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Research Articles

ORIGINAL RESEARCH

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The Possible Relationship Between Periodontitis and Alzheimer's Disease and the Beneficial Effects of Salvia Officinalis in an Experimental Rat Model: An Immunohistochemical Study

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Abstract

Objective

The present study aimed to investigate potential correlations between Alzheimer's disease (AD) and periodontitis in laboratory rat models with inflammation, the isoform of nitric oxide synthases (iNOS), sclerostin expression, and beneficial effects of *Salvia officinalis* (*S.officinalis*).

Material and Method

Eighty Wistar albino male rats were randomly divided into equal groups as controls (C), *S.officinalis* (S), periodontitis (P), and periodontitis+*S.officinalis* (PS), Alzheimer's disease (A), Alzheimer's disease+S. officinalis (AS), Alzheimer's disease+periodontitis (AP), and Alzheimer's disease+periodontitis+*S. officinalis* (APS) groups. While aluminum chloride (AlCl3) and d-galactose (D-gal) were intraperitoneally applied in an AD-like model, *S.officinalis* extract was administered by oral gavage. Sclerostin and iNOS expressions in periodontal tissues and amyloid-β (Aβ) in the hippocampus were evaluated together.

Results

Alveolar bone loss (ABL) was detected in Groups A and AS ($p<0.05$). There was no difference in ABL between Groups P and AP ($p>0.05$). iNOS and sclerostin expressions were detected in Group A. *S.officinalis* significantly decreased ABL, Aβ, and, in parallel, iNOS and sclerostin expressions in periodontal tissues in Groups PS and APS ($p<0.05$). The most marked sclerostin expression was observed in Group AP.

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Conclusion
Study findings revealed that periodontitis may increase Aβ in AD. Although ABL was not higher in Group AP than in Group P, the increase in iNOS and sclerostin expression in periodontal tissues in Group AP supports the pathogenetic association of AD with periodontitis. *S.officinalis* may be used as an immunomodulatory aromatic plant in the pathogenetic interaction of periodontal disease and AD.

Keywords: Alzheimer's disease, periodontitis, Salvia officinalis, iNOS, sclerostin.

Introduction

Bones play a vital role in the fundamental functions of the body (1). Bone emerges as a complex peripheral element able to communicate not only with peripheral organs but also with the brain, both indirectly through the peripheral nervous system and directly by releasing molecules that can cross the blood-brain barrier and act at the brain level (2). It has been reported that some proteins, such as osteocalcin, found in bone structure, cross the blood-brain barrier and have positive effects on anxiety, depression, and memory in Alzheimer's disease (AD) (3,4). Sclerostin, a glycoprotein, is released by osteocytes and inhibits osteoclastogenesis and bone formation by inhibiting the Wnt-β catenin pathway. Additionally, sclerostin keeps osteoclast-mediated bone resorption going by stimulating osteoblasts to produce the receptor activator of the NF-κB ligand (5). Sclerostin has pleiotropic effects, including the regulation of Wnt expression, synaptic plasticity, and memory, and is linked to the etiology of such neurodegenerative conditions as AD (1,6).

AD is a neurodegenerative health challenge leading to cognitive decline and dementia. Increased Amyloid -β (Aβ) and neurofibrillary tangle (NFY) levels are the main signs of AD (7). Inflammatory conditions are considered to contribute to the progression of AD. It was found that oxidative stress (OS) is an important factor initiating AD. It was reported that overexpression of reactive oxygen species (ROS) can induce the accumulation of Aβ, and there is an increase in the number of iNOS-positive neurons that accompany neuronal damage in AD patients (8). It was also pointed out that chronic inflammatory diseases, including periodontitis, could be of vital importance in the pathogenesis of AD (8).

Periodontitis, a long-term inflammatory condition, damages the alveolar bone (1). Dental plaque is a strong causative factor in the progression of the disease. The level of periodontal damage can be related to the produced inflammatory mediators, ROS, and nitrogen species (RNS), as well as environmental and genetic factors (9). The importance of OS in periodontal diseases, according to host and microbial stimulations, was revealed to activate ROS and RNS immediately (9). Nitric oxide (NO) plays a big role in the inflammatory reactions. (10). The nitric oxide synthase isoform (iNOS) is one of the variants of nitric oxide synthase (NOS). Studies revealed an increased level of iNOS in the inflamed periodontal tissues (10). Due to being a crucial negative regulator of bone formation, sclerostin prevents bone remodeling during periodontitis. Furthermore, an evidence-based study suggests that removing sclerostin appears to logically avoid resorption of the alveolar bone (11).

In recent years, several alternative treatment methods have been studied to inhibit OS, playing an important role in periodontitis and AD. *Salvia officinalis L.* (*S.officinalis*) is an aromatic plant known for its antioxidant, anti-inflammatory, and memory-enhancing effects with its high flavonoid and phenolic content (12). Based on the literature, pro-inflammatory cytokine and iNOS synthesis inhibition, anti-inflammatory, and antioxidant effects of various Salvia species have been determined (13). It has been shown that *S.officinalis* may provide therapeutic effects for periodontal infections (12). The present study, thus, aimed to evaluate the possible association between periodontitis and AD through its impact on periodontal tissues and hippocampus, and the beneficial effects of S.officinalis on the two diseases.

Material and Method

The present study was conducted with 80 Wistar albino rats randomly classified into eight groups: the controls (C), S.officinalis (S), periodontitis (P), periodontitis+S.officinalis (PS), Alzheimer's disease (A), Alzheimer's disease+S.officinalis (AS), Alzheimer's disease+periodontitis (AP), and Alzheimer's disease+periodontitis+*S.officinalis* (APS), in the Experimental Animal Production and Research Laboratory of Süleyman Demirel University, Isparta.

In the AD-like model, aluminum chloride (AlCl3) (Merck, Darmstadt, Germany) (10 mg/kg/day) and d-galactose (D-gal) (Sigma, Taufkirchen, Germany)

(150 mg/kg/day) intraperitoneally and *S.officinalis* extract (100 mg/kg/day) by oral gavage were administered for 21 days (14).

Periodontitis was induced by a 3.0 silk ligature (RT-ED, Shandong, China) to maxillary 2nd molar teeth in the last 14 days of the experiment. After the sacrification of the rats, Aβ deposition in the hippocampus, iNOS expression, and sclerostin in periodontal tissues were evaluated histomorphometrically and histopathologically.

S.officinalis extract was prepared in The Department of Pharmacognosy, Faculty of Pharmacy, Isparta Applied Sciences University, Isparta. Firstly, the leaves of the plant were separated and ground into powder. We kept it in ethyl alcohol (80%) in a shaker for 48 hours. Then we filtered the liquid part and evaporated the liquid until it dried with a rotavapor and put the remaining dry fraction in solution (0.5%) with carboxymethyl cellulose (Akbel Kimya, Bursa, Türkiye). *S.officinalis* extract (100 mg/kg/day) was administered through oral gavage for 21 days. The rats were euthanized by transcardial perfusion with cold phosphate-buffered saline pH 7.3 under deep anasthesia of xylazine (Rompun 2%, Bayer, İstanbul, Türkiye) 10mg/kg and ketamine HCl (Ketasol 10%, Richter, Pharma, Wels, Austria) 90 mg/kg body weight.

After the euthanasia, in addition to the brain, the maxilla was taken from each rat. The hippocampus was removed from the brain. The maxilla was separated into two halves along the sutura palatina.
Histomorphic method

The right maxillary halves were dissected from gingival tissue and incubated in 3% hydrogen peroxide (Natural H2O2, Istanbul, Türkiye) for 24 hours (15). To determine the cementoenamel junction (CEJ), 1% methylene blue (Noratex Chemistry, Istanbul, Türkiye) was kept for one minute, washed under running water, and dried. The alveolar bone level was measured at six points in the buccal and palatal areas between the CEJ and the alveolar crest (16). The samples were photographed with a digital camera (Leica DLUX3, Wetzlar, Germany), fixed to a stereomicroscope (Leica S4E Stereomicroscope, Wetzlar, Germany), and transferred to the computer environment (4X). The measurements were standardized using the ImageJ program (1.53f, Maryland, USA).

Histopathological Method
Histopathological and histomorphometric evaluations were made in the left maxillary halves and the hippocampus. The maxillary samples were decalcified with a 0.1 M EDTA solution for two weeks. After fixation in the 10% neutral formalin solution for two days, the samples of the tissues were then routinely processed and embedded in paraffin wax. The five-micron thickness sections were taken from the samples by a rotary microtome (Leica RM2155, Leica Microsystems, Wetzlar, Germany). Following drying the slides, the sections were placed on coverslip plates, stained with hematoxylin-eosin (HE), and examined under a light microscope. As a result, the histopathological alterations were blinded during grading.

Immunohistochemical Examinations
The tissue samples of the jaws, drowned on the polylysine slides, were immunostained with Aβ (beta Amyloid 1-42 antibody, bs-0076R, Bioss Antibodies Inc., Massachusetts, USA), iNOS (Rabbit anti-iNOS polyclonal antibody, bs2072R, Bioss Antibodies Inc., Massachusetts, USA), and sclerostin (anti-sclerostin antibody, (ab63097, Abcam, Cambridge, UK)) by the streptavidin-biotin technique.

The biotinylated secondary antibody and streptavidin–alkaline phosphatase conjugate were used for immunohistochemistry after the sections were incubated with the primary antibodies for 60 minutes. The secondary antibody was prepared using the EXPOSE Mouse and Rabbit Specific HRP/DAB detection IHC kits (Abcam®, Cambridge, MA, USA). The antigens were shown by using diaminobenzidine (DAB) as the chromogen. The primary antiserum was replaced with antibody dilution solutions for the negative controls. Blinded samples were used for each examination. Each slide was investigated for immunopositivity, and the percentage of immunopositive cells in each group was determined by counting 100 cells in 10 distinct fields for each section at a magnification of X40. The outcomes of the image analyzer were applied to the statistical analyses. The Database Manual Cell Sens Life Science Imaging Software System (Olympus Co., Tokyo, Japan) was used to carry out the morphometric analyses.

Statistical Analysis
Each variable was given as mean±standard deviation (SD). The ANOVA and Duncan tests were utilized to compare the histopathological and immunohistochemical scores among the groups. The Statistical Package for Social Sciences (SPSS) for Windows 15.0 program was used to perform the statistical analyses (SPSS Inc., Chicago, IL, USA). A p-value of <0.05 was accepted to be significant.

Results

Histomorphometric Findings

The histomorphometric findings are shown in Table 1. Alveolar bone loss (ABL) was seen in all the groups, except for Groups C and S, showing no statistical difference in histomorphometric measurements. It was noted that there was a statistical significance in terms of ABL in Groups AS and A, compared to Group C. The highest mean ABL was found in Groups P and AP. After adding S. officinalis extract to P and AP applications (Groups PS and APS), ABL was statistically significantly found to be decreased ($p<0.05$).

Histopathological Findings in Periodontal Tissues:

On the histopathological examination, normal gingival mucosae were observed at the interdental area in Groups C and S. Slight erosions were commonly observed at the interdental papilla in Group A. *S.officinalis* treatment was detected to decrease both the number and severity of the ulcers in Group AS. Periodontitis led to marked inflammation and a decrease in the height of the papilla in the periodontitis-induced group. The slight epithelization was also noticed in some rats' interdental areas in that group. The most marked inflammatory reaction was noticed in Group AP; however, a severe inflammatory reaction was also extended to the tooth roots in this group. The epithelization was generally very slight or absent in interdental areas in the group. *S.officinalis* treatment was seen to decrease the inflammatory reaction and induce the epithelization in both Group PS and Group APS (Figure 1).

Immunohistochemical Findings in the Hippocampus

In the immunohistochemical examination of hippocampal tissues for Aβ, while significant Aβ expression was noted in group A, the most severe Aβ expression was observed in the AP group. Aβ expression in the hippocampus was negative in the C and S groups. Mild Aβ expression was found in the hippocampus of 2 rats in group P. Aβ expression was not detected in any rat in the PS group. It was noted that Aβ expression decreased in the APS group compared to the AP group (Figure 2). Table 2 shows the findings of Aβ positive cells in the hippocampus in the groups. Immunohistochemical findings in the periodontal tissues

The immunohistochemical examination of sclerostin revealed negative-to-slight intracytoplasmic expressions in alveolar bone in Groups C and S. Slight-to-moderate expressions were also observed in

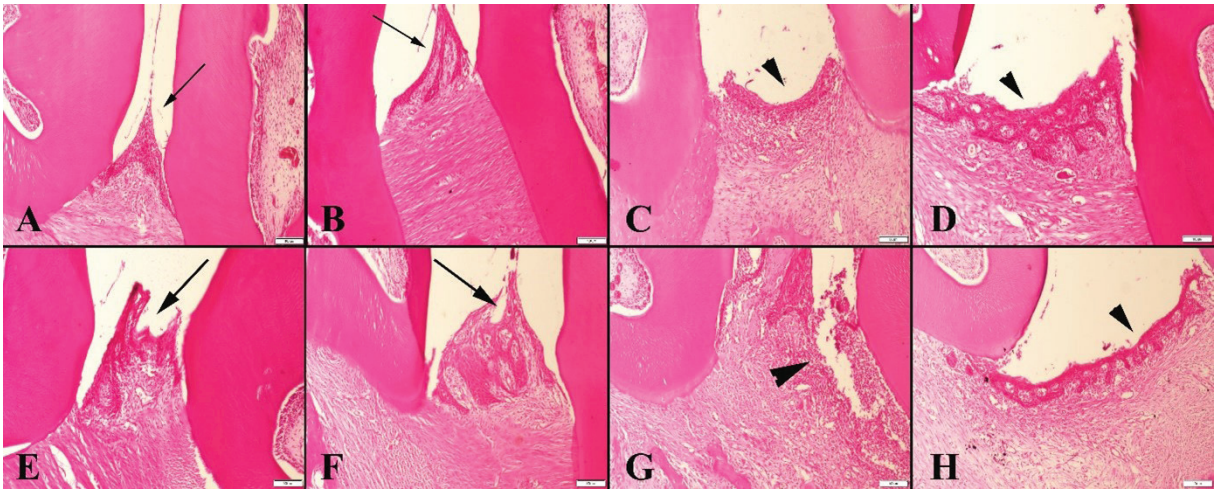

Figure 1

Representative histopathological appearances in the groups. (A) Normal interdental papilla (thin arrow) in Group Control. (B) Normal gingival mucosa in interdental papilla (thin arrow) in Group S. (C) Marked inflammatory reaction, ulcer, and slight epithelization at the interdental area (arrow head) in Group P. (D) Moderate amelioration, decreased inflammatory reaction, and increased epithelization (arrow) in Group PS. (E) Slight erosions at the interdental papilla (thick arrow) in Group A. (F) Decreased inflammation and amelioration in erosive reaction (thick arrow) in Group AS. (G) Very severe inflammatory reaction and severe ulcer (arrow head) in Group AP. (H) Decreased inflammation and increased epithelization in Group APS. HE, scale bars 100µm.

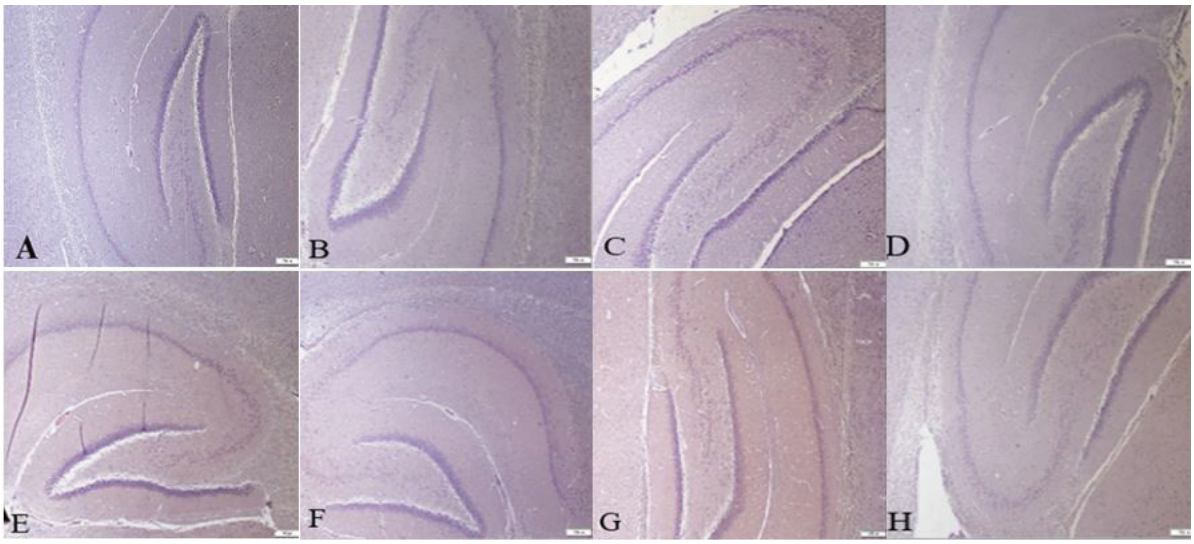

Figure 2

The Aβ expression in the hippocampus in the groups. (A) Negative Aβ expression in the hippocampus in Group C. (B) Aβ negative expression in hippocampus tissue in Group S. (C) Mild Aβ expression in hippocampus tissue in Group P. (D) Negative Aβ expression in hippocampus tissue in Group PS. (E) Increased Aβ expression in hippocampus tissue in Group A. (F) Negative Aβ expression in Group AS. (G) Significantly increased Aβ expression in hippocampus tissue in Group AP. (H) Decreased Aβ expression in the hippocampus tissue in Group APS. Streptavidin Biotin Peroxidase method, scale bars 200 µm.

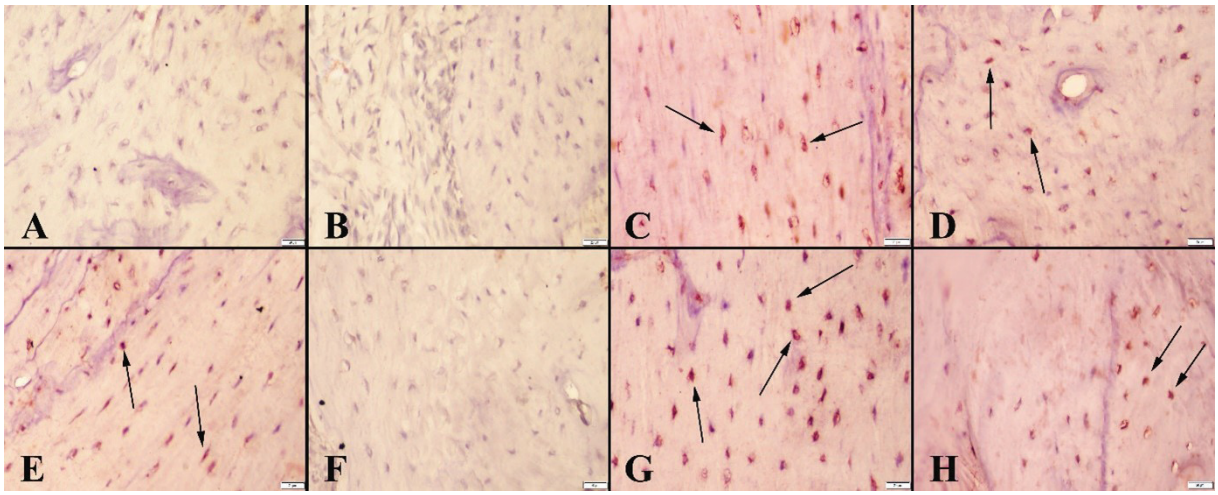

Figure 3

Sclerostin immunohistochemistry findings in the alveolar bone in the groups. (A) Negative expression in Group C. (B) No expression in Group S. (C) Increased immunoreaction (arrows) in Group P. (D) Decreased immunoexpression (arrows) in Group PS. (E) Slight expression (arrows) in Group A. (F) Decreased expression in Group AS. (G) Marked expression (arrows) in Group AP. (H) Decreased immunoreaction in Group APS. Streptavidin biotin peroxidase method, scale bars 20µm.

Table 1 ABL, iNOS, and sclerostin-positive cell examinations in alveolar bones

Groups	ABL	iNOS	Sclerostin
C (n=10)	0.179±0.006^{a}	5.70±0.42^{ef}	1.10±1.19^{k}
S (n=10)	0.187±0.014^{a}	4.90±0.50^{e}	0.80±0.78^{k}
P (n=10)	0.927±0.033^{d}	27.0±1.05^{i}	42.70±6.54^{l}
PS (n=9)	0.624±0.036^{c}	10.11±0.42^{g}	20.22±1.98^{m}
A (n=10)	0.340±0.027^{b}	19.20±0.93^{h}	11.80±2.74^{n}
AS (n=10)	0.318±0.015^{b}	9.80±0.42^{fg}	8.90±1.19^{n}
AP (n=9)	0.845±0.061^{d}	39.67±1.93^{j}	61.11±3.44^{o}
APS (n=10)	0.696±0.016^{c}	23.50±1.02^{i}	35.00±3.81^{p}

*Values are presented as mean±standard deviation. **Data with different superscripts indicate significant differences from each other (p>0.05). *** The same symbols used for different parameters differ from each other. A: Alzheimer's disease, ABL: Alveolar bone loss, AP: Alzheimer's disease+periodontitis, APS: Alzheimer's disease+periodontitis+ *S.officinalis*, AS: Alzheimer's disease+*S.officinalis*, C: Control, iNOS: Isoform of nitric oxide synthases, P: Periodontitis, PS: Periodontitis+*S.officinalis*, S: *S.officinalis*

Table 2 Aβ positive cell examinations in the hippocampus

		95% CI for Averages				
Groups	**n**	**Mean mm± SE**	**Lower Limit**	**Upper Limit**	**Minimum**	**Maximum**
C	10	0.00±0.00^{a}	0.00	0.00	0	0
S	10	0.00±0.00^{a}	0.00	0.00	0	0
P	10	1.20±0.36^{a}	0.39	2.01	0	3
PS	9	0.00±0.00^{a}	0.00	0.00	0	0
A	10	23.60±1.35^{d}	20.54	26.66	19	30
AS	10	7.20±0.81^{b}	5.36	9.04	2	10
AP	9	42.56±1. 63^{e}	38.81	46.30	38	53
APS	10	13.20±0.73^{c}	11.55	14.85	10	17

a, b, c, d: The averages of the group having the same letter are not different from each other (p>0.05). A: Alzheimer's disease, AP: Alzheimer's disease+periodontitis, APS: Alzheimer's disease+periodontitis+ S.officinalis, AS: Alzheimer's disease+*S. officinalis*, C: Control, iNOS: Isoform of nitric oxide synthases, P: Periodontitis, PS: Periodontitis+*S.officinalis*, S: *S.officinalis*

Group A. The induction of periodontitis brought about a marked increase in sclerostin expression in the P, AP, and APS groups. *S.officinalis* treatment decreased the expression in Group AS, compared to Group A. The most marked increase was observed in Group AP. The treatment of salvia was observed to decrease the expression in treated groups (Figure 2).

At the examination of the iNOS immunostained slides, mild-to-very slight expressions were noticed in Groups C and S; while increased expressions were observed in Groups P, A, and AP, a decrease in expression was seen in Groups PS, AS, and APS (Figure 3). iNOS and sclerostin-positive cells were immunohistochemically examined in each group, and

the findings are demonstrated in Table 1.

Discussion

Periodontitis induction by ligature is a suitable model for investigating the host responses during the development of periodontitis (17). Toker et al. (18) observed that in the model where periodontitis was induced by the ligature, ABL reached the highest level on the 11th day. Additionally, Molon et al. (19) found a significant increase in ABL and proinflammatory cytokine expression within the first 14-day period in terms of the measurements on the 1st, 3rd, 5th, 7th, 14th, and 21st days, and recorded no significant alterations in ABL and inflammatory process between the findings on those days. In our study, the induction of periodontitis was performed for 14 days with 3-0 silk sutures in the maxillary second molar teeth of the rats (20). Periodontitis developed in all rats undergoing the induction, and the distance between CEJ and ABL was evaluated histomorphometrically.

Studies revealed that cholinergic system disruptions, OS, and memory problems similar to AD occur with the use of combined AlCl3 and D-gal (21,22). The combined use of AlCl3 and D-gal increases Aβ levels in the cortex and hippocampus (21). Al+3 cholinergic in the CNS is known to lead to dysfunction (21). In various animal studies, aging-like changes, including learning and memory problems, were stated to increase the production of ROS while decreasing the activities of antioxidant enzymes after the administration of D-gal (21,22). In our study, AlCl3 (10 mg/kg) and D-gal (150 mg/kg) were intraperitoneally applied for 21 days to create an AD-like model (14). The gold standard is the neuropathological evaluation of Aβ and NFYs (23,24). In our study, Aβ was examined in the hippocampal tissue to determine the pathological characteristics of AD. No memory or behavioral analysis tests were performed on the rats. In all AD-induced groups, increased Aβ expression was detected in the hippocampus.

Periodontitis may have vital importance in the neuroinflammatory process through the direct invasion of periodontal pathogens, bacterial products activating microglia, or pro-inflammatory cytokines produced by the host response (25). It was reported that chronic periodontitis was likely to increase the development of AD (26). Kamer et al. (27) reported the relationship between the load of Aβ detected through the positron emission tomography scanning and the severity of increased periodontitis in cognitively normal elderly individuals. The accumulation of Aβ increased in the hippocampus, in mice with transgenic AD when periodontitis induction was added, (28,29). However, in the study by Holmer et al. (8), ABL was detected further in individuals with poor cognitive performance, compared to the healthy controls. Ishida et al. (29) also found ABL further in periodontitis and higher levels of interleukin-1β and tumor necrosis factor-α in brain tissues in transgenic AD mice, compared to the controls. In the study by Kantarcı et al. (28), ABL was reported although periodontitis was not induced in transgenic AD mice; however, the authors pointed out that the rate of periodontitis induction in transgenic AD mice was not greater than that of ABL in only periodontitis-induced mice (28).

The detection of Aβ expression in two rats in Group P demonstrated that the chronic inflammatory nature of periodontitis may have contributed to neuroinflammation, although AD was not induced in our study, and thus, periodontitis may have caused the accumulation of Aβ. Additionally, compared to Group C, the histomorphometric examination of the maxillary halves showed a significant rate of ABL, supported by the increased hyperemia and inflammation in Group A. Even though no difference was detected between the mean ABL in Groups P and AP, more severe inflammation findings were found in the histopathological evaluation, consistent with those detected by Kantarcı et al. (28), which may be due to the impact of AD on periodontitis.

S.officinalis has memory-enhancing effects, as well as antimicrobial, antioxidant, anti-inflammatory, antinociceptive, and antimutagenic effects (30). It is also known that, as well as its high flavonoid and phenolic content, *S.officinalis* repairs DNA damage caused by free radicals and prevents the reactions leading to lipid peroxidation (31). In the study by Kolac et al. (32), S.officinalis was reported to decrease the parameters of OS.

In the light of the literature, however, we encountered no study exactly examining the relationship between *S.officinalis* and periodontitis. Even so, various reports have investigated that the other members of the salvia family, such as *S.sclarea*, *S.officinalis*, and S.miltiorrhiza, showed a decreasing effect on ABL in periodontitis (33,34). In our study, it was histopathologically determined that hyperemia was significantly reduced in *S.officinalis* in the hippocampus of the rats. The accumulation of Aβ detected in Group A was seen as a negative expression in Group AS. However, the extract of *S.officinalis* was applied along with the induction of AD in Group APS, and periodontitis was found to decrease the expression of Aβ. Our findings were consistent with those defined in the literature.

Synthesized via iNOS as an oxidant, NO is of crucial importance in host defense and homeostasis. Leitao et al. (35) revealed that ABL was prevented in rats with the inhibitor of NO synthesis in periodontitis.

Nevertheless, Pan et al. (36) stated that the degree of periodontal disease in periodontal tissues was correlated with an increase in iNOS expression. Increased levels of iNOS in periodontal tissues in those with periodontitis cause an increase in NO levels (37). Despite its neuroprotective mechanism at low concentrations, higher concentrations of NO are potentially neurotoxic to brain cells (38). It is believed that iNOS may be connected to the pathophysiology of AD and that patients with AD have a marked increase in the number of iNOS-positive neurons in their brains (38). Haas et al. (39) found that iNOS increased the expression of mRNA in brain tissues in AD. The close location of iNOS-positive microglial cells with the accumulation sites of Aβ suggests that Aβ is a contributor to the activation of microglial cells (39).

In our study, iNOS expression was determined to be increased in periodontal tissues with both AD and periodontitis induction. Based on our findings, while AD could modulate the inflammatory response in periodontal tissues, the higher rate of iNOS-positive inflammatory cells in periodontal tissues in both diseases reflected the increased rate of OS. In different studies, Salvia strains are stated to be able to reduce iNOS expression (33,40). However, there was no study in the literature evaluating the expression of iNOS with *S.officinalis*. Our findings revealed that *S.officinalis* significantly reduced the expression of iNOS in periodontal tissues in periodontitis and induction of AD, a similar finding to the antioxidant effects of other Salvia species.

Sclerostin is a critical factor with its negative regulator impacts on bone formation, thereby inhibiting bone remodeling in the development of periodontitis. Furthermore, data suggest that removing sclerostin appears to logically prevent the resorption of the alveolar bone (12). In most studies, it was revealed that the monoclonal antibody treatment with sclerostin can contribute to bone strength by promoting bone formation and reducing the resorption of the bone (41). In addition, sclerostin antibodies show a good performance in implants by enhancing the osseointegration and bone regeneration around the bone implants and dental implants, suggesting a possible therapeutic strategy to shorten patients' healing periods by accelerating bone regeneration after the placement of implants (11). The suppression of Wnt appears to play a critical role in the neurodegeneration development due to the progression of AD (4). Therefore, it is not surprising that sclerostin, an antagonist to Wnt signaling, was negatively associated with cognitive changes, almost exclusively expressed in the skeleton. In our study, the most marked increase in the expression of sclerostin was observed in Group AP, and *S.officinalis* treatment decreased the expression of sclerostin. Particularly, the regulation of the sclerostin expression is a novel therapeutic strategy for regulating bones for oral health and is likely to be a positive contributor to future research for general health.

In conclusion, we consider that our findings will contribute to future studies in which the pathogenetic interaction of periodontal disease and AD, and different aromatherapy approaches will be evaluated, and so *S.officinalis* will be benefited as an immunomodulatory aromatic plant.

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Conflict of Interest Statement
The authors declare no conflicts of interest.

Ethical Approval
The present study was carried out under the ethical standards of the research committee of the institution by the 1964 Helsinki Declaration and its later amendments or comparable ethical standards, after the decision of the Süleyman Demirel University Animal Experiments Local Ethics Committee of the university (Approval number: 24/10/2019, 12/03).

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Availability of Data and Materials
The data of the study can be obtained through the corresponding author upon reasonable request.

Artificial Intelligence Statement
The authors declare that no type of artificial intelligence program has been used in writing of this manuscript, nor for the creation of figures, graphics or tables.

Authors Contributions
İY: Conceptualization; Project administration; Formal analysis; Investigation; Validation; Writing-original draft.
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ORIGINAL RESEARCH

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The Diagnostic Importance of RDWLR, PDWLR, and Immature Granulocytes in Colorectal Cancer

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Abstract

Objective
A complex inflammatory response forms against the local effects of malignancy. In the differentiation of colorectal cancer from benign colorectal polypoid lesions, inflammatory biomarkers such as platelet to lymphocyte ratio (PLR), neutrophil to lymphocyte ratio (NLR), and Red Cell Distribution Width (RDW) have previously provided promising results. This study aimed to investigate the results of immature granulocytes (IG) and as yet unstudied biomarkers such as nucleated red blood cells (NRBC), platelet distribution width (PDW), and Mean Platelet Volume (MPV) with new inflammatory indexes such as the RDW-lymphocyte ratio (RDWLR) and the PDW-lymphocyte ratio (PDWLR).

Material and Method
The hematological biomarkers were compared between 269 patients with colorectal benign polyps and 57 patients with colorectal adenocarcinoma. To determine the sensitivity and specificity of the biomarkers related to the neoplastic characteristics of the colorectal polyps, the Receiver Operating Characteristics (ROC) curve analysis was performed.

Results
The mean NRBC count, IG count, and rates were similar in both groups. In the colorectal carcinoma group, PDW was lower (p=0.004) and RDW was higher (p=0.018). The NLR, PLR, RDWLR, and PDWLR values were significantly higher in the colorectal carcinoma group (p<0.001, p<0.001, p<0.001, p=0.003, respectively). In the ROC analysis for the differential diagnosis of colorectal cancer from colorectal polyp, the AUC values of NLR, PLR, and RDWLR were determined to be higher (0.714, 0.719, 0.720) with sensitivity and specificity of 73.68% and 64.31%, 64.91% and 72.75%, and 69.09 and 69.78%, respectively. The AUC value of PWDLR was determined to be 0.625, with a sensitivity of 73.21% and specificity of 52.81%.

Conclusion
The diagnostic importance of NLR and PLR in colorectal cancer was confirmed, and RDWLR is a promising new parameter. IG has no significant diagnostic value.

Keywords: Colorectal cancer, colorectal polyp, immature granulocyte, red cell distribution width to lymphocyte ratio, platelet distribution width to lymphocyte ratio, diagnosis

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Introduction

Colorectal cancer is the third most frequently seen cancer, but the cure rates have increased significantly with early detection (1). Colorectal polypoid lesions are precursors of colorectal adenocarcinoma. The incidence of colon adenomatous polyps in individuals aged >50 years is estimated to be 24-50% (2). Colorectal cancers develop from a polyp, but malignancy is present in less than 1% of colon polyps (3). Therefore, the morphological and histopathological identification of colorectal polyps is important. Currently, colorectal cancer screening is performed using colonoscopy, and polyps that do not have a morphologically obvious malignant appearance can only be distinguished with polypectomy and histopathological examination. However, colonoscopy cannot be used as a routine screening program as it is both costly and troublesome for the patient. The fecal occult blood test, which is used in colorectal cancer screening, has low sensitivity (4). Moreover, patients who have undergone polyp excision with colonoscopy require more frequent colonoscopy follow-up afterwards (5). Therefore, there is a clear need for a new, easily accessible, low-cost method that can be widely used in the differential diagnosis of colorectal cancer and colorectal benign polyps.

A complex inflammatory response forms against the local effects of malignancy. This inflammatory response is known to have been observed at the initial stage of the tumour, and in progression or recurrence (6, 7). Biomarkers such as platelet to lymphocyte ratio (PLR), neutrophil to lymphocyte ratio (NLR), and Red Cell Distribution Width (RDW) have been studied in the differentiation of colorectal cancer from benign colorectal polypoid lesions, and promising results have been shown in the literature (8, 9).

RDW is a reflection of the heterogenity of red blood cell size (10, 11). Immature granulocytes (IG) refer to an increase in the ratio of immature granulocytes in circulation (12, 13). Mean Platelet Volume (MPV) is a marker of the mean thrombocyte volume, and is accepted as a differentiating characteristic of the rate and stimulation of thrombocyte production. In destructive thrombocytopenia, there are high MPV levels, and in hypoproliferative thrombocytopenia, there are low MPV levels (14). Platelet distribution width (PDW) is a marker of variation in thrombocyte size (14). There is increasing information that all these parameters show reactive characteristics in inflammation.

Hemogram is an accessible, low-cost test that can be performed in every centre. However, questions have arisen about biomarkers such as IG, nucleated red blood cells (NRCB), PDW, and MPV, which are routinely studied in the hemogram in the differentiation of colorectal cancer from colorectal polypoid lesions (8, 15). This study aimed to support the information related to biomarkers such as PLR, NLR, and RDW in the differential diagnosis of colorectal invasive carcinoma and colorectal benign polyps, and to contribute to the medical literature with the results of as yet unstudied biomarkers such as immature granulocytes (IG), NRBC, PDW, and MPV and new inflammatory indexes such as the RDW-lymphocyte ratio (RDWLR) and the PDW-lymphocyte ratio (PDWLR).

Material and Method

The study included 326 patients diagnosed with colorectal polyps during colonoscopy in the Endoscopy Unit of a tertiary-level hospital between 01.01.2023 and 31.12.2023. The data were screened retrospectively by examining the patient files in the hospital information system. From the results of the histopathological examination of the colorectal polyps, the cases were separated into two groups as Group A, containing patients with colorectal benign polyps, and Group B, containing patients with colorectal adenocarcinoma. The hematological biomarkers were compared between these two groups. Patients were excluded from the study if they had a current diagnosis of or a history of colorectal cancer, or a history of inflammatory bowel disease or hereditary polyposis syndromes. Other study exclusion criteria were defined as a current malignancy other than colorectal cancer, pregnancy, the use of anti-aggregants or anticoagulants, a recent history of blood transfusion, the presence of any gastrointestinal, inflammatory, hepatobiliary, cardiac, pulmonary, or hematological disease determined during colonoscopy, or age <18 years.

The full blood count taken routinely before colonoscopy examination was used for the hematological biomarkers. Peripheral venous blood samples were obtained from the patients for the full blood count, and the samples were centrifuged for 15 minutes at room temperature. Using a Sysmex XN series analyzer (Sysmex, Kobe, Japan), measurements were taken of white blood cell count (WBC), neutrophil count, lymphocyte count, platelet count, hemoglobin value (g/dl), immature granulocyte count and ratio, platelet distribution width (PDW), red cell distribution width (RDW), nucleated red blood cell count (NRBC), and mean platelet volume (MPV).

The neutrophil lymphocyte ratio (NLR) was calculated by dividing the neutrophil count by the lymphocyte count, the platelet lymphocyte ratio (PLR) by dividing the platelet count by the lymphocyte count, the RDW lymphocyte ratio (RDWLR) by dividing the RDW by the lymphocyte count, and the PDW lymphocyte ratio (PDWLR) by dividing the PDW by the lymphocyte count.

Approval for the study was granted by the Ankara Etlik City Hospital Ethics Committee Ethics Committee (decision no: AEŞH-BADEK-2024-195, dated: 28.02.2024). All the study procedures complied with the Helsinki Declaration and local ethics criteria.

Data obtained in the study were analyzed statistically using SPSS v. 26.0 software (SPSS Inc., Chicago, IL, USA) and MedCalc v. 22.023 (MedCalc software, Ostend, Belgium). Conformity of numerical variables to normal distribution was examined with visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov and Shapiro-Wilk tests). Descriptive statistics were stated as mean±standard deviation (SD) values for continuous variables with normal distribution and as median (minimum-maximum) values for those not showing normal distribution. Categorical variables were stated as numbers (n) and percentages (%). The Student's t-test was applied to data with normal distribution, the Mann-Whitney U-test to data not normally distributed, and the Pearson Chi-square test to categorical data. A value of $p<0.05$ was accepted as statistically significant. To determine the sensitivity and specificity of the biomarkers related to the neoplastic characteristics of the colorectal polyps, Receiver Operating Characteristics (ROC) curve analysis was performed, and cut-off values were determined.

Results

Evaluation was made of a total of 326 patients as 269 (82.5%) in the colorectal benign polyp group (Group A) and 57 (17.5%) in the colorectal carcinoma group (Group B). The comparisons of the clinical characteristics and inflammatory parameters of the patients are shown in Table 1. The mean age of the colorectal carcinoma group (66.28±10.49 years) was determined to be significantly older than that of the benign polyp group (59.81±11.36 years) ($p<0.001$). The gender distribution was similar in the two groups, with more males in both groups.

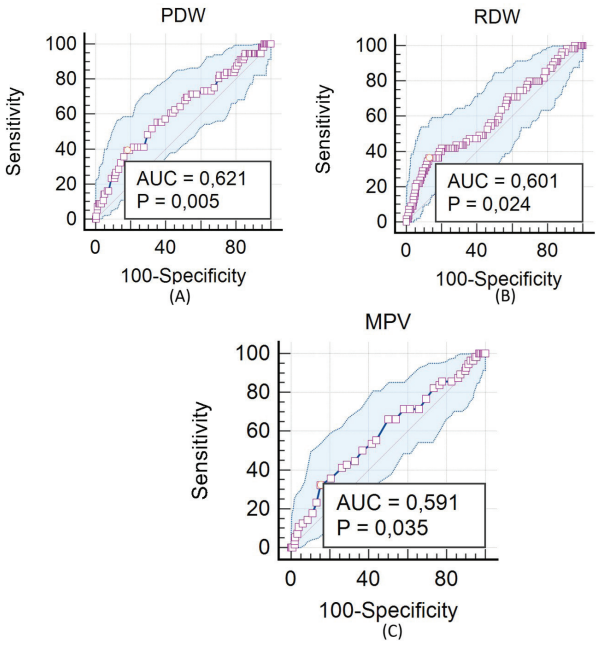

Figure 1
(A) ROC curve and AUC of PDW, (B) ROC curve and AUC of RDW, (C) ROC curve and AUC of MPV

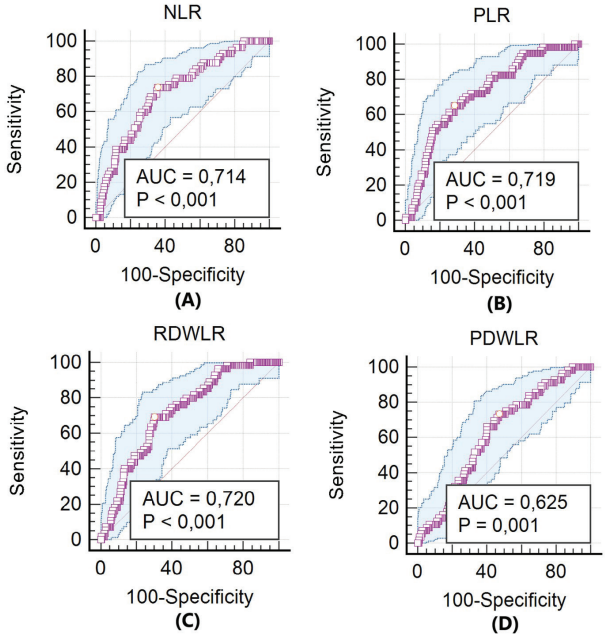

Figure 2
(A) ROC curve and AUC of NLR, (B) ROC curve and AUC of PLR, (C) ROC curve and AUC of RDWLR, (D) ROC curve and AUC of PDWLR

Colorectal benign polyps were seen more often in the rectum (40.9%), and colorectal carcinomas were seen more often in the left colon (descending+sigmoid colon) (43.9%) ($p=0.038$). No statistically significant difference was determined between the two groups in respect of WBC and neutrophil count ($p=0.800$,

Table 1 The comparisons of the clinical characteristics and inflammatory parameters of the patients

	Colorectal Benign Polyp n=269 (82,5 %) (Group A)	Colorectal Carcinoma n=57 (17,5 %) (Group B)	P value	Confidence interval of difference
Age	59,81 ± 11,36	66,28 ±10,49	**<0,001**	-9,68 to -3,24
Gender (male)	150 (55,8%)	34 (59,6)	0,591	
Polyp localization			**0,038**	
Ascendent	61 (22,7%)	12 (21,1%)		
Transverse	29 (10,8%)	3 (5,3%)		
Descendent + sigmoid	69 (25,7%)	25 (43,9%)		
Rectum	110 (40,9%)	17 (29,8%)		
WBC	7,60 (2,74 – 20,00)	7,34 (3,46 – 12,86)	0,800	
Neutrophil	4,28 (0,48 – 13,64)	4,66 (2,12 – 10,70)	0,067	
Lymphocyte	2,34 ± 0,85	1,81 ± 0,56	**<0,001**	
Platelet	263,00 (59,00 – 576,00)	276,00 (147,00 – 649,00)	0,132	
HGB	14,00 (6,60 – 18,60)	12,25 (8,70 – 16,30)	**<0,001**	
Immature granulocytes	0,02 (0,00 – 0,53)	0,02 (0,01 – 0,13)	0,914	
immature granulocytes %	12,20 (11,70 – 22,50)	14,10 (11,60 – 26,80)	0,352	
PDW	12,10 (8,30 – 20,30)	11,20 (8,40 – 16,60)	**0,004**	
RDW	43,90 (32,20 – 73,80)	44,50 (38,90 – 77,30)	**0,018**	
NRBC	0,00 (0,00 – 2,00)	0,00 (0,00 – 0,30)	0,841	
P-LCR	29,25 ± 7,33	26,66 ± 7,33	**0,017**	0,46 to 4,70
MPV	10,52 ± 0,91	10,24 ± 0,89	**0,037**	0,01 to 0,54
NLR	1,91(0,19 – 15,86)	2,66 (1,24 – 8,82)	**<0,001**	
PLR	113,22 (25,11 – 787,50)	171,42 (52,69 – 600,93)	**<0,001**	
RDWLR	19,02 (7,72 – 119,50)	26,39 (13,74 – 107,31)	**<0,001**	
PDWLR	5,36 (1,87 – 28,75)	6,62 (3,44 – 23,27)	**0,003**	

HGB: hemoglobin, PDW: platelet distribution width, RDW: Red Cell Distribution Width, NRBC: nucleated red blood cells, P-LCR: platelet larger cell ratio, MPV: Mean Platelet Volume, NLR: neutrophil to lymphocyte ratio, PLR: platelet to lymphocyte ratio, RDWLR: RDW-lymphocyte ratio, PDWLR: PDW-lymphocyte ratio

p=0.067, respectively). The mean lymphocyte count was determined to be statistically significantly greater in the colorectal benign polyp group (2.34 ± 0.85 10^3/μL vs. 1.81 ± 0.56 10^3/μL; p<0.001). The mean platelet counts were similar in both groups. The median hemoglobin value was determined to be statistically significantly lower in the colorectal carcinoma group [14.00 (6.60-18.60) g/dl vs. 12.25(8.70-16.30) g/dl] (p<0.001).

The median NRBC count and the IG count, and the ratio were similar in both groups. In the colorectal carcinoma group, PDW was lower (p=0.004) and RDW was higher (p=0.018). The platelet large cell ratio (P-LCR) and MPV, which are parameters related to the measurements of platelet size, were determined to be statistically significantly lower in the colorectal carcinoma group (p=0.017, p=0.037, respectively). Statistically stronger p-values were determined for the inflammatory parameters when

Table 2 ROC-AUC analyses and Cut-off values of inflammatory biomarkers evaluated by complete blood count

	ROC-AUC	Cut-off Value	sensitivity	spesifity	P value
PDW	0,621 (0,566 to 0,674)	≤10,5	39,29	82,02	0,0050
RDW	0,601 (0,545 to 0,655)	>48,2	36,36	86,94	0,0241
MPV	0,591 (0,535 to 0,645)	≤9,6	32,14	84,64	0,0353
NLR	0,714 (0,662 to 0,763)	>2,25	73,68	64,31	<0,0001
PLR	0,719 (0,666 to 0,767)	>140,42	64,91	71,75	<0,0001
RDWLR	0,720 (0,668 to 0,768)	>23,35	69,09	69,78	<0,0001
PDWLR	0,625 (0,570 to 0,678)	>5,57	73,21	52,81	0,0008

PDW: platelet distribution width, RDW: Red Cell Distribution Width, MPV: Mean Platelet Volume, NLR: neutrophil to lymphocyte ratio, PLR: platelet to lymphocyte ratio, RDWLR: RDW-lymphocyte ratio, PDWLR: PDW-lymphocyte ratio

the values were proportional to the lymphocyte count. The NLR, PLR, RDWLR, and PDWLR values were determined to be statistically significantly higher in the colorectal carcinoma group ($p<0.001$, $p<0.001$, $p<0.001$, $p=0.003$, respectively).

ROC analysis was performed to investigate the diagnostic value of the parameters that showed a significant difference in the paired comparisons between the two groups (Table 2). PDW, RDW, and MPV were significant parameters in the paired comparisons. The results of the ROC analysis showed that the area under the curve (AUC) values of NLR, PLR, and RDWLR were higher (Figures 1 and 2). A cutoff value of >2.25 for NLR had an AUC of 0.714 with 73.68% sensitivity and 64.31% specificity (Figure 2A). A cutoff value of >140.42 for PLR had an AUC of 0.719 with 64.91% sensitivity and 72.25% specificity (Figure 2B). A cutoff value of >23.35 for RDWLR had an AUC of 0.720 with 69.09% sensitivity and 69.78% specificity (Figure 2C). A cutoff value of >5.57 for PDWLR had an AUC of 0.625 with 73.21% sensitivity and 52.81% specificity (Figure 2D).

Discussion

Colorectal cancer usually occurs with dysplasia of colorectal polyps over time and transformation to malignant processes (16). The incidence of polypoid lesions in the colon increases with advancing age (2). However, malignancy is present in less than 1% of colon polyps (3). Patients who have undergone colonoscopy polypectomy are at risk of again developing colonic polyps or malignancy, and therefore, colonoscopy follow-up must be more frequent than for patients at average risk (5, 17, 18). Moreover, colonoscopy is not an easily accessible method as it is high-cost, requires specialist personnel, and patient compliance with colon cleaning before the procedure (9). The fecal occult blood test has low sensitivity (19). Therefore, there is a need for new, easily accessible, low-cost methods with high patient compliance that can be used in the differential diagnosis of colon polyps from colon adenocarcinoma.

During the development of cancer, inflammatory cytokines and growth factors are expressed from malignant cells, and at the same time, an inflammatory response against the malignancy forms in the host (20, 21). Prolonged inflammation is present together with malignancy. The relationship between colorectal cancer and some inflammatory markers has become a matter of debate in some studies (15, 22, 23).

Malignant cells cause neutrophilia by releasing granulocyte colony-stimulating factor (23). Neutrophils then cause extracellular matrix remodelling, angiogenesis, and mutagenesis, and suppress the T-lymphocyte response (24, 25). All these effects lead to tumour growth and the development of metastasis. Lymphocytes prevent tumour maturation and are responsible for the cytotoxic effect directed at malignant cells (8, 26). The NLR shows the balance between the pro-tumour inflammatory status and the anti-tumour immune status, and an elevated NLR disrupts the oncological results and decreases survival. With an increase in NLR, poor oncological results are seen in intestinal malignancies such as colon, pancreas, and stomach cancers (8, 23, 27-29). In the current study, the lymphocyte count was

determined to be lower in the colorectal cancer group, and in parallel with the literature, the NLR had high sensitivity and specificity in the ROC analysis.

By passing the submucosal barrier, malignant cells create a systemic inflammatory response, and many pro-inflammatory cytokines are released (30). Platelets are both carriers of these cytokines and are affected by them, and an increase in the number. As in many other malignancies, PLR is elevated in patients with colorectal cancer compared to healthy individuals, and the current study results support this finding (23, 31). MPV is a marker of mean platelet volume and shows the change in inflammation status. In literature, MPV is high in different cancer types such as gastric cancer and hepatocellular cancer (32, 33). However, in colorectal cancer, low MPV has been associated with malignancy and decreased survival, and the current study results were consistent with this (30, 34, 35).

The PDW value is obtained with the measurement of variation in thrombocyte size, immature thrombocytes in inflammation, and there is increased destruction of mature thrombocytes at the same time. The relationship between PDW and cancer has not been able to be fully clarified. In parallel with the current study, there are studies in the literature showing low PDW in colorectal cancer (30, 36). However, just as there are studies showing that low PDW is associated with better survival, there are other studies stating that there is no diagnostic significance (34, 37). The PDWLR was evaluated for the first time in the current study. It was determined to be higher in the colorectal carcinoma group, but a high ROC-AUC value did not emerge. The low PDW and high PDWLR in the current study colorectal carcinoma group can be attributed to the significantly low lymphocyte count. The ratio of the two parameters, which were low in the colorectal carcinoma group, can be considered to have prevented the emergence of a diagnostic parameter. Therefore, there is a need for further, more detailed studies of the value of PDW and PDWLR in the differential diagnosis of colorectal cancer and colorectal benign polyps.

RDW is a parameter for which high values are measured in inflammatory processes. Since the diagnostic importance was shown in solid tumours, there have been few studies showing that it could be useful in the differentiation of colorectal cancer from colon polyps (38). In parallel with the literature, RDW in the current study was determined to be high in the colorectal cancer group, but the AUC value of 0.621 in the ROC analysis was not satisfactory. Therefore, proportioning with the lymphocyte count, which was shown to be low in colorectal cancer, was considered. The RDWLR value was determined to be higher in the colorectal cancer group than in the colon polyp group, and a satisfactory diagnostic value of AUC 0.720 was obtained in the ROC analysis. The diagnostic value of RDWLR has been investigated in only one previous study. Huang et al. reported that there was diagnostic importance (9). There is one study related to the prognostic importance of RDWLR, and the results of that study showed significance in the prediction of 3-year disease-free survival (39). It can be said that RDWLR is a remarkable parameter in the diagnosis of colorectal cancer, but further studies of larger series are.

The immature granulocyte count is a parameter in the full blood count, and its diagnostic importance in inflammation has been shown. It is elevated in inflammatory conditions such as sepsis, pancreatitis, and appendicitis (13). Except for bladder and breast cancer, there are no data related to its diagnostic importance in solid organ cancers (40, 41). According to the results of our study, there was no diagnostic significance of IG in the differentiation of colorectal cancer from colorectal benign polyps. This is the first and only study on this subject in the literature.

There were some limitations to this study. A group of healthy individuals could not be included for comparison because of the retrospective nature of the research. A second limitation was that the number of patients in the colorectal cancer group was lower than that of the colorectal benign polyp group, and this could have reduced the statistical power of the results.

Conclusion

The results of this study confirmed the diagnostic importance of NLR and PLR in colorectal cancer. RDWLR is a promising new parameter in the differential diagnosis of colorectal cancer from colon polyps. However, IG was not found to have any significant diagnostic value. There remains a need for further investigation of these findings with larger series.

Conflict of Interest Statement

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Ethical Approval

Approval for the study was granted by the Ankara Etlik City Hospital Ethics Committee (decision no: AEŞH-

BADEK-2024-195, dated: 28.02.2024). All the study procedures complied with the Helsinki Declaration and local ethics criteria.

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Authors Contributions
ÜÖ: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing-original draft.

AÖ: Investigation; Data curation; Formal analysis; Writing- review & editing.

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ORIGINAL RESEARCH

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Understanding the Night Shift Stress of Nurses: A Point Prevalence Study

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Abstract

Objective
This study aims to investigate the stress levels and stressors experienced by nurses during the night shift.

Material and Method
This point prevalence study was designed as a descriptive and cross-sectional study, and it was conducted with 73 nurses in a university hospital. The study data were collected with a personal information form that was designed by the researchers and a nurse stress identification form.

Results
It was concluded that the stress levels of nurses were above the average, with 106 points (27 lowest and 164 highest). Nurses who were frequently working at night shifts were found to have higher stress levels compared to those working less at night shifts. Besides, it was also noted that those who were satisfied with their jobs had lower stress levels. The most significant stress sources for nurses were understaffing, heavy workload, and limited career opportunities.

Conclusion
It was consequently reported that this study presented evidence based data on nurses' stress levels and their stress sources as well as potential precautions to prevent job stress. It was recommended to regularly conduct point prevalence studies to determine the levels of stress and stressors to develop solution based approaches.

Keywords: Job stress, nurse, nursing, shift work.

Introduction

Nurses make vital contributions the universal health care in terms of protecting and improving public health and preventing diseases. For nurses who comprise more than half of the medical personnel around the world, the job poses a significant stress source (1,2). Job stress is commonly defined as a state of negative emotional status, distress, or anxiety caused by the stress factors at a job (3,4).

Job stress proves to be a serious issue that affects nurses' health and efficiency (5). Kane (2009) found that stress considerably increases psychosomatic

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disorders like anger, anxiety, forgetfulness, gastrointestinal problems, backache, stiff neck, and shoulders (6). Applebaum et al. (2010) similarly reported that stress was among the major causes of quitting a job for nurses (7). Additionally, stress results in lower quality of nursing care and increases costs for medical institutions by causing nurses to get sick and therefore to miss workdays and take sick leaves (6). Virtanen et al. (2009) correlated higher levels of job stress and long work hours with the increase in hospital infections (8). Moreover, stress is known to decrease concentration, decision-making, and assessment skills while adversely affecting care quality (9).

Nurses frequently work at night shifts to provide 7/24 care for individuals from a variety of age groups, genders, and cultural backgrounds (3,10). However, nurses working at night shifts have limited sources in terms of personnel backup and equipment, and receive considerably less managerial support in comparison to those working at the day shift (11). Therefore, it has been widely reported that nurses had higher levels of stress at night shifts (12,13). In a study comparing stress levels of day shift nurses and night shift nurses, it was stated that cortisol levels of nurses working at night shifts were found to be higher at the end of their shifts, which confirmed the correlation between higher levels of cortisol and physiological stress (12). Furthermore, nurses working at night shifts reported more sleeping disorders, attention deficiency, and concentration problems, and lower job performance (14). In relevant studies, it was also found that nurses working at night shifts had considerable stress and that increasing levels of stress negatively influenced their health and well-being (15-17).

It has been suggested that acute stress in nurses can lead to psychosomatic disorders and medical errors, and cause feelings of compassion fatigue, fear, sadness, and grief (18-20). However, it has also been reported that long-term exposure to stress may also cause chronic problems such as burnout, posttraumatic stress, grief, and disorders (6,9,21). In this regard, acute stress must be prevented and carefully managed so as not to result in devastating chronic stress.

It has been widely reported that relevant studies have compared the stress levels of day shift nurses and night shift nurses (12,13) and focused on job stress sources of nurses (4,6,9,22). Nevertheless, no particular study has been reported on the stress sources of nurses working at night shifts and evaluating the acute stress of night shift nurses. This study, therefore, aimed to investigate the stress levels of nurses working at night shifts and their stress sources. It was suggested that the results of this study would contribute to the scientific literature and help to improve solution based strategies and approaches.

Materıal and Method

Study Design

This point prevalence study was designed as a descriptive and cross-sectional study.

Sample

The study universe was composed of 450 nurses working in Turkey at a university hospital. The researchers did not use a sampling method, and they aimed to conduct the study with 80 nurses working at the night shift on that day. And they completed the study with 73 nurses who consented to participate in the study.

Data Collection Tools

The study data were collected with a personal information form, a stress identification form, and a stress sources form via face to face interviews on 15.11.2021.

The personal information form was designed by the researchers in line with the recent literature, and it basically interrogates the demographic characteristics and the worklife variables that affect their stress levels (4,13,16).

The nurse stress identification was designed by Üstün and Kanbay (2009) in order to map out the stress factors for the job environment of nurses (23). The form originally included 18 questions, but the question regarding their losses was taken out with the permission of the authors. Therefore, the form used in this study had 17 questions. The questions in the form are scaled between 0-10, and higher points indicate higher levels of stress. The points for each stress factor are evaluated as low between 0-3, moderate between 4-7, and high between 8-10. The Cronbach's alpha reliability value of the scale was calculated as 0.88.

In addition to the stress identification form, the stress sources form was designed by the researchers based on the recent literature and their own job experiences in order to determine the stress factors that nurses may encounter at work (4,6,9,22).

Validity and Reliability of the Questionnaire

Eight nurses were asked to fill in the questionnaire form in order to test the clarity of the form. According

to the results of the pilot study, the items No windows in nurses' rest rooms and Inadequacy to cover own needs (nutrition, toilet, sleep, etc.) were added to the "Stress Sources of Nurses" form with the suggestions of the nurses. The questionnaire form was finalized after their suggestions. These nurses were not included in the main study.

Study Procedure

The study data were collected by the researchers during a night shift (16:00-08:00) after conducting a pilot study and being granted the necessary permissions from the institution. The participant nurses were asked to fill in the questionnaire form considering the conditions only on that particular day.

Statistical Analysis

The study data were analyzed with SPSS 20.0 (SPSS Inc., Chicago, IL, USA). In the study, the fitness of the data to the normal distribution was evaluated with the Shapiro-Wilk and Kolmogorov-Smirnov tests. The researchers also used conducted from descriptive statistical methods (mean, standard deviation, frequency) as well as Mann-Whitney U and Kruskal-Wallis H tests to correlate study groups. For the correlative analysis of study environment and stress scores, Spearman Correlation Analysis was used. The significance level was taken as $p<0.05$.

Results

The Descriptive Characteristics of Nurses

The descriptive and clinical characteristics of the participants' nurses, including their demographic characteristics and work life, are given in Table 1 and Table 2. Accordingly, it was noted that 13.7%

Table 1 Descriptive characteristics of nurses (n=73)

Characteristics	n	%
Age		
22-24 years old	14	19.2
25-29 years old	26	35.6
30-39 years old	23	31.5
40 years old and over	10	13.7
Gender		
Female	58	79.5
Male	15	20.5
Educational Status		
High school	14	19.2
College	9	12.3
Undergraduate	41	56.2
Graduate	9	12.3
Marital Status		
Married	41	56.2
Single	32	43.8
Having Children		
Yes	37	50.7
No	36	49.3
Dependant Family Members		
Yes	12	16.4
No	61	83.6

Table 2 Clinical characteristics of nurses (n=73)

Characteristics	n	%
Job Experience		
1-5 years	25	34.2
6-10 years	23	31.6
11 years and over	25	34.2
Experience in Their Current Unit		
≤ 1 years	32	43.9
2-5 years	25	34.2
6 years and over	16	21.9
Unit		
Internal diseases	20	27.4
Surgery	16	21.9
Intensive care	25	34.3
Operation room	3	4.1
Outpatient	3	4.1
Emergency room	6	8.2
Satisfied with Their Unit		
Satisfied	46	63
Not Satisfied	27	37
Satisfied with Their Job		
Satisfied	58	79.5
Not satisfied	15	20.5
Work Order		
Day	3	4.1
Night	6	8.2
Shift (Day + Night)	64	87.7
Their Shift Preference if They had a Choice		
Day	38	52.1
Night	17	23.3
Shift	18	24.6
Monthly Number of Night Shifts	11.18 ± 2.73	
Weekly Work Hours	51,63 ± 10,08	
Feeling Anxious About the Care of Their Children When Working at Night Shifts		
Yes	34	46.5
No	39	53.5
Feeling Anxious About the Care of Their Dependant Family Members When Working at Night Shifts		
Yes	17	23.3
No	56	76.7

Table 3 Factors that identify nurses' stress levels (n=73)

Stress Factors		Low		Moderate		High	
		n	%	n	%	n	%
1	Heavy workload	5	6.8	13	17.8	55	75.4
2	Clash with doctors	27	37	27	37	19	26
3	Clash with other nurses	33	45.2	23	31.5	17	23.3
4	Clash with head nurses	38	52.1	15	20.5	20	27.4
5	Changes in clinic rules and policies	25	34.2	27	37	21	28.8
6	Unsupportive seniors	37	50.7	15	20.5	21	28.8
7	Overcrowded units	11	15.1	16	21.9	46	63
8	Fear of making mistakes	37	50.7	20	27.4	16	21.9
9	Clinic environment (physical conditions, noise, lights, etc.)	18	24.7	17	23.3	38	52.1
10	Understaffed nurses	4	5.5	8	11	61	83.5
11	Limited career opportunities	8	11	13	17.8	52	71.2
12	Verbal or physical assault	13	17.8	20	27.4	40	54.8
13	Professional risks	8	11	19	26	46	63
14	Patients and their relatives (attendans, their behaviors, etc.)	10	13.7	22	30.1	41	56.2
15	Losses of patients (patient arrests)	24	32.8	21	28.8	28	38.4
16	Feeling insufficient	48	65.7	18	24.7	7	9.6
17	Understaffed assisted services	9	12.3	14	19.2	50	68.5

*0-3 points low, 4-7 points moderate, 8-10 points high

of the nurses were 40 years old and older, 79.5% of them were female, 56.2% of the nurses were married, 50.7% of them had children, 83.6% of them had no dependent family member, and 56.2% of the participants had an undergraduate degree. It was further reported that 34.2 of the nurses in the study had a nursing experience of 1-5 years or 11 years or over job experience, 43.9% of them were working in their current unit for less than a year, 34.3% of the participants were working in the intensive care units, 63% of the nurses were satisfied with their current unit, 79.5% of them were satisfied with their jobs, 87.7% of them working in shifts, and finally 52.1% of them would prefer to work day shifts if they had a choice. It was also found that the monthly number of night shifts was 11.18 ± 2.73, and their average weekly working hour were 51.63 ± 10.08 (Table 1, Table 2).

Stress Sources of Nurses

Factors that identify nurses' stress levels are given in Table 3, which demonstrates that the participant nurses had higher levels of stress due to understaffed nurses (83.5%), heavy workloads (75.4%), limited career opportunities (71.2%), understaffed assisted services (68.5%), overcrowded units and professional risks (63%), patients and patient relatives (56.2%), verbal or physical assaults (54.8%), and clinic environment (52.1%), (Table 3). Moreover, the major sources of stress for nurses were found to be low wages (80.8%), insufficient break times (80.8%), no windows in nurses' rest rooms (67.1%), lack of medical equipment and other equipment (61.6%), and finally, inadequacy to cover their own needs (nutrition, toilet, sleep, etc.), (57.5%), (Table 4).

A Correlative Analysis between Nurses' Descriptive Characteristics and Total Stress Scores

A correlational analysis between nurses' descriptive Characteristics and total stress scores is demonstrated in Table 5. The total stress score of nurses was reported to be 106 (27-164), which indicated that the participant nurses had above the average stress.

Table 4 Stress sources of nurses (n=73)

Situations*	n	%
Lack of medical equipment and other equipment	45	61.6
Providing care for terminal patients	24	32.9
Arrest interventions	24	32.9
Being called for urgently	19	26
Low wages	59	80.8
Insufficient break times	59	80.8
New patient admissions	37	50.7
No windows in nurses' rest rooms	49	67.1
Inadequacy to cover own needs (nutrition, toilet, sleep, etc.)	42	57.5

*Nurses chose more than one option.

Table 5 Correlation between the descriptive characteristics of nurses and total stress scores

Characteristics	Stress Total Score 106 (27-164)
Satisfied with their jobs	
Satisfied	98.50 (27-164)
Not satisfied	120 (93-162)
Test value	U: 257.000
p	0.015
Monthly Number of Night Shifts	11.18 ± 2.73
Test value	r: 0.259
p	0.027

*U: Mann Whitney U Test, r: Spearmen Correlation

It was further found that the total stress scores of nurses were correlated with their satisfaction with their jobs (U: 257.000, $p<0.05$) and the monthly number of night shifts (r: 0.027, $p<0.05$). In other words, the participant nurses who were satisfied with their jobs and had a lower number of night shifts also had lower stress levels. On the other hand, there was no correlation between total stress scores and nurses' other descriptive characteristics ($p>0.05$) (Table 5).

Discussion

It has been widely acknowledged that working night shifts could be a stressful experience for nurses (12). Nurses might be exposed to a variety of stress sources besides the natural stress caused by night shifts. Therefore, this point prevalence study was carried out to investigate the stress levels and stress sources of nurses on night shifts. The results of

the study showed that nurses had above-average stress (106 points). The top three stress sources of nurses with regard to their job stress scores were understaffing, heavy workloads, and limited career opportunities. In a relevant study on the stress levels of medical personnel working on day and night shifts, it was reported that those who were working on night shifts were noted to have higher levels of stress in comparison to those who were working on day shifts, which, nevertheless, didn't yield a significant statistical difference (13).

The descriptive characteristics of nurses who participated in the study were analyzed and it was reported that only 13.7% of nurses were 40 years old and older, 68.5% of them had an undergraduate degree, a majority of them didn't have a dependent family member (83.6%) or children (49.3%). Similarly, Buja et al. (2013) pointed out that nurses who were working night shifts were much younger, more educated, and had no dependents or children (16). They also noted that nurses working night shifts had less job experience, and they were either single or divorced. The results of our study indicated that the nurses who participated in the study had a variety of job experiences, and a majority of them were married, which was also found to be weak indicators for determining the stress levels of nurses working night shifts. It was concluded that it stemmed from the fact that individual characteristics were neglected since the number of nurses was not sufficient to maintain a work plan.

The most frequent stress source for nurses in our study was understaffing. It was considered to be unsurprising considering the recruitment of nurses in Turkey and other countries. According to the OECD Health Statistics Report 2024, around 60% of European Union-27 countries report a shortage of nurses (24). On the other hand, Powell (2013) conducted a study on the nursing experiences of nurses who were working night shifts, which included a nurse's statement that "I felt we are not important enough to think that we need more personnel". It suggested that it was of utmost significance to hire more nurses and to support them while managing the stress levels of nurses (25).

Heavy workload was the second most frequent stress source for the nurses in our study. Buja et al. (2013) argued that nurses who worked night shifts didn't have only a higher workload but also lower decision-making authority, which eventually increased the stress levels of nurses (16). In a similar study, it was indicated that nurses working night shifts had to make critical decisions without having a chance to consult with head nurses or doctors (22). The results of our study urged that certain stress sources, such as understaffed nurses and heavy work, should not be considered only numerical data but rather crucial significant factors that would affect nurses' health, care quality, and safety.

The nursing profession requires a professional education and expertise. However, nurses need more respect and independence as well as better payment to overcome substantial challenges (heavy workload, being treated as only allied healthcare personnel, working too many hours, and limited career opportunities) (26). In our study, the participant nurses reported that the main sources of stress for them were low wages (80%) and limited career opportunities (71%), which was noteworthy in the sense that it highlighted the concerns of nurses regarding professional development and decent payment.

In the university hospital where our study was conducted, nurses were working in shifts (night-day). During the night shift when the study data were collected, almost one fourth of the nurses preferred to work at night shifts and more than half of the nurses especially preferred to work at only day shifts if they had a choice, which resulted from the fact that most of the nurses in the study were married with children. The study results demonstrated that the participant nurses with children had concerns about the care of their children during night shifts. It was similarly stated that personal and familial factors had a profound influence over nurses' shift choices (27). In a study conducted in Iran, it was also reported that nurses and society in general had a negative attitude towards working at night shifts (28). It was further noted in a relevant study that medical personnel who voluntarily worked at shifts had higher levels of job satisfaction, and they reported fewer complaints about working at shifts (29). It would be reasonable to argue that none of these work orders can be ideal, but it would be much more favorable for the employees if they were given an opportunity to choose their shifts (30).

The International Labor Organization (ILO) and the American Nurses Association (ANA) equally recommend that nurses should not work more than 40 hours a week, paid or otherwise (31,32). Nurses in Turkey are recruited as civil servants according to the Civil Servants Law No. 657 and the Labour Code No. 4857. The weekly work hours of nurses were determined as 40 hours according to the Civil Servants Law and 45 hours according to the Labor Code. To

compensate for overtime work, nurses are granted daily leaves in line with their extra working hours (33). Caruso (2014) reported that working more than 40 hours a week adversely affected nurses' health and patient safety (17). The results of our study showed that nurses' weekly working hours were higher than the recommended hours (51,63 ± 10,08). Besides, the number of night shifts a month ranged from 8 to 14. The results of our study also indicated that the weekly working hours of nurses did not significantly affect stress levels, but the increasing frequency of night shifts was correlated with stress levels of nurses. The results of our study further suggested that nurses who worked more frequently at night shifts had higher levels of stress when compared to those who did not, which could refer to the fact that they had fewer days off due to frequent night shifts, and they often suffered fatigue. It was similarly reported that nurses who worked fewer hours had lower levels of stress and they more successfully managed stress (34). It was stated in a relevant study on the influences of working hours on the stress levels of nurses that the stress levels of nurses increased with the working hours and that 15% of nurses who worked more than 40 hours a week had higher stress levels (35).

It has also been noted that senior nurses, especially those who were 40 years old and over, were more affected by the effects of night shifts, and that they could not adjust to the conditions of night shifts and reported sleeping disorders (36). The results of our study indicated that age and job experience were not correlated with stress levels and nurses' stress sources. The results were also confirmed by a study conducted by Kane (2009), who found that age or seniority did not deliberately reduce the stress levels of nurses (6). However, in a relevant qualitative study, it was argued that senior nurses who worked night shifts were not supported by inexperienced nurses and felt quite isolated. It was additionally suggested that they had difficulties in making decisions at work and could not cope with such uncertainties, which eventually caused higher levels of stress (19,37).

A majority of the participant nurses found their units overcrowded and inconvenient in terms of clinic environment (physical conditions, noise, light, etc.), unventilated (as there were no windows in nurses' rest rooms), which caused high levels of stress. In a similar study, it was reported that receiving sunlight at least 3 hours a day resulted in less stress and higher levels of satisfaction (15). Moreover, most of the nurses in our study complained about the lack of medical equipment and other equipment as a source of stress, which might stem from their concerns about ensuring the patient's safety and optimum care quality.

It was also noted that the participant nurses worked 8 hours in day shifts and 16 hours in night shifts. It is vitally important to establish a work environment to best respond to the needs of nurses while managing the stress levels of nurses who were working for long hours at night shifts. More than half of the nurses in our study stated that they were unable to cover their own physical needs, such as nutrition, toilet, sleep, etc., which consequently enhanced their stress. In a study on sleep quality, it was reported that night shifts negatively affected sleep quality and medical personnel working in shifts had lower levels of sleep quality in comparison to those who worked only day shifts (13). It was considered that nurses were unable to take care of their own needs because of understaffed in the units, heavy workloads, and longer work hours at night shifts.

Recent studies on the stress sources of nurses have usually focused on the physical conditions of workplaces. On the other hand, it can be presumed that future studies would certainly take into consideration both job related factors and other factors that affect the stress levels of nurses to produce a more sophisticated identification of stress sources (1). The study results also showed that the nurses' perception of stress sources was not correlated with having a dependent in the family or having children. Although it has been previously reported that nurses had a "secondary stress source" when they had to care take care of children, the elderly, or their family members outside of their work, these secondary sources haven't been widely analyzed in nursing studies so far (38).

More than half of the nurses in our study reported the attitudes of patients or their relatives and verbal or physical assault as a major source of stress. Nurses play a crucial role in providing health care, and they have to deal with the demands of patients and their relatives, which may not be included in their job description. Sometimes, conflicts may arise when nurses do not, or can not, meet the expectations of demanding patients, which naturally causes even more stress for nurses. It has also been noted that some patients or their relatives tend to be aggressive and violent (6). The results of our study indicated that stress management should address the needs of patients and their relatives as well as medical personnel.

Vicente et al. (2016) reported that the main cause of stress for nurses when providing quality care was the feeling of inadequacy to meet challenging

expectations, to deal with insufficient resources, and to perform within limited periods (22). The nurses in our study reported several stress sources, but they did not regard themselves as inadequate to work night shifts. However, a majority of nurses stated that they were generally satisfied with their jobs. It was further found that those who were satisfied with their jobs had lower stress levels, which can be evaluated as a result of the fact that nurses were capable of coping with stress and they were satisfied with their jobs.

Conclusion and Recommendations

Valizadeh et al have concluded that stress levels of nurses are, and should be, a major concern for a healthy nursing practice and quality patient care (5). The stress levels of nurses were found above the average with major stress sources like understaffed, heavy work load and limited career opportunities.

It is recommended that stress sources and stress levels of nurses can be easily managed, provided that hospital management and nursing managers attentively listen to the nurses' demands, support them, and integrate them into the decision-making processes. For instance, nurses' preferences and needs can be taken into account when organizing shifts in the hospital. Additionally, the physical work environment can be ameliorated to reduce the stress levels of nurses. Considering that the nursing profession already causes a large amount of stress, they can be incorporated into education programs focusing on coping with stress. Such measures will certainly prevent acute stress situations from becoming more devastating chronic stress situations .

This study provided substantial evidence-based data to explore stress levels and stress sources of nurses and to ensure legal or institutional regulations. Given that stress sources at work may vary depending on the institutional structure of medical institutions, these institutions are advised to regularly implement point prevalence studies in order to assess stress sources and to develop solution-based approaches.

Study Limitations

The stress levels of nurses were based on their self-report rather than the results of biochemical blood analysis. Therefore, the reliability of the study data was limited by the reliability of their statements. This study was implemented in only one hospital, with the participation of nurses who were working on the night shift when the study was carried out by the researchers. Thus, the results represented only those who participated in the study.

Conflict of Interest Statement

Written informed consent to participate and publish was obtained from all individual participants included in the study.

Ethical Approval

The permission to implement the study was granted by the Süleyman Demirel University Research and Application Hospital (Date:15/12/2021, Issue: E-26515734- 605.01-181076) and the Board of Ethics at the Süleyman Demirel University Faculty of Medicine (Date: 15/09/2021, Issue: 72867572-050.01.04-114341). All nurses were informed about the purposes of the study, and the researchers sought oral consent from all nurses who consented to participate in the study. To use forms, the researchers also asked for the permission of the author. The study was conducted in line with the principles of the "Helsinki Declaration".

Consent to Participate and Publish

Written informed consent to participate and publish was obtained from all individual participants included in the study.

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Authors Contributions

HCE: Conceptualization; Methodology; Formal analysis; Writing - Original draft preparation; Writing - Reviewing and Editing.

TC: Conceptualization; Methodology; Formal analysis; Writing - Original draft preparation; Writing - Reviewing and Editing.

AO: Methodology; Writing - Original draft preparation; Investigation; Writing - Reviewing and Editing.

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ORIGINAL RESEARCH

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Impact of Educational Status and Number of Children on Weight Loss Outcomes After Laparoscopic Sleeve Gastrectomy

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Abstract

Objective
Laparoscopic sleeve gastrectomy (LSG) is a successful method to achieve sustained weight loss and reduce morbidity and mortality. However, outcomes vary widely based on several factors, including preoperative childbirth history and educational status. The purpose of this research is to evaluate the influence of the number of children and educational level before surgery on the weight reduction results in female patients who had LSG.

Material and Method
We analyzed data from 70 female patients who underwent LSG between January 2017 and January 2022. Patients were stratified into groups based on the number of children (0 vs. ≥1) and educational status (primary or secondary school vs. high school or university). The primary outcome measures were the percentage of excess weight loss (%EWL) and percentage of total weight loss (%TWL) at 1-year post-surgery.

Results
At 1 year, the mean %EWL was 79.3, and the mean %TWL was 36.2. No differences were observed in %EWL and %TWL between patients with and without children. However, patients with high school or university education had significantly better %EWL and %TWL outcomes compared to those with less education.

Conclusion
Education level positively influences weight loss outcomes following LSG, while the number of children does not appear to significantly impact postoperative weight loss. These findings suggest that LSG can be equally effective in women regardless of their childbirth history, but higher education levels may contribute to better surgical outcomes.

Keywords: educational status, sleeve gastrectomy, number of children, weight loss

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Introduction

Among bariatric surgical procedures, laparoscopic sleeve gastrectomy (LSG) is a successful method to achieve sustained weight loss (WL) and reduce morbidity and mortality (1,2). WL following LSG varies depending on many factors, ranging from the number of children a patient has to socioeconomic status, education level, and health literacy (3-5).

Considering the relationship between pregnancy and weight gain, it can be anticipated that women with more children enter surgery with a higher preoperative body mass index (BMI) (3). Each additional pregnancy-related weight gain can lead to a higher BMI and poorer bariatric surgery (BS) outcomes. Therefore, knowing the full history of pregnancies is crucial (6). Education level is another factor that influences WL. As patients' education levels and thus their awareness of BS increase, improvements in WL outcomes are observed (5).

Limited studies are showing the impact of preoperative educational status and number of children on postoperative outcomes in BS. Therefore, our primary aim is to compare the influence of the preoperative number of children and educational status on WL outcomes in female patients after BS.

Materıal and Method

Patients who underwent LSG between January 2017 and January 2022 and were followed up in our hospital's obesity center were included in the study. The study evaluated the outcomes of 70 female patients with available preoperative data and 1-year postoperative follow-up data. The ethics committee of the hospital gave its approval (Approval number: 2024/24, 26/06/2024). The study was conducted by the principles outlined in the Declaration of Helsinki. Data were retrospectively collected using the hospital information system and the national health database (e-nabız). The number of preoperative children, adherence to dietary recommendations, and education level parameters were obtained from patient histories. Patient height, weight, BMI, age, percentage of excess weight loss (%EWL) and percentage of total weight loss (%TWL), comorbidities, surgery date, and surgical reports were evaluated through the system. Patients were subjected to subgroup analyses based on educational status (primary or secondary school vs. high school or university) and the number of children.

Patients with incomplete 1-year data, those who did not adhere to dietary recommendations, patients whose surgical reports were not accessible through the database, patients who had adopted children, and male patients were excluded from the study. Patients who were lost to postoperative follow-up or had physical activity restrictions were excluded from the study. Additionally, patients who did not regularly attend dietitian follow-ups were not included.

Surgical Procedure

LSG was performed using the classic 5-port technique. Patients with a bougie size of 36–38 French were included in the study. Only those whose transection was initiated 4–6 cm from the antrum were eligible for inclusion.

Statistical Analysis

An analysis of the normal distribution of continuous data was conducted using the Shapiro-Wilk test. An analysis was conducted on categorical variables using the Pearson’s chi-square test to investigate differences between groups, and on continuous covariates using the Mann-Whitney U test. A statistical significance level was defined as a p-value below 0.05. Analyses were performed using SPSS software developed by IBM in Armonk, NY, USA.

Results

Seventy female patients participated in the study. The average age of the patients was 41.8 years. The mean weight was 124.2 kg, and the mean BMI was 47.5. Only 20 of the patients had no children. At the end of the first year, the mean %EWL was 79.3, while %TWL was 36.2. Table 1 presents the demographic indicators, comorbidities, and weight-related outcomes of the patients. None of the patients with diabetes were using insulin. All patients were on metformin therapy.

The comparison of parameters between groups based on childbearing status is shown in Table 2. Patients without children were younger. Patients with children had higher preoperative BMI values. Hypertension was more common in patients without children. One-year %EWL and %TWL values in the two groups contained similar results.

The comparison of parameters between groups based on education level is presented in Table 3. Patients with high school or university education were younger and had fewer children. Additionally, in this group, preoperative weight and BMI were lower. Diabetes mellitus was less common in the group with high school or university education. Most importantly, patients with high school or university education had

Table 1 Demographic Data, Comorbidities, and Weight-Related Data

Age, mean (SD), year	41.8 ± 10.4
Educational Status (Primary or middle school/High School or University)	37/33
Number of children, n **0** **1** **2** **3** **4** **5+**	 20 12 19 10 5 4
Comorbidities Hypertension Diabetes	 19 29
Weight (preoperative) (kg)	124.2 ± 16.3
BMI (preoperative) (kg/m^2)	47.5 ± 5.4
Weight (one year after LSG)(kg)	79.5 ± 14.2
BMI (one year after LSG)(kg)	30.3 ± 4.4
EWL (one year after LSG) (%)	79.3 ± 15.8
TWL (one year after LSG) (%)	36.2 ± 6.1

BMI:Body mass index, EWL:Excess weight loss, TWL:Total weight loss

Table 2 Comparative Analysis of Groups Based on Whether Patients Have Children

	No children (n=20)	**≥ 1 Child (n=50)**	**P value**
Age, median, year	35 (19-53)	43 (27-61)	0.03
Weight (preoperative) (kg)	127.5 (93-158)	120 (94-154)	0.621
BMI (preoperative) (kg/m2)	46.1 (37.2-57.4)	47.0 (38.0-60.1)	0.308
EWL (one year after LSG) (%)	81.0 (35.9-107.9)	78.2 (43.9-106)	0.474
TWL (one year after LSG) (%)	36.4 (20.2-45.0)	37.0 (20.8-50.8)	0.938
Educational Status (Primary or middle school/High School or University)	6/14	31/19	0.019
Comorbidities Hypertension (Present/Absent) Diabetes (Present/Absent)	 0/20 5/15	 19/31 24/26	 0.001 0.108

BMI:Body mass index, EWL:Excess weight loss, TWL:Total weight loss

Table 3 Comparative Analysis of Groups Based on Educational Status

	Primary or Middle School (n=37)	High School or University (n=33)	P value
Age, median, year	46 (19-61)	40 (25-58)	0.009
Weight (preoperative) (kg)	126 (98-158)	120 (93-139)	0.003
BMI (preoperative) (kg/m2)	48.0 (38.0-60.1)	43.8 (37.2-55.0)	0.012
EWL (one year after LSG) (%)	78.1 (35.9-104.6)	86.8 (66.0-107.9)	<0.001
TWL (one year after LSG) (%)	35.0 (20.2-47.7)	37.0 (29.1-50.8)	0.015
Children (≥ 1 child/no children)	31/6	19/14	0.019
Comorbidities Hypertension (Present/Absent) Diabetes (Present/Absent)	 13/24 23/14	 6/27 6/27	 0.178 <0.001

BMI:Body mass index, EWL:Excess weight loss, TWL:Total weight loss

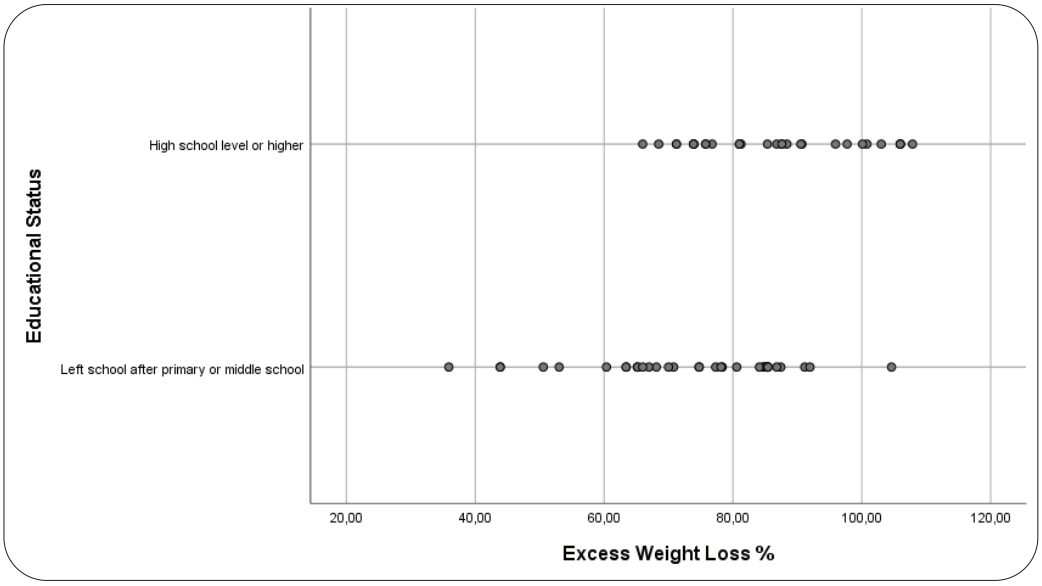

Figure 1
The relationship between education status and 1-year %EWL outcomes

better %EWL and %TWL outcomes at the end of one year.

The 1-year %EWL results were superior in patients with a high school or university education. The relationship between education status and 1-year %EWL outcomes is illustrated in Figure 1.

Discussion

Many factors influence WL after BS. The objective of this research was to examine the influence of the number of children during surgery and the degree of education on WL after BS. This research is significant since it is among the first investigations on the impact

of educational status and the number of children before surgery on the effectiveness of WL after LSG. In the current literature, successful WL after LSG is defined as a loss of more than 50% EWL in the medium term. Studies have shown that most patients achieve %EWL levels of over 50% by the sixth month following LSG (5,7). In our study, when evaluating 1-year outcomes, all patients reached successful %EWL results.

The impact of preoperative pregnancies on preoperative BMI and %EWL remains unclear. Pregnancy induces various metabolic changes, such as weight gain, insulin response, and insulin sensitivity. However, these changes often return to pre-pregnancy levels within a few years after childbirth (8). Therefore, the time elapsed since childbirth may affect WL after BS. It is well known that many women have their first child in their 20s (8). The mean age of the patients included in the research was 41.8 years, suggesting that more than a year had passed since childbirth, minimizing the metabolic effects of pregnancy. Women who had children before undergoing bariatric surgery had a lower average weight and BMI compared to those without children. This result is consistent with the study by Hill et al (9). Additionally, we found no statistically significant difference in %EWL between patients with and without children. This finding is inconsistent with the study by Hecht et al (3). The discrepancy could be due to the time interval between pregnancy and surgery, or could be attributed to racial differences.

Another factor we examined in our study was education level. Patients with high school education or higher had lower preoperative weight and BMI, similar to findings in other studies (5,10). Moreover, %EWL and %TWL outcomes were higher in the group with high school education or higher, consistent with the study by Dilektaşlı et al (5). These results may be because patients with higher education levels better understand and adhere to long-term follow-up and dietary recommendations. Additionally, individuals with lower education levels are reported to have a higher incidence of mental health disorders (11). Considering the positive correlation between eating disorders, mood disorders, and obesity, lower education levels could lead to poorer WL outcomes (12). Recent studies have shown that psychiatric disorders contribute to poor WL outcomes and an increased need for revisional BS (13,14).

Although higher education levels do not always correlate with higher health literacy, they often do (15). As health literacy increases, lower preoperative BMI and higher %EWL levels are observed (4,15). Patients are given lifestyle modification and dietary recommendations both before and after surgery. The understanding and implementation of these lifelong changes are more feasible for patients with higher education levels, which likely leads to better long-term WL outcomes in this group. In contrast to Mahoney et al.'s study, which found a positive correlation between preoperative hypertension and diabetes mellitus, and education level, our study had the opposite results (4). Barcelo et al. also found a negative correlation between diabetes mellitus and education level (16). This discrepancy might be due to a lower level of knowledge about healthy eating.

One of the limitations of our study is that it has a relatively small sample size. Another limitation is that historical data (such as the number of children) were obtained directly from the patients. No health literacy questionnaires were administered to the patients. Finally, the uneven distribution of diabetes prevalence and age between groups may also introduce bias.

Conclusion

In conclusion, this study showed that patients with higher education levels had lower preoperative BMI and higher %EWL-%TWL values. The number of children before surgery did not affect weight loss. These findings suggest that BS can be equally effective in women regardless of their childbirth history, but higher education levels may contribute to better surgical outcomes. Although it is not possible to change the level of education before surgery, educating patients on health literacy may be beneficial in achieving better bariatric surgery outcomes.

Conflict of Interest Statement
The authors declare no conflicts of interest.

Ethical Approval
Ethics approval was obtained from the Non-Invasive Clinical Research Ethics Committee of the Gaziantep City Hospital (Date: 26.06.2024, No 2024/24). The study was conducted by the principles outlined in the Declaration of Helsinki.

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The authors declare that they have not used any type of generative artificial intelligence for the writing of this

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Authors Contributions

ET: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing- original draft.

BT: Conceptualization; Formal analysis; Funding acquisition;

BG: Investigation; Methodology; Project administration;

EK: Resources; Supervision; Validation; Writing-review & editing.

EC: Investigation; Validation; Writing- original draft.
HCY: Formal analysis; Investigation; Visualization; Writing- original draft.

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ORIGINAL RESEARCH

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The Mediating Role of Statistical Anxiety in the Relationship Between Statistical Attitudes and Statistical Self-Efficacy Beliefs of Students Taking Biostatistics Courses: A Path Analysis

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Abstract

Objective

In this study, the mediating relationship among statistical self-efficacy beliefs, statistical anxiety, and statistical attitudes of students taking biostatistics courses was examined. The population of our study consisted of undergraduate, graduate, and doctoral students taking biostatistics courses.

Material and Method

The study population comprised a total of 85 students, with 51 at the undergraduate level and 34 at the graduate and doctoral levels. Data for our study were collected at three different stages before the course (Pre), in the 8th week of the course (Intra), and at the end of the course (Post). The participation rates were as follows: Pre-course: 74 (87%), Intra-course: 59 (69%), and Post-course: 62 (73%). The "Statistical Self-Efficacy Belief Scale," "Statistical Anxiety Scale," and "Statistical Attitude Scale" were used to collect data in the research. The mediating role between statistical self-efficacy beliefs, statistical anxiety, and statistical attitudes was analyzed using the regression-based Bootstrapping technique.

Results

Analysis results showed that statistical anxiety had a mediating effect on the relationship between statistical attitudes and statistical self-efficacy beliefs before the course (p=0.013). At the 8th week of the course, a mediating effect of statistical anxiety was found in the relationship between statistical attitudes and self-efficacy beliefs (p=0.033). At the end of the course, no mediating effect of statistical anxiety was found in the relationship between statistical attitudes and self-efficacy beliefs (p=0.298).

Conclusion

Our study demonstrated that during the biostatistics course, statistical anxiety had a mediating effect on the relationship between statistical attitudes and statistical self-efficacy beliefs at the beginning and during the course; however, this effect disappeared at the end of the course. As a result of the research, the mediating role among statistical self-efficacy beliefs, statistical anxiety, and statistical attitudes of students taking biostatistics courses was found to be significant.

Keywords: Biostatistics, Anxiety, Attitude, Self-efficacy

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Introduction

Statistics is a mathematics-based science that interprets data collected to measure both quantitative and qualitative variables by reporting them through graphs or tables. Statistical science, a foundational field including areas like data mining, econometrics, actuarial science, and biostatistics, encompasses common research methods used in positive sciences, including economics, medicine, sociology, and engineering (1). To address the growing need for statistical science in many fields, including social sciences and health sciences, statistics has increasingly been integrated into the curricula of undergraduate and associate degree programs. During statistical education, performing statistical calculations directly related to mathematics and interpretation of the obtained results may lead to anxiety among students (2).

Statistical anxiety is not derived from the complexity of methods used for statistical analyses, but rather stems from attitudinal factors. It is a situational type of anxiety that emerges when dealing with statistics, observed while performing tasks such as data collection, data analysis, and interpretation of analysis results. Although related to mathematics, it is distinct from mathematical anxiety (3, 4). Factors leading to statistical anxiety in students are grouped under three main categories: 1. Personal factors consist of psychological and emotional attitudes and behaviors (perception, self-esteem, learning styles, and general anxiety level). 2. Situational factors are directly related to the course (the instructor's teaching style and terminology used in class, the pace of the course). 3. Environmental factors refer to individual-specific situations (gender, age, academic department, experiences in mathematics courses) (5).

Attitude, defined as the tendency to react positively or negatively towards an object, person, institution, or event, refers to an individual's willingness to adopt or reject the statistical learning process when it comes to statistics (6). It has been determined that students' negative attitudes towards statistics courses increase statistical anxiety (7). For students with high levels of statistical anxiety, this situation is considered a factor that negatively affects academic achievement (8). Self-efficacy belief plays a role in statistical learning as it affects a student's perception of their capacity to understand and complete statistical tasks (9).

In the literature, studies are extending from the past to the present that use different scales to determine students' levels of statistical anxiety and attitude (12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 5). These studies have revealed that negative attitudes towards statistics, statistical anxiety, and low levels of statistical self-efficacy affect students' well-being and overall academic performance. There is a limited number of studies investigating statistical anxiety, attitudes, or statistical self-efficacy. To increase the knowledge base in this field, it is crucial to investigate more deeply the interaction between statistical anxiety, attitudes, and self-efficacy (23).

In the health sector, statistics hold great importance in research planning, implementation, conducting statistical analyses, interpreting results, and preparing quality scientific publications (10). Due to the need for biostatistics in health sciences, it is extremely important to eliminate students' negative attitudes (11). Awareness of the opinions and attitudes related to the biostatistics course by instructors will help students participating in this course to better understand the content and engage with the course more positively (10).

The study aims to examine the mediating role of statistical anxiety in the relationship between statistical attitudes and statistical self-efficacy beliefs of students taking a biostatistics course. In addition, the scales to be used were applied to the students at different times during the biostatistics course to determine the relationships between them.

Material and Method

Data Collection Tools

In our study, the demographic data of the students was obtained. Additionally, scales were used to assess their status regarding the biostatistics course. In our study, the Statistical Self-Efficacy Belief Scale, the Statistical Anxiety Scale, and the Statistical Attitude Scale were used to determine the students' status towards the biostatistics course.

Statistical Self-Efficacy Belief Scale (SSEB)

The Statistical Self-Efficacy Belief Scale, developed by Finney and Schraw in 2003 and adapted to Turkish by Sevimli and Aydın (2017), is specifically developed for the Statistics course and used to evaluate the self-efficacy beliefs of students or instructors regarding this course (27, 28). The scale, which is prepared in a 6-point Likert-type format, consists of 14 items. The Cronbach's Alpha values of the scale in our study were calculated as Pre: 0.965, Intra: 0.935, and Post: 0.962.

Statistical Anxiety Scale (SAnx)

The Statistical Anxiety Scale, developed by Faber, Drexler, Stappert, and Eichhorn for graduate students,

was adapted to Turkish by Güler (2019) and consists of 17 items (29, 30). Statistical anxiety arises during the process of data collection, data analysis, and interpretation of analysis outputs. This anxiety affects the student's ability to understand articles, analyze data, and interpret the results. The Total Cronbach's Alpha values of the scale in our study were calculated as Pre: 0.920, Intra: 0.908, and Post: 0.945.

Statistical Attitude Scale (SAtt)
Robert and Bilderback (1980) developed a 34-item Statistical Attitude Scale and administered it to three groups of graduate students taking an introductory statistics course (12). Many studies have been conducted to measure statistical attitudes. One such study was developed by Köklü in 1994 (31). The statistical attitude scale developed by Köklü consists of 28 items. The scale was developed to reveal students' attitudes towards statistics. The Total Cronbach's Alpha values of the scale in our study were calculated as Pre: 0.897, Intra: 0.909, and Post: 0.935.

Study Design
The scales used to determine the students' status towards the biostatistics course were administered at three different time points. The biostatistics course was planned for 14 weeks.

The administration times of the scales:

Pre: Before the biostatistics course (Pre)

Intra: 8th week of the biostatistics course (Intra)

Post: Final week of the biostatistics course (Post)

The data were collected by administering the scales through a Google Form at the pre-, intra-, and post-test points.

Statistical Analysis
Sample Size
The population of our study consisted of undergraduate, master's, and doctoral students taking the biostatistics course. At the undergraduate level, there were 58 students, and at the master's and doctoral levels, there were 30 students, making a total population of 88 students. Our study aimed to reach the entire population. In our study, the participation rates were: Pre: 75 (85.2%), Intra: 59 (67.0%), and Post: 62 (70.4%) participants.

Statistical Analysis
The data is presented in terms of mean, standard deviation, median, minimum, maximum, percentage, and frequency. The normality of distribution for continuous variables was assessed using the Shapiro-Wilk test, Kolmogorov-Smirnov test, skewness, and kurtosis (with Lilliefors correction). For comparisons between quantitative variables, Pearson and Spearman correlation tests are used, depending on the normality of distribution. For comparisons between two independent groups, the Independent Samples t-test is used if the normality assumption is met, and the Mann-Whitney U test will be used if it is not. For comparisons of continuous variables among more than two independent groups, ANOVA is used if the normality assumption is met, and the Kruskal-Wallis test is used if it is not. Post-hoc tests after ANOVA used Tukey's test if the variances are homogeneous, and Tamhane's T2 test if they are not. For post-hoc tests after Kruskal-Wallis, the Kruskal-Wallis 1-way ANOVA (k samples) test is used. For comparisons of more than two dependent group variables, Repeated Measures ANOVA is used if the normality assumption is met, and the Friedman test is used if it is not. Post-hoc tests after Repeated Measures ANOVA used Tukey's test if the variances are homogeneous, and Tamhane's T2 test if they are not. For post-hoc tests after Friedman, the Friedman 2-way ANOVA by ranks (k samples) test is used. The significance of indirect effects in the model used to determine the relationships between the scales is tested using the structural equation modeling bootstrapping method. Multivariate normality was assessed using Mardia's test, which evaluates skewness and kurtosis and is widely used in SEM analyses. To address potential deviations from normality, bootstrapping with 1000 resamples was applied. The path coefficients (β) of the model were calculated. All analyses were performed using IBM SPSS 20 and JAMOVI 2.2.2 statistical software. Statistical significance set at $p<0.05$.

Results

The participants' mean age (±SD) was 23.7±3.09 years (21–36), and 83.0% were female. The descriptive statistics of the scores of the scales and the sociodemographic characteristics of the students are shown in Table 1.

In the comparison of gender and total scores of the scales, no statistically significant difference was found between the total scores of the scales between genders ($p>0.05$). When the education level of the participants was compared with the total scores of the scales, a statistically significant difference was found only between the total score of the Pre SAtt scale ($p=0.008$). There was no statistically significant difference between education level and other scale

Table 1 Sociodemographic characteristics of participants and descriptive statistics of the scores of the scales

		N	%
Gender	Female	73	83.00%
	Male	15	17.00%
Educational Level	Undergraduate Student	58	65.90%
	Master's Student	20	22.70%
	Doctoral Student	10	11.40%
		Mean ± SD	Median (Min-Max)
Pre SSEB (n=75)		33.76 ± 13.48	34 (14-68)
İntra SSEB (n=58)		45.81 ± 11.15	46.5 (27-82)
Post SSEB (n=62)		45.15 ± 12.91	43.5 (15-75)
Pre Satt (n=75)		80.31 ± 13.05	78 (50-110)
Intra SAtt (n=58)		83.64 ± 12.99	84 (57-111)
Post Satt (n=62)		81.52 ± 17.65	82 (24-118)
Pre Sanx (n=75)		32.73 ± 9.83	30 (18-68)
Intra SAnx (n=58)		29.36 ± 7.42	29 (17-46)
Post Sanx (n=62)		29.79 ± 9.10	28 (17-51)

Pre SSEB: Prior to The Statistical Self-Efficacy Belief Scale, Intra SSEB: Intra to The Statistical Self-Efficacy Belief Scale, Post SSEB: Post to The Statistical Self-Efficacy Belief Scale, Pre Satt: Prior to The Statistical Attitude Scale, Intra SAtt: Intra to The Statistical Attitude Scale, Post Satt: Post to The Statistical Attitude Scale, Pre Sanx: Prior to The Statistical Anxiety Scale, Intra SAnx: Intra to The Statistical Anxiety Scale, Post Sanx: Post to The Statistical Anxiety Scale

total scores (p>0.05). When the correlation of the total scores of the scales with age was analysed, it was found that there was a correlation only with the total score of the Pre-SHT scale (r=0.329; p=0.004). No statistically significant correlation was found between the other scale total scores and age (p>0.05) (Table 2).

The results in Table 3 showed the internal consistency levels for three different scales (SSEB, SAnx, and SAtt), and all of them showed high levels of reliability. For the Statistics Self-Efficacy Belief Scale, Cronbach's Alpha values and McDonald's Omega were very high in the pre-course (0.965 – 0.967), in-course (0.935 – 0.936), and post-course (0.962 – 0.964) measurements. This indicates that the scale reliably measures participants' self-efficacy beliefs. Similarly, Cronbach's Alpha values and McDonald's Omega values for the Statistics Anxiety Scale were also high in the pre-course (0.920 – 0.922), in-course (0.908 – 0.911), and post-course (0.945 – 0.948) measurements, indicating that the scale consistently assessed participants' anxiety tendencies. The Statistics Attitude Scale shows a similar reliability, providing reliable results with pre-course (0.897 – 0.904), intra-course (0.909 – 0.919), and post-course (0.935 – 0.945) values. In general, the high Cronbach's Alpha values obtained in all scales indicate that the internal consistency of these measurement tools and their consistency among the participants are high, and therefore the results are reliable and valid (Table 3). The results of the goodness of fit analysis of the CFA model for the scales used and the path model are presented in Table 4 (Table 4).

According to the results of Table 5, the analyses show that there are significant differences between the stages in statistical self-efficacy belief, anxiety, and attitude scales. In terms of statistical self-efficacy belief, there were significant differences between pre, intra, and post measurements (F=31.00, p<0.001). This shows that there was a significant increase in students' self-efficacy beliefs during the course. In the evaluation of the statistics anxiety scale, significant differences were found between the pre- and Intra measurements (F=3.23, p=0.045), indicating that there were improvements in anxiety levels. In the results of

Table 2 Comparisons of total scale scores with Age, Educational Level and Gender

Correlations (Age)									
	Pre_SSEB	İntra_SSEB	Post_SSEB	Pre_SAtt	Intra_SAtt	Post_SAtt	Pre_SAnx	Intra_SAnx	Post_SAnx
r	0.061	0.012	-0.017	0.329	0.244	0.094	-0.011	-0.22	-0.022
p	0.601	0.926	0.898	0.004	0.065	0.468	0.925	0.097	0.863
N	75	58	62	75	58	62	75	58	62
	Educational Level								
	Undergraduate Student		Master's Student		Doctoral Student				
	Mean ± std	Medyan (min-max)	Mean ± std	Medyan (min-max)	Mean ± std	Medyan (min-max)	H	p	
Pre_SSEB	33.6 ± 13.66	32.5 (14-68)	35.18 ± 14.45	35 (14-57)	31.75 ± 11.25	31.5 (14-48)	0.367	0.832	
Intra_SSEB	46.28 ± 11.74	48 (27-82)	43 ± 10.37	42 (28-66)	48.5 ± 5.51	49 (42-54)	1.695	0.429	
Post_SSEB	45.89 ± 13.15	43 (19-75)	44.36 ± 11.32	47 (26-57)	39.75 ± 17.44	44 (15-56)	0.053	0.974	
Pre_SAtt	77.14 ± 12.6	75 (50-110)	87.88 ± 11.54	87 (71-109)	84 ± 12.59	81.5 (72-109)	9.657	0.008	
Intra_SAtt	82.77 ± 12.94	84 (57-111)	83.36 ± 13.18	86 (66-107)	93.75 ± 11.76	89.5 (85-111)	2.551	0.279	
Post_SAtt	79.34 ± 18.85	78.5 (24-118)	86.5 ± 12.91	90.5 (60-104)	88 ± 16.69	86 (71-109)	2.241	0.326	
Pre_SAnx	31.8 ± 8.57	29 (19-56)	34 ± 11.81	34 (18-68)	35.88 ± 12.92	33 (20-63)	1.172	0.556	
Intra_SAnx	29.77 ± 7.49	29 (17-46)	28.73 ± 6.71	29 (17-42)	26.75 ± 9.91	25 (17-40)	0.741	0.69	
Post_SAnx	30.36 ± 8.73	28 (18-51)	30.57 ± 9.83	28.5 (20-51)	20.75 ± 7.5	17 (17-32)	4.822	0.09	
	Gender								
	Female		Male						
	Mean ± std	Medyan (min-max)	Mean ± std	Medyan (min-max)	t		p		
Pre_SSEB	32.7 ± 13.42	32.5 (14-68)	39.91 ± 12.68	42 (16-58)	-1.657		0.102		
İntra_SSEB	44.76 ± 10.84	46 (27-82)	53.43 ± 11.16	60 (41-66)	-1.977		0.053		
Post_SSEB	45.62 ± 12.77	43 (19-75)	42.33 ± 14.12	44 (15-60)	0.704		0.484		
Pre_SAtt	79.22 ± 12.4	78 (50-109)	86.64 ± 15.52	84 (68-110)	-1.766		0.082		
Intra_SAtt	83.37 ± 12.82	84 (57-111)	85.57 ± 15.11	79 (72-107)	-0.417		0.678		
Post_SAtt	79.91 ± 17.26	81 (24-112)	91 ± 17.93	93 (67-118)	-1.773		0.081		
Pre_SAnx	33.44 ± 10.07	30.5 (19-68)	28.64 ± 7.39	29 (18-40)	1.51		0.135		
Intra_SAnx	29.31 ± 7.6	28 (17-46)	29.71 ± 6.52	29 (17-37)	-0.133		0.895		
Post_SAnx	30.28 ± 8.89	28 (17-51)	26.89 ± 10.34	24 (17-51)	1.035		0.305		

Mean ± std = Mean ± standard deviation; Median (min–max) = Median and range; t = Independent samples t-test statistic; p = Significance level ($p < .05$ considered statistically significant); r = Pearson correlation coefficient; H = Kruskal-Wallis H test statistic; N = Sample size.

Table 3 Cronbach's Alpha Values and McDonald's Omega of the Scales

Cronbach's α – McDonald's ω			
	Statistics Self - Efficacy Belief Scale (Item=14)	**Statistical Anxiety Scale (Item=17)**	**Statistics Attitude Scale (Item=28)**
	Total	Total	Total
Pre	0.965 – 0.967	0.920 – 0.922	0.897 – 0.904
Intra	0.935 – 0.936	0.908 – 0.911	0.909 – 0.919
Post	0.962 – 0.964	0.945 – 0.948	0.935 – 0.945

Pre: Prior to the biostatistics course; Intra: 8th week of the biostatistics course; Post: Final week of the biostatistics course.

Table 4 Results of model fit indexes

		Fit Measures							Test for Exact Fit		
		CMIN/DF	CFI	TLI	SRMR	RMSEA	RMSEA 90% CI Lower	RMSEA 90% CI Upper	χ^2	df	p
Statistics Self - Efficacy Belief Scale (Item=14)	**Pre**	1.68	0.956	0.942	0.043	0.096	0.064	0.125	116	69	<.001
	Intra	2.09	0.874	0.833	0.078	0.135	0.104	0.167	144	69	<.001
	Post	1.94	0.923	0.899	0.049	0.124	0.092	0.155	134	69	<.001
Statistical Anxiety Scale (Item=17)	**Pre**	2.41	0.780	0.733	0.094	0.138	0.117	0.159	270	112	<.001
	Intra	1.63	0.836	0.800	0.082	0.104	0.076	0.130	183	112	<.001
	Post	1.40	0.931	0.917	0.058	0.080	0.048	0.109	157	112	0.003
Statistics Attitude Scale (Item=28)	**Pre**	1.95	0.651	0.613	0.119	0.113	0.100	0.126	664	341	<.001
	Intra	2.16	0.639	0.600	0.140	0.140	0.126	0.154	737	341	<.001
	Post	1.94	0.766	0.741	0.134	0.123	0.109	0.137	661	341	<.001
Path Model (Pre_SAtt ⇒Pre_ SAnx ⇒Pre_SSEB)	**Pre**	9.62	1.000	1.000	0.000	0.000	0.000	0.000	28.866	3	<.001
	Intra	13.53	1.000	1.000	0.000	0.000	0.000	0.000	40.575	3	<.001
	Post	15.09	1.000	1.000	0.000	0.000	0.000	0.000	45.274	3	<.001

CMIN/DF: Chi-square/degree of freedom; RMSEA: Root mean square of approximation; SRMR: Standardised root mean square residual; CFI: Comparative fit index

Table 5 Comparison between pre, intra and post measurements of the scales

		N	Mean ± SD	Median (Min-Max)	F	p	post-hoc
Statistics Self - Efficacy Belief Scale (Item=14)	Pre	75	33.8 ± 13.48	34 (14-68)	31.00	<0.001	Pre-Intra, Pre-Post
	İntra	58	45.8 ± 11.15	46.5 (27–82)			
	Post	62	45.1 ± 12.91	43.5 (15-75)			
Statistical Anxiety Scale (Item=17)	Pre	75	80.3 ± 13.05	78 (50-110)	3.23	0.045	Pre-Intra
	İntra	58	83.6 ± 12.99	84 (57-111)			
	Post	62	81.5 ± 17.65	82 (24-118)			
Statistics Attitude Scale (Item=28)	Pre	75	32.7 ± 9.83	30 (18-68)	3.31	0.042	Pre-Intra
	İntra	58	29.4 ± 7.42	29 (17-46)			
	Post	62	29.8 ± 9.10	28 (17-51)			

F: One Way ANOVA test, p: p-value

the statistics attitude scale, significant changes were observed between pre and Intra (F=3.31, p=0.042), but no significant changes were observed between Pre and Post the course. These results emphasize that an effective statistics education can have positive effects on student achievement, especially by strengthening self-efficacy beliefs, and the importance of structured support for anxiety and attitude management (Table 5).

According to the results of Table 6, the correlations between the variables were analyzed. When we look at the non-significant correlations, we observe that

Table 6 Correlation analysis of the scales

Correlation Matrix										
		Pre SSEB	**Intra SSEB**	**Post SSEB**	**Pre SAtt**	**Intra SAtt**	**Post SAtt**	**Pre SAnx**	**Intra SAnx**	**Post SAnx**
Pre SSEB	**r**	—								
	p	—								
Intra SSEB	**r**	**0.440**	—							
	p	0.001	—							
Post SSEB	**r**	0.109	**0.372**	—						
	p	0.440	0.015	—						
Pre SAtt	**r**	**0.275**	0.245	**0.272**	—					
	p	0.017	0.083	0.051	—					
Intra SAtt	**r**	**0.287**	**0.402**	0.235	**0.618**	—				
	p	0.041	0.002	0.134	<.001	—				
Post SAtt	**r**	0.163	0.266	**0.484**	**0.441**	**0.621**	—			
	p	0.247	0.089	<.001	0.001	<.001	—			
Pre SAnx	**r**	**-0.433**	**-0.457**	-0.042	**-0.384**	**-0.438**	0.030	—		
	p	<.001	<.001	0.768	<.001	0.001	0.835	—		
Intra SAnx	**r**	**-0.406**	**-0.452**	**-0.320**	**-0.642**	**-0.594**	**-0.419**	**0.512**	—	
	p	0.003	<.001	0.039	<.001	<.001	0.006	<.001	—	
Post SAnx	**r**	-0.114	**-0.387**	**-0.368**	-0.238	**-0.299**	**-0.560**	0.193	**0.493**	—
	p	0.421	0.011	0.003	0.090	0.054	<.001	0.171	<.001	—

r: Spearman's Correlation Coefficient, p: p-value.

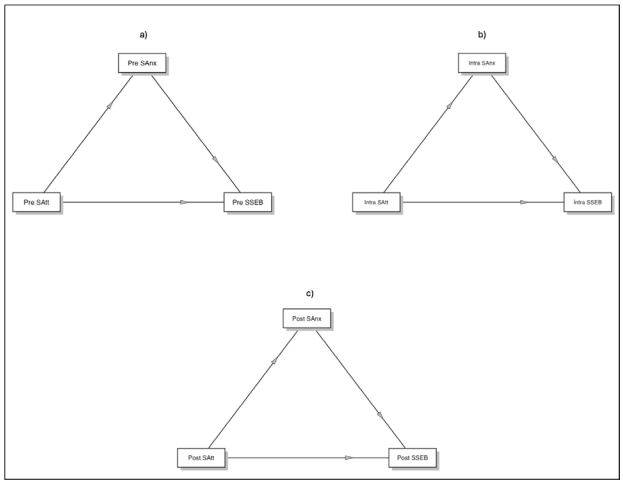

Figure 1
Path models
a): Mediation model of the prior of the biostatistics course, b): Mediation model of the 8th week of the biostatistics course, c): Mediation model of the final week of the biostatistics course.

Table 7 Mediation models results

Time	Type	Effect	Estimate	SE	95% C.I. Lower	95% C.I. Upper	β	z	p
Pre	Indirect	Pre_SAtt ⇒ Pre_SAnx ⇒Pre_ SSEB	0.152	0.0613	0.0323	0.272	0.148	2.49	0.013
	Component	Pre_SAtt ⇒ Pre_SAnx ⇒Pre_ SSEB	-0.289	0.0803	-0.4466	-0.132	-0.384	-3.6	< .001
		Pre_SAnx⇒ Pre_SSEB	-0.527	0.1533	-0.8272	-0.226	-0.384	-3.44	< .001
	Direct	Pre_SAtt ⇒ Pre_ SSEB	0.132	0.1154	-0.0942	0.358	0.128	1.14	0.253
	Total	Pre_SAtt ⇒ Pre_ SSEB	0.284	0.1154	0.0582	0.511	0.275	2.46	0.014
Intra	Indirect	Intra_SAtt ⇒ Intra_SAnx ⇒ Intra_SSEB	0.168	0.0789	0.0134	0.3226	0.196	2.13	0.033
	Component	Intra_SAtt ⇒ Intra_SAnx ⇒ Intra_SSEB	-0.34	0.0603	-0.4579	-0.2214	-0.594	-5.63	< .001
		Intra_SAnx ⇒ Intra_SSEB	-0.495	0.2149	-0.9159	-0.0734	-0.329	-2.3	0.021
	Direct	Intra_SAtt ⇒ Intra_SSEB	0.177	0.1228	-0.0638	0.4176	0.206	1.44	0.15
	Total	Intra_SAtt ⇒ Intra_SSEB	0.345	0.1041	0.1409	0.5489	0.402	3.31	< .001
Post	Indirect	Post_SAtt ⇒ Post_SAnx ⇒ Post_SSEB	0.0578	0.0555	-0.051	0.167	0.0791	1.04	0.298
	Component	Post_SAtt ⇒ Post_SAnx	-0.2888	0.0542	-0.395	-0.182	-0.5602	-5.32	< .001
		Post_SAnx ⇒ Post_SSEB	-0.2003	0.1886	-0.57	0.169	-0.1413	-1.06	0.288
	Direct	Post_SAtt ⇒ Post_SSEB	0.2956	0.0972	0.1051	0.486	0.4044	3.04	0.002
	Total	Post_SAtt ⇒ Post_SSEB	0.3535	0.0819	0.1929	0.514	0.4835	4.31	< .001

SE: Standard Error, 95% C.I. Lower: 95% Confidence Interval Lower Bound, 95% C.I. Upper: 95% Confidence Interval Upper Bound, β: Beta coefficient, Z: Z-score, p: p-value, Pre SSEB: Prior to The Statistical Self-Efficacy Belief Scale, Intra SSEB: Intra to The Statistical Self-Efficacy Belief Scale, Post SSEB: Post to The Statistical Self-Efficacy Belief Scale, Pre Satt: Prior to The Statistical Attitude Scale, Intra SAtt: Intra to The Statistical Attitude Scale, Post Satt: Post to The Statistical Attitude Scale, Pre Sanx: Prior to The Statistical Anxiety Scale, Intra SAnx: Intra to The Statistical Anxiety Scale, Post Sanx: Post to The Statistical Anxiety Scale.

there is no statistically significant relationship between Intra SSEB and Pre SAtt (p=0.297), Pre SSEB and Post SAtt (p=0.372), and Pre SAtt and Post SAtt (p = 0.913). On the other hand, the variable pair with the highest significance among the relationships was Intra SAnx and Intra SAtt (r=-0.618, p<0.01), showing a strong inverse correlation between these two variables. The relationship with the lowest significance was found between Post SSEB and Intra SSEB (r=0.203, p=0.01), and this relationship was found to

be a weak but significant correlation in the positive direction (Table 6).

The aim of the study was to examine the mediating role of statistical anxiety in the relationship between statistical attitudes and statistical self-efficacy beliefs of students taking biostatistics courses. Therefore, a theoretical framework was developed as shown in Figure 1.

According to Table 7, as a result of the bootstrapping analysis performed with the data obtained before the course, it is understood that the indirect effects in the model are statistically significant. Statistical anxiety mediates the relationship between statistical attitude and statistical self-efficacy beliefs (p=0.013). This effect was estimated as 0.152, indicating that statistical attitude has a significant indirect effect on Statistical self-efficacy beliefs through statistical anxiety. Looking at the percentages of explanatory power within the model, there is a significant negative relationship between statistical attitude and statistical anxiety (β = -0.384, p < 0.001). Similarly, there is a significant negative relationship between statistical anxiety and statistical self-efficacy beliefs (β = -0.384, p < 0.001). The direct effect of statistical attitude on statistical self-efficacy beliefs is not statistically significant (β = 0.128, p = 0.253), but the total effect is significant (β = 0.275, p = 0.014). These findings suggest that the total effect of statistical attitude on statistical self-efficacy beliefs is strengthened through the path mediated by statistical anxiety. There are no differential mediation effects across the other conditions (Table 7).

As a result of the bootstrapping analysis conducted in the 8th week of the course, it is seen that the indirect effects in the model are statistically significant. Intra-anxiety scale has a mediating effect on the relationship between statistical attitude and statistical self-efficacy beliefs (p=0.033), and this effect was estimated as 0.168. This shows that statistical attitude has a significant indirect effect on statistical self-efficacy beliefs through statistical anxiety. When the explanatory percentages in the model are analyzed, it is seen that there is a statistically significant negative relationship between statistical attitude and statistical anxiety (β = -0.594, p < 0.001). There is also a significant negative relationship between statistical anxiety and statistical self-efficacy beliefs (β=-0.329, p=0.021). The direct effect of statistical attitude on statistical self-efficacy beliefs was not statistically significant (β=0.206, p=0.15), but the total effect was significant (β=0.402, p<0.001). These findings suggest that the total effect of statistical attitude on statistical self-efficacy beliefs is strengthened through the mediated path of statistical anxiety. There were no different mediation effects in the relationships between the other variables (Table 7).

As a result of the bootstrapping analysis conducted with the data obtained after the course, the statistical significance levels of the indirect effects in the model were evaluated. The mediating effect of statistical anxiety in the relationship between statistical attitude and statistical self-efficacy beliefs was not statistically significant (p=0.298), and the effect was estimated as 0.0578. This shows that the mediating role of statistical anxiety is weak. When the explanatory percentages in the model are analyzed, it is seen that there is a significant negative relationship between statistical attitude and statistical anxiety (β=-0.5602, p<0.001), but there is no significant relationship between statistical anxiety and statistical self-efficacy beliefs (β=-0.1413, p = 0.288). In contrast, the direct effect of statistical attitude on statistical self-efficacy beliefs was statistically significant (β=0.4044, p = 0.002), and the total effect was also significant (β=0.4835, p<0.001) (Table 7). These findings indicate that statistical anxiety does not mediate, but the direct and total effects of statistical attitude on statistical self-efficacy beliefs are significant. There are no mediating effects among other variables.

Discussion

The findings of the study comprehensively reveal how statistics education affects students' self-efficacy beliefs, anxiety levels, and attitudes. The high Cronbach's Alpha values obtained indicate that the internal consistency of the scales used is robust and provides reliable measurements across participants. The pre-course (0.965), intra-course (0.935), and post-course (0.962) values for the Statistics Self-Efficacy Scale and the pre-course (0.920), intra-course (0.908), and post-course (0.945) values for the Statistics Anxiety Scale emphasize the reliability both between the scales and over time. The Statistics Attitude Scale shows a similar reliability with pre-course (0.897), intra-course (0.909), and post-course (0.935) values.

In terms of self-efficacy, a significant increase was observed in students' self-efficacy beliefs during the course (F=31.00, p<0.001). This indicates that the information and experiences in the teaching process strengthened students' beliefs about their capacities. In terms of statistics anxiety, significant differences between pre- and intra-course measurements (F=3.23, p=0.045) indicate that an effective teaching strategy was effective in reducing anxiety. Attitudes

towards statistics changed between pre- and intra-course measurements (F=3.31, p=0.042), but there were no significant changes between intra- and post-course measurements, indicating the limits of the initial attitudinal improvements.

Mediation effects analysis reveals that statistical anxiety plays an important role in the relationship between statistical attitude and statistical self-efficacy beliefs. In the pre-course analysis, the mediating effect of statistical anxiety was found to be significant (p=0.013, effect=0.152); in the analysis at the 8th week of the course, this effect became more evident (p=0.033, effect=0.168). After the course, this mediating effect was not statistically significant (p=0.298). This suggests that anxiety should be managed effectively during the training.

As a result, while statistics education positively affects students' self-efficacy beliefs (β=0.275, p = 0.014), it is understood that anxiety levels need to be managed to sustain this effect. It is recommended that anxiety management and attitude development strategies be used together in the training process because the overall effect of statistical attitude on statistical self-efficacy beliefs is strengthened through the mediation of anxiety. Using these findings, educators can make instructional strategies more effective by further integrating student-centered approaches and psychological support systems. Such integrated approaches may have more positive outcomes on student achievement and educational effectiveness.

At the end of our study, it was found that statistical anxiety played a mediating role in the model between students' attitudes towards statistics and their statistical self-efficacy beliefs at the beginning and middle of the course, but did not play a mediating role in the model at the end of the course. Our result shows that statistical anxiety in students decreases over time, and students start to learn statistics. Similar results were found in the literature. While Akyüz and Topcu (2022) stated that attitude positively affected self-efficacy, Bourne et al. (2024) and Hernandez de la Hera et al. (2023) showed that statistical anxiety was negatively related to self-efficacy (32, 33, 34). Peiro-Signes et al. (2021) emphasised the reducing effect of self-confidence on anxiety (35). Amirgholami et al. (2023) supported the complexity of these relationships by addressing the mediating role of self-efficacy (36).

Limitation

Even though the study's conclusions offer significant insights, it is crucial to acknowledge several limitations. The sample used in this study was selected from a single university population, which presents limitations. As a result, care should be taken when interpreting the findings' generalizability.

It should be prepared without subheadings.

Conflict of Interest Statement

All authors have no conflicts of interest to declare.

Ethical Approval

Ethical approval was obtained from "Atatürk University Faculty of Medicine Clinical Research Ethics Committee" (date: 31/103/2022; number: B.30.2.ATA.0.01.00/244).

Consent to Participate and Publish

Written informed consent to participate and publish was obtained from all individual participants.

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Availability of Data and Materials

Data available on request from the authors.

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The authors declare that they have not used any type of generative artificial intelligence for the writing of this manuscript, nor for the creation of images, graphics,tables, or their corresponding captions.

Authors Contributions

KK: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing- original draft.

SG: Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation.

DÖE: Investigation; Resources; Supervision; Validation; Writing-original draft.

ŞB: Formal analysis; Investigation; Visualization; Writing-original draft.

DBD: Funding acquisition; Resources; Supervision; Writing-review & editing.

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ORIGINAL RESEARCH

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Evaluation of Sexual Health Literacy in Midwifery Students

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Abstract

Objective

Midwifery students need to have sufficient knowledge and equipment about sexual health to provide accurate guidance to individuals during lifelong service provision. Therefore, our study aims to evaluate the sexual health literacy levels of midwifery students.

Materials and Methods

This study was designed as a descriptive study. Students (n=282) who were attending the midwifery department of a public university between May and June 2024 and who volunteered to participate in the study were included in the study. A form was created using Google Forms, and demographic and descriptive characteristics of the participants, such as age, gender, class, family type, and questions about sexual health, were collected with this form. The Sexual Health Literacy Scale was also sent to the participants using Google Forms.

Results

In the study, it was determined that the mean total score of the Sexual Health Literacy Scale of midwifery students was 48.78±13.05. The mean score of Sexual Knowledge sub-dimension of the scale was 36.86±11.23 and the mean score of Sexual Attitude sub-dimension was 11.91±4.62. Accordingly, it was determined that the students had a moderate attitude towards sexual health literacy, and the factors affecting it were income status, housing status, and family approach. In addition, knowing the concept of sexual health literacy, finding the level of knowledge sufficient, and being able to provide sexual and reproductive health education affect the mean total score of the students.

Conclusion

In line with these findings, it should be aimed to strengthen students' knowledge and attitudes in this field by including more educational content that will increase sexual health literacy in midwifery programs. It is recommended that interactive methods, such as simulation training and case discussions, should be added to the programs in addition to theoretical course hours in order to increase practical applications and experiences in sexual health education and to equip students with ethical dilemmas, cultural sensitivity, and evidence-based practices in the cases they encounter.

Keywords: Midwifery, Student, Sexual Health Literacy

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Introduction

Sexual health is an important health field that directly affects the quality of life of individuals. The World Health Organisation (WHO) defines sexual health as 'the positive enrichment and strengthening of personality, communication and love by addressing sexual life as a whole in terms of physical, spiritual, mental and social aspects (1). Sexual health is possible with a respectful and positive approach towards sexuality, free from violence and discrimination (2). Negative sexual health outcomes such as unwanted pregnancies and sexually transmitted infections may occur via risky sexual behaviours, which are more frequently encountered in young people and university students (3,4). University women aged 20-24 years have the highest rate of unwanted pregnancy among all age groups, with a rate of 81 per thousand (5). In addition, according to the Centers for Disease Control and Prevention (CDC), approximately half of women in the 15-24 age group are reported to contract STIs each year. It is known that 19% of all new Human Immunodeficiency Virus (HIV) diagnoses in 2021 will be made by young people aged 13-24 (6). Bakır and Beji's study revealed that 64% of university students did not receive sexual health education (7). In Erenoğlu and Bayraktar's study, it was reported that 33.4% of the students had not received any sexual health education before and that the lack of information resulting from this reason could lead to risky sexual behaviours (8). Lack of knowledge about risky sexual behaviours is related to the improvement of sexual function by training sexual skills (5). The protection and continuity of health can be gained by accessing health information and eliminating the lack of information and therefore, health literacy is an important issue (9,10). The ability of individuals to access correct information about sexual health and to use this information correctly constitutes the basis of sexual health literacy. Health literacy is the ability of individuals to access health information, understand, evaluate, and use this information for a healthy life. It also includes the ability to think critically about personal and social health needs and to communicate effectively (11). Sexual health literacy provides understanding and application of sexual health information, reduces the risk of Sexually Transmitted Infections (STIs), and also provides various benefits beyond health (12). Health literacy is increasing among young people. The reason for this is that if health literacy is integrated into the early stages of life, the possibility of individuals adopting healthy lifestyles in later ages can be maximised (13).

The midwifery profession has a critical role in the provision of sexual and reproductive health services to the community. In addition, since they take an active role in the protection and development of women's health, it is important that they follow the current literature on sexual health (14). It is necessary to determine the attitudes, values, beliefs, and knowledge level of midwifery students who are professional midwife candidates about sexual and reproductive health during their undergraduate education (15). Although the projects carried out by the Ministry of Health and non-governmental organizations aiming to improve sexual and reproductive health and increase the utilization of services are positive steps, the need to expand and strengthen the scope of sexual health courses in the undergraduate education of professional groups that will directly serve in this field, such as midwifery, and to support them with practical training comes to the fore. It is of vital importance for midwives to have sufficient knowledge and equipment about sexual health to provide correct guidance to the individuals they will encounter in their professional lives. However, their level of knowledge and literacy skills in this field may not always be at the desired level. This situation creates the need to evaluate the current status of sexual health literacy among midwifery students. This study aims to evaluate the sexual health literacy levels of midwifery students. In this way, suggestions for the education processes of midwifery students can be developed, and they can be supported to contribute to society as individuals who are more aware of sexual health when they graduate.

Material and Method

This descriptive and cross-sectional study was conducted to evaluate the level of sexual health literacy of midwifery students. Ethics committee approval of the study was obtained from Suleyman Demirel University Health Sciences Ethics Committee on 29-4-2024 with decision number 75/6. The purpose of the study was explained to the students in the sample group in detail, and they were assured of the confidentiality of the information to be obtained from them. Participants were asked to participate in the study voluntarily. The students participating in the study were informed about the purpose of the study, the process, and the questionnaire, and their informed consent was obtained. For the use of the scale, permission was obtained via e-mail from Üstgörül (2022), who developed the scale. The population of the study consisted of 323 midwifery students continuing their undergraduate education in the midwifery department of a public university. The study was completed with 282 midwifery students who volunteered to participate in the study. Socio-demographic data form and Sexual Health Literacy Scale were used during data

collection. Socio-demographic data form was created by the researchers by reviewing the literature and consisted of 18 questions (3-8,15). The Sexual Health Literacy Scale was developed by Üstgörül in 2022. The scale aims to measure the sexual health literacy of individuals aged 18 years and over. This five-point Likert-type scale consists of 17 items. The scale has two sub-dimensions as 'Sexual Knowledge' and 'Sexual Attitude'.

There are 12 items in the sexual knowledge sub-dimension, and the lowest score is 12 points, and the highest score is 60 points. The sexual attitude subscale consists of five items and is reverse-coded. The lowest score to be obtained from this section is 5, and the highest score is 25; and high scores are considered to have a negative attitude towards sexual health knowledge.

The items in the scale are graded as strongly disagree (1), strongly agree (5). The increase in the sub-dimensions and general scores of the Sexual Health Literacy Scale indicates that the sexual health literacy of individuals is high. The Cronbach's Alpha value of the scale was found to be 0.88 (16). The data were sent to the students with an online form prepared on Google Forms between May and June 2024, and they were asked to fill it out. Statistical analyses of the study were performed with SPSS 27.0 (IBM Inc., Armonk, NY, USA). Descriptive statistics were presented as frequency (percentage ratio) for categorical data and mean±SD for numerical measurements. The conformity of the scale score and the scores of the sub-dimensions to normal distribution was checked by the Kolmogorov-Smirnov test. One-way Analysis of Variance was used to compare the scale scores according to demographic characteristics. Tukey HSD post-hoc test was preferred for significant results. $P<0.05$ was considered statistically significant.

Results

The mean age of the research group was 21.05±1.86. The distribution of sociodemographic characteristics of the students is shown in Table 1. Of the midwifery students, 67.0% were graduates of Anatolian High School, and 99.3% were single. 35.8% of the students spent most of their lives in the district, and 81.9% of them had a nuclear family. 63.8% of the students stayed in dormitories and 24.8% in apartments. 41.8% of the midwifery students provide their income with family support. Of the participants, 64.9% of the mothers and 46.1% of the fathers were primary school graduates.

The distribution of midwifery students' knowledge about sexual health is shown in Table 2. 72% of midwifery students stated that information about sexual health was not discussed in the family. 98.9% of the students found sexual health education necessary, 81.9% had not heard the concept of sexual health literacy before, and 51.1% found the level of sexual and reproductive health knowledge sufficient. It is seen that 26.3% of the participants received information about sexual and reproductive health from health personnel, 36.2% of them found health personnel to be the safest source of information, and 35.2% of them found health personnel to be the first source they would apply to when they had problems. 66% of the students state that they are not qualified to provide sexual health education, and 66.7% of them state that they are not qualified to provide reproductive health education.

Table 3 shows the distribution of the mean scores of the total and sub-dimensions of the sexual health literacy scale. The total mean score of the Sexual Health Literacy Scale was found to be 54.94±11.16, and the mean score of the Sexual Knowledge sub-dimension of the scale was 36.86±11.23, and the mean score of the Sexual Attitude sub-dimension was 18.08±4.62.

The distribution and comparison of the mean scores of the scale total and sub-dimensions of the students according to their socio-demographic characteristics are given in Table 4. There is a statistically significant difference between the income status of the participants and the sexual health literacy scale total score and sexual knowledge sub-dimension score ($p=0.023$; $p=0.017$). Students living in dormitories have higher sexual health literacy levels than students living with their families. The sexual health literacy level and sexual knowledge level of students who were employed were found to be lower than those who received scholarships, loans, and family support. There is no statistically significant difference between the level of sexual health literacy, sexual knowledge level, and sexual attitude level with the school graduated from, marital status, place of residence, family type, mother's education level, and father's education level. Table 5 shows the distribution and comparison of the mean scores of the scale total and sub-dimensions of the students according to their knowledge about sexual health. A statistically significant difference was found between the approach of the students' families to issues related to sexual health and the level of sexual health literacy ($p=0.010$) and sexual knowledge ($p=0.022$). Sexual health literacy level and sexual knowledge level were found to be higher in the families of the students whose families shared information than in the families

Table 1 Distribution of descriptive characteristics of students

Characteristics	n	%
Class		
Class 1	79	28.0
Class 2	63	22.3
Class 3	58	20.6
Class 4	82	29.1
Graduated school		
Anatolian High School	189	67.0
Vocational High School	21	7.4
Private High School	20	7.1
Other	52	18.4
Marital status		
Married	2	0.7
Single	280	99.3
Where do you spend most of your life?		
Metropolitan	76	27.0
City	74	26.2
District	101	35.8
Village	31	11.0
Family type		
Core Family	231	81.9
Extended Family	42	14.9
Fragmented family	9	3.2
Accommodation Status		
Dormitory	180	63.8
Home	26	9.2
Apart	70	24.8
Family	6	2.1
Income status		
I get a Scholarship	101	35.8
Getting a loan	56	19.9
Family support	118	41.8
I work in any job	7	2.5
Education level of your mother		
Primary education	183	64.9
High School	68	24.1
University and above	23	8.2
Literate	8	2.8
Education level of your father		
Primary education	130	46.1
High School	101	35.8
University and above	45	16.0
Literate	6	2.1

Table 2 Distribution of midwifery students' information about sexual health

Sexual health-related characteristics	n	%
What is your family's approach to issues related to sexual health?		
Information about sexuality is given and discussed within the family.	67	23.8
Information about sexuality is not discussed within the family.	203	72.0
It is forbidden to talk about sexuality within the family.	3	1.1
When a question is asked about sexuality within the family, topics are closed.	9	3.2
Do you think sexual health education is necessary?		
Yes	279	98.9
No	3	1.1
Have you heard of the concept of sexual health literacy before?		
Yes	51	18.1
No	231	81.9
Do you find your level of knowledge about sexual and reproductive health sufficient?		
Yes	144	51.1
No	138	48.9
Where or from whom did you get information about sexual and reproductive health?		
Family	37	13.1
Social environment	71	25.2
Internet-based access resources	64	22.7
Written resources	36	12.8
Healthcare personnel	74	26.3
Where do you think you can get the safest information about sexual and reproductive health?		
Family	21	7.5
Social environment	6	82.1
Internet-based access resources	25	8.9
Written resources	83	29.4
Healthcare personnel	147	52.1
What is the first source you will turn to when you encounter a problem related to sexual health?		
Family	53	18.8
Social environment	13	4.6
Internet-based access resources	94	33.3
Written resources	23	8.2
Healthcare personnel	99	35.2
Do you consider yourself qualified to provide sexual health education?		
Yes	96	34.0
No	186	66.0
Do you consider yourself qualified to provide reproductive health education?		
Yes	94	33.3
No	188	66.7

Table 3 Distribution of the mean scores of the sexual health literacy scale and its subscales

Sexual Health Literacy Scale	Minimum	Maximum	Mean ±SD
Sexual Knowledge	12	66	36.86 ±11.23
Sexual Attitude	5	25	18.08 ± 4.62
Total Scale	17	85	48.78 ± 13.05

of the students in which sexuality-related topics were covered. A statistically significant difference was found between the level of sexual knowledge (p=0.043) and whether the students had heard the concept of literacy before. The sexual health literacy levels of the students who had heard the concept of literacy before were higher. A statistically significant difference was found between the level of sexual and reproductive health literacy (p=0.014) and sexual attitude level (p=0.009). The sexual health literacy level and sexual attitude level of those who found the sexual and reproductive health knowledge level sufficient were higher than those who did not. A statistically significant difference was found between the level of competence to provide sexual health education and sexual health literacy level (p=0.001), sexual knowledge level (p=0.001), and sexual attitude level (p=0.031). The sexual health literacy level, sexual knowledge level, and sexual attitude level of the students who considered themselves competent to provide sexual education were higher than the students who did not. A statistically significant difference was found between the level of competence to provide reproductive health education and the level of sexual health literacy (p=0.001) and sexual knowledge (p=0.001). The sexual health literacy level and sexual knowledge level of the students who considered themselves competent to provide reproductive health education were higher than the students who did not consider themselves competent to provide reproductive health education. No significant difference was found between the level of sexual health literacy, sexual knowledge, and sexual attitudes, and the level of sexual health literacy, sexual knowledge and sexual attitudes with the status of considering sexual education necessary, the source of sexual and reproductive health information, the safe source and the first applied source.

Discussion

Sexual health problems can be observed in all age groups, especially in young women (17). Considering that sexual life starts in university years. It is extremely important to instill sexual health literacy in young women (18,19). This concept is more important, especially for midwifery students who are candidates for health personnel and who are closely interested in women's health (20).

In our study, the mean score of 'Sexual Health Literacy' was found to be 48.78±13.05, the mean score of the 'Sexual Knowledge' sub-dimension of the scale was 36.86±11.23, and the mean score of the 'Sexual Attitude' sub-dimension was 11.91±4.62.

In Doğan and Tuğut's study, the mean score of 'Sexual Health Literacy' was 49.27±11.20, the mean score of 'Sexual Knowledge' sub-dimension was 34.87±8.76, and the mean score of 'Sexual Attitude' sub-dimension was 14.40±5.26 (21). In Doğan's study, the mean total score of the scale was found to be 54.37±9.96, the mean score of the 'Sexual knowledge' sub-dimension was 32.22±9.00, and the mean score of the 'Sexual attitude' sub-dimension was 17.35±4.31 (22). In the study of Altınayak and Özkan, it was concluded that the mean total score of the scale was 50.66±8.41, the mean score of the 'Sexual Knowledge' sub-dimension was 34.50±8.00, and the mean score of the 'Sexual Attitude' sub-dimension was 16.16±3.84 (23). The studies in the literature are similar to our study.

In this study, it was found that the most common source used by midwifery students to access sexual health information was health personnel (26.3%). This finding partially contradicts the result of Vamos et al. (24), who reported that the internet was the primary source of information for university students. However, the main reason for this difference may be due to the significant difference in the target populations of the two studies. Vamos et al. (24) conducted their study on all university students. The interdisciplinary differences of the students in this group may also diversify the methods of accessing sexual health information. In particular, students studying in non-health fields may have limited awareness or shyness in accessing expert support on the subject, which may increase their

Table 4 Distribution and comparison of the mean scores of the total and sub-dimensions of the scale according to the socio-demographic characteristics of the students

Property	Sexual Knowledge	Sexual Attitude	Total Scale
	Mean ±SD	Mean ±SD	Mean ±SD
Graduated school			
Anatolian High School	36.57±11.57	12.04±4.90	48.61±13.62
Vocational High School	35.61±13.85	10.76±3.30	46.38±15.36
Private High School	35.65±10.21	11.15±3.88	46.80±11.60
Other	38.88±9.05	12.23±4.29	51.11±10.18
T;P	0.514;0.765	0.743;0.527	0.953;0.415
Marital status			
Married	43.00±9.89	10.50±6.36	53.50±3.53
Single	36.81±11.24	11.92±4.62	48.74±13.09
T;P	0.600;0.439	0.189;0.664	0.262;0.609
Where do you spend most of your life?			
Metropolitan	38.89±11.43	12.01±4.83	50.90±13.55
City	35.47±10.57	12.32±4.86	47.79±12.50
District	36.45±12.06	11.41±4.49	47.87±13.64
Village	36.51±9.08	12.35±3.96	48.87±10.97
T;P	1.264;0.287	0.687;0.561	0.814;0.487
Family type			
Core Family	37.00±11.57	11.90±4.63	48.91±13.45
Extended Family	35.04±9.99	11.92±4.78	46.97±11.61
Fragmented family	42.12±4.91	12.50±4.40	54.62±7.02
T;P	0.963;0.411	0.099;0.960	0.814;0.487
Accommodation Status			
Dormitory	38.07±10.89	11.97±4.68	50.05±12.34
Home	34.19±10.16	11.42±4.53	45.61±12.17
Apart	35.27±12.26	11.92±4.53	47.20±14.75
Family	30.50±8.73	12.16±5.49	42.66±13.88
T;P	2.334;0.074	0.412;0.745	1.878;0.133
Income status			
I get a Scholarship	38.50±10.75 [a]	11.94±4.71	50.44±12.27[a]
Getting a loan	36.35±11.58	12.01±4.63	48.37±13.44
Family support	36.38±11.22	11.96±4.61	48.35±13.21
I work in any job	25.14±9.13	10±3.82	35.14±11.93[a]
T;P	3.454;0.017*	0.412;0.745	3.226;0.023*
Education level of your mother			
Primary education	36.73±11.39	11.69±4.63	48.42±12.98
High School	37.61±11.36	12.63±4.77	50.25±13.90
University and above	35.73±10.99	11.52±4.29	47.26±13.19
Literate	36.62±8.07	12.12±4.29	48.75±6.08
T;P	0.187;0.905	0.742;0.528	0.433;0.729
Education level of your father			
Primary education	36.30±10.70	12.30±4.38	48.60±12.23
High School	37.37±12.06	11.60±4.85	48.98±13.79
University and above	37.71±11.02	11.60±4.89	49.31±13.97
Literate	33.83±11.26	11.33±4.58	45.16±13.22
T;P	0.404;0.750	0.550;0.648	0.192;0.902

*: Significant at 0.05 level according to One-Way ANOVA test. a: The difference between categories with exponential letters is significant according to Tukey HSD post-hoc test, Test values are given as F statistics.

Table 5 Distribution and comparison of the mean scores of the total and sub-dimensions of the scale according to the students' knowledge about sexual health

Property	Sexual Knowledge	Sexual Attitude	Total Scale
	Mean ±SD	Mean ±SD	Mean ±SD
What is your family's approach to issues related to sexual health?			
Information about sexuality is given and discussed within the family.	40.38±11.11	18.55±4.97	59.94±10.75
Information about sexuality is not discussed within the family.	35.80±11.21	17.88±4.55	53.68±11.11
It is forbidden to talk about sexuality within the family.	36.66±2.08	18.66±5.50	55.33±4.50
When a question is asked about sexuality within the family. topics are closed.	34.55±9.97	18.77±3.73	53.33±11.12
T;P	2.607;0.022*	0.308;0.511	2.392;0.010*
Do you think sexual health education is necessary?			
Yes	39.91±11.25	18.06±4.65	54.98±11.18
No	31.66±10.01	19.33±1.15	51.00±10.44
T;P	0.862;0.389	0.605;0.669	0.605;0.545
Have you heard of the concept of sexual health literacy before?			
Yes	39.27±12.48	18.37±5.23	57.64±12.28
No	36.32±10.89	18.01±4.49	54.34±10.84
T;P	2.021;0.043*	0.726;0.468	1.865;0.062
Do you find your level of knowledge about sexual and reproductive health sufficient?			
Yes	37.74±12.33	18.71±4.73	56.45±11.81
No	35.94±9.91	17.42±4.44	53.36±10.25
T;P	1.865;0.062	2.624;0.009*	2.452;0.014*
Where or from whom did you get information about sexual and reproductive health?			
Family	35.48±11.06	17.00±4.85	52.48±9.39
Social environment	35.32±11.03	17.84±4.55	53.16±11.76
Internet-based access resources	37.20±10.85	17.95±4.40	55.15±10.65
Written resources	37.83±12.83	18.55±5.11	56.38±11.63
Healthcare personnel	38.25±11.06	18.72±4.52	56.98±11.38
T;P	3.061;0.398	0.179;0.382	2.263;0.180
Where do you think you can get the safest information about sexual and reproductive health?			
Family	34.23±11.73	16.61±4.67	50.85±11.19
Social environment	34.83±13.39	19.00±4.33	53.83±15.02
Internet-based access resources	35.48±11.12	17.68±4.46	53.16±8.96
Written resources	38.27±11.64	18.53±4.76	56.80±12.41
Healthcare personnel	36.75±10.89	18.06±4.58	54.82±10.50
T;P	3.640;0.326	0.750;0.441	2.631;0.106
What is the first source you will turn to when you encounter a problem related to sexual health?			
Family	33.75±10.70	18.18±4.35	51.94±10.68
Social environment	34.53±13.76	19.92±4.46	54.46±13.26
Internet-based access resources	37.90±10.69	17.78±4.83	55.69±10.77
Written resources	34.60±13.17	18.91±5.02	53.52±11.81
Healthcare personnel	38.36±10.95	17.86±4.50	56.23±11.24
T;P	2.206;0.084	0.565;0.468	3.964;0.202
Do you consider yourself qualified to provide sexual health education?			
Yes	39.43±13.34	18.73±5.22	58.17±12.75
No	35.53±9.74	17.74±4.26	53.27±9.88
T;P	3.616;0.001*	0.158;0.031*	3.570;0.001*
Do you consider yourself qualified to provide reproductive health education?			
Yes	39.67±13.33	18.25±5.48	57.92±12.58
No	35.45±9.76	17.99±4.14	53.45±10.09
T;P	4.082;0.001*	0.001;0.275	3.351;0.001*

*: Significant at 0.05 level according to One-Way ANOVA test

tendency towards anonymous and accessible sources such as the internet. In contrast, midwifery students have opportunities for direct interaction with health personnel in clinical settings due to their education in the field of health. Their academic curriculum may cover sexual health topics, and they tend to obtain this information from professional authorities. In their search for reliable sources, they may prefer expert opinion over questionable content on the internet. In our study, it was concluded that marital status did not affect the sexual health literacy levels of students. Studies in the literature show that the sexual health literacy levels of single students are lower (25,26). In our study, it was concluded that the income status of the students affected the level of sexual health literacy. Similar results are seen in studies in the literature. It is thought that income status and the sources of income can also affect the level of sexual health literacy by affecting situations such as social and cultural factors, family support, and communication. (23,25). It is believed that income level and the sources of income can influence social and cultural factors, family support, and communication, which in turn can also affect the level of sexual health literacy. In our study, it was concluded that the educational level of the mother and father did not affect the level of sexual health literacy in students. The findings of our study show that there is no significant relationship between parents' level of education and students' sexual health literacy. This suggests that sexual health literacy is mostly influenced by factors such as family cultural values, attitudes towards sexual health, and social norms. In light of these findings, it appears that interventions to increase sexual health literacy should be aimed at improving families' approaches to sexual health issues and communication skills rather than focusing only on the level of formal education. Similar results were also observed in Doğan's study (22). In some studies, it was reported that parental education level affected the level of sexual knowledge and sexual health literacy in students (27,28). The findings of our study reveal that dormitory students have high levels of sexual health literacy. When the main reasons underlying this situation are examined, it is seen that the socialization opportunities offered by the dormitory environment play a critical role. Students living in dormitories are in a wider social network and experience a more intense information-sharing process with their peers. This process creates an environment that facilitates obtaining information on sensitive issues such as sexual health. In addition, health education and information seminars organized in many dormitories are also thought to have an impact on this result. In these seminars, topics such as sexual health, prevention methods, and sexual rights are addressed, which increases students' level of knowledge in this area.

The findings of our study show that demographic factors such as the type of high school graduated from, place of residence, and family type did not make a statistically significant difference in sexual health literacy, knowledge level, and attitudes. These results can be explained by the fact that the current sexual education programs in our country remain at the basic level and the curricula exhibit a standard approach. The fact that there were no significant differences in the content and scope of sexual health education between high school types may have led to the limited effect of this variable. In today's digital age, the internet and social media platforms facilitate access to sexual health information, largely eliminating the traditional barriers of geographical location in obtaining information. This can be considered an important factor preventing a significant difference in sexual health literacy between urban and rural areas. Our findings on family structure, on the other hand, suggest that sexual health literacy is more related to qualitative factors such as the quality of communication within the family and parents' approach to the issue. It is seen that the attitudes and behaviors of family members towards discussing sexual health issues are more determinant than structural characteristics such as a traditional extended family or a nuclear family. In our study, sexual health literacy and sexual knowledge levels of those who talked about sexual health issues in their families were found to be higher than those who did not. Similar results were found in the study by Doğan and Tuğut (21). The reason for this is that parents' communication and relationships with their children have a significant effect on sexual health literacy (29). Environments where sexual health issues can be openly discussed within the family are a critical factor that facilitates children's acquisition of healthy information on these issues. When this communication process is not limited to the transfer of biological information, but also includes issues such as social values, ethical principles, and personal boundaries, it helps young people to form their sexual identities more healthily. The expansion of sexual health education within the family will have positive results not only at the individual level but also at the societal level. This approach will contribute to raising individuals with high sexual health literacy, who are at peace with themselves and their environment, and who can make healthy decisions. Considering the pioneering role of the midwifery profession in public health, equipping midwife candidates in this field should be considered as a strategic investment in terms of public health. In our study, it was observed

that the sexual health literacy and sexual attitudes of the students who considered their level of knowledge about sexual health sufficient were high. Similarly, in the study of Yeşil and Apak, it was reported that the sexual health literacy and sexual attitudes of students with an adequate sexual health knowledge level were high (26). Since the population of our study was midwifery students. The theoretical and practical courses they took may suggest that they are adequate in terms of their level of knowledge. In our study, the mean scores of the students who had previously heard the concept of sexual health literacy were found to be higher. Yeşil and Apak's study also shows similar results (26). Future midwives' knowledge of the concept of sexual health literacy has an important effect on both their individual health and public health (20).

In this study, the sexual health literacy level of the students who considered themselves competent to provide education on sexual and reproductive health was found to be high. The midwifery profession has an important role in providing sexual and reproductive health services. In this case, it has been reported that midwives and midwife candidates should increase their sexual health literacy levels and provide training on sexual and reproductive health (30,31). The results of our study revealed that there was no statistically significant relationship between students' perception of sexual education as necessary, the sources from which they obtained information, and their level of trust in these sources, and their sexual health literacy, knowledge level, and attitudes. This finding indicates that even information sources perceived as reliable may not always provide accurate and sufficient information. As a matter of fact, this discrepancy between the reliability of the sources and the accuracy of the information they provide is an important factor limiting the development of sexual health literacy. In terms of the effectiveness of sexual health education, the fact that the content of education remains at the basic level and is limited to superficial information prevents students from gaining in-depth knowledge on the subject. This situation suggests that the quality and scope of education are determinants of sexual health literacy rather than the presence of education. Especially in a complex and multidimensional subject such as sexual health, it seems inevitable that training in which only basic information is provided will be insufficient. The impact of social and cultural factors should not be ignored. Sociocultural barriers in society may limit the development of sexual health literacy, regardless of the quality of educational content.

The Limitations of the Study

Since the study was conducted in a single center and included midwifery students at a specific university, its findings on sexual health literacy cannot be generalized to all midwifery students in our country. The data used in the study were collected through a questionnaire, and their attitudes towards sexual health literacy are limited to the scope of the scale used. The sample selection in this study was based on “volunteerism”, which may cause selection bias. Furthermore, students' sexual health knowledge and attitudes may be shaped not only by the education they receive but also by their cultural and social environment. The influence of these factors may not have been adequately considered in this study.

Conclusion

Increasing sexual health literacy in midwifery students is of great importance for both individual and social welfare. Since midwives focus on women's health and obstetric services, increasing the level of knowledge and awareness of students about sexual health contributes to the development of healthy individuals and communities. In conclusion, strengthening sexual health literacy in midwifery students will increase their professional skills, enable them to provide a more qualified and effective health service, and improve public health in the long term.

Having sufficient knowledge about sexual health is of great importance for midwifery students to provide effective guidance to individuals in their professional lives. The data obtained showed that there were deficiencies, especially in the sexual knowledge levels of the students, and this situation indicates that measures should be taken to increase sexual health literacy in educational processes.

According to the findings of the study, the most important factors affecting the sexual health literacy levels of students are income level, housing status, and family approach. It was determined that the income level and housing status of the students positively affected their sexual health literacy.

It was found that students' knowledge of this concept and their perception of themselves as competent in this field made a great contribution to the increase in sexual health literacy scores. In addition, it was determined that their competence to provide sexual and reproductive health education also positively affected their sexual health literacy scores. This shows that midwifery students' ability to provide education on these issues in their future professional lives can also improve their sexual health literacy.

In line with these findings, midwifery programmes should include more training content that will increase sexual health literacy. It should aim to strengthen the knowledge and attitudes of students in this field by providing more information about sexual and reproductive health.

Sexual health education plays a critical role in helping students develop informed and healthy sexual behaviors. In this context, the most effective intervention method is considered to be age-appropriate and gradual sexual health education integrated into the standard curriculum. Such systematic education from an early age can contribute to the prevention of risky behaviors by ensuring that students acquire the right information promptly. In today's digital age, diversification of education methods is of great importance. Online learning platforms provide students with easy access to sexual health information and offer interactive learning experiences. These platforms can adapt to students' learning speeds and offer the opportunity to reach wider audiences by overcoming the limitations of traditional classroom environments. Digital tools, especially those supported by up-to-date and scientific content, can facilitate students' access to accurate information. In addition, increasing practical applications and experiences in sexual health education can reinforce the level of knowledge of students.

Finally, the findings of this study suggest that further research is needed to improve the sexual health literacy levels of midwifery students and that it would be beneficial to adopt multidisciplinary approaches in this regard. It may positively affect public health if educational institutions and health authorities develop a common understanding in this field and provide comprehensive and effective sexual health education to midwifery students.

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Conflict of Interest Statement
The authors declare that there is no conflict of interest.

Ethical Approval
Ethics committee approval of the study was obtained from Suleyman Demirel University Health Sciences Ethics Committee on 29.04.2024 with decision number 75/6. The study was conducted in accordance with the principles set forth in the Declaration of Helsinki.

Consent to Participate and Publish
The students participating in the study were explained about the purpose, process and questionnaire of the research and their informed consent was obtained.

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SÖ: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing-original draft.

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ORIGINAL RESEARCH

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The Protective Role of Gilaburu in Amiodarone-Induced Testicular Damage: Immunohistochemical Evaluation via the TNF-α Pathway

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Abstract

Objective
This study aimed to investigate the protective effects of Gilaburu (GL) in mitigating testicular damage induced by the antiarrhythmic drug Amiodarone (AD) in rats.

Material and Methods
Rats were randomly assigned to four groups: Group Control: received no treatment. Group Amiodarone (AD): received AD (100 mg/kg, intraperitoneally). Group Amiodarone + Gilaburu (GL): received both AD (100 mg/kg, intraperitoneally) and GL (100 mg/kg, orally). Group Gilaburu: received GL alone (100 mg/kg, orally). Following a 10-day experimental period, the animals were euthanized. The left testis was harvested for histological and immunohistochemical examinations, while the right testis and epididymis were collected for testicular weight measurement, sperm isolation, count, and motility analysis.

Results
AD administration results in significant testicular damage, as evidenced by histopathological alterations such as irregularly shaped seminiferous tubules, a reduced number of spermatogenic cells, degeneration, and pyknotic nuclei. The highest testicular weight, sperm count, progressive motility, and total motility values were recorded in the control group, whereas the GL group exhibited values comparable to those of the control, with no statistically significant difference observed between them. In contrast, the AD and AD+GL groups demonstrated significant reductions in histopathological analysis, Tumor necrosis factor (TNF-α) stainings, spermiogram analysis ($p < 0.05$). However, the co-administration of GL with AD mitigated these adverse effects, reducing testicular damage.

Conclusion
The findings of this study indicate that Gilaburu has a protective effect against AD-induced testicular damage, potentially attributed to its potent antioxidant properties.

Keywords: Amiodarone; gilaburu, testis; TNF- α; immunohistochemical analysis

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Introduction

Amiodarone (AD) is a benzofuran derivative characterized by a phenol moiety with two covalently bound iodine atoms (1,2). It is recognized as the longest-acting and most broad-spectrum antiarrhythmic drug, extensively utilized for treating arrhythmias in patients with heart failure (1,3-7). In addition to being highly favoured clinically, AD administration has also been associated with side effects such as hepatic dysfunction, pulmonary complications, thyroid abnormalities, and urogenital disorders (2). In certain cases, these adverse effects may necessitate the discontinuation of treatment. The likelihood of side effects tends to increase with higher doses and prolonged exposure. However, administrating lower doses has been shown to mitigate toxicity while maintaining clinical effectiveness (5, 8). In most tissues commonly affected by the drug's side effects, amiodarone toxicity is believed to be partially attributable to the sequestration of the drug and its metabolite (7, 9).

While pharmacological interventions are designed to target the affected organs, they may inadvertently exert detrimental effects on other organs, potentially resulting in additional health complications during the recovery process. This scenario often encourages patients to seek alternative treatments rather than relying on conventional pharmaceuticals, including herbal remedies, which are perceived to have fewer adverse effects.

Plants and their derived pure chemicals have been used in the treatment of various diseases. Among such natural alternatives, GL (Viburnum opulus L.) is a plant endemic to the Kayseri province in Turkey, recognized for its potent antioxidant properties. It contains compounds such as proteins, ellagic acid, organic acids like catechin, quercetin, ferulic acid, phenolic compounds, sugars, and essential vitamins (10). The red, cluster-shaped fruit is harvested in the autumn and can be consumed either in its raw form or as a juice extract (11-13).

Gilaburu has been traditionally utilized for centuries in the treatment of a wide range of ailments, including circulatory, respiratory, reproductive, and digestive disorders. Its flavonoid composition endows it with notable antiproliferative, antiallergic, antiviral, and anti-inflammatory properties. As a result, it is widely employed in managing conditions such as rheumatism, hypertension, diabetes, and urinary incontinence (11-13).

The scientific literature on GL remains limited, specifically investigating its effects on testicular health. In the present study, the therapeutic potential of GL as an antioxidant, believed to have both preventive and curative effects against AD-induced testicular toxicity, was evaluated through immunohistochemical analysis of the TNF-α pathway which is a cytokine that mediates many of the metabolic responses after tissue injury and regulating different cellular processes pertinent to spermatogenesis (14).

Material and Method

This experimental study was evaluated based on quantitative results.

Experimental Desing

This study utilized a total of 32 male Wistar rats, each weighing between 300 and 350 grams, which were randomly assigned to four groups. The animals were housed under standardized laboratory conditions, including controlled humidity, a 12-hour light/dark cycle, and a temperature of 25°C for 10 days. They were granted continuous ad libitum access to food and water for the entire duration of the experiment.

The dosage of AD (Cordarone / 3 ml I.V, Sanofı Aventıs) was established as 100 mg/kg, based on existing literature (15). The dose was calculated based on the body weight of each rat and administered intraperitoneally at a consistent time during the study. GL (Viburnum Plus, Talex Pharma) powder extract was prepared at a concentration of 100 mg/kg, dissolved in 10 ml of physiological saline, and administered via oral gavage. The dosage and administration method were determined with established guidelines for dosage calculation and stock solution preparation in experimental animal studies (7, 11). The animals were randomly assigned to four groups; Group I (Control) received no treatment. Group II (AD) was administered AD at a dosage of 100 mg/kg, intraperitoneal injection. Group III (AD + GL) received the same dose of AD (100 mg/kg, intraperitoneally) along with GL (100 mg/kg, orally). Group IV (GL) was administered only GL (100 mg/kg, orally).

At the conclusion end of the experimental period, the rats were humanely euthanized under anesthesia, which was induced by intraperitoneal administration of xylazine (10 mg/kg) and ketamine (90 mg/kg) (Ketamine HCl Ketasol 10%, 10 ml RICHTER Pharma-xylazine HCl, Rompun 2%, BAYER). Following euthanasia, the testicular tissues were harvested and fixed in 10% neutral buffered formalin for subsequent histological analysis. Tissue sections were subjected to histological evaluation using hematoxylin-eosin (H-E)

staining and immunohistochemical analysis targeting TNF-α. Additionally, the right testicular tissues were weighed, and the epididymis was processed for sperm isolation, enumeration, and motility assessment.

Histochemical Analyses

Testicular tissue samples were washed in water overnight, followed by sequential dehydration in ethanol and clearance in xylene. Subsequently, the tissues were embedded in paraffin. The paraffin-embedded samples were sectioned at a thickness of 4 µm. The sections were treated with three different xylol series for 30 min each to remove the paraffin from the sections. Afterwards, the tissues were rehydrated by putting them through a series of alcohols, from high grade to low grade. Subsequently, routine hematoxylin-eosin (H-E) staining was performed. After staining was completed, the sections were put through a series of alcohols for full tissue dehydration. Then, entellan was dripped on the xylol-polished tissues, the coverslips were glued, and the sections were evaluated under a microscope. Histopathological evaluation was conducted under a photomicroscope (Olympus CX21 FS), and findings were systematically graded according to the scoring system established by Refaiy et al (16, 17). Histopathological findings were graded and evaluated with a photomicroscope by using the semi-quantitative method according to as follows:

(-) (negative score): No structural changes

(+) (1 positive score): Light structural changes

(++) (2 positive score): Middle structural changes

(+++) (3 positive score): Serious structural changes

Immunohistochemical Analyses

Polyclonal TNF-α primary antibody (rabbit anti-TNF-α antibody, Abcam, ab220210, Cambridge, USA) was used for immunohistochemical examination. The primary antibody was diluted 1:100 in antibody dilution fluids. For immunohistochemical staining, the sections were deparaffinized and rehydrated by treating with xylol and alcohols as described in the histopathology method. The sections were then washed in water for 10 min and incubated in hydrogen peroxide, were then boiled in citrate buffer solution. After this, the sections were washed with phosphate buffer saline (PBS). Afterwards, a series of sections were incubated separately with the primary antibody. This step was carried out at +4 °C overnight. All the sections were then washed in PBS, then biotinylated serum was dripped onto the tissues. Subsequently, they were stained with freshly prepared DAB (3,3 diaminobenzidine) chromogen to make the reaction visible. The sections were then counterstained using hematoxylin. Then, they were dehydrated with alcohols and placed in xylol, dripped with entellan, and covered with coverslips. Sections were scored from 0 to 3 according to the density of staining (0, absence of staining; 1, light; 2, middle; and 3, intense) (16, 17).

(-,) (0 negative score): No immune staining,

(+) (1 positive score): Light immune staining,

(++) (2 positive score): Middle immune staining,

(+++) (3 positive score): Intense immune staining.

Testis weight, Sperm Isolation, Count, and Motility

The right testes of each group were weighed. The epididymis from the same group was meticulously dissected using scissors and a needle, then placed in 2 mL of Tris stock solution (trizma base, citric acid, D fructose, SIGMA) (18) at 37°C and incubated for 10–12 minutes in a petri dish. Following incubation, 1 mL of the solution was collected for sperm analysis. Sperm count and motility were assessed using an IX70 inverted microscope with a Macler chamber (Olympus, Tokyo, Japan) (19).

Statistical Analyses

Mann-Whitney U test were conducted as described in (20, 21) via SPSS 18 software for all analyses. All findings were considered significant at $p<0.05$.

Results

Histochemical Results

Hematoxylin and eosin (H&E) staining of testicular tissue sections revealed a significant difference between the control and experimental groups (Group AD and AD+G) ($p<0.05$). While the testicular tissues in the control group exhibited a normal histological structure (Fig. 1A-D), those in Group AD and AD+GL demonstrated notable histopathological alterations, including irregularly shaped seminiferous tubules, a reduced number of spermatogenic cells, degeneration, and pyknotic nuclei. It was observed that these histopathological abnormalities were attenuated in Group AD+GL following GL administration ($p<0.05$) (Fig.1 B-C). Structural changes were assessed using the semi-qualitative grading method (17), Table 1.

Immunohistochemical Results

Immunohistochemical analysis revealed that TNF-α staining was weak in the Control Group and GL Group

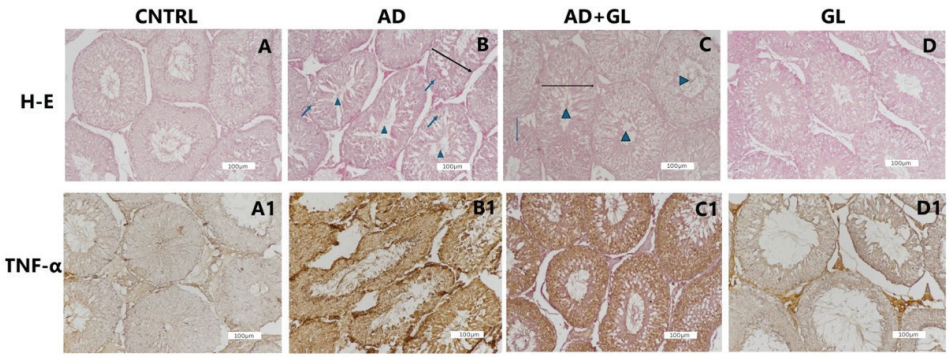

Figure 1

Histochemical (H&E) and immunohistochemical (TNF-α) staining of testicular tissues from control and experimental groups.
A-D: Histochemical staining results. B-C: Seminiferous tubules appear irregular (black arrows), with fewer spermatogenic cells (blue triangles), degeneration, and pyknotic nuclei (blue arrows).
A1-D1: Immunohistochemical staining results. B1: Positive staining for TNF-α, X20.

Table 1 Evaluation of Histopathological Findings Between All Groups

Histopathological Findings	CNTRL Group	AD Group	AD+GL Group	GL Group
Irregularly Shaped Seminiferous Tubules	-/+	+++	++/+++	-/+
A Reduced Number of Spermatogenic Cells	-	+++	++	-/+
Degeneration	-	++	+/-	-
Pyknotic Nuclei	-/+	++	+	-
Mononuclear Cell Infiltration	-/+	+/++	+	-/+

Values were presented as means ± S.D. The relationships between groups and results of immunohistochemical degress were assessed by Mann-Whitney U.
CNTRL: Control, AD: Amiodarone, GL: Gilaburu. (-) (negative score): No structural changes, (+) (1 positive score): Light structural changes, (++) (2 positive score): Middle structural changes, (+++) (3 positive score): Serious structural changes.

Table 2 TNF- α Marking Degrees Between All Groups

TNF- α	CNTRL Group	AD Group	AD+GL Group	GL Group
	-/+	++	+/++	-/+

Values were presented as means ± S.D. The relationships between groups and results of immunohistochemical degress were assessed by Mann-Whitney U.
CNTRL: Control, AD: Amiodarone, GL: Gilaburu, TNF- α: Tumor Necrosis Factor Alpha,
(-) (negative score): No immune staining, (+) (1 positive score): Light immune staining, (++) (2 positive score): Middle immune staining, (+++) (3 positive score): Intense immune staining.

Table 3 Statistical Analysis Results of Testis Weight, Sperm Isolation, Count, and Motility Among Groups.

	CNTRL Group	AD Group	AD+GL Group	GL Group	P value
Parameters	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	
Testis Weight (g)	3.69 ± 0.47[a]	2.60 ± 0.54[b]	2.79 ± 0.52[c]	3.56 ± 0.37[a]	0.0040
Sperm Count (x10^{6}ml)	17.88 ± 3.68[a]	9.13 ± 8.33[b]	13.88 ± 4.45[c]	17.12 ± 4.09[a]	0.0031
Progressive Motility (%)	32.62 ± 4.96[a]	10.00 ± 2.28[b]	16.12 ± 2.64[c]	32.75 ± 7.03[a]	0.0010
Non- Progressive Motility (%)	18.62 ± 4.34[a]	17.95 ± 3.78[a]	18.01 ± 8.26[a]	18.15 ± 7.05[a]	0.1400
Total Motility	51.25 ± 6.78[a]	25.17 ± 3.54[b]	38.75 ± 7.69[c]	50.50 ± 11.78[a]	0.0056

Values were presented as means ± S.D. The relationships between groups and results of immunohistochemical degress were assessed by Mann-Whitney U. CNTRL: Control, AD: Amiodarone, GL: Gilaburu, TNF- α: Tumor Necrosis Factor Alpha. a, b, c; different characters indicate statistically significant differences in the same column, p<0.05, p<0.001.

(Fig.1 A1-D1). A comparative evaluation between Group AD and AD+GL indicated that receptor staining was highest in Group AD, while a moderate reduction in staining was observed in Group AD+GL (p<0.05) (Fig.1 B1-C1), Table 2.

Testis Weight, Sperm Isolation, Count, and Motility Results

The control group exhibited the highest testicular weight, sperm count, progressive motility, and total motility values. While these parameters in the GL group were comparable to those of the control group, a significant decline was observed in the AD group, with an even more pronounced reduction in the AD+GL group substantial decrease was detected in the AD group (p <0.05). However, the evaluation of non-progressive motility did not reveal a significant difference, p = 0.14, Table 3.

Discussion

Amiodarone exerts significant effects on molecular processes, and multiple studies have explored its impact on various organs (3). In recent years, the GL plant (Viburnum opulus) has gained increasing attention (7, 11, 12). However, no studies to date have specifically examined the effects of GL on amiodarone-induced testicular damage.

Tabuu et al. investigated the impact of AD by administering varying doses (20–200 mg/kg) of AD to female rats and reported a dose-dependent induction of ovarian damage (3). In the present study, administration of 100 mg/kg AD resulted in testicular tissue damage. Similarly, Sakr et al. induced testicular damage using amiodarone (AD, 18 mg/kg) and examined the protective effects of grapefruit juice (27 mL/kg). Their histological and molecular analyses indicated a reduction in testicular damage in the groups that received grapefruit juice (5). In another study, Kagan et al. evaluated the effects of low-dose (20 mg/kg) and high-dose (200 mg/kg) AD administration on testicular tissue damage and apoptosis in rats. Following a 14-day period, histopathological analyses revealed severe testicular damage, with the highest degree of deterioration observed in the high-dose group, followed by the low-dose group. Moreover, immunohistochemical staining for caspase-3 and caspase-8 confirmed the presence of apoptosis, which was most pronounced in the high-dose group, followed by the low-dose group (22) Consistently, in the present study, administration of a single dose of AD (100 mg/kg) (15) led to detectable testicular tissue damage, confirming the deleterious effects of AD on testicular health.

Furthermore, immunohistochemical staining for TNF-α revealed increased expression in the AD-treated groups, indicating an inflammatory response associated with AD exposure. Additionally, Altun et al. demonstrated that the GL plant exerted hepatoprotective effects in rats by stabilizing elevated glucose levels and reducing them. They attributed this effect to the bioactive compounds present in the extract, such as glycosides and polyphenols (13).

Sarıözkan et al. investigated the effects of GL (100 mg/kg) on testicular damage induced by docetaxel and paclitaxel, reporting significant histological and cytological alterations, including increased Bax pro-apoptotic immunopositive cell scores in testicular and spermatozoa tissues. Their findings indicated that GL supplementation alleviated taxane-induced damage to the reproductive system in male rats (11). Similarly, in the present study, the administration of 100 mg/kg GL was found to mitigate tissue damage. The results demonstrated that GL exerted a protective effect by reducing histopathological alterations, preserving testicular weight, and improving sperm count, progressive motility, and total motility values. Since no previous studies have specifically investigated the effects of GL on testicular weight, sperm count, progressive motility, and total motility in the context of AD-induced toxicity, direct comparisons with existing literature were not possible.

Haiyu et al. observed that AD significantly reduced the survival rate of atrial myocytes, markedly decreased superoxide dismutase (SOD) activity, and increased the rates of cell apoptosis rates, as well as the levels of IL-1β, IL-6, malondialdehyde (MDA), and TNF-α (23). In a study conducted by Lu et al., administration of AD at doses ranging from 0 to 400 mg/kg did not elevate serum TNF-α levels in rats treated with AD alone. However, a significant increase in serum TNF-α concentration was observed in rats treated with LPS. These findings suggest that the elevated TNF-α levels in AD/LPS co-treated rats were not solely due to an additive effect. Instead, amiodarone AD likely potentiated TNF-α production or impaired its clearance when induced by LPS (2). In the present study, TNF-α was similarly investigated in the context of AD-induced testicular damage. However, it was assessed through positive staining via immunohistochemical analysis in tissue samples. The results confirmed that AD alone induced significant testicular damage.

In this study, testicular damage was induced solely by AD. Immunohistochemical analysis for TNF-α, a key mediator of accurate inflammation, revealed positive staining, indicating the presence of an inflammatory response. The pronounced TNF-α staining observed in the AD group confirmed AD-induced inflammation. Conversely, the reduced staining in the AD+GL group suggests that GL mitigates the inflammation. These immunohistochemical findings validated the toxic effects of AD, aligning with previously reported literature.

Gilaburu, an endemic plant of Turkey, has been extensively studied for its diverse activities in the literature and is gaining increasing recognition in the health sector. The absence of prior research investigating the effects of GL fruit extract on amiodarone-induced testicular toxicity underscores the significance of this study.

Based on the histological, immunohistochemical, and spermiogram parameters evaluated in this study, the findings suggest that incorporating GL into the diet, with careful consideration of appropriate dosage, may offer potential health benefits.

Conflict of Interest Statement

The authors declare no conflicts of interest.

Ethical Approval

Ethical approval was obtained from the Süleyman Demirel University Animal Experiments Ethics Committee of (HADYEK) on 15.09.2022, 06/69, and all procedures were carried out in strict accordance with established ethical guidelines.

Consent to Participate and Publish

Written informed consent to participate and publish was obtained from all individual participants included in the study.

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Availability of Data and Materials

The data supporting the findings of this study are available from the authors upon reasonable request.

Artificial Intelligence Statement

The authors declare that they have not used any generative artificial intelligence for the writing of this manuscript, nor for the creation of images, graphics, tables, or their corresponding captions.

Authors Contributions

MÖ: Conceptualization, Formal Analysis, Investigation, Validation, Visualization, Writing-Original Draft.

NK: Data Curation, Investigation, Methodology.

DUK: Investigation, Writing-Original Draft

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ORIGINAL RESEARCH

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Assessment of Gallbladder Epithelial Lesions and Clinicopathological High-Risk Patient

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Abstract

Objective

Recognizing epithelial pathologies in the gallbladder and understanding the accompanying clinicopathological features may be useful in determining the decision for cholecystectomy. However, this distinctive selection in gallbladder patients has not been fully elucidated yet. In our series, we aimed to investigate the relationship between epithelial pathologies of the mucosa and clinicopathological findings in cholecystectomy materials and evaluate them in the light of the literature.

Material and Methods

We designed a retrospective cross-sectional study in which we re-evaluated Hematoxylin & Eosin-stained slides of 852 cholecystectomy materials operated in our hospital for two years. Mucosal pathologies were grouped as papillary hyperplasia (PH), pyloric metaplasia (PM), intestinal metaplasia (IM), epithelial dysplasia (ED), and cancer. We obtained the demographic data from the electronic archive system of our hospital. The chi-square test and Fisher's exact test were used, and $p < 0.05$ was considered significant.

Results

The mean age of the patient was 53.17 ± 13.48. PH, PM, IM, PM+IM, ED, and invasive cancer were detected in 11.38%, 8.92%, 11.15%, 07.04%, 3.16%, and 0.47% of all cases, respectively. The mucosal pathologies were mostly observed over 50 years ($p < 0,05$). The mass-forming lesion could not be detected among dysplasia cases ($p < 0.0001$).

Conclusion

Age was found to be an essential factor for gallbladder epithelial changes in our study. We argue that taking additional macroscopic samples would be more effective in the definitive diagnosis of gallbladder epithelial pathologies in this age group, and being more careful when deciding on the indication for cholecystectomy in the over-50 age group.

Keywords: Gallbladder, Mucosal pathologies, Cholecystectomy, Diagnosis, Demographic findings

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Introduction

Cholecystectomy is one of the most commonly encountered procedures in routine surgical pathology practice. The primary clinical indication for cholecystectomy is cholelithiasis. However, a broad spectrum of mucosal pathologies, ranging from chronic inflammation and metaplasia to intraepithelial neoplasia and even carcinoma, can be incidentally identified during routine histopathological evaluation. While these epithelial changes are typically identified on hematoxylin and eosin-stained sections, additional diagnostic techniques may be required in suspicious cases. Before histopathological diagnosis, clinical and demographic data alone are insufficient for accurately classifying patients at risk for epithelial lesions. Therefore, a detailed histopathological examination of cholecystectomy specimens is essential to identify, characterize, and define these patient groups.

Over the past 20 years, epithelial changes in the gallbladder have received significant attention. While numerous clinical and etiological factors, such as age, gender, cholelithiasis, and inflammatory processes, have been proposed to influence epithelial changes, many of these associations remain controversial (1–4). In addition to established criteria for patient selection for cholecystectomy, it is crucial to define the clinicopathological features associated with mucosal pathologies of the gallbladder. Enhancing the macroscopic evaluation of cholecystectomy specimens by increasing the number of tissue samples examined is an effective approach for identifying these pathologies (3,5).

This study aims to investigate the relationship between mucosal pathologies in cholecystectomy specimens and associated clinicopathological findings.

Material and Method

Patients' Characteristics and Histopathological Features

This study included 896 cholecystectomy specimens submitted to the clinical pathology department of Buca Seyfi Demirsoy Education and Research Hospital between January 1, 2023, and the end of September 30, 2024. Clinical, pathological, and demographic data of all patients were retrieved from the pathology department's electronic database, and H&E sections were reevaluated. Cases were excluded if paraffin blocks or Hematoxylin and Eosin (H&E)-stained slides were unavailable in the pathology archive or if macroscopic data were incomplete. As a result, we examined 852 patient specimens.

The macroscopic evaluation in our laboratory followed a standardized procedure. For all cholecystectomy specimens without a preoperative diagnosis of malignancy, six samples were taken from the surgical neck margin, fundus, and corpus for routine assessment as two paraffin blocks. If epithelial dysplasia was identified during the initial microscopic examination, the specimen was re-evaluated macroscopically, and additional samples were taken. This approach has been taken as suggested by Roa et al. (6). This additional sampling was not routinely performed for cases with metaplasia.

Without knowing the clinical data, the pathologist reviewed all H&E-stained slides using light microscopy for this study under the current literature (6). Mucosal pathologies were classified as papillary hyperplasia (PH), pyloric metaplasia (PM), intestinal metaplasia (IM), low- and high-grade intraepithelial neoplasia, or invasive carcinoma. Cases presenting both low- and high-grade dysplasia were categorized as epithelial dysplasia. The presence and absence of pathological lesions in the table were evaluated as present or absent as a result of histopathologic examination, and were not further categorized. Macroscopic findings were categorized based on the presence or absence of stones and mass-forming lesions (exophytic masses ≥ 0.2 cm). Histological findings were similarly classified for the presence or absence of acute and chronic inflammation. Clinical, demographic, and macroscopic data were retrieved from the hospital's archival records.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics v21.0. The association between demographic features and mucosal pathological characteristics was assessed using the chi-square test. Fisher's exact test was applied for comparisons of categorical variables. A p-value <0.05 was considered statistically significant in all analyses.

Results

Firstly, we evaluated the similarities between the selected and excluded case groups to exclude the selection bias. We had a result that the 44 cases excluded had similar demographic and pathological data, and did not affect the general demographic characteristics of the selected case group.

The mean age of the patients was 53.17 ± 13.48 years, and 80.04% were women. Macroscopic examination revealed cholelithiasis in 745 cases (87.44%) and mass-forming lesions in 45 cases (5.28%). Mucosal pathologies were observed in 359 cases (42.13%).

Table 1 The distribution of current histopathological features according to age and gender

	Age		P value	Gender		P value
	50	≥50		F	M	
Papillary hyperplasia	43 (44.33%)	54 (55.67%)	**0.001**	77 (79.38%)	20 (20.62%)	**0.160**
Pyloric metaplasia	29 (38.16%)	47 (61.84%)		61 (80.26%)	15 (19.74%)	
Intestinal metaplasia	20 (21.05%)	75 (73.96%)		83 (87.36%)	12 (12.64%)	
Epithelial dysplasia	5 (18.52%)	22 (81.48%)		22 (81.48%)	5 (18.52%)	
Pyloric and Intestinal metaplasia	11 (18.33%)	49 (81.67%)		52 (86.67%)	8 (13.33%)	
Invasive carcinoma	0 (00.00%)	4 (100.0%)		4 (100.0%)	0 (00.00%)	
Cholelithiasis	307 (41.21%)	438 (58.79%)	**0.503**	595 (79.86%)	145 (20.14%)	**0.398**
Mass-form lesions	19 (42.33%)	26 (57.77%)	**0.856**	40 (88.89%)	5 (11.11%)	**0.120**
Acute inflammation	60 (36.13%)	90 (63.87%)	**0.196**	101 (67.33%)	49 (32.66%)	**0.072**
Chronic inflammation	349 (41.95%)	483 (58.05%)	**0.713**	651 (78.25%)	181 (21.75%)	**0.506**

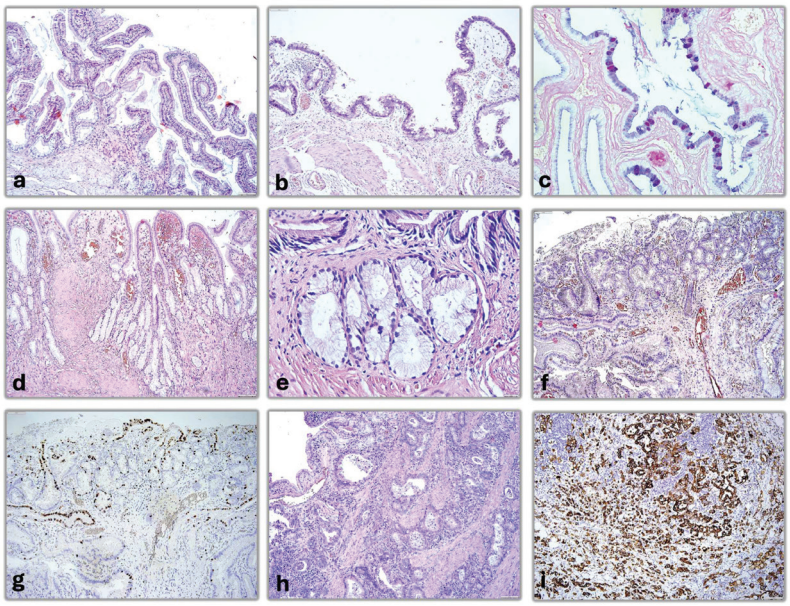

Figure 1
The histomorphological features of gallbladder mucosal lesions a) Papillary hyperplasia (PH), (Hematoxylin&Eosin) H&E, x10; b-c) Intestinal metaplasia (IM), H&E (b) and (Periodic acid schiff-alcian blue) PAS+AB histochemical staining (c), x10; d, e) Ploric metaplasia (PM), H&E, x10 (d) and X40 (e); f-g) Low-grade epithelial dysplasia, H&E (f) and Ki-67 proliferation index immunohistochemical staining (g), x10; h-i) Invasive adenocarcinoma, H&E (h), and Cytokeratin 7 (CK7) immunohistochemical staining (i) x20.

Among these, papillary hyperplasia (PH) was identified in 97 cases (11.38%) (Figure a), pyloric metaplasia (PM) in 76 cases (8.92%) (Figures d-e), intestinal metaplasia (IM) in 95 cases (11.15%) (Figures c,d), PM+IM in 60 cases (07.04%), low-grade dysplasia in 27 cases (3.16%) (Figures f-g), and invasive carcinoma in 4 cases (0.47%) (Figures h-i). None of the patients had high-grade dysplasia as an isolated finding; all cases of high-grade dysplasia were observed in the mucosa adjacent to invasive carcinoma.

Relationship Between Age and Mucosal Pathologies

A majority of patients with mucosal pathologies were older than 50 years. Specifically, 54 (55.67%) of patients with PH, 47 (61.84%) with PM, 75 (73.96%) with IM, 49 (81.67%) with PM+IM, 22 (81.48%) with epithelial dysplasia, and all four patients with carcinoma (100%) were over 50 years of age. A statistically significant association was found between the presence of mucosal pathologies and age (p = 0.001) (Table).

Relationship Between Gender and Mucosal Pathologies

Among the patients with mucosal pathologies, 77 (79.38%) of 97 with PH, 61 (80.26%) of 76 with PM, 83 (87.36%) of 95 with IM, 52 (86.67%) of 60 with PM+IM and 22 (81.48%) of 27 with dysplasia, all Invasive carcinoma (4 (100.0%)) were women. However, no statistically significant relationship was found between gender and the occurrence of PH (p = 0.697), PM (p = 0.211), IM (p = 0.332), PM+IM (p = 0.282), or dysplasia (p = 0.408). All four carcinoma cases occurred in women. However, this finding was insufficient for statistical analysis due to the limited number of carcinoma cases. Furthermore, when dysplasia and carcinoma were analyzed as a single group, no significant relationship was detected between gender and dysplastic changes (p = 0.408). The distribution of histopathological features and mucosal pathologies across all cholecystectomy specimens is summarized in the Table.

Relationship Between Macroscopic Features and Mucosal Pathologies

Cholelithiasis was identified in 80.2% of patients with PH, 84.9% with PM, 60% with IM, 88% with PM+IM, 78.6% with dysplasia, and 100% with carcinoma. However, no statistically significant relationship was found between cholelithiasis and mucosal pathologies, including PH (p = 0.404), PM (p = 0.125), IM (p = 0.194), PM+IM (p = 0.204), dysplasia (p = 0.371), and carcinoma (p = 0.482).

A total of 45 cases exhibited macroscopic mass-forming lesions, of which 26 (57.77%) were cholesterol polyps, 7 (15.55%) were PM, 2 (4.44%) were IM, 6 (13.33%) were PH, and 4 (8.88%) were invasive carcinoma. No macroscopic mass-forming lesion was observed in epithelial dysplasia cases. While no significant relationship was found between mass-forming lesions and PH (p = 0.726) or PM (p = 0.791), it was significant that none of the patients with dysplasia had a mass-forming lesion (p < 0.0001).

Relationship Between Mucosal Pathologies

Chronic inflammatory infiltration was more common than acute inflammation in patients with PH (p = 0.026) and dysplasia (p = 0.001).

Among the 97 patients with PH, 21 also had metaplasia, with 19 of these cases exhibiting PM and 2 showing IM. One patient with both PH and IM also had carcinoma. Invasive carcinoma was not observed in any patient with PM, whereas two patients with IM were diagnosed with carcinoma. Due to the limited number of cases with these mucosal pathologies, statistical analysis was not applied to these findings.

Discussion

Cholecystectomy is one of the most commonly performed procedures in general surgery. For instance, approximately 750,000 cholecystectomies are conducted annually in the United States (7), 25,000 in the Netherlands (8), and an estimated 40,000–60,000 in Turkey (9). There is ongoing debate regarding the necessity of routine histopathological examination for cholecystectomy specimens that lack visible macroscopic pathology. Nonetheless, the widely accepted view is that routine histopathological evaluation should be performed on all cholecystectomy specimens (9–12). This recommendation stems from the fact that gallbladder tumors do not always form easily noticeable masses, and most cancers cannot be detected through clinical or radiological methods. Furthermore, it is well-documented that a small proportion of gallbladder tumors develop without forming a visible mass and are only incidentally discovered during histopathological examination (13–16). Benign epithelial metaplastic changes and low- or high-grade intraepithelial dysplasia in the gallbladder typically do not produce mass-forming lesions, making incidental detection during pathological evaluation crucial (9). In our study, we detected two cases of invasive tumors penetrating the muscle layer of the gallbladder (pT1 b according to WHO 2019) incidentally. The reported rate of incidental tumors in the literature is 0.15%–0.3 %, consistent with our 0.46% findings (13,14,16). Additionally, epithelial dysplasia cases, like the two cancer cases we identified, showed no

evidence of macroscopic mass lesions. These results further support routine histopathological examination of cholecystectomy specimens, as advocated in the literature (10–12,17,18).

The molecular mechanism of subsequent epithelial changes along the line of malignancy is not clear, because diagnosis can be generally obtained incidentally (6,19,20). Some molecular pathway abnormalities can be detected to this day, such as the mucin family members and the P53 pathways (6,18–20).

A thorough macroscopic evaluation is essential to identify premalignant or malignant lesions that do not form masses. Additional sections must be taken if suspicious epithelial abnormalities are detected during the initial histopathological examination. This step is critical, as additional sampling can reveal carcinoma. Consequently, pathologists must be aware of patient groups at higher risk for gallbladder epithelial lesions to detect accompanying malignancies or associated mucosal pathologies, because not all samples can be subjected to extensive examination during routine pathology examination.

Papillary hyperplasia (PH) in the gallbladder is categorized as either primary or secondary. Epithelial dysplasia is more likely to develop in the context of secondary PH, which is often associated with chronic cholecystitis, adenomatous hyperplasia, or other inflammatory conditions (21). However, there is limited evidence in the literature to confirm this relationship. In our study, chronic inflammation was present in 93.8% of PH cases, suggesting that most of our cases were secondary PH. However, only two cases of PH were associated with low-grade intraepithelial neoplasia, and three cases were associated with intestinal metaplasia (IM). No statistically significant relationship was found between PH and dysplasia or metaplasia.

When comparing our findings on PH to the existing literature, we found limited information and no established diagnostic criteria for PH. This lack of standardization may make it challenging for pathologists to identify PH consistently. Additionally, the malignant potential of PH remains unclear in the current literature. Larger case studies involving PH are needed to clarify diagnostic criteria and establish a standardized macroscopic approach for evaluating these lesions.

The most common pathological diagnoses in cholecystectomy specimens are cholelithiasis and chronic cholecystitis. Chronic cholecystitis is diagnosed based on morphological criteria such as dominant mononuclear cell infiltration, fibrosis, and, in some cases, metaplastic changes (21). Chronic inflammation can be a key factor in the development of metaplastic changes in the gallbladder. In our study, 231 out of 832 cases with chronic inflammation demonstrated metaplastic changes. Pyloric metaplasia (PM) was the most common type of metaplasia observed in our study, which is consistent with the literature. While PM is less frequently associated with precancerous potential, intestinal metaplasia (IM) has a much stronger link to cancer. In our study, low-grade dysplasia was observed in 8.66% of PM cases but in 28% of IM cases (17). Notably, IM was observed in the mucosa adjacent to invasive cancer in two cases, further supporting the association between IM and cancer despite the small number of cases (17).

Akki et al. suggested that when cholecystectomy specimens contain IM or dysplasia, the entire gallbladder should be examined histopathologically (22). They also recommended additional sampling for patients with low-grade dysplasia, although this was deemed unnecessary for cases with IM. In contrast, Adsay et al. argued that two additional samples should be taken in cases of IM (5). In our study, additional sections were obtained for cases with epithelial dysplasia and IM. The relatively low rates of low-grade dysplasia in our study may be attributed to the lack of additional sampling. Our findings, consistent with those of Esendağlı, Adsay, and Akki et al., underscore the importance of additional sampling in cases with epithelial changes (5,9,22).

The most common indication for cholecystectomy is cholelithiasis (3,8). Gracia and Ransohoff followed 123 patients with asymptomatic gallstones for over ten years and found that none developed cancer (23). However, other studies have reported a strong association between gallstones and gallbladder cancer (3,7,24,25). In our study, 80.24% of PH cases, 60% of IM cases, 78.57% of dysplasia cases, and all cancer cases were associated with cholelithiasis. However, no statistically significant relationship was found between the presence of gallstones and mucosal pathologies. Metaplasia, dysplasia, and cancer were observed more frequently in our group over 50 years of age, and this finding was statistically significant. These data are consistent with the literature, and we think age should be considered before cholecystectomy.

One study investigating the relationship between the demographic characteristics of gallstones (such as size, family history, and duration of stone presence) and cancer development showed that these factors

might contribute to cancer risk (26). While gallstones are believed to increase the incidence of cancer, most individuals with cholelithiasis do not develop gallbladder cancer in their lifetime (21). Although all cancer cases in our study were associated with cholelithiasis, the small sample size and lack of demographic data on gallstones limit the scope of our conclusions. Further large-scale studies are needed to investigate the potential effects of gallstones on the gallbladder mucosa and their relationship with mucosal changes, incorporating demographic characteristics for a more comprehensive understanding.

Gallbladder diseases are more prevalent in women, with the incidence reportedly increasing after the age of 50 (2,21,27,28). In our study, we also observed a predominance of female patients. However, statistical analysis did not reveal a significant relationship between female gender and mucosal pathologies. Our findings support existing literature indicating that female gender alone is not a determinant for cholecystectomy and does not necessitate changes in macroscopic evaluation methods (2–4,21,28).

Metaplasia, dysplasia, and cancer were observed more frequently in patients over the age of 50 in our study, a statistically significant finding. This aligns with previous reports, suggesting that age is a critical factor to consider before recommending cholecystectomy. Since mucosal pathologies and some cancers often do not form easily visible macroscopic masses, we propose that cholecystectomy specimens from patients over 50 years of age should be sampled more extensively during histopathological examination. Conversely, given that more than half of the patients in our study were over the age of 50, routinely increasing the number of samples for all such cases may not be economical or practical, particularly as even routine histopathological examinations for every cholecystectomy specimen are debated in the literature (15,16,27), but our results prove otherwise.

Although our study has a large population, it is limited to the comparisons between dysplasia and cancer because the number of these lesions is small and limited in this population. Additional studies are needed to compare the prevalence and distribution of epithelial dysplasia in the larger population. Since imaging methods are limited in detecting this type of epithelial lesion of the gallbladder, it is not possible to plan a prospective study at present. In this regard, our findings suggest that a more careful decision for cholecystectomy, including cancer markers, in patients over 50 years of age, may enable the future determination of risky patients.

It is claimed that if high-grade dysplasia is detected anywhere in the cholecystectomy specimen and the surgical margins are positive, the remaining part of the bile duct may be at risk for cancer development (18). These patients should be under close clinical follow-up. In conclusion, patient age should be considered not only when determining the indication for cholecystectomy but also during macroscopic pathological examination. For example, for patients over 50 years old, a more meticulous macroscopic evaluation and more extensive sampling of the gallbladder can be recommended, or the option of examining the entire cholecystectomy material may be considered, as done by Koshiol et al. (20).

In summary, based on the results of our study and the existing literature, further studies involving larger cohorts are needed to elucidate the stone-mucosal damage-cancer pathway.

Epithelial changes and cancer are more commonly detected in patients over 50 years of age. This demographic factor must be taken into account when determining cholecystectomy indications and during macroscopic pathological evaluation.

Conflict of Interest Statement
The authors declare no conflicts of interest regarding this article's research, authorship, or publication. The paper’s content and writing are solely the authors’ responsibility.

Ethical Approval
The Ethics Committee of the University of Izmir Democracy, Buca Seyfi Demirsoy Education and Research Hospital approved using primary tissue samples in this study (Reference Number: 2024/348, Date: December 30, 2024). This study adhered to the principles of the Helsinki Declaration and its subsequent amendments.

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Authors Contributions

OO: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing-original draft.

AI: Data curation; Formal analysis; Investigation; Validation.

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