particularly those over 65, are crucial to emphasize the importance of INR monitoring and the potential consequences of non-adherence. For non-valvular atrial fibrillation patients, a temporary shift towards newer, more user-friendly DOAC compared to warfarin may be warranted during pandemic periods. Additionally, further development of home INR monitoring systems is crucial. These systems could involve either point-of-care devices for patients' home use or healthcare provider-initiated home blood sampling for laboratory analysis. Ultimately, a heightened awareness of these pandemic-related risks among patients and healthcare professionals is essential for optimal patient outcomes.

Strengths and Limitations

A major strength of this study is that it provides empirical evidence of the 'collateral damage' caused by the pandemic, demonstrating that public health restrictions negatively impacted chronic disease management independently of the virus itself.

This study has limitations. The retrospective design prevented us from capturing data on patient outcomes, such as bleeding and thrombosis. Additionally, the single-centre design resulted in a relatively small sample size, potentially limiting the generalizability of our findings.

CONCLUSION

This study investigated the impact of pandemic-related restrictions on warfarin users. We observed a significant decline in TTR during the pandemic, potentially leading to adverse consequences for patients with subtherapeutic INR levels. Additionally, INR monitoring intervals were significantly extended. These findings were particularly concerning in the elderly population. This study highlights patients' challenges in maintaining therapeutic warfarin levels during the pandemic. Our findings underscore the potential risks associated with disruptions to healthcare access during public health emergencies like pandemics or natural disasters. It is crucial to develop strategies, informed by data from this study, to ensure optimal warfarin monitoring during such events.

Ethics Approval and Consent to Participate

This study was approved by the Inonu University Health Sciences Non-Interventional Clinical Research Ethics Committee (Decision No:2025/7288; date: 25.03.2025). 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. 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: İA; Study Design: İA; Supervision: EY; Funding: GS, İA; Materials: İ.A, GS; Data Collection and/or Processing: İA, EY, GS; Statistical Analysis and/or Data Interpretation: EY, İA; Literature Review: EY; Manuscript Preparation: GS; and Critical Review: İA, EY, GS.

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(6):692-703 Research Article

DOI: https://doi.org/10.18621/eurj.1793048 *Dermatology*

Clinical and Dermoscopic Features of Erythema Dyschromicum Perstans: A Case Series

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Abstract:

Objective: Erythema dyschromicum perstans (EDP) is a rare acquired dermal melanosis that clinically overlaps with lichen planus pigmentosus and Riehl's melanosis. Its diagnosis relies on integrating clinical, dermoscopic, and histopathological features. To characterize the clinical, dermoscopic, and histopathological spectrum of EDP in a 14-patient case series and to highlight the diagnostic contribution of dermoscopy.

Methods: A retrospective review was conducted of 14 biopsy-proven EDP patients diagnosed between 2023 and 2025 at a secondary care dermatology center. Demographic data, clinical distribution, dermoscopic findings, histopathology, and treatments were systematically analyzed.

Results: Patients had a mean age of 41.4 years (range 21–69); 12 were female and 2 male, with Fitzpatrick skin types III–IV. Mean disease duration was 2.4 years (range 4 months–10 years). Lesions most often involved the trunk (71.4%), with additional sites including the face, upper arms, axillae, inframammary folds, and neck. Most patients were asymptomatic; two reported mild pruritus. Dermoscopy, available in ten cases, revealed a bluish-gray granular background in 90%, darker blotches in 40%, faint blurred vascular structures in 40%, and perifollicular accentuation in 20%; a diffuse cloud-like pattern was noted in one case. Histopathology consistently showed basal vacuolar alteration, pigment incontinence, and dermal melanophages, with Fontana–Masson confirming dermal melanin in selected specimens. Treatments included isotretinoin in four patients, dapsone in three, topical agents in four, while three remained untreated due to loss to follow-up.

Conclusion: This series documents both sexes and rarely reported sites of EDP, including a pregnancy-associated case, and highlights reproducible dermoscopic clues that support histopathologic confirmation and aid in distinguishing EDP from other acquired dermal melanoses. Larger prospective multicenter studies are warranted to clarify therapeutic approaches and long-term outcomes.

Keywords: Erythema Dyschromicum Perstans, Dermoscopy, Histopathology, Hyperpigmentation, Acquired Dermal Macular Hyperpigmentation

Erythema dyschromicum perstans (EDP) is a chronic acquired pigmentary disorder characterized by slowly progressive ash-gray macules, most often on the trunk, neck, face, and proximal extremities. First described by Ramirez in 1957 as ashy dermatosis (AD) [1], EDP and AD have frequently been used interchangeably. However, a 2018 global consensus highlighted overlaps with lichen planus pigmentosus (LPP) while supporting their distinction based on inflammation, erythema, and

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distribution [2, 3]. EDP occurs worldwide but is more frequently recognized in Latin America and Asia. It typically affects young to middle-aged adults, with occasional pediatric cases, shows a slight female predominance, and is more readily identified in darker phototypes (III–V) [4–8]. Clinically, lesions are asymptomatic, symmetrically distributed gray to blue-gray macules or patches with ill-defined borders; an erythematous rim may indicate early activity, but pruritus is uncommon [6, 8].

The etiology of this condition remains unclear. Proposed triggers include infections, chemicals, drugs, and endocrinopathies, while HLA-DR4 positivity in Mexican Mestizo patients suggests genetic susceptibility [9, 10]. The ashy hue reflects the Tyndall effect of dermal melanin after basal cell injury and pigment incontinence. Histopathology typically shows basal vacuolar change, interface dermatitis, and dermal melanophages [8–10]. Key differential diagnoses include LPP, idiopathic eruptive macular pigmentation (IEMP), post-inflammatory hyperpigmentation, and Riehl's melanosis (RM). Dermoscopy usually demonstrates a bluish-gray to brown structureless background with fine granular "speckled" dots, while vascular and follicular changes are minimal and vessels are faint or out-of-focus [11–14].

Therapeutic responses are variable. Dapsone and clofazimine may yield partial improvement, topical tacrolimus and newer agents show emerging promise, but persistence for years is common in adults. In contrast, spontaneous resolution can occur in children [12, 15–17].

This study aimed to describe the clinical and dermoscopic features of EDP in 14 patients and to highlight dermoscopic clues that differentiate EDP from other acquired dermal melanoses, supplemented by histopathology and therapeutic outcomes.

METHODS

This retrospective case series included 14 patients (12 females, 2 males) diagnosed with EDP between 2023 and 2025 at the Department of Dermatology, Nizip State Hospital, Gaziantep, Turkey. Diagnosis was based on the integration of characteristic clinical morphology, dermoscopic features, and histopathological confirmation. Patients with concomitant pigmentary disorders (e.g., melasma, post-inflammatory hyperpigmentation) or incomplete clinical/dermoscopic data were excluded. Cases with histological features favoring LPP (e.g., band-like lichenoid infiltrates) were also excluded.

Demographic variables (age, sex, and Fitzpatrick skin phototype), disease duration, lesion distribution, symptoms, dermoscopic findings, histopathological features, and treatment details were systematically recorded. Dermoscopy was performed with a polarized dermoscope at ×10 magnification (DermLite DL5; 3Gen, USA), and images were reviewed by a dermatologist for pigmentary, vascular, and follicular structures.

Punch biopsies were obtained from representative lesions in all patients. Histopathology confirmed basal vacuolar alteration, pigment incontinence, and dermal melanophages, while Fontana–Masson staining was applied in selected cases to demonstrate dermal melanin deposition.

Given the small sample size, data were analyzed descriptively without inferential statistics. The study was approved by the Bezmialem Vakıf University Scientific Research Ethics Committee (Approval No: 2025/347; Date: 23 September 2025; Document No: E-54022451-050.04-210711) and was conducted in accordance with the principles of the Declaration of Helsinki.

Case Presentations

Ten female patients with dermoscopic documentation (Cases 1–10) are presented below; the remaining four patients (Cases 11–14) had similar clinical and histopathological features without dermoscopic images.

CASE 1. A 29-year-old woman (Fitzpatrick skin type IV) presented with a 6-month history of asymptomatic slate-gray macules on the upper and lower back (Figure 1a). Dermoscopy showed a bluish-gray structureless background with fine speckled granules and perifollicular halos (Figure 1b). Histopathology confirmed basal vacuolar changes with dermal melanophages, consistent with EDP. Oral dapsone 100 mg/day was started after G6PD screening.

CASE 2. A 63-year-old woman (skin type IV) presented with a 12-month history of progressive gray-

brown facial hyperpigmentation (Figure 1c). Dermoscopy showed a homogeneous brown-gray background with fine bluish-gray speckled granules, without follicular or vascular changes (Figure 1d). Histopathology demonstrated pigment incontinence with mild perivascular lymphocytic infiltration. Oral dapsone 100 mg/day was initiated.

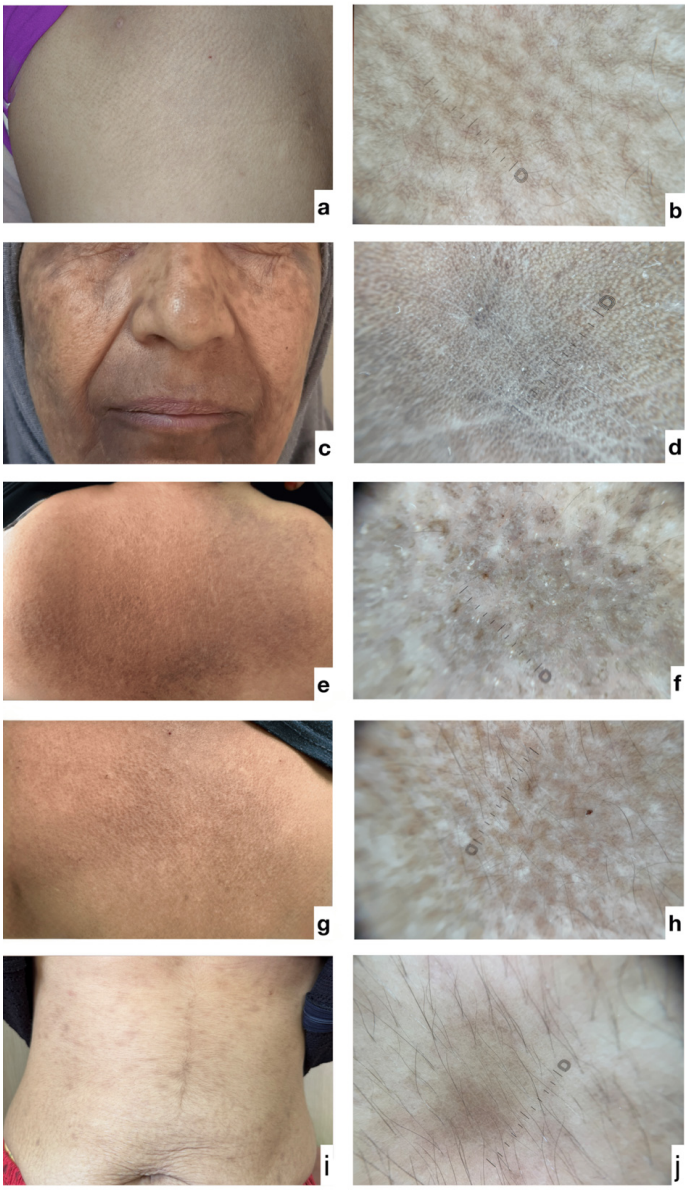

FIGURE 1. Clinical and dermoscopic features of erythema dyschromicum perstans (Cases 1–5) (a, b) Case 1, upper and lower back: bluish-gray background with perifollicular halos. (c, d) Case 2, face: homogeneous speckled bluish-gray granules. (e, f) Case 3, upper back: diffuse bluish-gray granules with interspersed brown areas. (g, h) Case 4, upper back: coarse and fine bluish-gray granules with perifollicular accentuation. (i, j) Case 5 – Trunk: homogeneous gray-brown "cloud-like" structureless pigmentation without vascular structures.

CASE 3. A 49-year-old woman (skin type III) had an 8-year history of asymptomatic slate-gray patches on the upper back (Figure 1e). Dermoscopy showed a bluish-gray structureless background with irregular speckled granules and occasional light-brown areas (Figure 1f). Histopathology confirmed basal vacuolar change with pigment incontinence and dermal melanophages. Oral dapsone 100 mg/day was prescribed.

CASE 4. A 44-year-old woman (skin type III) had a 10-year history of hyperpigmented macules on the upper back (Figure 1g). Dermoscopy showed a bluish-gray background with coarse and fine granules and perifollicular accentuation, with follicular openings preserved; faint blurred linear vessels were occasionally observed (Figure 1h). Histopathology confirmed basal vacuolar change with pigment incontinence and dermal melanophages. Oral isotretinoin 30 mg/day was initiated.

CASE 5. A 24-year-old woman in her second pregnancy (12 weeks) presented with a 6-month history of asymptomatic, slowly progressive gray-brown macules on the trunk. Lesions were symmetrically distributed, ill-defined, and non-scaly. Dermoscopy showed a homogeneous gray-brown diffuse cloud-like pattern without vascular structures. Histopathology demonstrated basal vacuolar change with increased basal pigmentation and dermal melanophages, consistent with EDP. Due to pregnancy, she was treated with topical centella asiatica and a low-potency corticosteroid.

CASE 6. A 51-year-old woman (skin type IV) presented with an 8-month history of hyperpigmented patches in the axillae and inframammary folds (Figure 2a). Dermoscopy showed a brown-gray background with an irregular granular pattern and scattered darker blotches, with a few blurred linear vessels (Figure 2b). Histopathology confirmed basal vacuolar change with pigment incontinence and numerous dermal melanophages. Oral isotretinoin 20 mg/day was initiated.

CASE 7. A 69-year-old woman (skin type III) presented with a 4-month history of slate-gray to brown macules clustered along the midline of the trunk (Figure 2c). Dermoscopy showed a diffuse gray-brown background with irregular darker blotches and fine focal granules, without vascular or follicular

changes (Figure 2d). Histopathology confirmed basal vacuolar change with pigment incontinence and numerous dermal melanophages. Oral isotretinoin 20 mg/day was initiated.

CASE 8. A 58-year-old woman (skin type IV) had a 3-year history of progressive slate-gray to brown facial hyperpigmentation, predominantly on the cheeks and forehead (Figure 2e). Dermoscopy showed a brown-gray background with numerous fine bluish-gray granules in a homogeneous speckled pattern, without vascular or follicular changes (Figure 2f). Histopathology confirmed basal vacuolar change with pigment incontinence and dermal melanophages. She was treated with topical pimecrolimus and oral isotretinoin 30 mg/day.

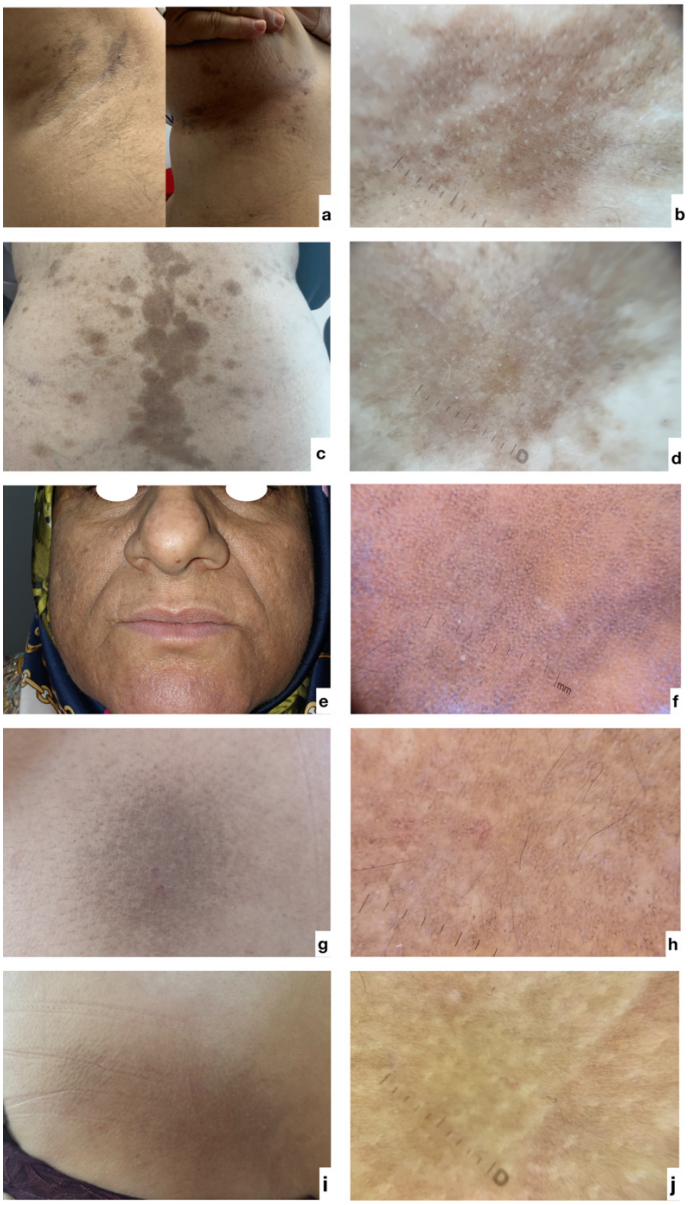

FIGURE 2. Clinical and dermoscopic features of erythema dyschromicum perstans (Cases 6–10) (a, b) Case 6, axillae and inframammary folds: irregular brown-gray granules with scattered darker blotches. (c, d) Case 7, trunk: diffuse gray-brown background with irregular blotches. (e, f) Case 8, face: brown-gray background with fine bluish-gray speckled granules. (g, h) Case 9, upper back: gray-brown granular background with superimposed darker blotches. (i, j) Case 10, mid- to lower back: slate-gray macules with gray-brown background and fine speckled granules with focal irregular blotches.

CASE 9. A 32-year-old woman (skin type III) had a 2-year history of asymptomatic slate-gray macules on the upper back (Figure 2g). Dermoscopy showed a gray-brown background with fine speckled granules and irregular darker blotches, without vascular or follicular changes (Figure 2h). Histopathology confirmed basal vacuolar change with pigment incontinence and dermal melanophages. She was started on topical 4% hydroquinone.

CASE 10. A 30-year-old woman (skin type III) had a 3-year history of asymptomatic slate-gray macules on the mid- and lower back (Figure 2i). Dermoscopy showed a bluish-gray to brown background with fine speckled granules and scattered darker blotches, without vascular or follicular changes (Figure 2j). Histopathology confirmed basal vacuolar change with pigment incontinence and dermal melanophages. The patient did not return for follow-up, and no treatment was initiated.

Additionally, four patients (Cases 11–14) showed similar clinical and histopathological findings but lacked dermoscopic documentation. Their demographic and clinical characteristics are summarized in Table 1.

Statistical Analysis

All analyses were descriptive. Continuous variables were reported as mean and range (minimum–maximum), and categorical variables as counts and percentages. No inferential statistical tests were performed due to the study design and sample size.

RESULTS

Fourteen biopsy-proven patients with EDP were included. The mean age was 41.4 years (range 21–69);

TABLE 1. Clinical and Demographic Characteristics of 14 Patients with Erythema Dyschromicum Perstans

Patient No.	Age (years)	Sex	Skin photo type	Disease duration	Lesion location(s)	Symptoms	Treatment
1	29	F	IV	6 mo	Upper and lower back	–	Dapsone
2	63	F	IV	12 mo	Face	Mild cosmetic concern, no pruritus	Dapsone
3	49	F	III	8 y	Upper back	(long-standing, asymptomatic)	Dapsone
4	44	F	III	10 y	Upper back	Occasional mild pruritus	Isotretinoin
5	24	F	IV	6 mo	Trunk (abdomen, back)	–	Centella asiatica topical preparation + low-potency corticosteroid (due to pregnancy)
6	51	F	IV	8 mo	Axillae + Inframammary folds	–	Isotretinoin
7	69	F	III	4 mo	Upper and lower back	–	Isotretinoin
8	58	F	IV	3 y	Face (cheeks + forehead)	–	Pimecrolimus cream + oral isotretinoin 30 mg/day
9	32	F	III	2 y	Upper back	–	Topical 4% hydroquinonecream (initial treatment)
10	30	F	III	3 y	Mid- and lower back	–	None (patient did not return for treatment after biopsy confirmation)
11	47	M	III	2 y	Upper back	Occasional mild pruritus	None (patient did not return for treatment after biopsy confirmation)
12	35	M	IV	1 y	Back and upper arms	–	None (patient did not return for treatment after biopsy confirmation)
13	21	F	III	1 y	Back and upper arms	–	Topical agents (hydroquinone, tretinoin, hydrocortisone)
14	28	F	III	1 y	Neck	Cosmetic concern, no pruritus	Topical agents (hydroquinone, tretinoin, hydrocortisone)

The table outlines age, sex, skin phototype, disease duration, lesion distribution, and treatment details. F, female; M, male; mo, months; y, years; –, absent.

12 were female and 2 male. Fitzpatrick phototypes were type III in 8 and type IV in 6. Mean disease duration was 2.4 years (range: 4 months–10 years). Lesions most often affected the trunk (71.4%), with additional sites including the face (14.3%), upper arms (14.3%), flexural areas (7.1%), and neck (7.1%). Two (14.3%) patients reported mild pruritus (Table 1).

Dermoscopy was performed in 10 cases, revealing a bluish-gray granular background in 9 (90%), a diffuse cloud-like pattern in 1 (10%), darker blotches in 4 (40%), faint blurred vascular structures in 4 (40%), and perifollicular accentuation in 2 (20%). These case-based dermoscopic characteristics are presented in Table 2, while the overall frequency distribution of dermoscopic features is summarized in Table 3.

Histopathology in all patients revealed basal vacuolar alteration, pigment incontinence, numerous dermal melanophages, and a superficial perivascular lymphocytic infiltrate, while Fontana–Masson staining in selected biopsies confirmed dermal melanin deposition. Treatments included oral isotretinoin in 4 patients, oral dapsone in 3, and topical depigmenting or anti-inflammatory agents in 4, whereas 3 patients remained untreated due to loss to follow-up.

DISCUSSION

This case series contributes to the limited literature on EDP by highlighting its clinical and dermoscopic characteristics. In our cohort, lesions most often affected the trunk, face, mid- to lower back, axillae,

TABLE 2. Clinical and Dermoscopic Features of 10 Patients with Erythema Dyschromicum Perstans

Patient No.	Clinical morphology	Dermoscopic pigment pattern	Vascular features	Follicular changes
1	Slate-gray macules, back	Bluish-gray granules + light brown areas; perifollicular halos	None	Preserved
2	Gray-brown confluent patches, face	Speckled bluish-gray & light brown granules	None	None
3	Long-standing slate-gray patches, back	Diffuse bluish-gray granules; irregular pattern	Rare blurred vessels	None
4	Chronic brown-gray macules, back	Coarse + fine bluish-gray granules; perifollicular accentuation	Faint linear vessels	Preserved
5	Gray-brown, ill-defined macules on the trunk	Homogeneous diffuse gray-brown pigmentation, cloud-like pattern	None	None
6	Brown-gray patches, axillae/inframammary	Irregular brown-gray granules + darker blotches	Few blurred vessels	Absent
7	Slate-gray to brown confluent patches, trunk	Gray-brown background + darker blotches; fine speckled granules	Rare blurred vessels	Preserved
8	Brown-gray facial patches	Homogeneous bluish-gray fine granules	None	None
9	Ill-defined gray patches, back	Gray-brown background; fine granules + darker blotches	None	None
10	Slate-gray patches, mid/lower back	Bluish-gray to brown granules + darker blotches	None	None

The table summarizes clinical morphology, dermoscopic pigment patterns, and vascular/follicular features for cases with dermoscopy (cases 1–10).

TABLE 3. Frequency of Dermoscopic Features in 10 Patients with Erythema Dyschromicum Perstans

Dermoscopic feature	n (out of 10)	Frequency (%)
Bluish-gray granular background	9	**90%**
Diffuse cloud-like pattern	1	**10%**
Darker blotches / irregular hyperpigmented areas	4	**40%**
Faint or blurred vascular structures	4	**40%**
Perifollicular accentuation / perifollicular halos	2	**20%**

This table summarizes the prevalence of key dermoscopic findings among the 10 patients with available dermoscopic documentation. The bluish-gray granular background was the most consistent feature, whereas darker blotches, faint blurred vessels, and perifollicular accentuation were variably observed.

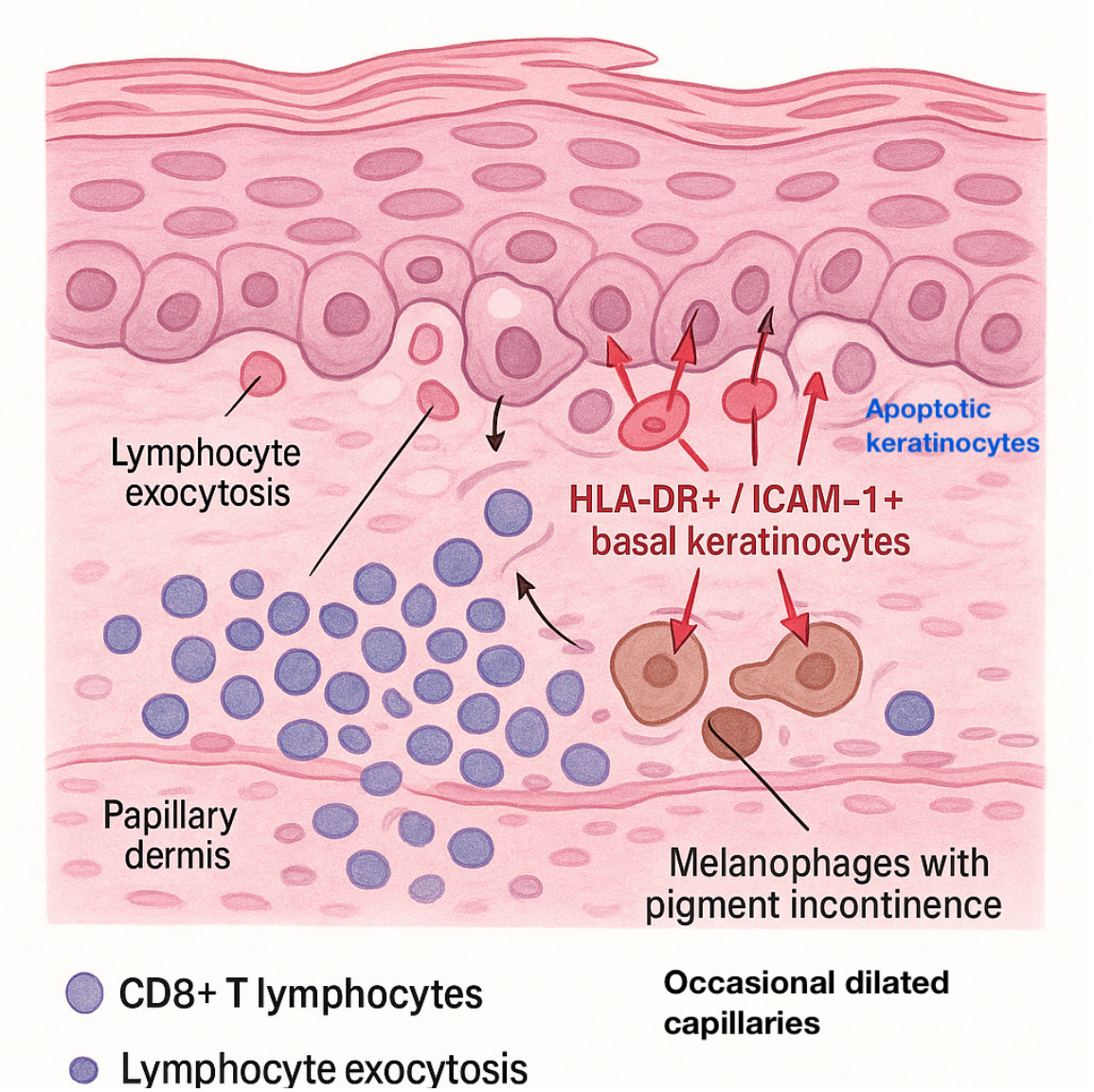

FIGURE 3. Proposed immunopathogenesis of erythema dyschromicum perstans. $CD8^+$ T lymphocytes infiltrate the papillary dermis and exocytose into the epidermis, targeting $HLA\text{-}DR^+/ICAM\text{-}1^+$ basal keratinocytes. This process induces keratinocyte apoptosis and pigment incontinence, with dermal melanophages engulfing released melanin. Occasional dilated capillaries may also be present (Created with BioRender.com).

TABLE 4. Comparative Clinical, Dermoscopic, and Histopathologic Features of Acquired Dermal Macular Hyperpigmentation (ADMH) Spectrum Disorders

Feature	EDP/AD	LPP	IEMP
Age at onset	Young adults; children possible	30–50 years	Children/adolescents
Sex distribution	No clear predilection (slight female bias in some series)	No predilection	No predilection
Skin phototype	More common in darker phototypes (III–V)	Darker phototypes	Lighter
Symptoms	Usually asymptomatic, mild pruritus rare	Mild pruritus frequent	Asymptomatic
Lesion morphology	Large, slate-gray/blue-gray macules and patches; often symmetric	Brownish-gray macules, smaller and more diffuse	Small, dark brown well-defined macules
Borders	Ill-defined, may have raised erythematous rim (active stage)	Ill-defined	Well-defined
Distribution	Trunk most common; face and neck; proximal extremities; rarely axillae	Face and neck most common, flexures, trunk	Trunk and extremities
Mucosal involvement	Absent	Rare	Absent
Dermoscopy	Homogeneous bluish-gray "cloud-like" background with fine–coarse curvilinear granules; uniform distribution; perifollicular/acrosyringeal accentuation absent; vessels minimal.	Brown–gray dots/globules in reticular or hem-like pattern; irregular distribution; perifollicular/acrosyringeal accentuation prominent; pseudonetwork ± telangiectatic vessels.	Brown macules, no specific dermoscopic pattern
Histopathology	Basal vacuolar change, dermal melanophages, superficial perivascular infiltrate	Interface dermatitis, basal vacuolar change, melanophages, sometimes lichenoid	Epidermal hypermelanosis, dermal melanophages
Clinical course	Chronic, persistent; rarely resolves in adults; children may remit	Chronic, treatment-resistant	Often resolves spontaneously within months–years

EDP, erythema dyschromicum perstans; AD, ashy dermatosis; LPP, lichen planus pigmentosus; IEMP, Idiopathic eruptive macular pigmentation. EDP is characterized by bluish-gray macules with minimal vascular features, LPP by brownish-gray globules with perifollicular accentuation, and IEMP by small well-defined brown macules without vascular changes.

and inframammary folds, consistent with prior reports that identified the trunk, extremities, and face as the most frequent sites [18]. The female predominance and disease duration ranging from months to over a decade parallel previous observations that EDP may present either as a recent eruption or a long-standing condition. All patients had Fitzpatrick skin types III–IV, supporting its higher prevalence in darker phototypes [6, 8, 19]. Nationally, only isolated cases and small series with similar demographic features have been described [20–23]. Most patients were asymptomatic, although a minority reported mild pruritus, a symptom far less frequent and severe than in LPP [18]. Importantly, our cohort also included a pregnancy-associated case, an uncommon presentation likely linked to hormonal and immunological changes during gestation [21].

Pathogenetically, EDP appears to be an immune-

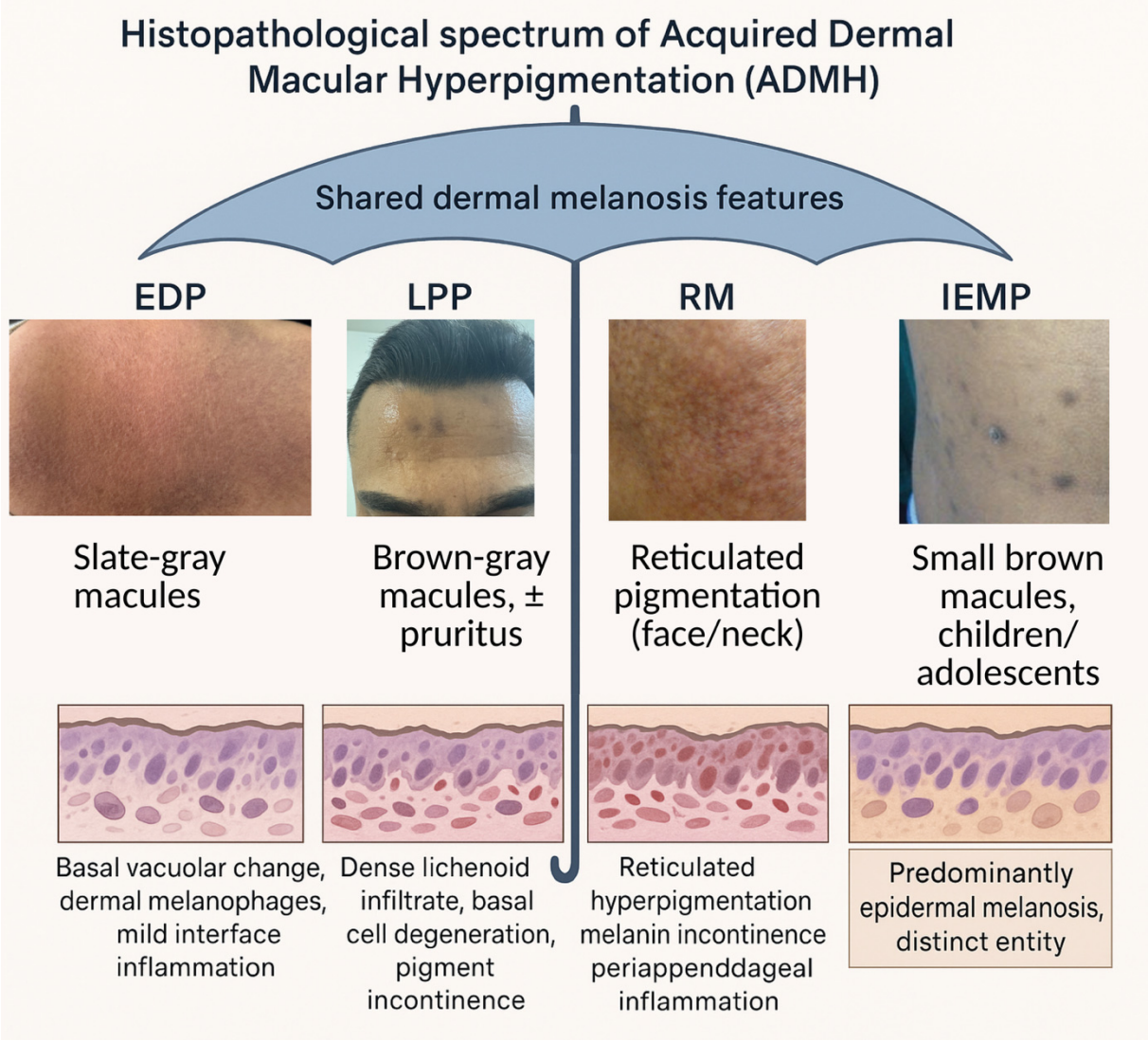

FIGURE 4. Histopathological spectrum of acquired dermal macular hyperpigmentation (ADMH). Schematic illustration demonstrating the clinical and histopathological features of erythema dyschromicum perstans (EDP), lichen planus pigmentosus (LPP), and RM under the shared umbrella of dermal melanosis, whereas Idiopathic eruptive macular pigmentation (IEMP) is represented as a distinct predominantly epidermal entity. Clinical clues are shown above, with the corresponding histopathological correlates depicted below (Created with BioRender.com).

mediated interface dermatitis. Infiltration of dermal $CD8^+$ T lymphocytes and HLA-DR/ICAM-1 expression on basal keratinocytes support a cytotoxic T-cell–driven injury at the dermoepidermal junction, leading to basal vacuolar change, keratinocyte apoptosis, and pigment incontinence [24]. Similar to other acquired dermal macular hyperpigmentation (ADMH) entities, infections and drugs, including EGFR and tyrosine kinase inhibitors, may act as triggers or amplifiers of this immune response [25]. The proposed immunopathogenesis is illustrated in Figure 3.

EDP is currently considered part of the spectrum of ADMH, which also includes LPP and RM because of their overlapping features [26]. On dermoscopy, EDP typically presents as a diffuse bluish-gray background with fine granular dots. In contrast, LPP shows larger brownish-gray globules with perifollicular accentuation, whereas RM is characterized by a pseudonetwork with telangiectatic

vessels [27]. A comparative overview of these entities is provided in Table 4 [28].

In our series, dermoscopy most often revealed a bluish-gray, cloud-like background with fine to coarse speckled granules, present in 90% of cases, sometimes admixed with light-brown areas or darker blotches (40%). Perifollicular accentuation was uncommon (20%), and faint blurred vascular structures were observed in 40% but lacked diagnostic significance. None of the patients showed peripheral erythema, supporting the indolent nature of long-standing lesions. By contrast, Elmas *et al*. [29] reported erythematous halos and vascular structures in early cases, reflecting active inflammation. This suggests that such findings are transient and stage-dependent. Our observations are consistent with reports describing the heterogeneity of EDP dermoscopy, including the distinctive "Wagyu beef-like" appearance noted by Tomii *et al*. [30]. They also reinforce the distinction from lichen planus pigmentosus, characterized by larger brown-gray globules with perifollicular accentuation, and from Riehl's melanosis, which typically shows pseudonetworks with telangiectatic vessels [31, 32]. Histologically, the bluish-gray granularity corresponds to basal vacuolar alteration, pigment incontinence, and dermal melanophages, as documented in earlier series [18, 33], situating EDP within the broader spectrum of acquired dermal macular hyperpigmentation (Figure 4) [34]. In addition, compared with LPP - which typically exhibits a denser band-like lichenoid infiltrate - EDP shows a milder interface dermatitis with relatively preserved adnexal structures [14, 27, 33]. AD is often histologically indistinguishable from EDP, supporting their placement within the same ADMH spectrum [2, 3].

Therapeutic outcomes remain variable. In our patients, short-term responses to dapsone and isotretinoin were modest, consistent with prior reports of partial or transient efficacy [16, 35]. Topical tacrolimus has shown benefits in isolated cases [15], and combination regimens or novel agents, such as topical ruxolitinib, may offer future promise [36, 37]. Nevertheless, relapses are frequent and no standardized treatment exists, highlighting the need for individualized management and long-term follow-up.

The originality of this series lies in the inclusion of both sexes and the documentation of rarely reported involvement sites such as the axillae, inframammary folds, arms, and neck, alongside detailed clinicodermoscopic correlations.

Strengths and Limitations

The study is limited by its small sample size, single-center and retrospective design, single-observer dermoscopic assessment, and short follow-up, which restrict generalizability and render the findings descriptive and hypothesis-generating rather than statistically generalizable or conclusive. Nevertheless, this represents one of the largest single-center EDP series reported from Turkey and provides valuable regional data. Larger multicenter studies with standardized dermoscopic evaluation and longer follow-up are needed to refine diagnostic criteria, clarify prognostic markers, and guide treatment strategies.

CONCLUSION

This case series demonstrates that dermoscopy offers consistent non-invasive clues for the diagnosis of EDP, particularly the characteristic bluish-gray granular or cloud-like background and the variable presence of darker blotches and faint blurred vessels. These patterns align with the uniform histopathologic findings of basal vacuolar alteration, pigment incontinence, and dermal melanophages. By documenting uncommon involvement sites and a pregnancy-associated case, our study expands the recognized clinical spectrum of EDP and reinforces its placement within the acquired dermal macular hyperpigmentation spectrum.

Ethics Approval and Consent to Participate

This study was approved by the Bezmialem Vakıf University Scientific Research Ethics Committee (Decision No: 2025/347; date: 23.09.2025). 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. Throughout the course of this study, we strictly adhered to the World Medical Association Declaration of Helsinki and Good Clinical and Laboratory Practice standards. All

participants provided written informed consent before their inclusion 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: GK; Study Design: GK; Supervision: GK; Funding: GK; Materials: GK; Data Collection and/or Processing: GK; Statistical Analysis and/or Data Interpretation: GK; Literature Review: GK; Manuscript Preparation: GK; and Critical Review: GK.

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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(6):704-707 Case Report

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Cardio-Hematology Crossroads: A Diffuse Large B-Cell Lymphoma Case Presenting with a Cardiac Mass

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Abstract:

Cardiac involvement by diffuse large B-cell lymphoma (DLBCL) is a rare clinical entity that often presents with nonspecific symptoms, making early diagnosis particularly challenging. In this context, we report a case that highlights the diagnostic obstacles and the importance of early evaluation. A 58-year-old male presented with dyspnea, malaise, fingertip numbness, weight loss, and shoulder pain. Transthoracic echocardiography revealed a right atrial mass, prompting further assessment. Positron Emission Tomography-Computed Tomography demonstrated increased 18F-FDG (18F-fluorodeoxyglucose) uptake in multiple regions, including the cardiac musculature, raising suspicion for disseminated lymphoproliferative disease. The biopsy of the left superior cervical lymph node confirmed diffuse large B-cell lymphoma. The patient underwent six cycles of chemoimmunotherapy and achieved complete metabolic remission. This case underscores the need to consider lymphoid malignancies in the differential diagnosis of intracardiac masses and persistent cardiac symptoms without an obvious cause. Early use of multimodal imaging and prompt histopathological confirmation are critical for accurate diagnosis and appropriate treatment planning. Rapid initiation of immunochemotherapy may improve clinical outcomes and reduce the risk of severe cardiac complications.

Keywords: Diffuse Large B Cell Lymphoma, Cardiac Involvement, Extranodal Involvement, Chemotherapy

Cardiac masses are clinically significant findings because they may lead to severe complications and demand rapid identification of their origin. The most common causes include benign primary tumors such as myxomas, malignant secondary lesions deriving from metastatic disease, and non-neoplastic entities such as thrombi. Primary cardiac tumors are exceedingly rare (0.001% to 0.056% in autopsy series), whereas secondary cardiac tumors (metastases) are significantly more common, observed in 0.7% to 3.5% of general population autopsies and up to ~9% in cancer patients [1, 2]. Similar case reports of rare cardiac masses (including hemangiomas and infiltrative lipomas) have recently been published in this journal, illustrating diagnostic and imaging challenges in distinguishing benign from malignant lesions [3, 4].

Lymphomas are hematologic malignancies capable of spreading beyond lymphoid tissues and involving extranodal organs with a variety of clinical manifestations. Cardiac involvement is rare and often under-recognized, especially when nodal disease is

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minimal or absent. We present a case of diffuse large B-cell lymphoma (DLBCL) in which the initial presentation was a cardiac mass, and we compare this presentation with reports in the literature to highlight diagnostic challenges and implications for management.

CASE PRESENTATION

A 58-year-old male with no prior systemic disease presented to the cardiology clinic with a two-month history of malaise and right fingertip numbness, and a three-week history of dyspnea, weight loss, and chest pain. He was afebrile but reported night sweats. Physical examination was unremarkable. Complete blood count revealed anemia, lymphopenia, and increased neutrophils.

Following detection of a right atrial mass on transthoracic echocardiography, transesophageal echocardiography (TEE) confirmed its presence, along with left atrial wall thickening, mild mitral and tricuspid valve insufficiency, and pericardial effusion and thickening. Positron Emission Tomography-Computed Tomography (PET-CT) demonstrated pathologically increased 18F-FDG (18F-fluorodeoxyglucose) uptake in cardiac lesions, lymphadenopathies, and some other nodal and extranodal foci with increased FDG uptake throughout the body, consistent with advanced-stage disease (Figure 1).

Given the suspicion for malignancy, an excisional biopsy of a left infraclavicular lymph node was performed, and histopathology revealed "diffuse large B-cell lymphoma of the germinal center B-cell (GCB) phenotype". The patient received 3 cycles of R-daEPOCH (rituximab, dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin), and after confirmation of interim PET response, the treatment was extended to six cycles, which resulted in a complete metabolic remission. The patient is planned to continue treatment with high-dose methotrexate due to high-risk features for central nervous system relapse.

DISCUSSION

Cardiac lymphoma involvement has been reported in 8.7–25% of patients in autopsy studies and accounts for 9% of cardiac metastases [5]. Primary cardiac lymphoma (PCL) is rare, representing less than 0.5%

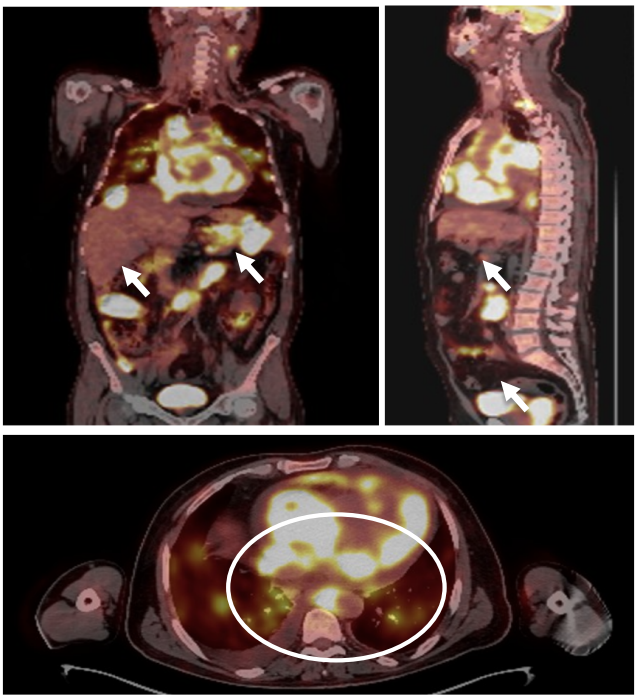

FIGURE 1. FDG PET/CT images in multiple planes demonstrate cardiac, nodal, and extranodal involvement.

of extranodal lymphomas and under 2% of resected cardiac tumors [2]. DLBCL is the most common subtype [2]. In this case, right atrial involvement and left atrial wall thickening matched anatomical patterns, while early cardiac involvement highlighted prompt detection.

Cardiac lymphoma is often asymptomatic or presents with nonspecific features, including heart failure, chest pain, arrhythmias, or pericardial effusion [5]. Conventional tests such as ECG and chest X-ray are generally insensitive [6]. Multimodal imaging is essential: transthoracic echocardiography (TTE) is usually the first screening tool (~60% sensitivity); TEE provides higher sensitivity (97% vs. 75.9%) in PCL [7]. In this patient, TEE accurately identified the right atrial mass. CT evaluates cardiac and extracardiac disease, while PET/CT confirms systemic involvement.

Differential diagnosis includes benign tumors, malignant primary tumors, mediastinal DLBCL invasion, and metastases [8]. Biopsy remains the gold standard [8,9]. Combined TEE and PET/CT enabled differentiation between cardiac infiltration and mediastinal involvement [7]. Prognosis is generally worse in mediastinal DLBCL with cardiac involvement due to delayed diagnosis [10].

Given the patient's high-risk profile, including an R-IPI score of 3 driven by elevated lactate dehydrogenase levels, advanced-stage disease, and multiple extranodal involvements, along with a GCB histological subtype, a more intensive immunochemotherapy regimen was favored. Accordingly, R-daEPOCH was selected over standard R-CHOP as the initial treatment approach [11]. R−CHOP (Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, and Prednisolone) chemotherapy achieves a 79% overall response rate in cardiac DLBCL, with median survival of 30 months; alternative regimens such as R−pola−CHP may improve progression-free survival. Rare but potentially fatal complications may occur, and imaging guidance reduces this risk [2]. Our patient received R−daEPOCH, resulting in complete metabolic remission and rapid clinical improvement, demonstrating the effectiveness of early chemoimmunotherapy.

Surgery or radiotherapy may be considered for hemodynamic compromise or progression. Prognostic factors include bone marrow and extranodal involvement, left ventricular infiltration, and immunodeficiency [2]. Early clinical suspicion, multimodal imaging, and prompt therapy allowed complete remission, illustrating how timely intervention favorably modifies prognosis in cardiac DLBCL.

CONCLUSION

Cardiac involvement was an early, pre-diagnostic sign of DLBCL, emphasizing the importance of high clinical suspicion. Multimodal imaging, including TEE and PET/CT, ensured accurate detection and evaluation of the cardiac mass. Timely CHOP-based immunochemotherapy led to complete remission, demonstrating that rapid recognition and treatment of cardiac lymphoma can yield beneficial outcomes.

Ethics Approval and Consent to Participate

A written informed consent was obtained from the patient for the publication of this case report. Prior to obtaining consent, the aims of the report and the information to be shared were clearly explained. The patient understood the scope of publication, was assured that all identifying details would be kept strictly confidential and willingly agreed to the use of clinical data 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: PT, OEÇ, LK; Study Design: PT, ZSY, OEÇ; Supervision: OEÇ, LK; Funding: N/A; Materials: N/A; Data Collection and/or Processing: PT, ZSY; Statistical Analysis and/or Data Interpretation: OEÇ, LK; Literature Review: PT, ZSY; Writer: PT, ZSY, OEÇ; and Critical Review: OEÇ, LK.

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