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The European Research Journal 2026;12(2):139-148 Original Article

DOI: https://doi.org/10.18621/eurj.1752916 *Medical Pathology*

The Effects of Nateglinide and Octreotide on the Uterus in Rats with Experimentally Developed Polycystic Ovary Syndrome: A Histopathological Study

Ömür Gülsüm Deniz[1], Pınar Kırıcı[2], Ebru Annaç[3], Selçuk Kaplan[4]

[1]Department of Histology and Embryology, Faculty of Medicine, Bolu Abant İzzet Baysal University, Bolu, Türkiye; [2]Department of Obstetrics and Gynecology, Faculty of Medicine, Malatya Turgut Özal University, Malatya, Türkiye; [3]Department of Histology and Embryology, Faculty of Medicine, Adıyaman University, Adıyaman, Türkiye; [4]Department of Obstetrics and Gynecology, Faculty of Medicine, Adıyaman University, Adıyaman, Türkiye

ABSTRACT

Objectives: It was aimed to investigate the histopathological effects of Nateglinide (NG) and Octreotide (OC) on uterine morphology in rats with experimentally induced polycystic ovary syndrome (PCOS).

Methods: Forty-two female Sprague-Dawley rats (10-12 weeks old, 340-360 g) were divided into six groups (n=7 per group) as Control, PCOS, PCOS+NG, NG only, PCOS+OC, OC only. PCOS was induced via daily oral administration of Letrozole (1 mg/kg) for 21 days. Treatment groups received NG (oral, 30 days) or OC (intraperitoneal, 0.1 mg/kg/day for 30 days). After the experiment, the uterus tissues of all rats were dissected and subjected to histopathological examinations after histological procedures.

Results: Histopathological analysis revealed significant uterine damage in the PCOS group compared to other groups ($P<0.01$). In contrast, the Control, NG-only, and OC-only groups showed normal uterine architecture with intact epithelium, organized glands, and normal stromal structure and there were no significant differences between related groups ($P>0.05$). Treatment with NG or OC in PCOS rats led to improved epithelial and glandular morphology and reduced Mast cell density, no evidence of edema, and inflammation was found in the connective tissue of these treated groups, suggesting partial improvement of PCOS-induced uterine pathology.

Conclusions: NG and OC treatments ameliorated PCOS-induced uterine histopathological changes, suggesting their potential to improve endometrial morphology. These findings may have implications for therapeutic strategies aimed at enhancing endometrial receptivity and highlighting the importance of addressing endometrial health in therapeutic strategies beyond ovarian treatment in PCOS patients.

Keywords: Polycystic Ovary Syndrome, Nateglinide, Octreotide, Uterus, Histopathology, Rat

Polycystic ovary syndrome (PCOS), a significant endocrinopathy affecting women of reproductive age, is not yet recognized as a critical health issue globally [1, 2]. It impacts approximately 6–13% of women in this demographic worldwide [3]. Prevalence, diagnosis, etiology, management, clinical practices, psychological implications, and prevention strategies are among the

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Corresponding author: Ömür Gülsüm Deniz, PhD., Assist. Prof., Phone: +90 374 254 10 00, E-mail: omur.denizomu@gmail.com, omur.deniz@ibu.edu.tr

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most complex aspects related to PCOS [2]. PCOS is characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology [1]. The pathophysiology of PCOS is primarily associated with insulin resistance and a high prevalence of visceral adiposity, regardless of obesity, leading to disruptions in hormonal communication between the hypothalamus, pituitary gland, and ovaries [4]. Women with this syndrome may experience infrequent menstrual periods or amenorrhea, along with elevated androgen levels. The ovaries typically develop numerous small follicles but do not ovulate regularly, leading to subfertility in those women who desire to conceive [5, 6]. Recent reports indicate that women with PCOS exhibit an increased inflammatory profile in the endometrium during the proliferative phase [7]. PCOS is associated with several comorbidities, including infertility, metabolic syndrome, obesity, impaired glucose tolerance, type 2 diabetes mellitus, increased cardiovascular risk, depression, obstructive sleep apnea, endometrial cancer, and metabolic dysfunction-associated steatotic liver disease [8, 9]. In addition, PCOS is a complex condition with a strong multigenic basis and notable epigenetic contributions. Genome-wide association studies have identified several genetic loci linked to PCOS, many of which are involved in insulin resistance; related studies have indicated that approximately 30% of patients with PCOS and endometrial lesions experience insulin resistance [10], ovarian steroid production, steroid hormone synthesis, adrenal cortisone reductase deficiency, and disruption of gonadotropin regulation [11].

PCOS disorder is driven by insulin resistance, hyperinsulinemia, and hyperandrogenism [12]. These factors disrupt the hypothalamic-pituitary-ovarian axis, leading to excess Luteinizing hormone (LH), impaired follicle-stimulating hormone (FSH) function, and altered ovarian steroidogenesis [13]. Theca cell overactivity and granulosa cell dysfunction contribute to anovulation and typical polycystic ovarian morphology. Elevated insulin and androgens reduce sex hormone binding globulin and alter insulin like growth factor (IGF)-1 activity, further impairing follicular development [14]. Peripheral conversion of androgens to estrogens, especially in adipose tissue, causes chronic endometrial stimulation, increasing the risk of hyperplasia. Low-grade inflammation and possible autoimmune responses may also play a role [15].

Histological findings from rat models revealed that PCOS rats exhibited numerous follicular cysts with deteriorating and thin granulosa layers [16]. In certain women with PCOS, there appears to be persistent exposure to estrogen in the endometrial lining during both the proliferative and secretory phases, accompanied by a diminished effect of progesterone during the secretory phase. This situation may impair endometrial receptivity and, over time, contribute to hyperplasia and cancer [7]. Due to the highly heterogeneous nature of PCOS, the existing clinical classification fails to accurately represent the underlying pathological mechanisms. The individualized and precise treatment of PCOS is challenging because of the insufficient understanding of its etiology and prognostic markers [17], there for there was a rationale for testing new drugs in PCOS rat models, especially their impact on uterine histology.

Nateglinide (NG), a derivative of D-phenylalanine, is classified as part of a novel group of insulinotropic agents characterized by their rapid onset and short duration of action. These agents have been designed to minimize the risk of hypoglycemia related to pharmacological management and to reduce the potential for pancreatic beta-cell exhaustion [18]. In addition, numerous other approved peptide drugs are derived from natural hormones [19], including octreotide (OC) which is a somatostatin analogue drug that blocks the exocrine and endocrine functions of the pancreas [20]. NG and OC mitigate atresia and degenerative follicular damage induced by PCOS via antioxidant mechanisms, and anti-inflammatory pathways [10].

NG and OC are two distinct medications that regulate serum glucose levels via different mechanisms. While several studies have investigated the metabolic and ovarian effects of these agents, little is known about their histopathological impact on the uterus in PCOS. In this context, an experimental PCOS model was created in this study, considering that these agents may be effective on the low-level inflammation, insulin resistance, and oxidative damage that play a role in the etiology of PCOS, and the therapeutic effects of these agents on the uterus were investigated histopathologically.

METHODS

Animals and Group Design

The animals were obtained from the Experimental Animal Research Center at Adıyaman University, Adıyaman, Turkey. The research began after obtaining approval from the Adıyaman University local Animal Experiments Ethical Committee (no. 2022/030 dated 26.05.2022). All experimental methods were conducted in accordance with the protocol guidelines. Forty-two female Sprague-Dawley rats, aged 10-12 weeks and weighing (340-360 g), were randomly assigned to six groups, each consisting of seven animals. To allow for acclimatization, all rats were housed without intervention for 7 days. Estrous cycles were monitored via vaginal smears, and only rats in the estrus phase were included. The groups were organized as follows:

(1) Control Group (n=7): Following surgical exposure of the ovaries and uterine horns, the abdominal wall was closed using 4-0 nylon sutures and no additional surgical or pharmacological intervention was applied (Sham).

(2) PCOS Group (n=7): PCOS was induced via oral administration of Letrozole (1 mg/kg/day) dissolved in 1% carboxymethylcellulose (CMC) and 0.9% saline for 21 consecutive days. At the end of the induction period, rats were anesthetized with intraperitoneal ketamine (50 mg/kg) and xylazine (10 mg/kg), and ovarian biopsies were taken. Successful PCOS induction was confirmed histologically by the presence of atretic follicles without granulosa cell stratification. After a 4-week recovery period, uterus tissues were dissected [21].

(3) PCOS+NG Group (n=7): NG was administered orally for 30 days to rats with confirmed PCOS [22]. After treatment, uterus tissues were dissected under anesthesia.

(4) NG only Group (n=7): Rats received oral NG for 30 days. At the end of the treatment period, uterus tissues were dissected under anesthesia.

(5) PCOS+OC Group (n=7): Rats with induced PCOS received intraperitoneal OC (0.1 mg/kg/day) for 4 weeks [22]. At the conclusion of treatment, uterus tissues were dissected under anesthesia.

(6) OC (only Group) (n=7): Rats received intraperitoneal OC (0.1 mg/kg/day) for 30 days [22]. Following treatment, uterus tissues were dissected under anesthesia.

Histopathological Assessment

Following the completion of the experimental application procedures, uterine tissue samples were collected and preserved for two weeks in a 10% formalin solution. Following the tissues' fixation procedure, a standard histological tissue follow-up using chemicals including alcohol, xylene, and paraplast was carried out. Next, tissue samples were transformed into blocks of paraffin. For histopathological analysis, 5 µm thin sections were taken from paraffin blocks. After being deparaffinized with xylene, the produced slices were stained using the Toluidine blue staining procedure and Hematoxylin-Eosin. A digital camera-attached microscope of the Carl Zeiss brand, the Axiocam ERc5 model, was used to assess the stained sections histopathologically. The morphological parameters were evaluated semi-quantitatively on a scale from 0 to 3. If no change occurred, the score was 0; if there was mild damage, the score was 1; if there was considerable damage, the score was 2; and if there was severe damage, the score was 3.

Statistical Analysis

SPSS version 21.0 analysis program was used in the statistical analysis of the numerical data of the groups obtained from our study. The conformity of the data to the normal distribution assumption was evaluated by the Shapiro-Wilk test. In comparing continuous variables specified by measurement, data conforming to normal distribution were evaluated using One-Way ANOVA and Bonferroni tests as post-hoc analysis. In the statistical evaluations, the difference was accepted as statistically significant when $P<0.05$.

RESULTS

Histopathological Findings

In the present study, when the uterine tissue was examined, a statistically significant increase was observed in the PCOS group compared to the control, PCOS+NG, and PCOS+OC groups in terms of vacuolization (control vs PCOS, P=0.003; PCOS vs PCOS+NG, P=0.004; PCOS vs PCOS+OC P=0.005; Figure 1A), glandular degeneration (control vs PCOS, P=0.002; PCOS vs PCOS+NG P=0.004; PCOS vs

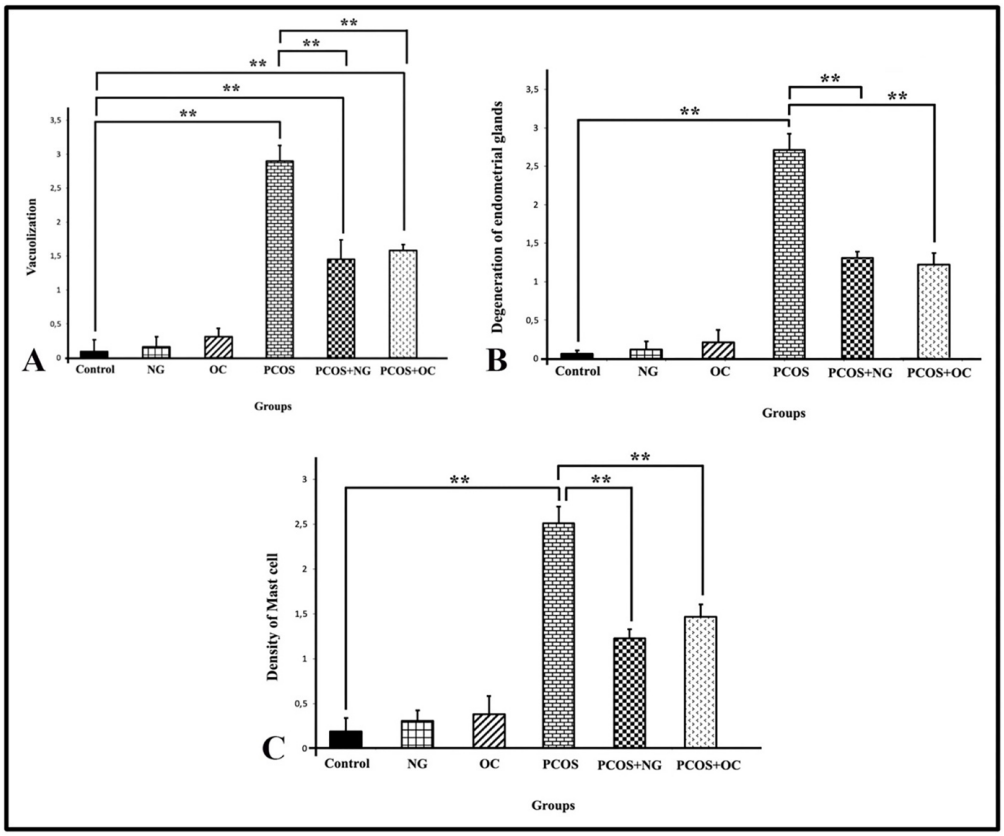

FIGURE 1. The graph shows vacuolization (A), degeneration of endometrial glands (B), and density of Mast cell (C) parameters in the uterus tissue belonging to all groups (n=7). Differences at the P<0.01 level are pointed out by "". NG, nateglinide; OC, octreotide; PCOS, polycystic ovary syndrome.**

PCOS+OC, P=0.003; Figure 1B) and Mast cell density (control vs PCOS, P=0.002; PCOS vs PCOS+NG, P= 0.005; PCOS vs PCOS+OC, P= 0.007; Figure 1C). In this context, it was determined that NG and OC statistically mitigated the effects of PCOS in terms of the evaluated parameters. In addition, no statistical significance was found among the control, NG, and OC groups in terms of vacuolization (control vs NG, P=0.390; control vs OC, P=1.000; NG vs OC, P=0.209), glandular degeneration (control vs NG, P=0.165; control vs OC, P=0.260; NG vs OC, P=0.535), and Mast cell density (control vs NG, P=0.188; control vs OC, P=0.130; NG vs OC, P=0.890). Furthermore, epithelium, gland structures and connective tissue areas in the uterine tissue were examined in detail. It was observed that the morphological structure was normal in the control, NG and OC groups. The single-layered prismatic epithelium of the endometrium and the loose connective tissue underneath it was found to be healthy. It was observed that the blood vessels and stromal cells in the connective tissue maintained their structural integrity (Figures 2A, B and C). In addition, it was also determined that the lumens of the uterine glands in the endometrium were clearly visible and the cells forming the gland epithelium were in a single-layered cuboidal epithelial structure (Figures 3A, B and C). Mast cell density in connective tissue was found to be normal (Figures 4A, B and C). No findings such as inflammation and hemorrhagic areas were observed in the tissue samples examined in the control, NG and OC groups.

While degeneration and vacuolization findings were observed in the epithelium of the endometrium layer in the PCOS group (Figure 2D), deformation was also detected in the cuboidal epithelial cells that form the structure of the endometrial glands. No edema, inflammation and hemorrhagic findings were observed in the connective tissue beneath the epithelium (Figure 3D). In addition, the increase in

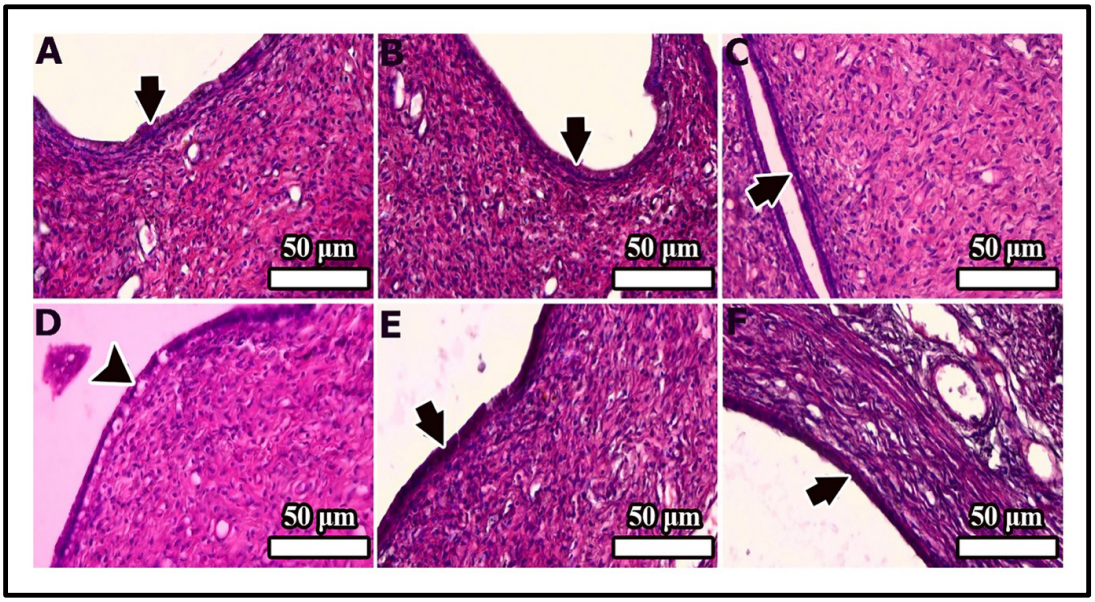

FIGURE 2. A, B, C, D, E and F show histological images at ×40 objective magnification of the Control, NG, OC, PCOS, PCOS+NG and PCOS+OC groups, respectively (Hematoxylin-Eosin staining). Black arrows show prismatic epithelium with normal structure; Black arrowhead shows vacuolization. NG, nateglinide; OC, octreotide; PCOS, polycystic ovary syndrome.

Mast cell density in connective tissue was remarkable (Figure 4D).

It was observed that the damage to the endometrium layer was minimized in the PCOS+NG and PCOS+OC groups. The single-layered columnar epithelium surrounding the lumen (Figures 2E and F) and most of the glands in the connective tissue (Figures 3E and F) were found to have normal morphology. Besides, there were no findings of edema, inflammation, and hemorrhagic areas in the

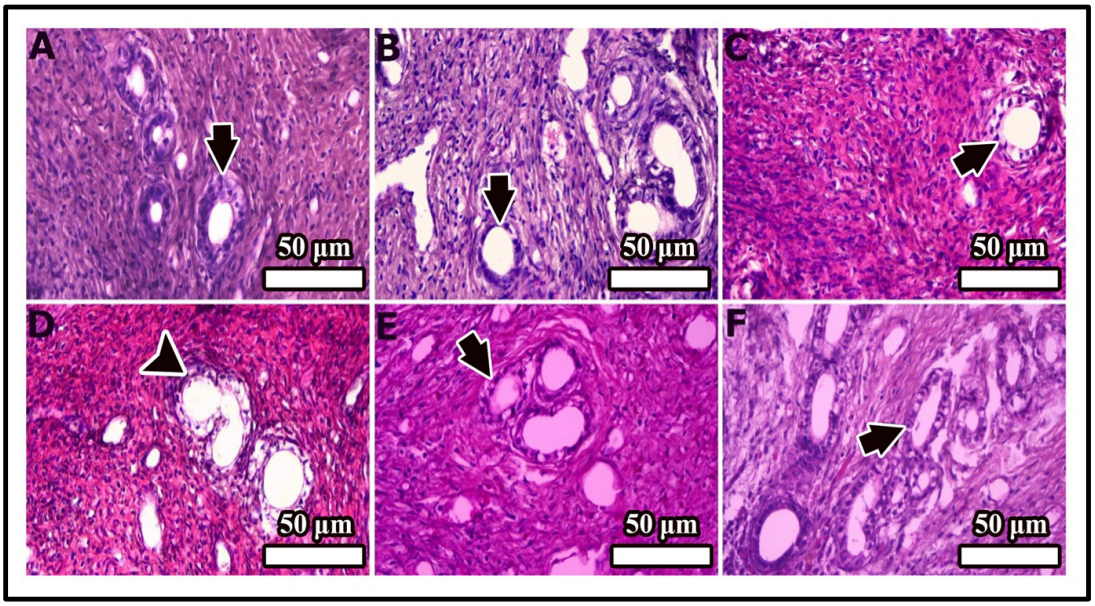

FIGURE 3. A, B, C, D, E and F show histological images at ×40 objective magnification of the Control, NG, OC, PCOS, PCOS+NG and PCOS+OC groups, respectively (Hematoxylin-Eosin staining). Black arrows show gland with normal structure; Black arrowheads show degenerated gland. NG, nateglinide; OC, octreotide; PCOS, polycystic ovary syndrome.

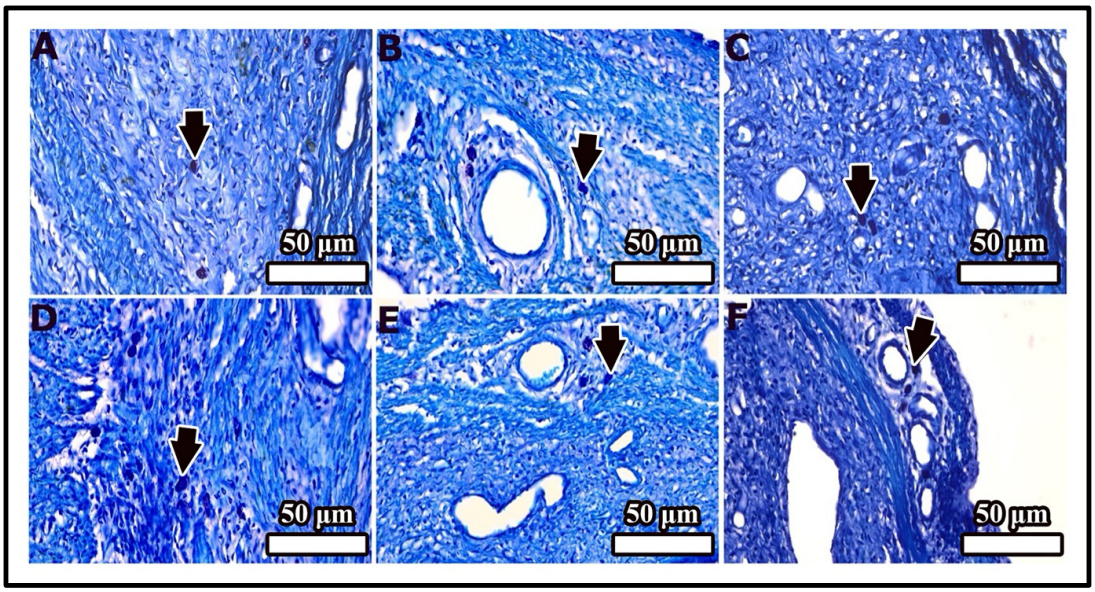

FIGURE 4. A, B, C, D, E and F show histological images at ×40 objective magnification of the Control, NG, OC, PCOS, PCOS+NG and PCOS+OC groups, respectively (Toluidine Blue staining). Black arrows show Mast cell. NG, nateglinide; OC, octreotide,; PCOS, polycystic ovary syndrome.

connective tissue beneath the epithelium. In addition, a decrease in Mast cell density in the connective tissue was detected compared to the PCOS group (Figures 4E and F).

DISCUSSION

Our experimental approach aimed to evaluate the histopathological effects of NG and OC on the uterus in rats affected by PCOS. Furthermore, the combination of a disease model (PCOS) and treatment controls (drug-only groups) facilitates a clear assessment of treatment efficacy in reversing PCOS-induced uterine pathology by comparing drug-treated PCOS groups with untreated PCOS controls. Also, our experimental design also allows for the evaluation of whether NG or OC induce independent uterine changes in the absence of PCOS by incorporating drug-only groups. This framework enables the differentiation between therapeutic effects and drug-related adverse outcomes, thereby providing a comprehensive basis for determining whether the observed histological changes are beneficial or iatrogenic.

The pathophysiology of PCOS is multifactorial, encompassing impaired pulsatility of gonadotropin-releasing hormone (GnRH), increased secretion of LH from the pituitary gland, elevated androgen levels [23], insulin resistance, obesity, and chronic low-grade inflammation [24]. Epidemiological indicate that 50-70% of cases of PCOS are linked to insulin resistance, frequently accompanied by compensatory hyperinsulinemia [25]. This association underscores a notable and prevalent pathological connection between insulin resistance and PCOS. Currently, there are four widely acknowledged phenotypes of PCOS as type A polycystic ovaries (PCO)+chronic oligo-anovulation+hyperandrogenism; Type B (oligo-anovulation+hyperandrogenism), Type C (PCO+ hyperandrogenism), and Type D (PCO+oligo-anovulation) [26]. Although insulin resistance is present across all phenotypes, insulin sensitivity varies depending on the specific type [27]. Energy metabolism plays a key role in maintaining normal endometrial function. Studies on the endometrium of patients with PCOS have shown that insulin resistance and hyperinsulinemia have a detrimental impact on endometrial physiology [28]. Specifically, the expression of insulin receptors, endometrial tissues exhibit the expression of molecules that are integral to insulin signaling pathways. However, in women with PCOS, the expression of insulin receptors, IRS proteins, AS160, PKC, and GLUT4 in the

endometrium is diminished and correlates with adverse reproductive outcomes [29].

Histopathological studies have demonstrated that PCOS induces significant alterations in uterine, including epithelial hyperplasia, glandular cystic dilation, stromal fibrosis, and increased inflammatory cell infiltration [30]. In this context, the histopathological evaluation of our investigation showed that both NG and OC mitigated PCOS-related damage to the uterine endometrium. They helped restore normal epithelial and glandular morphology and reduced mast cell accumulation in connective tissue, with no adverse tissue effects observed in drug-only groups. Both NG and OC have shown potential positive effects in reversing PCOS-induced uterine changes. These findings support the hypothesis that systemic metabolic and hormonal disturbances in PCOS significantly affect uterine histology. Interventions that target particularly insulin signaling and IGF-1 can partially restore uterine morphology and potentially improve fertility outcomes [24]. The possible role of insulin resistance and IGF-1 in the association between PCOS and endometrial cancer is supported by observations that endometrial dysfunction, causing miscarriages in PCOS, has been linked to insulin resistance and the associated elevation in serum IGF-1 levels in individuals with PCOS [31]. Given this connection, therapies that reduce insulin levels or modulate IGF-1 signaling could also mitigate the associated endometrial pathology. This view aligns with our findings: both drugs led to improved uterine histology in treated PCOS animals, with decreased mast cell density and normalized epithelial and glandular structures.

In the present study, the histopathological comparison among the study groups demonstrated significant differences between untreated PCOS animals and the other groups. In the control, NG-only, and OC-only groups, the uterine epithelium, gland structures, and connective tissue exhibited normal morphology. These findings suggest that neither NG nor OC induced histological changes in the absence of PCOS. In contrast, the group with PCOS displayed significant epithelial degeneration and vacuolization, accompanied by deformation of the cuboidal cells that comprise the endometrial glands. Although no edema, or hemorrhage was observed, the connective tissue showed an increase in mast cell density, pointing to an altered microenvironment consistent with PCOS-induced pathology. The treatment groups (PCOS+NG and PCOS+OC) exhibited a partial to near-complete restoration of normal uterine histology. The endometrial epithelium restored its columnar architecture, the gland structures appeared morphologically normal, and no pathological changes, such as hemorrhage or inflammation, were detected in the underlying connective tissue. Significantly, mast cell density was diminished in these groups compared to the untreated PCOS group, indicating a potential reversal of the local inflammatory or hormonal effects associated with PCOS. These findings align with literature linking hyperinsulinemia and elevated IGF 1 to endometrial thickening, hyperplasia, and infertility in PCOS [32, 33].

A study offers additional evidence supporting the use of NG as a therapeutic agent for type II diabetes. NG is a potent insulin secretagogue that can stimulate both KATP channel-dependent and -independent insulin secretion. Furthermore, it enhances the initiation and augmentation pathways for glucose-induced insulin release, indicating its potential effectiveness in both first- and second-phase insulin secretion [34]. So, NG's insulin-sensitizing action likely mitigates endometrial hyperplasia by reducing systemic insulin levels, improving endometrial stromal signaling pathways.

OC enhances the endocrinological environment in patients with PCOS without affecting the ovulation rate [35, 36]. Several studies have demonstrated the beneficial effects of OC in patients with PCOS [35, 37]. OC has been reported to be effective in normalizing ovarian IGF-1 and androgen secretions in patients with PCOS [36]. These findings highlight that targeting insulin/IGF-1 signaling may partially mitigate uterine damage caused by PCOS and underscore the need to broaden therapeutic objectives beyond ovarian function to encompass endometrial health, which is frequently neglected in the management of PCOS. This perspective is consistent with recent research that promotes metabolic interventions for addressing endometrial pathology linked to PCOS.

In summary, both NG and OC improved uterine histological architecture in PCOS rats, with distinct effects observed compared to the untreated PCOS

group and no changes in drug-only controls. This suggests that the observed benefits stem from targeted pharmacological effects rather than non-specific drug action or toxicity. These findings emphasize the need to expand PCOS treatment goals to include endometrial recovery, not just ovarian function, and highlight the promise of metabolic interventions in managing PCOS-related uterine pathology.

Strengths and Limitations

This study is among the limited experimental investigations assessing uterine tissue modifications linked to PCOS and the possible protective benefits of treatment drugs like nateglinide and octreotide at the histopathological level. The experimental design, comprising both PCOS and therapy agent-only groups, facilitated the distinction between disease-specific changes and treatment-related effects. All operations were performed under ethically authorized and standardized settings, with uniform sample sizes (n=7) in each group. Furthermore, the concurrent application of Hematoxylin-Eosin and toluidine blue staining facilitated a comprehensive analysis of epithelial, glandular, and stromal architectures.

The study indicated that endometrial diseases linked to PCOS may be partially reversible with therapy. Nevertheless, the study possesses specific limitations. The lack of morphometric or stereological quantification limited the quantitative validation of the histology results. Furthermore, the absence of immunohistochemical markers such as Ki-67, VEGF, and caspase-3 precluded the assessment of cellular proliferation, angiogenesis, and apoptotic processes. The absence of evaluation of functional fertility markers has generated ambiguity regarding the extent to which the noted histological enhancements correspond to reproductive recovery. Subsequent research employing stereological and molecular analyses, alongside immunohistochemical techniques, larger sample sizes, and extended follow-up, will elucidate the durability and functional relevance of the noted histological enhancements.

CONCLUSION

The current study demonstrates that both OC and NG significantly restore uterine histology in a letrozole-induced rat model of PCOS. While our study has findings highlight the importance of addressing endometrial pathology in PCOS models, there is a paucity of data regarding their impact on uterine histopathology. OC and NG play a therapeutic role in reversing uterine changes associated with the syndrome. Although these agents have demonstrated therapeutic effects in restoring stromal integrity and reducing inflammation, the underlying mechanisms need to be comprehensively confirmed by further studies. Given the integral role of the uterus in fertility and the known uterine alterations in PCOS, this represents a significant gap in literature. Moreover, most studies have focused on short-term outcomes, with limited exploration of long-term effects of post-treatment withdrawal. These findings underscore the promise of both medicines as viable therapeutic alternatives for addressing uterine abnormalities in PCOS, while stressing the need for thorough mechanistic and long-term research to validate their translational significance.

Ethics Approval and Consent to Participate

The present study was approved by the Adıyaman University Animal Experiments Local Ethics Committee (Decision No: 2022/030 and date: 26.05.2022). All experimental procedures were carried out accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

Data Availability

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

Authors' Contribution

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

Conflict of Interest

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

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Generative Artificial Intelligence Statement

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

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Therapeutic Effects of Alpha-Lipoic Acid on High-Dose Ibuprofen-Induced Renal Damage in Rats

Halime Tozak Yıldız[1]

[1]*Department of Histology and Embryology, Faculty of Medicine, Kırşehir Ahi Evran University, Kırşehir, Türkiye*

ABSTRACT

Objectives: Nonsteroidal anti-inflammatory drugs, particularly ibuprofen, are commonly used worldwide and can cause nephrotoxicity through prostaglandin inhibition, oxidative stress, and inflammatory activation. This study aimed to investigate the histopathological, fibrotic, and oxidative effects of high-dose ibuprofen administration and to evaluate the therapeutic potential of alpha-lipoic acid) in reversing these changes.

Methods: Twenty-eight male Wistar albino rats were randomly divided into four groups (n=7): Control, Alpha-lipoic acid (100 mg/kg), Ibuprofen (250 mg/kg), and Ibuprofen+Alpha-lipoic acid. Ibuprofen was administered orally for 21 days, while alpha-lipoic acid was given during the last 7 days. Histopathological changes were evaluated using Hematoxylin & Eosin, Masson's Trichrome, and Periodic Acid–Schiff staining. Fibrotic and inflammatory markers (TGF-β1, α-SMA, TLR-4) were assessed immunohistochemically. Oxidative stress was evaluated by measuring malondialdehyde levels and superoxide dismutase activity.

Results: Ibuprofen administration resulted in significant tubular degeneration, hydropic changes, necrosis, tubular dilatation, and hyperemia. Masson's Trichrome staining showed a significant increase in collagen deposition, while Periodic Acid-Schiff staining revealed glomerular and tubular basement membrane thickening. Immunohistochemistry demonstrated marked upregulation of TGF-β1, α-SMA, and TLR-4 ($P<0.001$). Biochemically, malondialdehyde levels were significantly increased ($P<0.01$) and superoxide dismutase activity was markedly decreased ($P<0.001$) compared to controls. Alpha-lipoic acid treatment significantly ameliorated these changes, reducing fibrosis and inflammatory marker expression and restoring malondialdehyde and superoxide dismutase levels toward normal ($P<0.05$).

Conclusions: Alpha-lipoic acid exerts renoprotective effects against ibuprofen-induced nephrotoxicity by reducing oxidative stress, modulating fibrotic pathways, and improving renal histoarchitecture, suggesting its potential as a therapeutic agent in drug-induced kidney injury.

Keywords: Ibuprofen, Alpha-Lipoic Acid, Nephrotoxicity, Renal Fibrosis, Oxidative Stress

Nonsteroidal anti-inflammatory drugs (NSAIDs), particularly over-the-counter agents such as ibuprofen (IBU), are widely used worldwide. This high level of consumption increases the absolute number of adverse drug events (ADEs) associated with NSAIDs in the general population [1, 2]. Although the nephrotoxicity of NSAIDs is generally considered to have a low

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Corresponding author: Halime Tozak Yıldız, PhD., Assist. Prof., Phone: +90 386 280 39 00, E-mail: htyildiz@ahievran.edu.tr, hhalimeyildiz@hotmail.com

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incidence, repeated exposure and high-dose administration can significantly contribute to kidney injury. NSAIDs reduce renal perfusion by inhibiting prostaglandin synthesis, leading to tubular ischemic injury, inflammation, and mitochondrial dysfunction; additionally, they increase oxidative stress at the tissue level, thereby promoting interstitial fibrosis [2–4]. An important factor underlying the kidney's organ-level susceptibility is its high perfusion: the kidneys receive approximately 20–25% of cardiac output, and this high blood flow facilitates the delivery of toxic agents to the tissue and enhances their metabolic effects, making the kidneys particularly vulnerable to drug-induced toxicity [5].

According to data from the Thai Food and Drug Administration (FDA)/ Health Product Vigilance Center (HPVC), drugs used for musculoskeletal disorders account for approximately 14% of reported adverse drug events (ADEs), with IBU and diclofenac among the top 15 drugs most frequently associated with ADEs. This highlights the widespread use of NSAIDs and their potential toxicity profile on a global scale [6]. The primary mechanism underlying NSAID-induced kidney injury involves COX-1 and COX-2 inhibition, leading to reduced prostaglandin production; this can result in renal vasoconstriction, acute kidney injury, and chronic fibrosis [7–10]. Transforming growth factor (TGF)-β1 signaling plays a central role in fibrogenesis by promoting fibroblast and myofibroblast activation, thereby enhancing extracellular matrix production and fibrosis [9, 10]. Furthermore, NSAID exposure increases reactive oxygen species (ROS) generation, elevating oxidative stress and facilitating cellular necrosis/apoptosis [11].

Alpha-lipoic acid (ALA) is a potent antioxidant that is soluble in both water and lipids; it supports cellular homeostasis through multiple mechanisms, including scavenging free radicals, reactivating endogenous antioxidants, chelating metal ions, and modulating inflammation [12, 13]. Exposure to agents such as IBU, which can induce kidney toxicity, increases oxidative damage, leading to interstitial fibrosis and functional impairment. ALA can protect renal function by reducing oxidative stress and inflammation in kidney tissue and by inhibiting TGF-β1 signaling, thereby preventing interstitial fibrosis [14, 15].

In conclusion, the multifaceted effects of ALA reducing oxidative stress, modulating inflammatory responses, and preventing fibrosis position it as a promising therapeutic agent for the prevention and treatment of kidney toxicity. This study aims to evaluate the histopathological, fibrotic, and oxidative effects of high-dose IBU on the kidney and to investigate the potential of ALA to prevent or reverse this damage.

METHODS

Ethics Approval and Experimental Procedures

All experimental procedures were carried out following the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments). Ethics approval was obtained from the Kırşehir Ahi Evran University Local Ethics Committee for Animal Experiments (decision no: 08/04, dated 24.04.2025).

Experimental Groups and Treatment Protocols

A total of 28 male Wistar albino rats, aged between 12 and 14 weeks, were included in the study. The animals were housed under controlled conditions at 25°C with a 12-hour light/dark cycle, provided with unrestricted access to water, and fed a standard laboratory diet at the Experimental and Clinical Research Center of Kırşehir Ahi Evran University, Kırşehir, Turkey.

The rats were randomly assigned into four experimental groups, with seven animals per group (n=7).

Control (C): Rats received 300 µL of corn oil orally for the last 7 days.

Alpha-lipoic acid (ALA): Rats were administered 100 mg/kg of alpha-lipoic acid (Sigma-Aldrich, Cas:1077-28-7, St. Louis, MO, USA) dissolved in 300 µL of corn oil via oral gavage during the last 7 days. [16]

Ibuprofen (IBU): Rats were given 250 mg/kg of ibuprofen (Brufen 400 mg, Turkey) orally via gavage for 21 consecutive days. [17].

Ibuprofen + Alpha-lipoic acid (IBU + ALA): Rats received 250 mg/kg of ibuprofen orally via gavage for 21 days, and during the last 7 days, 100 mg/kg of alpha-lipoic acid dissolved in 300 µL of corn oil was administered via oral gavage.

All procedures were performed at the same time of day to maintain consistency in experimental

conditions. On day 22, tissue samples were collected under general anesthesia, and the animals were euthanized (Figure 1).

Surgical Procedure

On the 22nd day of the experiment, all animals were anesthetized via intraperitoneal injection with ketamine hydrochloride (60 mg/kg) and xylazine hydrochloride (10 mg/kg, 2% solution). Under sterile conditions, a midline laparotomy was performed. Following the incision of the subcutaneous tissue and abdominal muscles, the left kidney was carefully excised. This kidney was fixed in formaldehyde for subsequent histopathological and immunohistochemical analyses. The right kidney was harvested, placed in Eppendorf tubes for biochemical assays, and stored at -80°C until further use

Histopathological Analysis

To histologically assess renal alterations in each experimental group, tissue samples collected at the end of the study were fixed in 10% formaldehyde. Following 72 hours of fixation, the tissues were rinsed under running water, dehydrated through a graded series of alcohols, cleared in xylene, and embedded in paraffin. Sections of 5 µm thickness were cut from the paraffin blocks and mounted onto slides (Leica, Autocut, 14051956472, Germany).

The slides were deparaffinized with xylene, rehydrated through a descending alcohol series (100%, 96%, 80%, 70%, 50%), and washed in water according to standard histological procedures. For general histological evaluation, sections were stained with Hematoxylin and Eosin (H&E) (Bio-Optica 05-06004/L Harris' Hematoxylin & Bio-Optica 05-10002/L Eosin Y 1%) and Periodic Acid-Schiff (PAS) (Best Lab, Turkey). After staining, the sections were dehydrated through an ascending alcohol series, cleared with xylene, and mounted with coverslips using Entellan. Histological examination was performed under a light microscope.

Renal Histopathological Scoring Method

Degenerative changes in the tubular and intertubular areas were assessed semi-quantitatively. In each kidney section, 10 different fields were

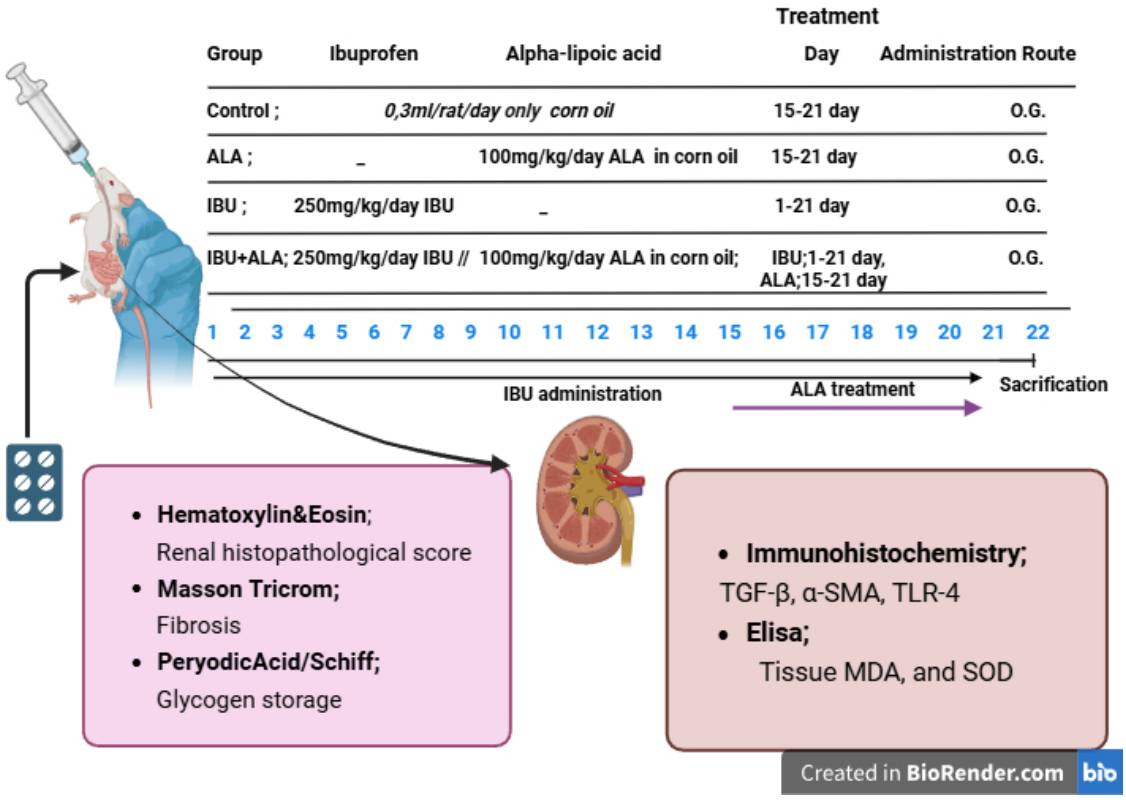

FIGURE 1. Schematic representation of the experimental design.

evaluated for each damage parameter, and average percentage values within each group were calculated. Histopathological changes were scored as follows: changes observed in less than 25% of tubular epithelial cells were scored as 1 (mild), 25–50% as 2 (moderate), 50–75% as 3 (severe), and 75–100% as 4 (very severe) (absence=0, mild=1, moderate=2, severe=3, very severe=4) [18].

Immunohistochemical Analysis

Immunohistochemical staining was performed using the Lab Vision™ UltraVision™ Large Volume Detection System (anti-polyvalent, HRP, TA-125-HL) in combination with the streptavidin-biotin-peroxidase method to assess TGF-β1, α-smooth muscle actin (α-SMA), and Toll-like receptor (TLR)-4 expression in kidney tissues. Five-micrometer sections from paraffin-embedded blocks were deparaffinized, rehydrated, and subjected to antigen retrieval in 5% citrate buffer (microwave, 600 W, 5 min). Endogenous peroxidase activity was blocked with 3% hydrogen peroxide (H_2O_2) for 20 min, and non-specific binding was prevented using blocking serum (room temperature, 10 min). Sections were incubated overnight at 4°C with primary antibodies: TGF-β1 (Proteintech, Cat. No. 21898-1-AP, 1:250), α-SMA (Proteintech, Cat. No. 80008-1-RR, 1:2500), and TLR4 (Proteintech, Cat. No. 19811-1-AP, 1:400). After washing, a biotinylated secondary antibody and streptavidin-peroxidase (Thermo Scientific, SHRP248-B) were applied, followed by DAB development (Lab Vision, TA-125-HL). The sections were counterstained with Mayer's hematoxylin, dehydrated through ascending alcohols, cleared in xylene, and mounted with Entellan. Images were captured from 20 fields under a light microscope, and immunoreactivity was quantified using ImageJ software (NIH, Washington, USA). [19]

Detecting oxidative stress indicators

Kidney tissues collected from all animals were stored at −80 °C. Prior to analysis, the tissues were homogenized and centrifuged, and the resulting supernatants were transferred to Eppendorf tubes for further use. Malondialdehyde (MDA; ELK-BIO, Catalog No: ELK10920) levels and superoxide dismutase (SOD; ELK-BIO, Catalog No: ELK8178) activity were measured using commercial ELISA kits according to the manufacturer's instructions. Optical densities were read using an ELISA reader (ELK808, BIOTEK), and the results were expressed as nmol/mg protein.

Statistical Analysis

The results obtained from the analyses were evaluated using the GraphPad Prism 9.0 statistical software. The Shapiro-Wilk test was performed to assess the normality of data distribution. For comparisons involving multiple groups, one-way analysis of variance (ANOVA) and the Kruskal-Wallis test were used. Post hoc analyses were conducted using the Bonferroni test following ANOVA and the Dunn test following the Kruskal-Wallis test, both of which identified significant differences between variables. A P-value of less than 0.05 was considered statistically significant for all analyses.

RESULTS

Alpha-Lipoic Acid Effectively Reduces Ibuprofen-Induced Renal Damage

In the renal tissues of the C and ALA groups, the glomeruli, cortex, and medullary tubules exhibited normal histological architecture. In the group administered IBU at a dose of 250 mg/kg for 21 days to induce nephrotoxicity, degenerative changes in the tubular epithelium, hydropic degeneration, necrotic tubular epithelial cells, tubular dilatation, and marked hyperemia in the intertubular and glomerular regions were observed. In the group treated with ALA during the last 7 days of IBU administration IBU+ALA, when compared with both the control and IBU groups, a marked reduction in tissue damage was detected. Restoration of normal histological architecture was observed in the stromal and parenchymal regions, with only a limited number of degenerative cells remaining (Figure 2).

In MT staining, increased collagen fiber density was observed as blue/purple areas in the IBU group, whereas this increase was milder in the IBU+ALA group. PAS staining revealed prominent thickening of glomerular and tubular basement membranes, along with intense staining and glycogen accumulation in the tubular epithelium in the IBU group. In contrast,

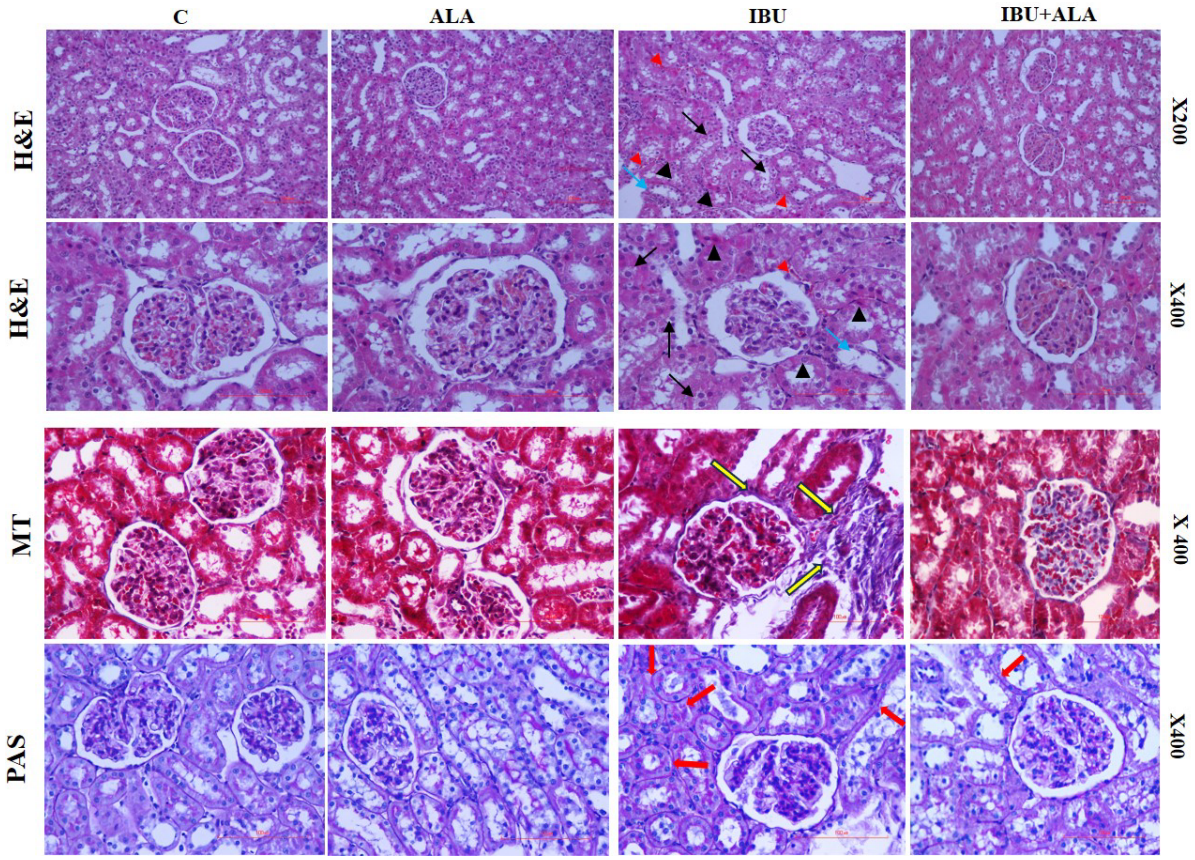

FIGURE 2. Histological sections of renal tissue (Nikon Eclipse Si, Tokyo, Japan; scale bar: 100 µm). Hematoxylin&Eosin (H&E) stained sections show preserved normal histological architecture of glomeruli, cortex, and medullary tubules in Control (C) and Alpha-lipoic acid (ALA)-only groups. In the Ibuprofen (IBU) group, marked histopathological changes are observed including necrotic tubular epithelial cells (black arrow), hydropic degeneration (black arrowhead), tubular dilatation (blue arrow), and widespread hyperemia in glomerular and intertubular regions (red arrowhead). In the IBU+ALA group, histological abnormalities are significantly reduced compared to the IBU group, with largely preserved tubular and glomerular structures and only limited presence of degenerative cells. Masson's Trichrome (MT) stained sections show intense collagen accumulation (yellow arrow) in the IBU group, which is significantly reduced in the IBU+ALA group. Periodic Acid-Schiff (PAS) staining reveals thickening of tubular and glomerular basement membranes, glycogen accumulation, and structural changes in Bowman's capsule (red arrow) in the IBU group, while these changes are minimal and similar to the control groups in the IBU+ALA group.

basement membrane thickness and glycogen accumulation in the IBU+ALA group were minimal and similar to the control groups (Figure 2).

Histopathological scoring revealed a marked increase in tubular degeneration, necrosis, dilatation, vascular hyperemia, collagen deposition, and glycogen accumulation in the IBU group compared with the C and ALA groups ($P<0.001$). Among these parameters, tubular degeneration and necrosis reached the highest scores, indicating severe IBU-induced nephrotoxicity. In contrast, co-administration of ALA (IBU+ALA group) significantly ameliorated these pathological changes ($P<0.01$), showing a clear reduction in tubular and vascular injury, as well as in collagen and glycogen accumulation. These findings suggest that ALA treatment effectively mitigated IBU-induced renal damage and partially restored normal histological architecture (Figure 3).

Alpha-Lipoic Acid Improves Ibuprofen-Induced Tubulointerstitial Fibrosis

In renal tissues, immunoreactivity of TGF-β1, a key marker of fibrosis, was minimal in C and ALA-only groups, with limited staining in glomerular and

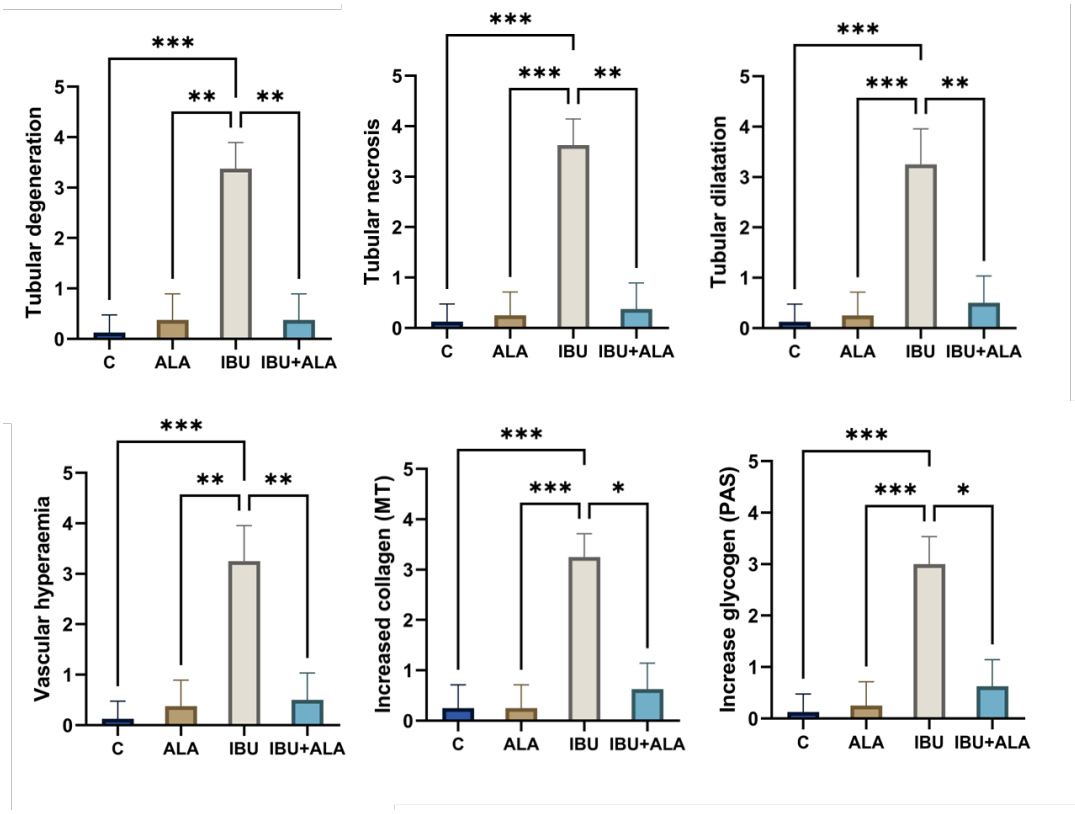

FIGURE 3. Histopathological scoring of renal tissue across experimental groups. Quantitative evaluation of tubular degeneration, tubular necrosis, tubular dilatation, vascular hyperemia, collagen deposition, and glycogen accumulation in Control (C), Alpha-lipoic acid (ALA), Ibuprofen (IBU), and IBU+ALA groups. Data are expressed as mean±standard deviation. *P<0.05, P<0.01, and *P<0.001 indicate statistically significant differences between the groups.

tubular areas. In the IBU group, TGF-β1 expression was significantly increased, particularly showing widespread cytoplasmic staining in the tubulointerstitial area. This increase was markedly reduced in the IBU+ALA group, with staining intensity approaching that of the control groups. Similarly, α-SMA immunoreactivity was very low in control and ALA groups but markedly elevated in glomerular and interstitial regions of the IBU group, indicating myofibroblast activation. ALA treatment significantly decreased α-SMA expression, bringing it close to control levels. TLR-4 expression also significantly increased in the IBU group, with strong cytoplasmic staining in glomerular, tubular, and interstitial areas, while the IBU+ALA group showed reduced and more limited TLR-4 staining. These findings suggest that ALA exerts a protective effect on renal tissue by suppressing IBU-induced fibrotic and inflammatory responses (Figure 4).

TGF-β1 and TLR-4 immunoreactivity remained low in the C and ALA groups but showed a statistically significant increase in the IBU group (P<0.001). This elevation was markedly reduced in the IBU+ALA group, approaching control levels (P<0.001). Similarly, α-SMA immunoreactivity was significantly elevated in the glomerular and interstitial regions of the IBU group (P<0.001); however, ALA treatment markedly suppressed this increase, restoring levels close to those of the controls (P<0.05). These findings indicate that ALA attenuates IBU-induced fibrotic and inflammatory responses, exerting an immunoregulatory effect in renal tissue (Figure 5).

Alpha-Lipoic Acid Reduces Oxidative Stress by Modulating MDA and SOD Levels

MDA, lipid peroxidation marker, was significantly elevated in the IBU group compared to the control (C) and ALA groups (P<0.001), indicating pronounced oxidative stress following IBU exposure. In contrast, MDA levels were significantly reduced in the IBU+ALA group compared to the IBU group (P<0.01), though they did not completely return to

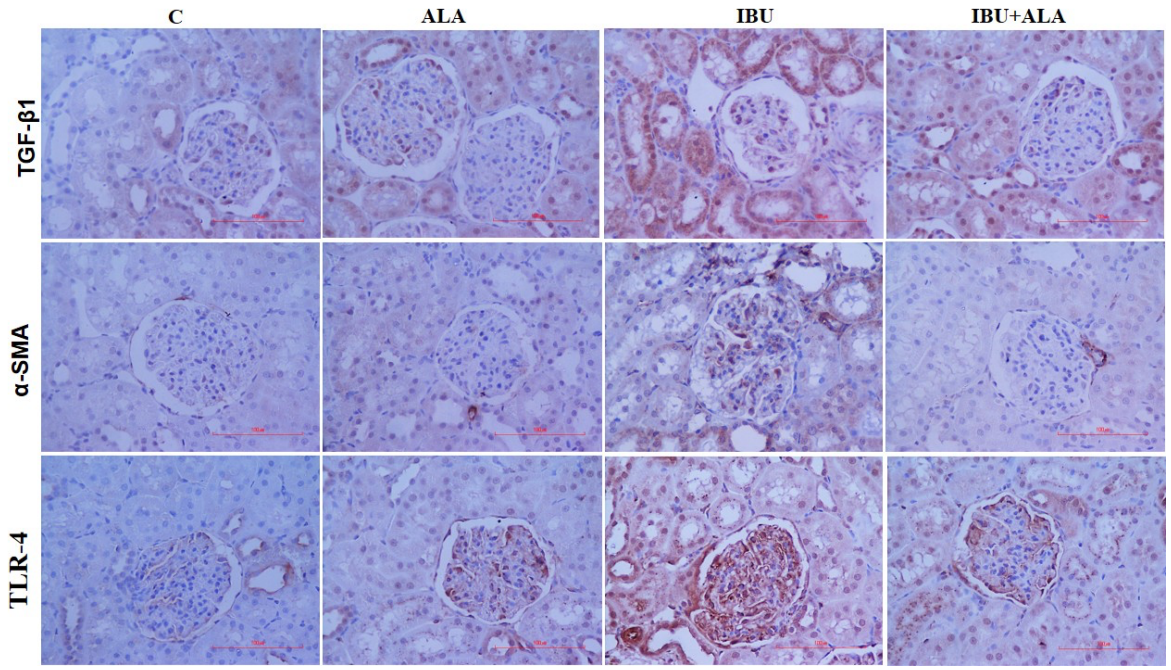

FIGURE 4. Immunohistochemical staining of TGF-β1, α-SMA, and TLR-4 in renal tissues (scale bar: 100 µm, Magnification: 400×). Minimal immunoreactivity was observed in Control (C) and Alpha-lipoic acid (ALA) groups. In the Ibuprofen (IBU) group, expressions of TGF-β1, α-SMA, and TLR-4 were significantly increased, with intense positive staining in tubulointerstitial areas. In the IBU+ALA group, these increases were markedly reduced, showing staining patterns similar to the control group.

control levels. Regarding antioxidant status, SOD activity was highest in the ALA group, showing a significant increase compared to control (P<0.001). IBU administration caused a marked reduction in SOD activity compared to both C and ALA groups (P<0.001), confirming suppression of endogenous antioxidant defenses. However, in the IBU+ALA group, SOD activity was significantly improved compared to the IBU group (P<0.001), approaching levels observed in the control group. These results

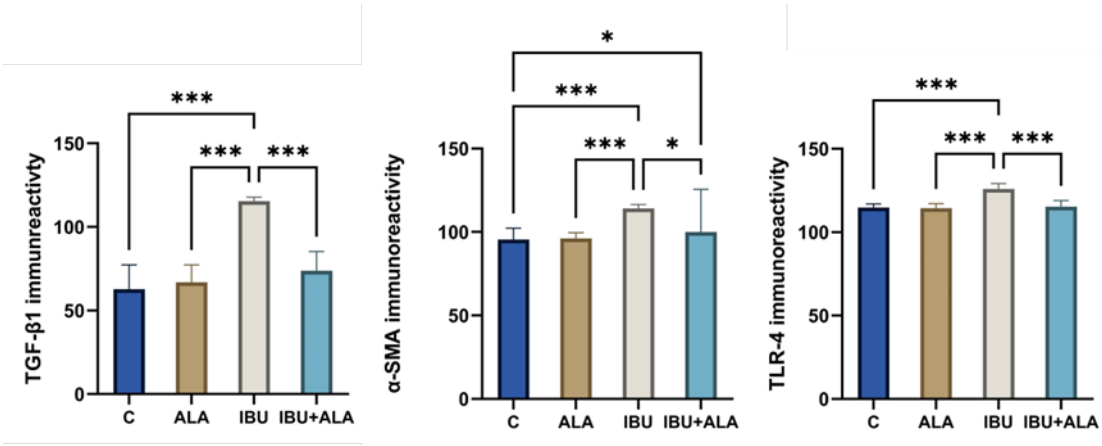

FIGURE 5. Immunohistochemical staining of TGF-β1, α-SMA, and TLR-4 in renal tissues (scale bar: 100 µm, Magnification: Quantitative analysis of TGF-β1, α-SMA, and TLR-4 immunoreactivity in renal tissues. Ibuprofen (IBU) treatment significantly increased the expression of all three markers (*P<0.001), while Alpha-lipoic acid (ALA) co-treatment markedly reduced these elevations, approaching control levels. Data are presented as mean±standard deviation. *P<0.05 and ***P<0.001 indicate statistically significant differences between groups.**

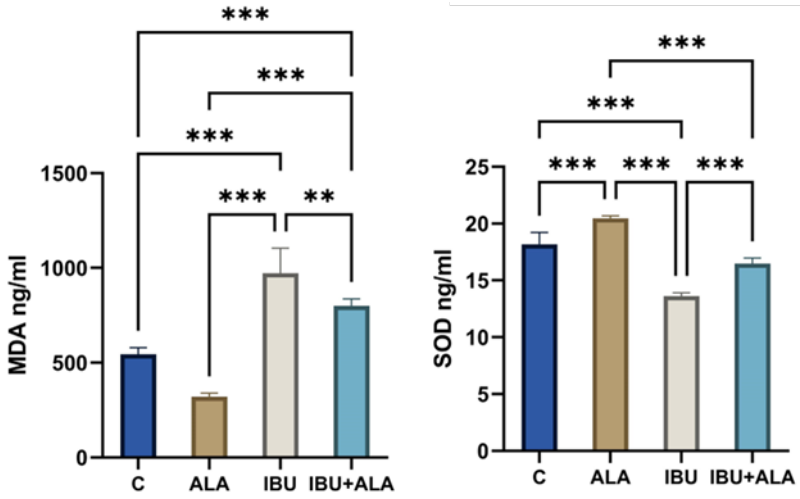

FIGURE 6. Renal tissue oxidative stress parameters. Quantitative analysis of malondialdehyde (MDA) levels and superoxide dismutase (SOD) enzyme activity. Data are presented as mean±standard deviation. **P<0.01, ***P<0.001 indicate statistically significant differences between groups.

collectively suggest that ALA supplementation attenuates IBU-induced oxidative damage by both reducing lipid peroxidation and restoring antioxidant enzyme activity (Figure 6).

DISCUSSION

In the present study, the nephrotoxic potential of high-dose ibuprofen (IBU, 250 mg/kg) and the protective effects of alpha-lipoic acid (ALA, 100 mg/kg) were comprehensively evaluated using histopathological, immunohistochemical, and biochemical approaches. Prolonged IBU exposure caused distinct renal injury characterized by tubular degeneration, necrosis, glomerular and interstitial congestion, and excessive collagen accumulation, accompanied by increased TGF-β1, α-SMA, and TLR-4 expression. These findings indicate that IBU promotes oxidative stress, inflammation, and fibrosis in renal tissue. Co-administration of ALA markedly mitigated these alterations, preserved renal architecture, reduced fibrotic and inflammatory responses, and restored antioxidant balance, as evidenced by lower MDA and higher SOD levels. These findings indicate that ALA exerts renoprotective effects against IBU-induced nephrotoxicity by modulating oxidative stress, inflammation, and fibrogenesis.

Histopathological analyses confirmed the severity of IBU-induced renal injury, showing tubular epithelial degeneration, necrosis, tubular dilatation, and pronounced hyperemia in glomerular and intertubular regions. These alterations align with the classical nephrotoxic profile of NSAIDs, which induce renal hypoperfusion and oxidative stress through prostaglandin depletion [2-5, 20-22]. Conversely, ALA administration preserved the structural organization of the renal parenchyma, markedly reducing necrosis and interstitial disorganization. In Masson's Trichrome staining, collagen accumulation was substantially lower, and basal membrane thickening and glycogen accumulation observed in the PAS staining were restored toward normal morphology. These improvements demonstrate ALA's capacity to maintain renal histoarchitecture and attenuate tissue injury, in agreement with previous studies reporting that ALA preserves renal morphology and reduces collagen accumulation in nephrotoxicity models [14, 23, 24].

The upregulation of TGF-β1 and α-SMA in the IBU group indicates activation of fibrogenic pathways responsible for tubular injury and interstitial remodeling. TGF-β1, a central mediator of renal fibrosis, activates both Smad-dependent and Smad-independent signaling to promote fibroblast-to-myofibroblast differentiation, leading to excessive collagen synthesis and extracellular matrix deposition [25]. α-SMA serves as a hallmark of myofibroblast activation and correlates with extracellular matrix accumulation in renal fibrosis

models such as diabetic kidney disease [26]. The suppression of TGF-β1 and α-SMA expression in the ALA-treated group suggests that ALA mitigates IBU-induced fibrogenesis by modulating the TGF-β1/Smad signaling cascade, consistent with previous evidence demonstrating the antifibrotic potential of ALA in renal injury models [26, 28].

Comprehensive reviews and recent studies have emphasized the renoprotective roles of ALA, including its ability to reduce oxidative stress, suppress inflammation, and modulate fibrogenic pathways; therefore, our finding that ALA partially reversed IBU-induced fibrosis is consistent with the established evidence in the literature [27, 29]. Lipid peroxidation mediated by ROS, reflected by elevated MDA levels, along with a decline in antioxidant defense systems particularly reduced SOD activity is widely recognized as an early indicator of renal injury. For instance, a recent study demonstrated that ALA significantly attenuated oxidative stress in an LPS-induced kidney injury model by reducing MDA and enhancing SOD activity [27]. In our study, IBU administration resulted in a marked increase in MDA levels and a significant decrease in SOD activity, suggesting enhanced lipid peroxidation and impaired endogenous antioxidant defense in renal tissue. These findings are consistent with reports from an iron-overload-induced nephrotoxicity model, where 100 mg/kg ALA treatment significantly reduced MDA levels and restored SOD and other antioxidant enzyme activities [30]. Moreover, in an LPS-induced renal oxidative stress model, ALA administration has been reported to reduce TBARS and other oxidative markers while enhancing antioxidant defense systems such as SOD and total glutathione [27]. Therefore, our findings confirm the capacity of ALA to mitigate ibuprofen-induced oxidative damage and are consistent with the growing body of evidence in the literature.

Strengths and Limitations

The study was conducted using a single animal species and sex, which may restrict generalizability. Moreover, specific intracellular signaling pathways underlying ALA-mediated protection were not directly investigated. The evaluation was limited to a single ALA dose and treatment duration, and functional renal biomarkers were not assessed. Future studies addressing these aspects would further strengthen the translational value of the results.

CONCLUSION

In conclussion, this study demonstrates that high-dose ibuprofen induces significant renal damage characterized by tubular degeneration, interstitial fibrosis, increased TGF-β1, α-SMA, and TLR-4 expression, as well as enhanced oxidative stress, as evidenced by elevated MDA levels and decreased SOD activity. Importantly, alpha-lipoic acid treatment significantly attenuated these changes, restoring renal histoarchitecture, reducing fibrosis and inflammatory marker expression, and improving oxidative balance. These findings highlight the potential of ALA as a therapeutic option for mitigating drug-induced nephrotoxicity and preventing progression toward chronic kidney disease. Future studies should focus on dose optimization and elucidation of the molecular mechanisms underlying its renoprotective effects.

Ethics Approval and Consent to Participate

This study was approved by the Kırşehir Ahi Evran University Animal Experiments Local Ethics Committee (Decision No: 08/4; date: 24.04.2025). All experimental procedures involving animals were conducted in accordance with the ethical standards of the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Data Availability

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

Authors' Contribution

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

Conflict of Interest

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

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Generative Artificial Intelligence Statement

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

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Comparative Outcomes of Surgery and Chemoradiotherapy After Neoadjuvant Chemotherapy in Stage II-III Bladder Cancer

Doğan Bayram[1], Öznur Bal[1], Alper Türkel[2], Serhat Sekmek[1], Emre Hafızoğlu[1], Berkan Karabuğa[2], Mutlu Doğan[2], Efnan Algın[1], Doğan Uncu[1]

[1]Department of Medical Oncology, Ankara Bilkent City Hospital, Ankara, Türkiye; [2]Department of Medical Oncology, Dr. Abdurrahman Yurtaslan Oncology Training and Research Hospital, Ankara, Türkiye

ABSTRACT

Objectives: Radical cystectomy following neoadjuvant chemotherapy is the standard treatment for Muscle-Invasive Bladder Cancer (MIBC). However, definitive chemoradiotherapy may represent a viable alternative in patients who are medically inoperable or decline surgery. This study aimed to compare the clinical outcomes of patients with stage II–III MIBC treated with neoadjuvant chemotherapy followed by either Radical cystectomy or CRT, the latter performed without maximal transurethral resection of bladder tumor (TURBT).

Methods: This retrospective study included 63 patients with stage II–III MIBC treated between December 2014 and March 2025 at two tertiary referral centers in Türkiye. All patients received neoadjuvant chemotherapy (NAC) prior to either surgery (n=39) or Chemoradiotherapy (CRT) (n=24). Clinicopathological, laboratory, and survival data were analyzed. Overall Survival (OS) and Event-Free Survival (EFS) were assessed using the Kaplan–Meier method and compared with the log-rank test. Cox regression was used to identify independent prognostic factors.

Results: The median age was 64 years, and 88.9% of patients were male. Comorbidities were more frequent in the CRT group (79.2% vs. 59%), though the difference was not statistically significant (P=0.099). Median OS was 46.5 months in the surgery group and 31.6 months in the CRT group (P=0.407), while median EFS was 30.1 and 21.0 months, respectively (P=0.375). Distant metastasis was the most common recurrence pattern (36.5%). Multivariate analysis identified comorbidity (Hazard ratio [HR] = 0.37, 95% CI: 0.17–0.80, P=0.012) and hemoglobin <12 g/dL (HR =0.53, 95% CI: 0.25–0.94, P=0.048) as independent predictors of poor survival.

Conclusions: NAC followed by either surgery or CRT provides comparable long-term disease control in patients with stage II–III MIBC. Although RC remains the gold standard for operable patients, CRT offers an effective curative-intent option for those unfit for surgery—even in the absence of maximal TURBT. Comorbidity and anemia were significant adverse prognostic factors, emphasizing the importance of individualized treatment selection in this patient population.

Keywords: Muscle-Invasive Bladder Cancer, Neoadjuvant Chemotherapy, Radical Cystectomy, Chemoradiotherapy, Comorbidity, Prognosis, Overall Survival, Event-Free Survival

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Corresponding author: Doğan Bayram, MD., Phone: +90 312 552 60 00, E-mail: drdoganb@gmail.com

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Urothelial carcinoma (transitional cell carcinoma) originates from the urinary bladder and represents the second most common malignancy of the genitourinary system following prostate cancer [1]. According to the 2025 estimates from the American Cancer Society, bladder cancer accounts for approximately 6% of all newly diagnosed cancers among men, ranking as the fourth most commonly diagnosed malignancy and the eighth leading cause of cancer-related death, responsible for around 4% of all cancer mortalities in males [2]. According to 2020 data, the incidence of bladder cancer in Türkiye exceeds 8.6 cases per 100,000 population [3].

Urothelial carcinoma constitutes the predominant histological type of bladder cancer. Variant histologic subtypes are frequently observed, particularly in high-grade tumors, and may include squamous, glandular, or sarcomatoid differentiation. In cases with mixed histologic features, these divergent elements are often associated with more aggressive tumor behavior and poorer clinical outcomes [4].

Bladder cancer staging is based on the American Joint Committee on Cancer (AJCC) TNM classification, which evaluates the local extent of the primary tumor, lymph node involvement, and the presence of distant metastases. Urothelial carcinoma may present as non–muscle-invasive, muscle-invasive bladder carcinoma (MIBC), or metastatic disease, each stage representing a continuum of tumor progression with distinct treatment strategies and prognostic implications. Invasion of the muscularis propria indicates the onset of muscle invasive disease, corresponding to stage T2 or higher [5].

The management of MIBC differs substantially from that of non–muscle-invasive disease, given its higher risk of progression and metastasis.
Cisplatin-based neoadjuvant chemotherapy (NAC) prior to radical cystectomy (RC) has demonstrated improved survival, enhanced pathological downstaging, and higher pathological complete response rates compared to RC alone. Administration of cisplatin-based chemotherapy before RC has been associated with superior survival outcomes, greater pathological downstaging, and an increased likelihood of achieving a complete pathological response relative to RC alone [6, 7]. Although RC remains the cornerstone of curative treatment for MIBC, bladder-preserving chemoradiotherapy (CRT) is increasingly used as an alternative in elderly, comorbid, medically inoperable, or surgery-refusing patients [8].

Our study aimed to compare the clinical outcomes of patients with stage II–III MIBC who received NAC followed by surgery with patients who, after neoadjuvant treatment, were not candidates for surgery due to comorbidities, medical inoperability, or personal preference and were managed with CRT.

METHODS

This retrospective study was conducted at two tertiary referral centers in Türkiye - the Medical Oncology Department of Ankara City Hospital and Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital - and included patients diagnosed with MIBC between December 2014 and March 2025. All patients were aged 18 years or older at the time of diagnosis. Clinicopathological and demographic data, including age, sex, smoking history, disease stage, histopathologic features, and treatment details, were retrieved from institutional medical records. Tumor staging was performed according to the criteria outlined in the 8th edition of the AJCC Staging Manual [5].

The study population consisted of patients with stage II–III MIBC who had received NAC followed by either surgery or CRT. In total, 63 patients were included, with 39 assigned to the surgery group and 24 to the CRT group. Patients who underwent maximal TURBT prior to CRT as part of trimodality therapy were excluded from the analysis to maintain treatment homogeneity.

Laboratory parameters were obtained from hospital electronic medical records at the time of diagnosis. Hematologic and biochemical parameters included hemoglobin (Hb), white blood cell (WBC) count, glomerular filtration rate (GFR), and lactate dehydrogenase (LDH). WBC count was categorized as <11,000/μL or ≥11,000/μL. Hemoglobin levels were classified as <12 g/dL or ≥12 g/dL to define the presence or absence of anemia. Renal function and potential kidney impairment were assessed using GFR, stratified as <60 or ≥60 mL/min/1.73 m². LDH levels were grouped as <225 or ≥225 U/L.

Overall Survival (OS) was described as the period

between the initial diagnosis and death from any cause or the last recorded follow-up, while Event-Free Survival (EFS) referred to the time from diagnosis to the first occurrence of recurrence, disease progression, or death.

Statistical Analysis

All statistical analyses were conducted using SPSS software version 22.0 (IBM Corp., Armonk, NY). Descriptive data were summarized as frequencies and percentages for categorical variables, and as medians with interquartile ranges (IQRs) for continuous variables. The Mann–Whitney U test was used to compare continuous variables, whereas categorical variables were evaluated using either Pearson's Chi-square or Fisher's exact test, as appropriate. Survival outcomes were analyzed using the Kaplan–Meier method with comparisons made by the log-rank test. Multivariable analyses were performed through the Cox proportional hazards model, and adjusted hazard ratios (HRs) with corresponding 95% confidence intervals (CIs) and p-values were reported. A P-value below 0.05 was considered statistically significant.

RESULTS

Baseline Characteristics

The median age of the entire cohort was 64 years (range: 42–82years). Patients in the CRT group tended to be older (median: 69 years) than those in the surgical group (median: 62 years), although the difference was not statistically significant (P=0.265). The majority of patients were male (88.9%), with similar sex distribution between groups (89.7% vs. 87.5%, P=0.783).

A history of smoking was observed in 77.8% of the patients, and there was no statistically significant difference between the groups (P=0.835). Comorbidities were observed in 66.7% of the entire cohort, being more frequent in the CRT group (79.2%) compared to the surgical group (59%), yet this did not reach statistical significance (P=0.099). The most common comorbidities were diabetes mellitus (28.5%), hypertension (26.9%), coronary artery disease (23.8%), and chronic obstructive pulmonary disease (17.4%). Chronic kidney disease was identified in 14.2% of patients, while cerebrovascular disease, rheumatoid arthritis, and Hodgkin lymphoma were rare.

Most patients had an ECOG performance score of 0–1 (77.8%), while only one (1.6%) patient had a score of 3. ECOG status did not differ significantly between groups (P=0.583). The median tumor size at presentation was 28 mm (range, 10–160 mm), comparable across groups (P=0.964).

The majority of patients (76.2%) had a WBC count below 11,000/μL, with no significant difference between the surgery and CRT groups (P=0.862). Anemia (hemoglobin <12 g/dL) was present in 34.9% of the overall cohort, more frequently in the CRT group (41.7%) than in the surgery group (30.8%), though this difference did not reach statistical significance (P=0.378).

Renal function, as assessed by GFR, was preserved (≥60 mL/min/1.73 m²) in 76.2% of patients, and the distribution between treatment groups was comparable (P=0.862). In contrast, serum LDH levels were significantly higher in the CRT group, with elevated LDH (≥225 U/L) observed in 50% of CRT patients compared to 23.1% in the surgery group (P=0.028).

Invasive urothelial carcinoma represented the predominant histology (87.3%), followed by micropapillary (7.9%), plasmacytoid (3.1%), and small cell neuroendocrine carcinoma (1.6%). All patients in the CRT group had conventional urothelial carcinoma, whereas variant histologies were seen exclusively in the surgery group (P=0.253).

Squamous differentiation was identified in nearly half of all patients (47.6%), being more frequent in the surgery group (56.4%) compared with CRT (33.3%) (P=0.075). Glandular differentiation was detected in 15.9%, and sarcomatoid differentiation in 11.1% of patients, with similar distribution between the two groups (P>0.05).

Based on preoperative imaging and pathological assessment, the most common T stages were T2b (27%) and T3b (23.8%), followed by T2a (23.8%), T4a (17.5%), and T3a (7.9%). The stage distribution was comparable between the two groups (P=0.074). Lymph node metastasis was present in 71.4% of patients, with the majority having N1–N2 involvement. N3 disease was detected in 10.3% of surgical patients but not in the CRT group. No statistically significant difference was observed

TABLE 1. Baseline Clinicopathological and Laboratory Characteristics of the Study Population

Variables	All patients (n=63)	Surgery group (n=39)	CRT group (n=24)	P-value
Age (years) (median)	64	62	69	0.265
Gender				0.783
Male	56 (88.9%)	35 (89.7%)	21 (87.5%)	
Female	7 (11.1%)	4 (10.3%)	3 (12.5%)	
Smoking history				0.835
None	14 (22.2%)	9 (23.1%)	5 (20.8%)	
Ex-smoker/ Current smoker	49 (77.8%)	30 (76.9%)	19 (79.2%)	
Comorbidity				0.099
Yes	42 (66.7%)	23 (59%)	19 (79.2%)	
No	21 (33.3%)	16 (41%)	5 (20.8%)	
Diabetes mellitus	18 (28.5%)	11 (28.2%)	7 (29.1%)	
Hypertension	17 (26.9%)	9 (23.1%)	8 (33.3%)	
Coronary artery disease	15 (23.8%)	6 (15.4%)	9 (37.5%)	
COPD	11 (17.4%)	5 (12.8%)	6 (25%)	
Chronic kidney disease	9 (14.2%)	4 (10.2%)	5 (20.8%)	
Cerebrovascular disease	3 (4.6%)	1 (2.5%)	2 (8.3%)	
Hodgkin lymphoma	1 (1.6%)	1 (2.5%)	0 (0%)	
Rheumatoid arthritis	1 (1.6%)	0 (0%)	1 (4.1%)	
ECOG				0.583
0	14 (22.3%)	12 (30.7%)	2 (8.3%)	
1	35 (55.5%)	19 (48.7%)	16 (66.7%)	
2	13 (20.6%)	7 (17.9%)	6 (25.0%)	
3	1 (1.6%)	1 (2.6%)	0 (0%)	
Tumor Size (mm)	28 (10-160)	29(11-160)	27 (10-8)	0.964
Histology				0.253
Invasive urothelial carcinoma	55 (87.3%)	31(79.5%)	24(100%)	
Micropapillary urothelial carcinoma	5(7.9%)	5(12.8%)	0(0%)	
Plasmacytoid urothelial carcinoma	2(3.1%)	2(5.1%)	0(0%)	
Small cell neuroendocrine carcinoma	1(1.6%)	1(2.6%)	0(0%)	
Squamous differentiation				0.075
Yes	30(47.6%)	22(56.4%)	8(33.3%)	
No	33(52.4%)	17(43.6%)	16(66.7%)	
Glandular differentiation				0.398
Yes	10(15.9%)	5(12.8%)	5(3.8%)	
No	53(84.1%)	34(87.2%)	19(79.2%)	
Sarcomatoid differentiation				0.582
Yes	7(11.1%)	5(12.8%)	2(8.3%)	
No	56(88.9%)	34(87.2%)	22(91.7%)	

TABLE 1 Continued. Baseline Clinicopathological and Laboratory Characteristics of the Study Population

Variables	All patients (n=63)	Surgery group (n=39)	CRT group (n=24)	P-value
Primary tumor T stage				0.074
T2a	15 (23.8%)	7 (17.9%)	8 (33.3%)	
T2b	17 (27%)	10 (25.6%)	7 (29.2%)	
T3a	5 (7.9%)	2 (5.1%)	3 (12.5%)	
T3b	15 (23.8%)	13 (3.3%)	2 (8.3%)	
T4a	11 (17.5%)	7 (17.9%)	4 (16.6%)	
Regional lymph nodes				0.133
N0	18 (28.6%)	11 (28.2%)	7 (29.2%)	
N1	19 (30.2%)	8 (20.5%)	11 (45.8%)	
N2	22 (34.9%)	16 (41.0%)	6 (25%)	
N3	4 (6.3%)	4 (10.3%)	0 (0%)	
TNM staging				0.315
Stage 2	11 (17.5%)	6 (15.4%)	5 (20.8%)	
Stage 3a	26 (41.3%)	13 (33.3%)	13 (54.2%)	
Stage 3b	26 (41.3%)	20 (51.3%)	6 (25%)	
WBC (/µL)				0.862
< 11.000	48 (76.2%)	30 (76.9%)	18 (75%)	
≥11.000	15 (23.8%)	9 (23.1%)	6 (25%)	
Hb (g/dL)				0.378
<12	22 (34.9%)	12 (30.8%)	10 (41.7%)	
≥12	41 (65.1%)	27 (69.2%)	14 (58.3%)	
GFR (mL/dk/1.73 m²)				0.862
<60	15 (23.8%)	9 (23.1%)	6 (25%)	
≥60	48 (76.2%)	30(76.9%)	18(75%)	
LDH (U/L)				**0.028**
<225	42 (66.7%)	30 (76.9%)	12 (50%)	
≥225	21 (33.3%)	9 (23.1%)	12 (50%)	

Data are shown as median (minimum-maximum) or n (%) where appropriate. COPD, chronic obstructive pulmonary disease; CRT, chemoradiotherapy; ECOG, Eastern Cooperative Oncology Group; GFR, glomerular filtration rate; Hb, hemoglobin; LDH, lactate dehydrogenase; TNM, tumor–node–metastasis; WBC, white blood cell count.
Statistically significant P-values are shown in bold.

between the two groups regarding lymph node stage (P=0.133). Overall, stage III disease was predominant (82.6%), with stage IIIa and IIIb observed in 41.3% each, while 17.5% of patients had stage II disease (P=0.315). The clinicopathological and laboratory characteristics of the patients are presented in Table 1.

Treatment Characteristics and Clinical Outcomes

Gemcitabine–cisplatin was the most commonly administered NAC regimen (68.3%), followed by gemcitabine–carboplatin (28.6%). One patient received ddMVAC, and another received cisplatin–etoposide. Cisplatin-based therapy was preferred in

TABLE 2. Treatment Characteristics and Clinical Outcomes of Patients According to Treatment Modality

	Surgery group	CRT group
Neoadjuvant chemotherapy regimen		
Gemcitabine+Cisplatin	29 (74.3%)	14 (58.3%)
Gemcitabine+Carboplatin	9 (23.1%)	9 (37.5%)
MVAC	0(0%)	1 (4.2%)
Cisplatin+Etoposide	1(2.6%)	0(0%)
Surgical procedure		
Radical cystectomy +/- Lymph node dissection	39 (100%)	
CRT Agent		
Gemcitabine		12 (50%)
Cisplatin		10 (41.7%)
Carboplatin		2 (8.3%)
Reason for omission of surgery		
Comorbidities		10 (41.7%)
Patient refusal		9 (37.5%)
Medically inoperable		5 (20.8%)
Pathologic response to neoadjuvant chemotherapy (in surgical group)		
Complete response	11 (28.2%)	
Partial response	18 (46.1%)	
Stable disease	7 (17.9%)	
Progression	3 (7.7%)	
Adjuvant chemotherapy in surgical group	12 (30.7%)	
Gemcitabine+Cisplatin	4/12 (33.3%)	
Gemcitabine+Carboplatin	3/12(25%)	
Nivolumab	4/12 (37.5%)	
Adjuvant radiotherapy	1/12 (8.3%)	
Recurrence/Progression		
Yes	23 (59%)	14(58.3%)
No	16 (41%)	10(41.7%)
Treatment at progression		
First-line systemic therapy	12/23 (52.2%)	9/14 (64.3%)
Surgery	3/23 (13.0%)	0/14 (0%)
No Treatment	8/23(34.8%)	5/14 (35.7%)
First-line systemic therapy agent		
Paclitaxel	6/12 (50%)	3/9 (33.3%)
Pembrolizumab	0 (0%)	3/9 (33.3%)
MVAC	2 (16.7%)	2/9 (22.2%)
Docetaxel	1 (8.3%)	0/9(0%)
Gemcitabine+Cisplatin	1 (8.3%)	1/9 (11.1%)
Gemcitabine +Carboplatin	1 (8.3%)	0/9(0%)
Carboplatin+Paclitaxel	1(8.3%)	0/9(0%)
Progression after first-line chemotherapy		
Yes	12/12 (100%)	8/9 (88.9%)
No	0 (0%)	1/9(11.1%)
Second-line systemic therapy		
Paclitaxel	1/12 (8.3%)	2/9 (22.2%)
No treatment	11/12 (91.7%)	7/9 (77.8%)

Data are shown as n (%). CRT, chemoradiotherapy; MVAC, methotrexate–vinblastine–doxorubicin–cisplatin.

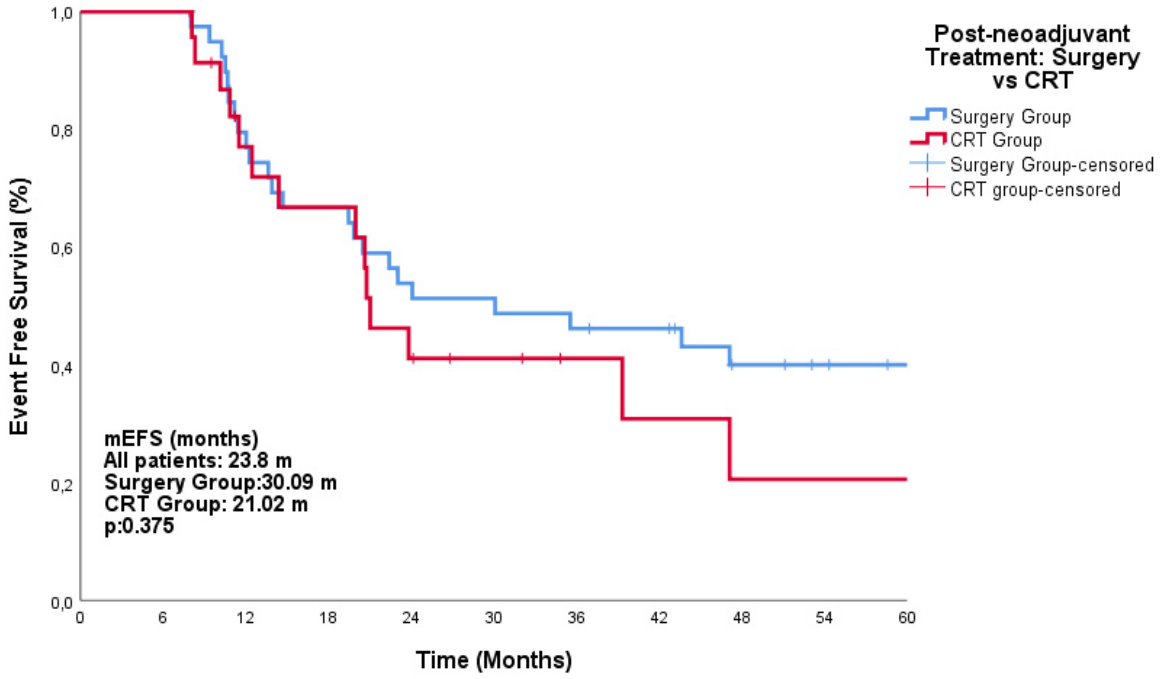

FIGURE 1. **Kaplan–Meier curves for event-free survival.**

the surgical group (74.3%), whereas carboplatin-based regimens were more frequently used in the CRT group (37.5%).

In the surgical cohort, all patients underwent radical cystectomy with or without pelvic lymph node dissection. In the CRT cohort, concurrent chemotherapy included gemcitabine in 50%, cisplatin in 41.7%, and carboplatin in 8.3%. Reasons for omission of surgery among CRT patients included comorbidities (41.7%), patient refusal (37.5%), and medical inoperability (20.8%).

Among patients who underwent radical cystectomy, complete pathological response (pT0) was achieved in 28.2%, while partial response occurred in 46.1%. Stable disease was observed in 17.9% and progression in 7.7%.

Twelve surgical patients (30.7%) received adjuvant chemotherapy. Among these, 33.3% received gemcitabine–cisplatin, 25% gemcitabine–carboplatin, and 37.5% nivolumab. One patient also received adjuvant radiotherapy (8.3%).

During follow-up, 37 (58.7%) of 63 patients developed recurrence or progression. The rate of recurrence was similar between groups (58.3% in surgery vs. 58.7% in CRT, P=0.96). The distribution of recurrence types was also comparable, with local recurrence in 12.7%, distant metastasis in 36.5%, and combined local plus distant recurrence in 9.5%.

Among those with disease progression, first-line systemic therapy was initiated in 52.2% of surgical and 64.3% of CRT patients. Surgery for recurrence was performed in 13% of surgical patients, whereas no surgery was performed in the CRT group. Approximately one-third of patients in both groups received no active treatment upon progression.

The most frequently administered first-line systemic therapies were paclitaxel (42%), MVAC (20%), and pembrolizumab (14%). Following first-line failure, only a minority proceeded to second-line treatment (8–22%), most commonly with paclitaxel. The treatment modalities and clinical outcomes of the patients are presented in Table 2.

Survival Outcomes

Among the entire cohort, recurrence occurred in 37 (58.7%) patients. The most common pattern was distant metastasis, observed in 36.5% of patients, followed by local recurrence in 12.7% and combined local plus distant recurrence in 9.5%. The distribution of recurrence types was similar between the surgery and CRT groups, with no statistically significant difference (P=0.96).

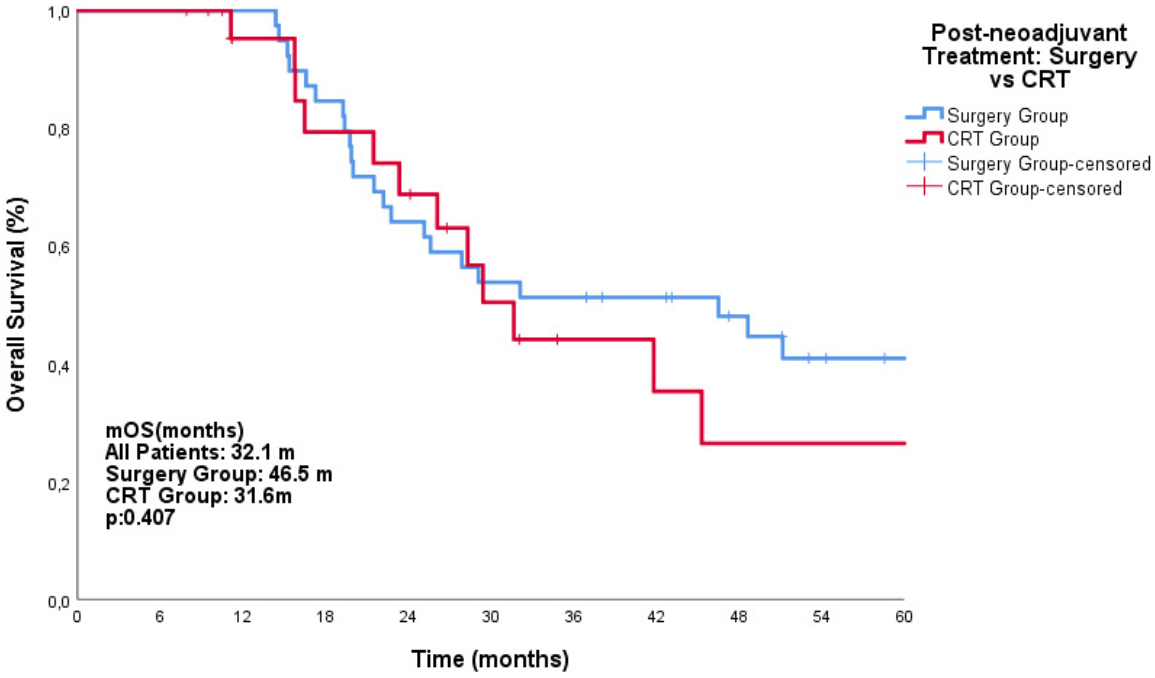

FIGURE 2. **Kaplan–Meier curves for overall survival.**

After a median follow-up of 58.5 months, mEFS was 23.8 months in the overall cohort. The mEFS was longer in the surgery group (30.1 months) than in the CRT group (21.0 months), though the difference was not statistically significant (P=0.375) (Figure 1). The mOS for all patients was 32.1 months, with mOS values of 46.5 months in the surgical group and 31.6 months in the CRT group (P=0.407) (Figure 2). The recurrence patterns, progression status, and survival outcomes (DFS and OS) of the patients are summarized in Table 3.

In univariate analyses, the presence of comorbidities was significantly associated with worse OS (P=0.011) and EFS (P=0.010). Multivariate Cox regression confirmed comorbidity as an independent prognostic factor for both OS (Hazard ratio [HR] = 0.37, 95% CI: 0.17–0.80, P=0.012) and EFS (HR = 0.34, 95% CI: 0.15–0.76, P=0.008). Hemoglobin level <12 g/dL was also predictive of poorer EFS (HR = 0.53, 95% CI: 0.25–0.94, P=0.048).

Stage, histologic subtype, nodal status, and type of neoadjuvant chemotherapy (cisplatin vs. carboplatin-based) were not significantly associated with survival outcomes. The results of the univariate

TABLE 3. **Patterns of Recurrence, Progression, and Survival Outcomes According to Treatment Modality**

	All patients	Surgery group	CRT group	P-value
Recurrence				
None	26 (41.3%)	16 (41%)	10 (41.7%)	0.96
Local	8 (12.7%)	5 (12.8%)	3 (12.5%)	0.265
Distant	23 (36.5%)	16 (41%)	7 (29.2%)	
Local and distant	6 (9.5%)	2 (5.1%)	4 (16.6%)	
mEFS (months)	23.8	30.09	21.02	0.375
mOS (months)	32.1	46.5	31.6	0.407

Data are shown as n (%). mEFS, median event-free survival; mOS, median overall survival; CRT, chemoradiotherapy.

TABLE 4. Univariate and Multivariate Analyses of Factors Associated with OS and EFS in the Patients

		mOS (months)	Univariate P-value	Multivariate P-value (HR)	mEFS (months)	Univariate P-value	Multivariate P-value (HR)
Gender	Male Female	31.6 N/A	0.075		23.0 N/A	0.226	
Age (years)	<64 >64	41.8 31.6	0.756		35.5 24.0	0.813	
Smoking	Yes No	31.6 45.3	0.488		24.0 20.6	0.984	
Comorbidity	Yes No	25.1 N/A	**0.011**	**0.012** **HR:0.37 (95.0% CI:0.17-0.806)**	19.9 N/A	**0.01**	**0.008** **HR:0.34 (95.0% CI:0.15-0.76)**
Stage	II III	62.5 32.1	**0.048**	0.221	30.0 23.8	0.886	
Lymph node metastasis	Yes No	31.6 46.5	0.921		23.1 30.2	0.989	
Squamous differentiation	Yes No	29.1 45.3	0.422		21.0 35.5	0.585	
Glandular differentiation	Yes No	31.6 41.8	0.588		24.1 23.0	0.885	
Sarcomatoid differentiation	Yes No	25.1 41.8	0.832		22.0 24.4	0.946	
Neoadjuvant chemotherapy	Cisplatin-based Carboplatin-based	29.4 N/A	0.229		23.0 N/A	0.186	
Post-neoadjuvant treatment	Surgery CRT	46.5 31.6	0.407		30.09 21.02	0.375	
WBC (/µL)	<11.000 ≥11.000	46.5 32.1	0.785		23.8 21.5	0.811	
Hb (g/dL)	<12 ≥12	25.1 48.6	**0.046**	0.148	19.8 43.6	**0.039**	**0.048** **HR:0.53 (95.0% CI:0.25-0.94)**
GFR (mL/dk/1.73 m²)	< 60 ≥60	31.6 45.3	0.983		22.4 30.9	**0.047**	0.205
LDH (U/L)	<225 ≥225	41.8 27.8	0.499		35.5 14.6	**0.037**	0.487

mOS, median overall survival; mEFS, median event free survival; Hb, hemoglobin; GFR, glomerular filtration rate; LDH, lactate dehydrogenase; WBC, white blood cell count; HR, hazard ratio; CI, confidence interval; CRT, chemoradiotherapy, NA, not available.
Statistically significant P-values are shown in bold.

and multivariate analyses evaluating prognostic factors for OS and EFS are summarized in Table 4. At the end of the study, 28 (44%) patients were alive.

DISCUSSION

This study evaluated the outcomes of patients with stage II–III MIBC who received NAC followed by either surgery or CRT. Notably, patients in the CRT group were those who did not undergo surgery due to comorbidities, medical inoperability, or refusal of surgical intervention. Furthermore, unlike most studies in the literature, patients in the CRT cohort did not undergo maximal TURBT. The primary endpoint of this study was to compare OS and EFS between the surgery and CRT groups. Although the surgery group demonstrated numerically longer OS and EFS, these differences did not reach statistical significance.

Radical cystectomy continues to be regarded as the standard curative approach for MIBC. The integration of neoadjuvant cisplatin-based chemotherapy into the treatment regimen has been shown to enhance long-term survival and increase the likelihood of pathological downstaging. In a landmark randomized trial by Grossman *et al*., involving patients with T2N0M0 to T4aN0M0 bladder cancer, the addition of cisplatin-based NAC to surgery resulted in a notable improvement in outcomes. Median survival was 77 months in the combination therapy group compared to 46 months in patients treated with surgery alone (P=0.06). Furthermore, survival benefit was strongly correlated with the absence of residual tumor in the cystectomy specimen, which was achieved significantly more frequently in the combination group than in the surgery-only group (38% vs. 15%, P<0.001) [9]. Similarly, the Advanced Bladder Cancer (ABC) Meta-analysis Collaboration reported an absolute 5% improvement in 10-year OS with the use of preoperative cisplatin-based neoadjuvant chemotherapy compared to surgery alone, further supporting the long-term survival benefit of incorporating systemic therapy before definitive local treatment [10].

In our study, the mOS and mDFS in the surgery group were 46.5 and 30.1 months, respectively, comparable to those reported in the aforementioned randomized trials and contemporary real-world series. The pathological complete response rate of 28.2% and partial response rate of 46.1% among surgically treated patients were consistent with prior reports indicating pCR rates between 25–35% following platin-based NAC [11]. These results reinforce the role of NAC in improving local control and eradicating micrometastatic disease, even in the setting of organ-confined tumors.

Unlike the majority of published studies on bladder-preserving therapy, the CRT cohort in our study consisted of patients who did not undergo TURBT before chemoradiotherapy. This distinction is noteworthy, as most prospective CRT trials have been based on the trimodality treatment protocol, which includes maximal TURBT followed by concurrent chemoradiotherapy. In those studies, complete TURBT was associated with improved local control and higher rates of bladder preservation, underscoring the importance of achieving adequate tumor debulking prior to radiotherapy [12, 13]. However, this conventional assumption has been challenged by recent evidence. In a multicenter real-world analysis, Avolio *et al*. compared patients with MIBC who underwent complete versus incomplete TURBT prior to chemoradiotherapy. Adjusted survival analyses revealed no significant differences in 5-year OS (48% vs. 52%), cancer-specific survival (64% vs. 61%), metastasis-free survival (43% vs. 46%), or DFS (32% vs. 35%) between the two groups, suggesting that the completeness of TURBT may not have a major impact on long-term oncologic outcomes when effective chemoradiation is administered [14].

In addition, data from the National Cancer Database (NCDB) further support the potential benefit of chemoradiotherapy in non-surgical settings. In this large retrospective analysis of 1,783 patients with clinically node-positive (cTanyN1–3M0) bladder cancer diagnosed between 2004 and 2013, outcomes of patients treated with chemotherapy alone (n=1,388) were compared with those receiving chemoradiotherapy (n=395). The study demonstrated a significantly longer median overall survival in the chemoradiotherapy group compared to chemotherapy alone (19.0 vs. 13.8 months; P<0.001), and this survival advantage remained significant after propensity score matching (P<0.001) [15]. These findings are consistent with our results, suggesting that definitive CRT may offer meaningful survival benefit even in patients who are not surgical

candidates, particularly when combined with effective systemic therapy.

Consistent with this, our CRT cohort achieved a median OS of 31.6 months and DFS of 21.0 months. While numerically lower than the surgical group, these outcomes were not statistically inferior (P=0.407 and P=0.375, respectively), supporting the notion that CRT remains a feasible curative-intent option even in patients without prior maximal resection.

Our findings further confirmed that baseline comorbidity burden and anemia were independent predictors of poor prognosis. Comorbidities were significantly more prevalent in the CRT group (79.2%) and were associated with inferior OS and EFS in multivariate analysis (HR 0.37 and 0.34, respectively). These observations are consistent with previous studies by Goossens-Laan *et al*. [16] and Megwalu *et al*. [17], which identified comorbidity and frailty as key determinants of treatment outcomes and long-term survival in patients with bladder cancer. Similarly, baseline anemia (Hb <12 g/dL) was associated with shorter DFS, in agreement with the findings of Chen *et al*. [18], who reported that anemic patients with bladder cancer have significantly shorter recurrence-free survival (RFS) and OS compared with non-anemic patients, and that anemia serves as an independent prognostic factor for both RFS and OS [18].

Strengths and Limitations

Taken together, our study highlights that NAC followed by either RC or CRT can provide meaningful disease control in patients with stage II–III MIBC. While RC remains the gold standard for operable patients, CRT offers a viable curative option for those medically inoperable or unwilling to undergo surgery - even in the absence of maximal TURBT. These findings suggest that treatment outcomes are not solely determined by the extent of initial tumor resection but also by systemic disease control and patient selection. The integration of advanced radiotherapy techniques and systemic agents, including immunotherapy, may further enhance bladder-preserving efficacy in such complex clinical scenarios.

The main limitations of this study include its retrospective design, relatively small sample size, and inherent selection bias. Patients in the CRT group tended to be older and had a higher burden of comorbidities compared to those who underwent surgery, along with less favorable baseline performance status. These factors may have contributed to the numerically shorter OS and EFS observed in the CRT group, potentially reflecting differences in patient characteristics rather than treatment efficacy. Molecular data (such as PD-L1 expression, DNA damage repair gene status, or tumor mutational burden) were unavailable, precluding analysis of predictive biomarkers for CRT response. Future multicenter prospective studies incorporating molecular profiling and standardized CRT protocols - including both maximal and non-maximal TURBT cohorts - are warranted to clarify the optimal patient population for bladder-preserving treatment after NAC.

CONCLUSIONS

In this real-world retrospective study, neoadjuvant chemotherapy followed by either surgery or CRT provided comparable survival outcomes in patients with stage II–III MIBC. Although the surgery group demonstrated numerically longer OS and EFS, these differences were not statistically significant. Comorbidity and baseline anemia were identified as independent prognostic factors associated with poorer survival, regardless of the treatment modality.

Importantly, unlike most reports in the literature, the CRT group in our study consisted of patients who did not undergo maximal TURBT prior to chemoradiation, yet achieved meaningful disease control. These findings suggest that CRT remains a viable curative-intent option for patients who are medically inoperable or decline surgery, even in the absence of maximal TURBT. Further prospective and biomarker-driven studies are warranted to refine patient selection and optimize outcomes for bladder-preserving approaches following neoadjuvant therapy.

Ethics Approval and Consent to Participate

This study was approved by the Ankara Bilkent City Hospital Clinical Research Ethics Committee No. 1 (Decision No: E1-23-4506; date: 27.12.2023). Additionally, the study's ethics approval was re-obtained on a multicenter basis: approval granted by Ankara City Hospital Ethics Committee (Date: July 30, 2025/Approval No: E1-25-1550). All procedures

were conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Informed consent was waived because of the retrospective nature of the study and the analysis used anonymous clinical data.

Data Availability

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

Authors' Contribution

Study Conception: DB; Study Design: DB, ÖB; Supervision: ÖB, DU, RE; Funding: DB; Materials: DB, AT; Data Collection and/or Processing: BK, SS; Statistical Analysis and/or Data Interpretation: DB, EA; Literature Review: EH, MD; Manuscript Preparation: DB; and Critical Review: ÖB, MD.

Conflict of Interest

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The author used artificial intelligence tools (ChatGPT, OpenAI) only for language editing and reference formatting. The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

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Evaluation of Cardiac Functions and Rhythm in Pediatric Patients Diagnosed with Chronic Renal Failure

Abdullah Akkuş[1], Fatih Şap[2], Bülent Ataş[3], Mehmet Burhan Oflaz[2], Tamer Baysal[1]

[1]Department of Pediatrics, Necmettin Erbakan University, Faculty of Medicine, Konya, Türkiye; [2]Department of Pediatrics, Division of Pediatric Cardiology, Necmettin Erbakan University, Faculty of Medicine, Konya, Türkiye; [3]Department of Pediatrics, Division of Pediatric Nephrology, Necmettin Erbakan University, Faculty of Medicine, Konya, Türkiye

ABSTRACT

Objectives: Chronic renal failure (CRF) represents a progressive and irreversible decline in renal function. Among individuals with end-stage renal disease (ESRD), cardiovascular disorders are recognized as the leading contributors to morbidity and mortality. The present study aimed to assess cardiac function in pediatric patients diagnosed with CRF.

Methods: This cross-sectional study included 30 pediatric patients with CRF who were under follow-up between October 2018 and August 2019, along with 30 age- and sex-matched healthy controls. The CRF group was further divided into two subgroups according to dialysis status. For all participants, conventional 12-lead electrocardiography was performed, and indices associated with atrial (P-wave dispersion, Pd) and ventricular (QT dispersion, corrected QT dispersion, Tp–e, Tp–e/QT, Tp–e/QTc) arrhythmia risk were recorded. Each subject also underwent a detailed echocardiographic evaluation, including both conventional and tissue Doppler measurements.

Results: Anthropometric characteristics and blood pressure values did not significantly differ between the patient and control groups. Apart from an increased mean corrected QT dispersion (QTcd), other electrocardiographic parameters were comparable between groups. Ejection fraction (EF) and fractional shortening (FS) values were lower in CRF patients compared with controls. LV mass index (LVMI) was significantly higher in the dialysis subgroup relative to the control group. Additionally, the myocardial performance index (Tei index) was markedly elevated in CRF patients ($P<0.001$).

Conclusions: These findings underscore the need for comprehensive cardiac evaluation in children with CRF. Nonetheless, larger-scale studies are warranted to confirm and expand upon these observations.

Keywords: Cardiac Functions, Child, Chronic Renal Failure, Echocardiography, Electrocardiography

Chronic Renal Failure (CRF) refers to a progressive and irreversible impairment of renal structure and function. It is characterized by a sustained decline in kidney performance, typically persisting for more than six months. Because CRF in the pediatric population often follows a progressive course, timely diagnosis and management are vital to prevent or delay further deterioration of renal function and the development of related complications [1, 2].

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Corresponding author: Abdullah Akkuş, MD., Assist. Prof., Phone: +90 312 552 60 00, E-mail: abdullahakkus29@gmail.com

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Among individuals diagnosed with CRF who progress to end-stage renal disease (ESRD) in adulthood, cardiovascular system (CVS) disorders constitute the leading causes of morbidity and mortality. The annual mortality rate due to cardiac diseases in the general pediatric population is reported to be below 3%. In children with ESRD, the primary causes of cardiac death are, in order of frequency, cardiac arrest, arrhythmias, and cardiomyopathy [3]. In addition to uremia, factors such as anemia, lipid abnormalities and various metabolic abnormalities including novel parameters such as asymmetric dimethylarginine, adiponectin, and homocysteine, are believed to contribute to cardiovascular damage and its progression [4, 5].

Earlier investigations have demonstrated that approximately 13% of children with ESRD exhibited congestive heart failure (CHF) at the onset of dialysis, and that cardiac events developed in nearly 34% of pediatric patients undergoing long-term dialysis [6]. Although the prevalence of CHF in children with mild to moderate renal insufficiency has not been clearly defined, studies indicate that left ventricular diastolic dysfunction can occur even in this group [7].

Systolic function is measured by ejection fraction (EF) and fractional shortening (FS). In patients with end-stage renal disease, left ventricular systolic and diastolic diameters, wall thickness, and cardiac output increase due to anemia, systemic hypertension, and volume overload, and EF indirectly decreases [8]. Diastolic function can be measured by the E/A ratio. An E/A ratio <1 is considered abnormal [9]. The E/E' ratio is measured from tissue Doppler measurements, and an E/E' ratio >10 is considered an indicator of diastolic dysfunction [10].

A prolonged QT interval is associated with sudden cardiac death and can be seen particularly in hemodialysis patients. QT dispersion is defined as the distance between the longest and shortest QT intervals on the electrocardiogram (ECG), and its prolongation indicates a higher risk of ventricular arrhythmia [11]. The Tp-e interval is the time between the peak and the end of the T wave. Tp-e, Tp-e/QTc. The Tp-e/QTc ratio has been shown to be an important arrhythmia index indicator [12].

The present study aimed to evaluate cardiac structure and function through echocardiography and to identify possible abnormalities in cardiac conduction using electrocardiographic assessment in pediatric patients diagnosed with CRF.

METHODS

Our study was conducted with a patient group of 30 pediatric CRF patients and a control group of 30 gender- and age-matched healthy individuals between October 2018 and August 2019. Participants were compared in regard to age, gender, hematological and biochemical parameters, anthropometrics, blood pressure values, and status of receiving dialysis (only in CRF patients). Heart examinations were performed on the same day. All individuals were subject to electrocardiographic and echocardiographic examinations. The patient group was further divided into two subgroups to analyze: those receiving and not receiving dialysis. The study was approved by the Institutional Review Board of our institution (approval number: 2018/1448).

In electrocardiographic evaluation, heart rate, PR interval, P wave duration (Pmin, Pmax), P dispersion (Pd), QT interval (QTmin, QTmax), QT dispersion (QTd), corrected QT (QTcmin, QTcmax), corrected QTc dispersion (QTcd), and Tp-e interval (Tp-e) were measured. Tp-e/QT and Tp-e/QTc ratios were calculated from the obtained data. Echocardiographic measurements were performed by an experienced pediatric cardiologist, and the measurements were recorded as the average of 3 consecutive measurements. Two-dimensional, M-mode, Pulsed Doppler, Continuous Flow (CW) Doppler, and Tissue Doppler data were obtained from the evaluated images for all cases.

Statistical Analysis

Statistical analyses were conducted using SPSS software, version 22. The normality of data distribution was assessed with the Kolmogorov–Smirnov test. Variables that did not follow a normal distribution were presented as mean ± standard deviation, while categorical variables were summarized as frequencies and percentages. Comparisons of nonparametric and categorical data were performed using the Mann–Whitney U test. A p-value of less than 0.05 was considered indicative of statistical significance.

TABLE 1. General Features of the Groups

	Patients (n=30)	Control (n=30)	P-value
Age (years)	11.47±4.64	11.59±4.46	0.921 †
Weight (kg)	32.88±15.53	42.01±18.81	**0.045** †
Height (cm)	133.67±27.76	144.55±25.77	0.066 ‡
Body mass index (kg/m^2)	17.27±3.00	18.83±3.84	0.162 ‡
Female/male	12/18	12/18	>0.999 §

Data are shown as mean±standard deviation or frequency where appropriate.
†Student t test, ‡Mann Whitney U test, §Fisher exact test. Statistically significant P-value is shown in bold.

RESULTS

A total of 60 participants were included: 30 CRF patients and 30 healthy controls. Table 1 summarizes the general features of the patient and control groups. Among CRF patients, the mean disease duration was 5.41±3.58 years, and 11 patients were on dialysis for 2.18±1.16 years. Dialysis patients were significantly older than non-dialysis patients (P=0.047) and had higher diastolic blood pressure (BP) compared with controls (P=0.019). A comparison of complete blood count results is given in Table 2. Urea, creatinine, potassium, and phosphorus levels were significantly higher, and GFR was lower in dialysis patients (P<0.05). Ferritin was also elevated in this group (P<0.05), while parathormone and vitamin D levels did not differ (P>0.05) (Table 3). In addition, lipid profiles of the groups were similar (P>0.05) (Table 4). QTcd was significantly higher in CRF patients than controls (P=0.018). PR interval was longer in dialysis patients than in patients not on dialysis (P<0.05). QTcd in non-dialysis patients exceeded that of controls (P<0.05), whereas other ECG parameters were similar across groups (P>0.05), and none were arrhythmic at the time of ECG examination (Table 5).

M-mode echocardiography revealed lower EF and FS values in CRF patients than controls (P=0.04 and P=0.02, respectively). Dialysis patients had greater IVSd and LVMI values (P<0.05). Although EF and FS remained within normal limits in non-dialysis patients,

TABLE 2. Complete Blood Count Results of the Patients

	All patients (n=30)	On Dialysis (n=11)	Not On Dialysis (n=19)	P-value
Leukocyte (/mm³)	1119.33±1578.6	7782.73±2260.81	13167.89±1968.31	0.395**
Neutrophil (/mm³)	4477±2219.59	4380.91±1737.93	4532.63±2500.07	0.860*
Lymphocyte (/mm³)	2907.33±1311.04	2429.09±771.04	3184.21±1489.37	0.052**
Neutrophil/lymphocyte	1.83±1.22	1.88±0.77	1.81±1.44	0.250**
Hgb (gr/dL)	11.69±1.96	11.75±2.07	11.65±1.95	0.894*
Hct (%)	34.75±5.31	35.05±5.68	34.58±5.24	0.821*
MCV (FL)	81.28±5.8	81.01±5.04	81.44±6.33	0.848*
MCH (pgr)	27.24±1.69	27.13±1.8	27.3±1.66	0.792*
MCHC (gr/dL)	33.55±1.41	33.48±1.42	33.58±1.45	0.852*
RDW (%)	13.65±1.12	14.11±1.06	13.38±1.1	0.086*
Platelets (/mm³)	305.7±75.89	285.18±65.11	317.58±80.72	0.420**

Data are shown as mean±standard deviation. P, values between patients receiving and not receiving dialysis.
*Student t test, **Mann-Whitney U test.

TABLE 3. Biochemistry Results of the Patient Groups

	All patients (n=30)	On Dialysis (n=11)	Not On Dialysis (n=19)	P-value
Urea (mg/dL)	95.33±53.03	132.01±61.76	74.1±33.38	**0.006****
Creatinine (mg/dL)	3.39±1.5	4.47±1.3	2.76±1.24	**0.001***
GFR ml/min /1.73 m^2	36±11.04	27.27±9.5	39.82±9.58	**0.009***
Sodium (mmol/L)	139.3±3.2	140±3.19	138.89±3.21	0.371*
Potassium mmol/L	4.71±0.62	4.44±0.41	4.87±0.67	**0.030****
Calcium (mg/dL)	9.55±0.9	9.41±1.36	9.63±0.5	0.703**
Phosphorus (mg/dL)	4.83±1.3	5.5±1.08	4.43±1.28	**0.027***
Albumin (gr/dL)	4.28±0.4	4.24±0.4	4.31±0.41	0.438**
Uric Acid (mg/dL)	6.16±1.43	6.29±1.32	6.08±1.52	0.710*

Data are shown as mean±standard deviation. P, values between patients receiving and not receiving dialysis.
*Student t test, **Mann Whitney U testi.
Statistically significant P-values are shown in bold.

they were lower than controls (P<0.05). LVPWd, Ao, and LA were higher in dialysis patients (P<0.05) (Table 6).

Pulsed Doppler findings showed lower Mitral E', E'/A', and Sm, and higher isovolumic contraction time (IVCT), contraction time (CT), isovolumic relaxation time (IVRT), and myocardial performance index (Tei index) in patients versus controls (P<0.05) (Table 7). For tissue Doppler of interventricular septum (IVS), peak early diastolic myocardial motion velocity (E'), systolic motion velocity (Sm), and E' to peak systolic atrial velocity (E'/A') were lower, while IVCT, IVRT, and Tei index were higher in patients versus controls (P<0.05) (Table 8).

Right ventricular Doppler parameters showed higher Tricuspid peak early diastolic filling velocity to early diastolic myocardial motion velocity (E/E'), IVCT, IVRT, and Tei index and lower CT and E'/A' in patients versus controls (P<0.05). Dialysis patients had higher Tricuspid E/E' and lower E'/A' ratios than non-dialysis patients (P<0.05) (Table 9).

By 2D echocardiography, left atrial volüme (LAV) and left atrial volume index (LAV index) were higher in patients (P<0.05). Four- and two-chamber lengths and areas were significantly greater in dialysis patients (P<0.05). Both dialysis and non-dialysis groups had higher LAV index values than controls (P<0.05) (Table 10).

Across all cases (n=60), LAV index correlated positively with Mitral E/E' (r=0.328, P=0.010; r=0.273, p=0.035). No correlations were found between Tei index and LAV, LAV index, or Tricuspid E/E' (P>0.05). IVS Tei index correlated positively with Mitral (r=0.520, P<0.001) and Tricuspid Tei

TABLE 4. Lipid Profiles of the Patient Groups

	All patients (n=30)	On Dialysis (n=11)	Not On Dialysis (n=19)	P-value
Cholesterol (mg/dL)	169.22±45.16	162.45±40.85	173.14±48.11	0.541*
Triglycerides (mg/dL)	132.58±90.28	145.71±74.01	124.98±99.62	0.250**
HDL (mg/dL)	47.46±14.63	43.75±12.21	49.61±15.76	0.471**
LDL (mg/dL)	91.41±31.81	86.67±26.24	94.16±35.02	0.544*

Data are shown as mean±standard deviation.
*Student t test, **Mann Whitney U testi.

TABLE 5. Electrocardiographic Results of Those Receiving and Not Receiving Dialysis and the Controls

	On Dialysis (n=11)	Not on dialysis (n=19)	Control (n=30)	P-value
Rate (bpm) †	89.64±16.84	97±21.71	87.2±14.71	P1=0.342 P2=0.654 P3=0.065
PR (ms) †	164±11.92	149.05±17.76	155.9±21.26	**P1=0.020** P2=0.241 P3=0.249
Pmin (ms) ‡	57.09±10.29	54.32±8.47	55.87±10.26	P1=0.431 P2=0.552 P3=0.934
Pd (ms) ‡	36±7.59	32.21±5.07	34.93±8.22	P1=0.185 P2=0.739 P3=0.237
Pmax (ms) ‡	93.09±10.44	86.53±9.26	91.47±11.4	P1=0.085 P2=0.717 P3=0.182
QTmin (ms) †	306.18±33.77	292.63±34.08	303.2±23.12	P1=0.301 P2=0.749 P3=0.202
QTd (ms) ‡	37.45±80.63	38.63±9.62	34.67±8.02	P1=0.740 P2=0.359 P3=0.152
QTmax (ms) †	343.64±35.57	331.79±38.01	337.87±26.65	P1=0.407 P2=0.578 P3=0.514
QTcmin (ms) †	371.47±35.77	366.62±26.13	363.83±19.98	P1=0.672 P2=0.391 P3=0.674
QTcd (ms) †	45.28±8.57	49.1±12.07	41.48±8.79	P1=0.365 P2=0.225 **P3=0.014**
QTcmax (ms) †	416.75±35.04	415.72±27.51	405.31±21.18	P1=0.407 P2=0.210 P3=0.142
Tp-e (ms) †	60.45±10.13	57.84±11.02	61.77±8.97	P1=0.525 P2=0.691 P3=0.179
Tp-e/QT†	0.18±0.04	0.19±0.02	0.19±0.02	P1=0.937 P2=0.407 P3=0.615
Tp-e/QTc (ms) †	0.15±0.03	0.15±0.03	0.16±0.02	P1=0.770 P2=0.453 P3=0.192

Data are shown as mean±standard deviation. Pd, P dispersion; Pmax, maximum P wave duration; Pmin, minimum P wave duration; QTcd, QTc dispersion, QTcmax; maximum QTc duration; QTcmin; minimum QTc duration; QTmax; maximum QT duration; QTmin, minimum QT duration; QTd, QT dispersion; Tp-e, the distance between peak and end-point of T wave; P1, those receiving and not receiving dialysis; P2, those receiving dialysis and controls; P3, those not receiving dialysis and controls. †Student t test, ‡Mann Whitney U test. Statistically significant P-values are shown in bold.

TABLE 6. M-Mode Findings of Those Receiving and Not Receiving Dialysis and the Control Group

	On Dialysis (n=11)	Not on dialysis (n=19)	Control (n=30)	P-value
IVSd (mm) †	8.63±1.42	7.58±1.23	7.66±1.25	P1=0.232 **P2=0.040** P3=0.820
LVEDD (mm) †	40.46±4.94	37.81±6.05	40.71±6.44	P1=0.228 P2=0.905 P3=0.122
LVESD (mm †	23.84±3.65	22.81±4.03	23.3±3.81	P1=0.491 P2=0.688 P3=0.667
LVPWd (mm) †	7.78±1.46	6.63±1.14	7.19±1.28	**P1=0.022** P2=0.211 P3=0.125
EF (%)†	72.3±4.34	70.93±4.09	74.06±2.38	P1=0.497 P2=0.107 **P3=0.001**
FS (%)†	41.03±3.56	39.61±3.24	42.52±2.13	P1=0.275 P2=0.108 **P3<0.001**
Ao (mm) †	20.45±2.68	18.11±3.04	18.69±2.64	**P1=0.042** P2=0.066 P3=0.481
LA (mm) †	24.94±2.85	22.19±2.94	22.91±3.21	**P1=0.019** P2=0.073 P3=0.435
LA/Ao†	1.23±0.09	1.24±0.14	1.23±0.09	P1=0.759 P2=0.885 P3=0.756
LVM†	101.84±37.66	76.59±32.03	92.54±39.98	P1=0.061 P2=0.507 P3=0.150
LVMI (g/ m^2) ‡	85.48±19.6	75.81±28.95	70.02±13.92	P1=0.112 **P2=0.006** P3=0.594

Data are shown as mean±standard deviation. Ao, aortic root; EF, ejection fraction; FS, fractional shortening; IVSd, end-diastolic interventricular septum thickness; LA, left atrial width; LA/Ao, left atrial width-to-aortic root; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVPWd, left ventricular end-diastolic posterior wall thickness; LVM, left ventricular mass; LVMI, left ventricular mass index.

†Student t test, ‡Mann Whitney U test. Statistically significant P-values are shown in bold.

TABLE 7. Comparison of Left Ventricular Pulsed and Tissue Doppler Findings of Those Receiving and Not Receiving Dialysis and the Control Group

	On Dialysis (n=11)	Not on dialysis (n=19)	Control (n=30)	P-value
Mitral E (m/s) ‡	0.93±0.24	0.88±0.3	1.02±0.6	P1=0.659 P2=0.805 P3=0.394
Mitral A (m/s) ‡	0.62±0.15	0.63±0.19	0.59±0.13	P1=0.933 **P2=0.027** P3=0.366
Mitral E/A‡	1.56±0.5	1.46±0.45	1.75±0.85	P1=0.589 P2=0.695 P3=0.151
Mitral E' (m/s) ‡	0.14±0.03	0.14±0.03	0.16±0.03	P1=0.854 P2=0.873 **P3=0.014**
Mitral A' (m/s) ‡	0.08±0.02	0.08±0.02	0.08±0.02	P1=0.558 P2=0.805 P3=0.265
Mitral E'/A'†	1.88±0.27	1.79±0.44	2.2±0.56	P1=0.558 P200.928 **P300.009**
Mitral E/E'†	6.85±2.1	6.86±3.67	6.69±5.46	P1=0.703 P2=0.074 P300.908
Mitral Sm (m/s)†	0.09±0.01	0.09±0.02	0.1±0.02	P1=0.695 **P2=0.010** **P3=0.032**
Mitral IVCT (ms)†	63.55±13.66	69.33±41.29	53.22±8.71	P1=0.735 **P2=0.012** **P3=0.017**
Mitral CT (ms) ‡	261.76±28.73	266.18±176.76	281.91±22.47	P1=0.471 **P2=0.049** **P3=0.003**
Mitral IVRT (ms) ‡	63.03±11.4	59.51±11.9	48.59±6.64	P1=0.435 **P2<0.001** **P3=0.001**
Mitral Tei index‡	0.49±0.08	0.48±0.18	0.36±0.05	P1=0.641 **P2<0.001** **P3<0.001**

Data are shown as mean±standard deviation. A, peak late diastolic filling velocity; A', peak systolic atrial velocity; CT, contraction time; E, peak early diastolic filling velocity; E', peak early myocardial motion velocity; IVCT, isovolumic contraction time; IVRT, isovolumic relaxation time; Sm, systolic myocardial velocity; Tei index, myocardial performance index. †Student t test, ‡Mann Whitney U test. Statistically significant P-values are shown in bold.

TABLE 8. Comparison of IVS Tissue DopplerFindings of Those Receiving and Not Receiving Dialysis and the Control Group

	On Dialysis (n=11)	Not on dialysis (n=19)	Control (n=30)	P-value
IVS E' m/s†	0.1±0.02	0.11±0.02	0.13±0.02	P1=0.420 **P2<0.001** **P3=0.001**
IVS A' m/s‡	0.08±0.01	0.07±0.01	0.07±0.01	P1=0.372 P2=0.139 P3=0.549
IVS E'/A'†	1.34±0.30	1.52±0.39	1.95±0.4	P1=0.691 **P2<0.001** **P3=0.007**
IVS Sm m/s‡	0.08±0.01	0.08±0.01	0.09±0.01	P1=0.051 **P2=0.046** **P3=0.002**
IVS IVCT ms‡	63.42±11.25	60.51±9.05	51.66±7.15	P1=0.184 **P2<0.001** **P3=0.001**
IVS CT ms‡	258.91±27.09	255.57±21.35	273.67±19.17	P1=0.524 P2=0.058 **P3=0.001**
IVS IVRT ms‡	67.33±11.47	63.46±10.71	48.62±8.02	P1=0.103 **P2<0.001** **P3<0.001**
IVS Tei index†	0.51±0.08	0.49±0.06	0.37±0.04	P1=0.115 **P2<0.001** **P3<0.001**

Data are shown as mean±standard deviation. A', peak systolic atrial velocity; CT, contraction time; E', peak early myocardial motion velocity; IVCT, isovolumic contraction time; IVRT, isovolumic relaxation time; Sm, systolic myocardial velocity; Tei index, myocardial performance index. P1, those receiving and not receiving dialysis; P2, those receiving dialysis and controls; P3, those not receiving dialysis and controls.

†Student t test, ‡Mann Whitney U test. Statistically significant P-values are shown in bold.

indices (r=0.651, P<0.001), while Mitral and Tricuspid Tei indices showed a weak positive correlation (r=0.417, P=0.001).

DISCUSSION

CRF is an irreversible condition with rising prevalence among children [13, 14]. Although pediatric mortality in end-stage kidney failure is lower than in adults [15], cardiovascular diseases remain the leading cause of death [16, 17], with arrhythmia being a major contributor [18]. Studies have reported arrhythmia in up to 25% of pediatric CRF patients [5].

In our study, dialysis patients were significantly older than non-dialysis patients, consistent with variations in disease onset, treatment adherence, and progression. Gender distribution (male 60%, female 40%) was comparable with prior studies [19-25]. The predominance of males may relate to higher rates of congenital renal and urinary anomalies such as obstructive uropathy or renal dysplasia.

TABLE 9. Comparison of Right Ventricular Pulsed and Tissue Doppler Findings of Those Receiving and Not Receiving Dialysis and the Control Group

	On Dialysis (n=11)	Not on dialysis (n=19)	Control (n=30)	P-value
Tricuspid E (m/s)†	0.66±0.1	0.64±0.2	0.59±0.11	P1=0.766 P2=0.097 P3=0.292
Tricuspid A (m/s)†	0.45±0.11	0.47±0.18	0.42±0.09	P1=0.682 P2=0.471 P3=0.204
Tricuspid E/A ‡	1.52±0.32	1.41±0.3	1.43±0.25	P1=0.348 P2=0.424 P3=0.485
Tricuspid E' (m/s) ‡	0.13±0.03	0.15±0.03	0.15±0.03	P1=0.155 P2=0.063 P3=0.809
Tricuspid A' (m/s)†	0.13±0.03	0.12±0.03	0.11±0.03	P1=0.341 P2=0.089 P3=0.434
Tricuspid E'/A' †	1.11±0.27	1.37±0.31	1.5±0.42	**P1=0.022** **P2=0.006** P3=0.274
Tricuspid E/E' †	5.07±1.1	4.2±0.95	3.93±0.95	**P1=0.030** **P2=0.002** P3=0.341
Tricuspid Sm (ms) †	0.13±0.02	0.13±0.02	0.13±0.02	P1=0.647 P2=0.894 P3=0.415
Tricuspid IVCT (ms) †	56.42±11.77	53.93±8.18	51.72±9.23	P1=0.672 P2=0.187 P3=0.399
Tricuspid CT (ms) †	258.45±33.59	250.89±17.49	275.89±20.26	P1=0.232 **P2=0.049** **P3<0.001**
Tricuspid IVRT (ms) ‡	60.91±16.14	56.47±6.57	50.06±8.88	P1=0.866 **P2=0.029** **P3=0.002**
Tricuspid Tei index ‡	0.46±0.11	0.44±0.06	0.37±0.06	P1=0.553 **P2=0.010** **P3<0.001**

Data are shown as mean±standard deviation. A, peak late diastolic filling velocity; A', peak systolic atrial velocity; CT, contraction time; E, peak early diastolic filling velocity; E', peak early myocardial motion velocity; IVCT, isovolumic contraction time; IVRT, isovolumic relaxation time; Sm, systolic myocardial velocity; Tei index, myocardial performance index. P1, those receiving and not receiving dialysis; P2, those receiving dialysis and controls; P3, those not receiving dialysis and controls. †Student t test, ‡Mann Whitney U test. Statistically significant P-values are shown in bold.

TABLE 10. Comparison of Left Atrial Area and Length Measurements of Those Receiving and Not Receiving Dialysis and the Control Group

	On Dialysis (n=11)	Not on dialysis (n=19)	Control (n=30)	P-value
4Ch area (cm^2) †	13.91±2.39	11.88±2.66	12.07±2.96	**P1=0.047** P2=0.072 P3=0.826
4Ch length (cm) †	4.73±0.49	4.49±0.56	4.28±0.59	P1=0.250 P2=0.028 P3=0.211
2Ch area (cm^2) †	12.73±2.47	10.85±2.64	11.04±2.87	P1=0.066 P2=0.092 P3=0.823
2Ch length (cm) †	4.54±0.54	4.19±0.56	4.07±0.63	P1=0.104 **P2=0.034** P3=0.511
LAV (cm^3) †	33.82±8.77	26.76±8.81	28.73±10.43	**P1=0.043** P2=0.158 P3:0.499
LAV index (ml/m^2) ‡	29.24±5.35	27.45±8.75	22.48±3.94	P1=0.545 **P2=0.001** **P3=0.006**

Data are shown as mean±standard deviation. LAV, sol atrial volume; LAV index, Left atrial volume/body surface area; 2Ch area, left atrial area on apical 2Ch view; 2Ch length, left atrial length on apical 2Ch view; 4Ch area, left atrial area on apical 4Ch view; 4Ch length, left atrial length on apical 4Ch view; P1, those receiving and not receiving dialysis; P2, those receiving dialysis and controls; P3, those not receiving dialysis and controls.

†Student t test, ‡Mann Whitney U test. Statistically significant P-values are shown in bold.

Hypertension is a frequent comorbidity among children with CRF and plays a significant role in the development of left ventricular (LV) hypertrophy and functional impairment [22]. Several studies have reported elevated diastolic blood pressure (BP) values in this population [23, 24], and our findings likewise revealed that diastolic BP was significantly higher in patients receiving dialysis compared with healthy controls.

Anemia, primarily resulting from inadequate erythropoietin production, remains a common complication of CRF [22, 25, 26]. A study conducted in the pediatric age group found that patients with CRF who were at the predialysis threshold also had a high prevalence of anemia. According to the United States Renal Data System records, 54.1% of pediatric hemodialysis patients had mean hemoglobin values less than 11 g/dL [22]. Studies have reported similar hemoglobin levels between dialyzed and non-dialyzed CRF patients [7]. Although mean hemoglobin concentrations in our study group were below normal reference ranges, no significant difference was observed between dialysis and non-dialysis subgroups. Electrolyte imbalances are also common in pediatric CRF. Previous investigations have documented elevated serum urea, phosphorus, and blood urea nitrogen (BUN) levels, accompanied by decreased calcium concentrations [7, 20, 27, 28]. Electrolyte imbalance and volume overload may alter ECG parameters. Previous studies reported prolonged PR intervals and QT abnormalities [29-31]. Heart rate measurements were comparable among the study groups, in agreement with some reports [24], though differing from others that identified increased rates in similar cohorts [20]. In our study, serum urea, creatinine, and phosphorus levels were higher,

whereas glomerular filtration rate (GFR) values were lower in the dialysis group, reflecting the severity of renal impairment. The reduced potassium levels observed were likely attributable to the dialysis process and concomitant medication use. Moreover, dialysis patients had longer PR intervals, while QTcd was significantly increased in CRF patients overall, especially in non-dialysis cases, consistent with arrhythmic risk reported in literature [29, 32-34].

Serum lipid profiles in our cohort remained within normal limits, which is consistent with previous pediatric studies reporting a relatively low prevalence of dyslipidemia in this age group [22].

Echocardiographic findings in the literature have varied [14, 21, 24, 35, 36]. We observed lower EF and FS values, though within normal limits, suggesting early systolic impairment due to volume overload. Structural indices (IVSd, LVPWd, LVMI) were higher in dialysis patients, paralleling reports linking increased LV mass to mortality in ESRD [38-41].
Left atrial (LA) and aortic (Ao) diameters were greater in dialysis patients, likely due to pressure and volume overload [21]. LAV and LAV index were elevated in CRF patients, especially in the dialysis group, consistent with adult studies identifying LAV index as a predictor of cardiovascular risk and mortality [42-44]. We therefore suggest that the LAV index may be a useful noninvasive marker for cardiac risk in pediatric CRF.

Diastolic dysfunction is often the earliest cardiac abnormality in CRF [3, 7, 45]. In our study, although mitral E and E/A ratios were reduced in patients compared with controls, these differences did not reach statistical significance, which may reflect an early or subclinical stage of myocardial involvement. This finding suggests that conventional Doppler parameters may not be sensitive enough to detect subtle diastolic impairment in the initial phases of CRF. Several studies have reported evidence of diastolic dysfunction even in the early stages of kidney failure, supporting the notion that myocardial relaxation abnormalities precede overt systolic impairment [45-48]. Consistent with these reports, our tissue Doppler analysis demonstrated significantly lower mitral E', Sm, and prolonged IVCT, CT, and IVRT, together with an elevated Tei index -indicating combined systolic and diastolic dysfunction that may not yet be apparent on conventional echocardiography. Similarly, IVS tissue Doppler imaging showed reduced E', E'/A', and Sm, and increased IVCT, IVRT, and Tei index [27, 49, 50], supporting subclinical LV dysfunction even when conventional Doppler was normal.

Right ventricular assessment revealed reduced tricuspid E'/A', elevated E/E', prolonged IVRT, and increased Tei index, especially in dialysis patients, consistent with early right ventricular dysfunction [38, 51]. Conventional Doppler failed to detect this, highlighting the greater sensitivity of tissue Doppler imaging in identifying biventricular impairment in CRF.

Strengths and Limitations

This study has several notable strengths that enhance the validity and clinical relevance of its findings. First, the simultaneous and comprehensive evaluation of cardiac structure, function, and electrical conduction using both advanced echocardiographic techniques (including M-mode, pulsed Doppler, and tissue Doppler imaging) and detailed electrocardiographic parameters provides an integrated assessment of cardiovascular involvement in pediatric patients with chronic renal failure (CRF). The inclusion of less frequently assessed ECG markers such as QT dispersion, Tp-e interval, and Tp-e/QTc ratio allows for a more refined evaluation of arrhythmogenic risk in this vulnerable population.

Second, the study design incorporated a well-matched control group with comparable age and sex distribution, strengthening the reliability of between-group comparisons. Additionally, stratifying CRF patients according to dialysis status enabled the identification of subclinical cardiovascular alterations associated with disease severity and renal replacement therapy, even in patients not yet receiving dialysis.

Finally, the study's focus on a pediatric cohort with varying stages of CRF, including non-dialysis patients, contributes valuable data to an area where pediatric evidence remains limited. The identification of early functional and structural cardiac changes before overt clinical manifestations highlights the potential role of routine cardiovascular surveillance in pediatric CRF and supports the clinical applicability of the findings.

Our study has several limitations. First, its cross-sectional and observational design precludes the

establishment of causal relationships. Second, the relatively small number of pediatric patients undergoing dialysis limited the ability to perform subgroup analyses based on dialysis modality.

CONCLUSION

In conclusion, regular electrocardiographic screening appears warranted for the early detection of potential CRF-related cardiac dysrhythmias in children. Our electro- and echocardiographic findings have the potential to address such clinically significant issues encountered in routine clinical practice through a noninvasive and cost-effective manner. We also believe that our findings contribute to the existing literature on the cardiac assessment of pediatric CRF patients. Nevertheless, further large-scale studies with greater number of dialysis patients, more dialysis modalities, comprehensive cardiac evaluations and longer follow-up periods are warranted to confirm and expand upon our results.

Ethics Approval and Consent to Participate

This study was approved by the Necmettin Erbakan University Meram Faculty of Medicine Non-Drug and Medical Device Research Ethics Committee (Decision No: 2018/71448-72; date: 29.06.2018). All procedures were conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from all individual participants included in the study.

Data Availability

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

Authors' Contribution

Study Conception: AA, FŞ; Study Design: AA, FŞ; Supervision: FŞ, BA, MBO, TB; Funding: N/A; Materials: AA, FŞ, BA; Data Collection and/or Processing: AA, FŞ; Statistical Analysis and/or Data Interpretation: AA, FŞ, MBO, TB; Literature Review: AA, MBO, TB; Manuscript Preparation: AA, FŞ; and Critical Review: FŞ, BA, MBO, TB.

Conflict of Interest

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

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Generative Artificial Intelligence Statement

The author used artificial intelligence tools (ChatGPT, OpenAI) only for language editing and reference formatting. The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

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The European Research Journal 2026;12(2):187-198 Original Article

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Impact of Comorbidities on Local Anesthetic Hypersensitivity: A Decade of Clinical Experience

Mehmet Kılınç[1], Fatih Çölkesen[2], Filiz Sadi Aykan[3], Recep Evcen[4], Tuğba Önalan[5], Fatma Arzu Akkuş[6], Eray Yıldız[7], Şevket Arslan[2]

[1]Division of Clinical Immunology and Allergy, Batman Training and Research Hospital, Batman, Türkiye; [2]Department of Internal Medicine, Division of Clinical Immunology and Allergy, Necmettin Erbakan University, Medical Faculty Hospital, Konya, Türkiye; [3]Division of Clinical Immunology and Allergy, Republic of Turkey Ministry of Health Ankara Gulhane Training and Research Hospital, Ankara, Türkiye; [4]Division of Clinical Immunology and Allergy, Republic of Turkey Ministry of Health Recep Tayyip Erdoğan University Training and Research Hospital, Rize, Türkiye; [5]Division of Clinical Immunology and Allergy, Health Sciences University, Beyhekim Training and Research Hospital, Konya, Türkiye; [6]Division of Clinical Immunology and Allergy, Republic of Turkey Ministry of Health Konya City Hospital, Konya, Türkiye; [7]Division of Clinical Immunology and Allergy, Republic of Turkey Ministry of Health Kahramanmaras Necip Fazil City Hospital, Kahramanmaraş, Türkiye

ABSTRACT

Objectives: Clinicians widely utilize local anesthetic (LA) agents in pain management and surgical interventions. Nevertheless, drug hypersensitivity reaction (HR) to their use continue to raise considerable concern among both clinicians and patients. The present study aims to investigate the relationship between local anesthetic hypersensitivity reactions (LA-HRs) and comorbid conditions in patients referred to our clinic.

Methods: Clinical data and test results of patients who were referred to our allergy clinic for evaluation of suspected LA-HR were retrospectively reviewed. Skin tests (ST) and subcutaneous provocation tests (SPT) were performed.

Results: A total of 200 patients were included in the study. The most common reason for referral was a suspected LA-HR (80%), followed by a history of HR to other drugs with a need for LA use during upcoming procedures (20%). Among 160 patients with suspected LA- HR, only 5.6% demonstrated confirmed hypersensitivity by positive ST and SPT. Of the 40 patients with a history of HR to other drugs, only 2.5% tested positive for LA-HR. A history of comorbidity was present in 70% patients. Patients with suspected LA-HR had a significantly higher prevalence of comorbidities compared to those with HR to other drugs. However, none of the patients who tested positive had a history of comorbidity.

Conclusions: Confirmed hypersensitivity risk among patients reporting LA-HR is very low. Although comorbidities were more common among patients reporting LA-HR, none of the patients with confirmed allergy had any comorbidities, underscoring the low confirmed hypersensitivity rate in this population.

Keywords: Local Anesthetics, Drug Hypersensitivity Reactions, Comorbidity, Skin Tests

Local anaesthetic (LA) agents are pharmacological agents widely used in many areas of daily clinical practice, including surgical procedures performed under LA, dental procedures, and childbirth. These drugs enable surgical procedures to be performed safely and painlessly. Most adverse drug reactions (ADRs)

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Corresponding author: Mehmet Kılınç, MD., Phone: +90 488 221 30 66, E-mail: drmehmetalerji@gmail.com

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associated with LAs have been reported to be related to mechanisms such as the adrenaline response, vasovagal syncope, or toxicity due to overdose [1]. However, patients often interpret such side effects as allergic reactions to LAs and express concern about the potential risk of allergy. Furthermore, vague clinical symptoms that arise following the administration of LAs may sometimes be misinterpreted as 'allergic reactions' by clinicians [2].

The incidence of allergy to LAs has not been definitively established [3]. However, it is estimated that the frequency of confirmed allergic reactions is approximately 1% and is quite rare [4]. It is clinically recommended that suspected drugs causing hypersensitivity reaction (HR) undergo a systematic diagnostic evaluation to identify the responsible agent and determine the potential risk of re-exposure [5]. Among patients with drug hypersensitivity reaction (DHR), the most frequently reported comorbid conditions include cardiovascular (CV) diseases (such as hypertension and coronary artery disease), asthma, and other respiratory disorders. These comorbidities are considered to influence not only the likelihood of developing HR but also the severity of their clinical manifestations [6]. Moreover, the presence of depression or other psychiatric disorders may amplify somatic complaints and contribute to an increased susceptibility to ADRs [7].

Inadequate investigation of suspected allergic reactions to LAs may cause anxiety and distress in patients and may also be a significant concern for clinicians. Therefore, many patients are referred to allergy clinics for diagnostic evaluation of possible allergic reactions to LAs. Allergic reactions to LAs occurring during anaesthesia or surgical procedures can lead to serious and life-threatening clinical conditions, particularly in cases of anaphylaxis. The limited characterization of the association between comorbidities and LA HR (LA-HR) warranted this investigation. In this context, the present study aims to analyze the association between LA-HR and comorbid conditions in patients referred to our allergy clinic.

METHODS

Study Design

This retrospective cohort study was conducted at Necmettin Erbakan University Medical Faculty Hospital, Konya, Turkey. The study protocol was approved by the University's Ethics Committee and conducted in accordance with the Declaration of Helsinki (decision no. 2023/4680). Patient files and the hospital database were systematically reviewed to identify eligible cases from January 2013 to November 2023. Cases were screened for complete medical data. The study included 200 patients aged ≥18 years with a history of LA-HR or other drug allergy who were referred to an allergy clinic outpatient clinic before LA administration and underwent LA skin testing (ST). Patients who refused LA ST, patients who could not be tested due to dermographism, and those with missing or insufficient data were excluded from the analysis (Figure 1).

Data Collection

Patient data were retrieved from medical records using a systematic approach: (1) demographic information and comorbid conditions were recorded; (2) details of the procedure during which the reaction occurred and referral reasons were noted; (3) clinical manifestations of drug HR, such as circulatory, respiratory, cutaneous, central nervous system, and gastrointestinal symptoms, were documented; (4) history of prior HR to LAs and/or other drugs was collected; and (5) results of tests performed with suspected or requested agents were gathered. Clinical manifestations included circulatory symptoms (palpitations, tachycardia, hypotension, shock, cardiac arrest), respiratory symptoms (chest tightness, dyspnea, wheezing, bronchospasm), cutaneous findings (rash, flushing, erythema, urticaria, angioedema), central nervous system signs (anxiety, malaise, sweating, dizziness, somnolence, syncope, loss of consciousness), and gastrointestinal symptoms (nausea, vomiting, abdominal pain). DHRs are classified as early type if they develop within one hour, and delayed type if they occur after one hour [5].

Skin Tests

The diagnostic approach was conducted in accordance with the recommendations of the ENDA/EAACI Drug Allergy Interest Group guidelines [8]. Diagnostic evaluation of allergic reactions to LAs in our clinic included ST, comprising

a skin prick test (SPT) and/or intradermal test (IDT). Patients were instructed to discontinue systemic antiallergic medications, such as antihistamines, leukotriene receptor antagonists, and corticosteroids, at least 1 week prior to testing. The drug yielding a positive skin test response was identified as the "culprit drug." LAs without vasoconstrictors were utilized in skin testing to minimize the risk of false-positive reactions, and all patients underwent subcutaneous challenge testing (SCT) [9]. Drugs tested in this study included lidocaine, mepivacaine, articaine, bupivacaine, and prilocaine. The SPT was initially performed using the undiluted (1/1) LA intended for clinical use. Sodium chloride (0.9%) served as the negative control, and histamine (1 mg/mL) as the positive control. Twenty minutes after administration, the diameters of the wheal and erythema were measured. A mean wheal diameter exceeding 3 mm compared with the negative control, accompanied by surrounding erythema, was considered a positive reaction. Patients with negative SPT results subsequently underwent IDT at 1/100 and 1/10 dilutions. Each concentration was evaluated 20 minutes after injection, and an increase in wheal diameter of ≥3 mm from the initial size, together with erythema, was interpreted as positive. When both SPT and IDT yielded negative results, SCTs were performed with gradually increasing doses of the LA (0.1 mL and 1 mL). The occurrence of cutaneous manifestations (such as urticaria or angioedema), respiratory symptoms (including cough, dyspnea, or wheezing), and/or cardiovascular findings (such as hypotension or tachycardia) within 20 minutes after provocation was regarded as a positive response. All ST procedures were conducted in a controlled clinical setting with full emergency preparedness. Resuscitation equipment, including epinephrine, oxygen, intravenous access materials, and monitoring devices, was available, and all procedures were supervised by physicians trained in anaphylaxis management. Patients were observed for standardized periods post-procedure (20–30 minutes after SPT/IDT and 30–60 minutes after SCT) in accordance with institutional and international safety guidelines. All steps were performed following established protocols to ensure patient safety and procedural reliability.

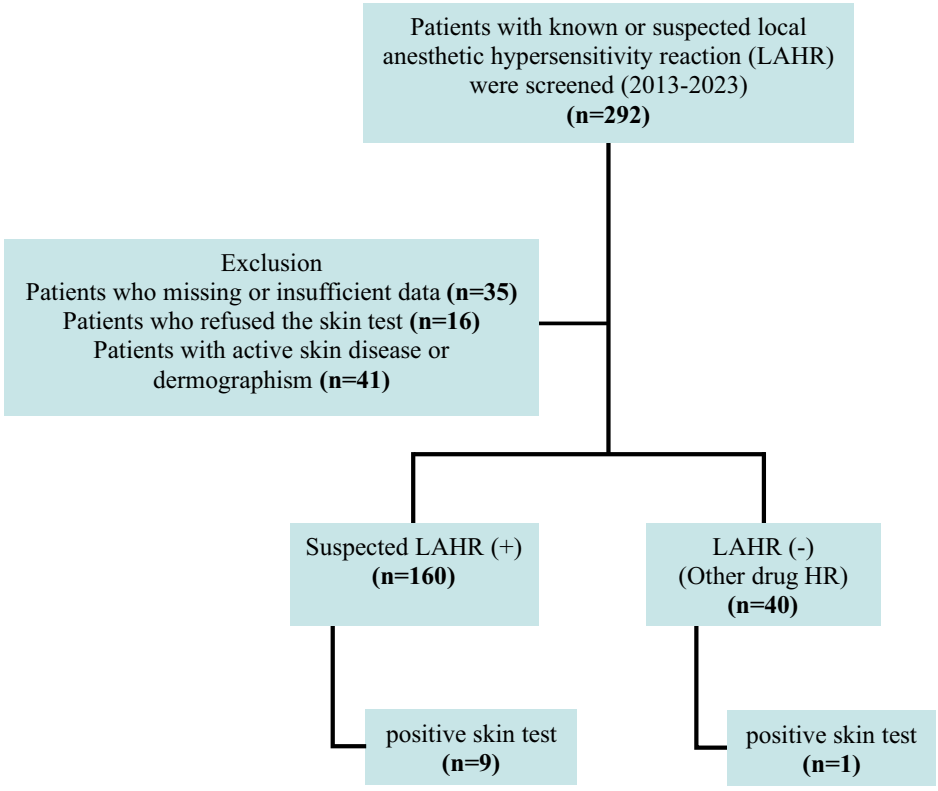

FIGURE 1. Flowchart depicting the inclusion pathway of the study participants.

Statistical Analysis

Statistical analyses were performed using SPSS for Windows, version 22.0 (IBM Corp., Armonk, NY, USA). Continuous variables are expressed as mean ± standard deviation or median (minimum–maximum) values, whereas categorical variables are presented as frequencies and percentages. Comparisons between categorical variables were performed using the Chi-square test. The distributional characteristics of continuous variables were evaluated using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Variables demonstrating normality were summarized as mean ± standard deviation, whereas those not meeting normality assumptions were presented as median values accompanied by the interquartile range (IQR). For group comparisons, the independent samples Student's t-test was applied to normally distributed data, while the Mann–Whitney U test was used for variables that exhibited non-normal distribution. The association between age and positive LA test results was assessed using logistic regression analys. A P-value <0.05 was considered statistically significant.

RESULTS

Demographic Characteristics of the Study Population

A total of 200 patients referred to our allergy clinic were included in the present study. Of these, 162 (81%) were female and 38 (19%) were male, with a mean age of 47±12.1 years (range, 25–70 years). Among the 200 patients assessed, 40 (20%) had a history of other allergic disorders, and 140 (70%) had comorbidities (Table 1). CV diseases were the most common comorbidity (n=71, 50.7%) (Table 1). The primary reason for referral was a suspected HR associated with procedures involving LAs (n=160, 80%). The secondary reason was a prior HR to other medications, with a need for LA administration in future interventions (n=40, 20%) (Table 1). Patients with a suspected history of LA-HR underwent testing with the implicated agents. Those with a history of HR to other medications were evaluated using the LAs needed for their planned procedures (Table 1). Among the 40 patients with a prior history of HR to drugs other than LAs, general anesthetic agents were the

TABLE 1. Clinical and Demographic Characteristics of the Study Population

Variable	Value
Age (years)	47±12.1
Sex	
Female	162 (81)
Male	38 (19)
Comorbidity	140 (70)
CV disease	71 (50.7)
Respiratory disease	52 (26)
Diabetes mellitus	36 (18)
Malignancy	7 (3.5)
Psychiatric disease	14 (7)
Other	17 (8.5)
Atopy	40 (20)
Asthma	24 (12)
Allergic rhinitis	8 (4)
Atopic dermatitis	2 (1)
Urticaria	6 (3)
Suspected LA-HR	160 (80)
History of Other Drug HR	40 (20)
General anesthetics	20 (50)
NSAIDs	8 (20)
Antibiotics	6 (15)
Radiocontrast agents	3 (7.5)
Others	3 (7.5)
Type of procedure	
Dentistry	120 (60)
Intra-articular procedures	24 (12)
Surgery	16 (8)
Skin testing /SCT	
Suspected LA	160 (80)
Requested LA	40 (20)
Lidocaine	16 (40)
Articaine	13 (32.5)
[a]Unknown	11 (27.5)
Latex sensitivity (skin test positive)	5 (2.5)

Data are shown as mean±standard deviation or n (%). CV, cardiovascular; LA-HR, local anesthetic hypersensitivity reaction; HR; hypersensitivity reaction; LA, local anesthetic; NSAIDs, nonsteroidal anti-inflammatory drugs; SCT, subcutaneous challenge testing.

[a]Prilocaine was tested.

most frequently implicated (n=20, 50%). Reactions during dental procedures were reported by 120 (60%) patients (Table 1). In most cases, the LA requested for upcoming procedures was lidocaine (n=16, 40%). When the specific LA was unknown to referring physicians, prilocaine was tested instead (n=11, 27.5%) (Table 1).

Clinical Features of Suspected Hypersensitivity to Local Anesthetics

In this study, 160 patients exposed to one or more LAs with suspected HR history were evaluated. The most frequently observed manifestations were cutaneous symptoms (n=120, 75%). Circulatory (n=96, 60%) and respiratory findings (n=80, 50%) followed. In most cases, lidocaine was the most commonly implicated agent (n=65, 40.6%). For 29 (18.1%) patients, the causative LA could not be determined (Table 2). Among all patients with suspected HR, 6 (3.75%) individuals required intramuscular adrenaline intervention (Table 2).

TABLE 2. Clinical Features of Suspected Drug Hypersensitivity to Local Anesthetics

Variable	n (%)
Symptoms of drug reactions	
Cutaneous (urticaria/angioedema, rash, flushing, etc.)	120 (75)
Circulatory (Hypotension, tachycardia, palpitations)	96 (60)
Respiratory (dyspnea, bronchospasm, etc.)	80 (50)
Gastrointestinal tract (nausea, vomiting)	40 (25)
CNS (syncope, anxiety, dizziness, etc.)	32 (20)
Anaphylaxis	6 (3.75)
Suspected LAs	
Lidocaine	65 (40.6)
Mepivacaine	17 (10.6)
Articaine	23 (14.3)
Bupivacaine	15 (9.3)
Prilocaine	11 (6.8)
Unknown	29 (18.1)
Treatment	
Adrenaline	6 (3.75)
No defined treatment	33 (20.6)
[a]Others	121 (75)

Data are shown as n (%). LAs, local anesthetics; CNS, central nervous system.

[a]Antihistamines, corticosteroids

Demographic and Clinical Findings of Patients with and without Hypersensitivity Reaction to Local Anesthetic Agents

Most of the patients in both groups were female (n=96, [60%] vs. n=27, [67.5%]), and their mean age was similar (46±11 vs. 40±10.5 years). There were no significant differences between the two groups in terms of sex (P=0.37) and mean age (P=0.28). One hundred and twenty (75%) patients with HR to LAs had one or more comorbidities, and the comorbidity was significantly higher than in patients with HR to other drugs (P=0.02) (Table 3). CV diseases were the most common comorbidity in patients with LA-HR and in patients with HR to other drugs (40% and 17.5%, respectively). CV diseases were significantly higher in patients with LA-HR than in the other group (P=0.03). Atopy was present in 36 (22.5%) patients with LA-HR and was significantly higher than in the other group (P=0.04). STs were performed on all patients. STs/SCT positivity was determined in 9 (5.6%) patients with LA-HR and 1 (2.5%) patient with HR to other drugs (Table 3).

Overview of Clinical Findings in Ten Patients Showing Positive Reactions to Local Anesthetics

Among 160 patients with suspected HR following procedures involving LAs, only nine (5.6%) had confirmed hypersensitivity to LAs as evidenced by positive ST/SCT. In contrast, 151 of these patients tested negative in all assessments. Additionally, only one patient with a prior history of HR to other medications demonstrated a positive response in ST/SCT. Of the ten patients with positive LA STs, the majority were female (n=7). Five patients had a history of reactions to LA injection accompanied by respiratory and/or circulatory symptoms. Two patients had skin symptoms. Four patients underwent SPT/SCT with lidocaine, three of whom were suspected agents (Table 4).

Logistic regression analysis revealed no significant association between age and positive LA

testing results (odds ratio (OR) = 1.07, 95% confidence interval, CI: 0.95–1.08, P=0.37).

Patients with positive and negative test results were evaluated comparatively. No statistically significant difference was observed in terms of age and gender (P=0.25 and P=0.44, respectively) (Table 5).

DISCUSSION

This study analysed a total of 200 patients tested with LAs, of whom 5% had a confirmed hypersensitivity to LA. Another notable finding of this study was that none of the patients who tested positive had a history of comorbidity. A study conducted with LA emphasised that confirmed hypersensitivity to LA is extremely rare (1%) [10]. Despite the widespread use of LAs in clinical practice, only a limited number of case reports have described immunoglobulin E (IgE)-mediated allergic reactions [11]. Furthermore, a previous study reported that none of the patients who underwent detailed evaluation for suspected LA-HR were confirmed hypersensitivities [12]. This study similarly supports that the risk of confirmed

TABLE 3. Comparison of Patients with and Without Hypersensitivity Reaction to Local Anesthetics

Variable	Patients with suspected LA-HR (n=160)	Patients with other drug HR[a] (n=40)	P-value
Age (years)	46±11	40±10.5	0.28
Gender			
Female	96 (60)	27 (67.5)	0.37
Male	64 (40)	13 (32.5)	
Comorbidity	120 (75)	20 (50)	**0.02**
CV disease	64 (40)	7 (17.5)	**0.03**
Respiratory diseases	47 (29.3)	5 (12.5)	0.15
Diabetes mellitus	32 (20)	4 (10)	0.83
Malignancy	5 (3.1)	2 (5)	0.91
Psychiatric diseases	12 (7.5)	2 (5)	0.72
[b]Other diseases	15 (9.3)	2 (5)	0.79
Atopy	36 (22.5)	4 (10)	**0.04**
Asthma	22 (13.7)	2 (5)	0.18
Allergic rhinitis	8 (5)	1 (2.5)	0.65
Atopic dermatitis	2 (1.25)	0	0.65
Urticaria	4 (2.5)	1 (2.5)	0.52
History of other drug HR	40 (25)	40 (100)	**0.02**
Clinical approach			
Skin tests	160 (100)	40 (100)	
LA Test Positivity	9 (5.6)	1 (2.5)	0.74
Latex hypersensitivity	4 (2.5)	1 (2.5)	0.45
[c]Antiseptic hypersensitivity	2 (1.25)	1 (2.5)	0.1

Data are shown as mean±standard deviation or n (%). CV, cardiovascular; LA-HR, local anesthetic hypersensitivity reaction; HR, hypersensitivity reaction; LA, local anesthetic.

[a]General anesthetics, nonsteroidal anti-inflammatory drugs, antibiotics, radiocontrast agents, iron preparations, chemotherapeutic agents, [b]Hypertension, thyroid disease, osteoporosis, [c]Chlorhexidine skin testings.

Statistically significant P-values are shown in bold.

TABLE 4. Clinical Findings in Ten Patients Demonstrating Test Positivity to Local Anesthetic

Patient	Sex	Age	Clinical Findings	Culprit Drug	History of other Drug Hypersensitivity (other than LA)	Comorbidity	Latex Sensitivity	Psychiatric condition
1	F	32	Cardiovascular Tachycardia Hypotension	Bupivacaine	No	No	No	No
2	F	35	Cutaneous Urticaria Angioedema	Lidocaine	No	No	No	No
3	M	40	Cardiovascular Hypotension Respiratory Dyspnea	Lidocaine	No	No	No	No
4	F	45	CNS Dizziness Respiratory Dyspnea	Mepivacaine	No	No	No	No
5	F	42	Respiratory Bronchospasm Cutaneous Rash Flushing	Articaine	No	No	No	No
6	M	47	GIS tract Nausea Vomiting CNS Dizziness	Bupivacaine	No	No	No	No
7	F	51	Cardiovascular Hypotension CNS Syncope	Prilocaine	No	No	No	No
8	M	49	GIS tract Nausea Vomiting	Lidocaine	No	No	No	No
9	F	52	Cutaneous Urticaria Angioedema	Articaine	No	No	No	No
10	F	38	No symptoms	[a]Lidocaine	Yes	No	No	No

F, female; M, male; LA, local anesthetic; CNS, central nervous system; GIS, gastrointestinal system.
[a]Requested local anesthetic

TABLE 5. Comparison According to Their Positive and Negative Test Outcomes

	Positive skin test	Negative skin test	P-value
Age (years)	40 (30-55)	42 (32-47.5)	0.25
Gender			
Female	7 (70)	155 (81.5)	0.44
Male	3 (30)	35 (18.5)	
Suspected LA-HR	9 (90)	151 (79.5)	0.38
History of other drug HR	1 (10)	39 (20.5)	0.25

Data are shown as median (interquartile range) or n (%). LA-HR, local anesthetic hypersensitivity reaction; HR, hypersensitivity reaction.

hypersensitivity to LA is low and that non-allergic reactions develop in most patients following LA administration. It has been reported that non-allergic reactions are frequently misclassified as LA allergy by physicians other than patients and allergy specialists [13].These patients are referred to allergy clinics to determine a safe LA agent prior to the planned procedure [14]. In order to confirm the presence of a genuine allergy, any suspicion of allergy must be carefully evaluated [15]. Despite the use of different methods in the diagnosis of drug allergies, the diagnostic process remains complex and difficult. Although the protocols used in studies vary, the majority are based on STs, which are considered the gold standard [16]. In this study, STs were also administered to all patients. In individuals who have experienced DHR in the past, the likelihood of reactions to LA increases [17]. It has been reported that patients with a history of allergic reactions to various drugs, particularly general anaesthetics, are at high risk of developing allergies to LAs [18]. A previous study conducted with LAs highlighted that the most common exposure in individuals with a history of HR associated with other drugs was to general anaesthetics [19]. Similarly, in this study, the most common history in patients with HR to other drugs was with general anaesthetic agents. Recent studies have shown that a history of DHR developing only after exposure to LAs may pose a risk for similar or more severe reactions [20, 21]. Another study has shown that a previous history of ADRs to LAs may be considered a risk factor in subsequent applications [14]. Current guidelines recommend that patients should only be tested if they have a history of LA-HR [8, 22]. This study also demonstrated that only one individual with a history of HR to other supportive medications had positive skin tests, ruling out IgE-mediated allergic reactions. In addition to drug-related factors, patient-related predisposing factors for DHR have also been identified. However, the role of atopy in the development of DHR remains a subject of debate [23]. Some studies suggest that atopy may be associated with LA-HR, but this potential link has not yet been confirmed in the literature [24, 25]. In a study examining the effect of atopic history on LA allergy test results, no significant relationship was found between atopic status and test positivity [26]. A previous study has shown that the likelihood of developing LA-HR is approximately five times higher in atopic individuals [23]. Similar to this study, individuals with a history of suspected LA-HR had a significantly higher prevalence of atopy. However, atopy was not detected in any of the test-positive patients. Prior evidence indicates that routine screening of asthma patients for potential LA-HR is not warranted [14]. In our study, asthma and chronic rhinitis were the most common atopic diseases. However, no significant increase was observed in asthma, chronic rhinitis or urticaria among individuals with LA-HR.

ADRs associated with LAs are generally characterised by skin reactions, respiratory, circulatory or neurological symptoms, and anaphylaxis may be observed in rare cases [4]. According to a study on LAs, circulatory manifestations constituted the most common clinical presentation, followed by cutaneous and central nervous system (CNS) involvement [19]. In this study, cutaneous manifestations were the most frequently observed symptoms, followed by circulatory disturbances.

Latex exposure is common during procedures that require the use of LAs [23]. The prevalence of latex

allergy in the general population is estimated to be approximately 4.3% [27]. Previous research has shown that latex sensitivity accounts for about 16.7% of hypersensitivity reactions observed during anesthetic procedures [28]. In the present study, latex sensitivity was identified in 2.5% of the study population, and this low rate may be related to the limited sample size.

Amide-type LAs undergo hepatic metabolism through microsomal enzyme systems, resulting in water-soluble metabolites that are subsequently eliminated by renal excretion [29].Comorbidities are critical determinants in the safe administration of LAs. Organ dysfunction is a key patient-related factor that increases the risk of systemic toxicity. Individuals with pre-existing cardiac disease are particularly vulnerable to the arrhythmogenic and myocardial depressant effects of LAs, whereas hepatic or renal impairment may slow drug metabolism and excretion, resulting in elevated plasma concentrations [30]. Heart failure reduces perfusion to organs responsible for the metabolism of LAs, such as the liver and kidneys, thereby impairing drug clearance and increasing the risk of systemic toxicity. Low cardiac output may also compromise drug absorption due to diminished tissue perfusion, resulting in higher concentrations of LAs reaching the central nervous system and elevating the risk of neurotoxicity [31]. In this study, the prevalence of comorbidities and a history of CV disease was significantly higher among individuals exhibiting suspected hypersensitivity to LAs.

One possible factor underlying the increased incidence of ADRs is polypharmacy. In such cases, pharmacokinetic and pharmacodynamic interactions among multiple drugs, particularly those involving cytochrome P450 enzymes, may enhance both drug efficacy and toxicity [32]. The increased prevalence of comorbidities leads to more frequent prescribing of medication, and the use of one or more drugs for each comorbidity results in polypharmacy. The risk of drug interactions associated with multiple drug use increases in individuals with more than one chronic condition [33]. The presence of comorbidities is a major contributor to polypharmacy, as multiple coexisting conditions often necessitate the concurrent use of several medications [34]. With an increasing number of prescribed medications, the likelihood and severity of ADRs and interactions also rise [35]. Estimates suggest that individuals using two medications have approximately a 13% risk of experiencing an ADR, whereas this risk escalates to 58% for those taking five drugs and to 82% for individuals using seven or more medications daily [36]. Similarly, in this study, the presence of comorbidities in the majority of the study population suggests that polypharmacy may have increased. However, none of the test-positive patients had comorbidities.

Recent evidence indicates that individuals with psychiatric disorders and those using psychoactive medications have a higher likelihood of experiencing suspected DHRs. Fluctuations in mood and heightened attention to bodily sensations may lead such patients to exaggerate perceived symptoms and, in some instances, attribute these reactions to the medication itself [37]. Previous research has demonstrated that individuals with psychiatric disorders exhibit a higher incidence of suspected DHRs than the general population [38]. Although findings across studies are not entirely consistent, several have suggested an association between anxiety and depression and a higher propensity for developing multiple drug intolerance syndrome [39, 40]. A recent study examining patients' concerns regarding drug allergies found that individuals with poorer overall and mental health were more likely to report such concerns. Moreover, these patients demonstrated a significantly higher likelihood of expressing worry about allergic reactions [41]. Psychiatric conditions - particularly anxiety, somatization tendencies, panic disorder, and chronic psychiatric illnesses - can contribute to the misdiagnosis of confirmed allergic reactions to LAs and may also increase the incidence of psychogenic, vasovagal, and behavioral responses. Additionally, systemic toxicity from LAs can induce CNS symptoms, which may manifest as psychotic-like presentations [42]. In this study, the prevalence of psychiatric disorders was higher among individuals exhibiting suspected HR to LAs. In this study, 7.5% of those with suspected LA-HR had a psychiatric disease, and none of those with a positive test had a psychiatric condition.

Strengths and Limitations

This study has several limitations. First, due to its

retrospective design, the data were derived from existing clinical records, which may be subject to missing information and potential bias. Second, as a single-centre study, the relatively small sample size may limit the generalisability of the findings. Variations in test protocol standardisation and the heterogeneity of patients clinical characteristics should also be considered when interpreting the results. Consequently, these findings warrant validation in larger, prospective, multicentre studies. Despite these limitations, this study reveals that confirmed HRs to LAs are rare and that the presence of comorbidities may be associated with suspected LA-HR histories but not directly linked to confirmed LA-HR, providing an essential perspective on the LA-comorbidity relationship.

CONCLUSION

LA-HRs occurring during anesthesia and surgical procedures, particularly anaphylaxis, are serious clinical events that can result in life-threatening complications and permanent sequelae. Patients with a history of suspected LA-HR may be reluctant to use these agents in future procedures due to potential risks. Consequently, many patients with suspected LA-HR are referred to allergy clinics to identify alternative agents that can be safely administered.

The present study demonstrates that, although a history of HR to LAs or other drugs represents a significant concern for patients and physicians prior to procedures requiring LAs, confirmed HRs are rare. Moreover, while a history of comorbidity was more frequent among individuals with suspected LA-HR, no comorbidity was observed in any of the patients who tested positive, suggesting that the relationship between comorbidity and confirmed LA-HR remains unclear. Further prospective multicentre studies are warranted to clarify this association.

Ethics Approval and Consent to Participate

This study was approved by the Necmettin Erbakan University Non-Drug and Non-Medical Device Research Ethics Committee (Decision No: 2023/4680-188; date: 15.12.2023). All procedures were conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Informed consent was waived because of the retrospective nature of the study and the analysis used anonymous clinical data.

Data Availability

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

Authors' Contribution

Study Conception: MK, FÇ, FSA; Study Design: MK, FÇ, RE; Supervision: MK, FSA, RE; Funding: MK; Materials: MK; Data Collection and/or Processing: MK, FSA, TÖ, RE; Statistical Analysis and/or Data Interpretation: MK, FAA, EY, TÖ; Literature Review: MK, EY, TÖ, FAA; Manuscript Preparation: MK, FAA, ŞA; and Critical Review: MK, EY, ŞA.

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Effects of Localized Arm Fatigue, General Fatigue, and Elbow Bracing on Shooting Accuracy and Shoulder Proprioception: A Randomized Crossover Study

Ukbe Şırayder[1], Oğuzhan Yılmaz[2]

[1]Department of Physiotherapy and Rehabilitation, Faculty of Health Sciences, Nuh Naci Yazgan University, Kayseri, Türkiye; [2]Department of Therapy and Rehabilitation, Vocational School of Higher Education, Nuh Naci Yazgan University, Kayseri, Türkiye

ABSTRACT

Objectives: This study aimed to comparatively investigate the effects of localized arm fatigue, general fatigue, and elbow brace use on proprioceptive accuracy and shooting accuracy in amateur female basketball players.

Methods: Fifty-two amateur female basketball players (mean age: 23.08±2.02 years) participated in a randomized crossover study. Participants underwent three experimental conditions: localized arm fatigue, general fatigue, and brace application, each tested on separate days with a 48-hour washout period between sessions. Proprioceptive accuracy was assessed using the Joint Position Sense Error (in degrees), and shooting accuracy was evaluated based on shot success percentage. Fatigue perception was measured using the Borg Rating of Perceived Exertion Scale. Statistical analyses included Friedman and Wilcoxon signed-rank tests with Bonferroni correction for multiple comparisons.

Results: Localized arm fatigue significantly decreased shooting accuracy (from 53.16% to 38.83%, P=0.014) and increased proprioceptive error (from 5.40° to 8.65°, P=0.003). General fatigue resulted in a moderate increase in proprioceptive error (from 4.05° to 5.35°, P=0.049) but did not significantly affect shooting accuracy (P=0.090). The use of an elbow brace improved proprioceptive accuracy (error reduction from 5.30° to 3.00°, P=0.035) and marginally enhanced shooting performance (increase from 48.33% to 54.83%, P=0.027). Strong negative correlations were found between proprioceptive degradation and shooting accuracy after localized fatigue (r= –0.787, P<0.001).

Conclusions: Localized arm fatigue impairs proprioception and shooting accuracy more severely than general fatigue. Elbow bracing mitigates these impairments, suggesting its use as an intervention to maintain technical performance under fatigue in basketball athletes.

Keywords: Proprioception, Localized Fatigue, Elbow Brace, Shooting Accuracy, Basketball, Crossover Study

Fatigue is a pervasive physiological phenomenon that affects various dimensions of athletic performance, including muscular strength, coordination, sensorimotor control, cognitive processing, and technical execution [1]. In sports that demand a combination of endurance, precision, and motor control - such as basketball - fatigue can disrupt not only physical outputs but also fine motor skills

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Corresponding author: Ukbe Şırayder, PhD., Assist. Prof., Phone: +90 352 324 00 00, E-mail: ukbesirayder@hotmail.com

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essential for successful skill-based actions [2]. One of the most fatigue-sensitive elements of basketball performance is shooting accuracy, which requires sustained motor precision and neuromuscular coordination under both physical and mental stress [3].

Numerous studies have documented that fatigue - whether systemic or task-specific - can lead to decrements in shooting accuracy, reaction time, and joint stability. Li *et al*. [4], in a recent meta-analysis, demonstrated that moderate physical fatigue significantly reduces two-point shot success, while severe fatigue impairs both two- and three-point accuracy. Furthermore, mental fatigue was found to compromise shooting accuracy, underscoring the multifactorial nature of fatigue-related performance decline. However, despite a growing body of evidence, the mechanisms through which different types of fatigue exert their effects remain insufficiently understood, particularly regarding localized versus systemic fatigue pathways.

Localized muscle fatigue - especially in the upper extremities - may disproportionately affect technical performance by impairing proprioception, a critical component of motor control. Proprioception enables athletes to perceive joint position and movement, allowing for precise execution of complex motor tasks. Shoulder joint proprioception, in particular, plays a central role in upper-limb-dominant movements such as shooting in basketball. Previous findings have indicated that fatigue impairs joint position sense, which may, in turn, deteriorate shooting mechanics and consistency [5-7].

At the same time, proprioceptive aids - such as elbow braces - have been suggested as a potential strategy to counteract fatigue-induced impairments. These braces provide mechanical support and stimulate cutaneous and joint mechanoreceptors, thereby potentially enhancing sensorimotor integration and joint awareness under fatigued conditions [8, 9]. Yet, despite promising theoretical frameworks, few controlled studies have directly evaluated the effectiveness of elbow bracing in mitigating performance deficits due to fatigue.

To date, most studies have either evaluated fatigue in general terms or failed to distinguish between different types of fatigue and their unique consequences. Although proprioception has often been evaluated under fatigue conditions, to the best of our knowledge, there are no randomized crossover studies in the literature that simultaneously examine these effects alongside interventions such as elbow bracing. Additionally, amateur female basketball players remain underrepresented in this research field, despite their growing participation in competitive sports.

Therefore, the aim of this study was to comparatively investigate the effects of localized arm fatigue, general fatigue, and elbow bracing on proprioceptive accuracy and shooting accuracy in amateur female basketball players. This study is novel in its design, as it simultaneously explores three distinct experimental conditions within the same participant group, providing a within-subject control for inter-individual variability. We hypothesized that localized arm fatigue would produce greater impairments in proprioception and shooting performance compared to general fatigue, and that the use of an elbow brace would mitigate some of these impairments. These findings could have practical implications for designing targeted fatigue management and proprioceptive training interventions in basketball and other precision-dependent sports.

METHODS

This study was conducted between February 2024 and January 2025 at Nuh Naci Yazgan University, Kayseri, Turkey. The sample size was determined through an a priori power analysis, which utilized preliminary data from a previous study conducted by our research team and presented at a national sports science conference [10]. In that preliminary study, a moderate effect size (r=0.38) was observed for the association between arm fatigue and shooting accuracy. The sample size calculation in the current study was based on this published conference data provided by the same research group. Based on this effect size, a two-tailed analysis with an alpha level of 0.05 and a desired power of 0.80 indicated that a minimum of 52 participants would be necessary. Participants were active basketball players, training at least three days a week, aged below 35, and free from health issues or injuries within the past year that could affect performance. Only individuals with a minimum of five years of experience in amateur-level basketball were included. Participants with any history of orthopedic

surgery were excluded. Participants were also screened to ensure no history of upper limb injuries or surgeries within the past 12 months. All participants confirmed current injury-free status through pre-study interviews and physical clearance by the supervising physiotherapist.

The study was conducted in accordance with the Declaration of Helsinki, and was approved by the Nuh Naci Yazgan University Scientific Research and Publication Ethics Committee (protocol code 2024/002-01, approval date: 12 February 2024).

Age, body weight, and height were recorded for each subject. Body mass index (BMI) was calculated using the body weight/height^2 (kg/m^2) formula.

Study Design

This study employed a randomized crossover experimental design, involving 52 amateur female basketball players actively engaged in regular training. Each participant underwent three distinct experimental conditions—localized arm fatigue, general fatigue, and elbow bracing - across three separate testing days, with the order of conditions randomized and counterbalanced to minimize order effects and learning bias. On each test day, only one condition was assessed to avoid carry-over fatigue or confounding influences, with a 48-hour washout period between sessions to ensure adequate recovery. By allowing each participant to serve as her own control under systematically varied sequences, this counterbalanced crossover structure reduced intra-subject variability, controlled for sequence-dependent effects, and enhanced both the internal validity and statistical power of the study (Figure 1).

Randomization was performed using a computer-generated random sequence (www.randomizer.org) to assign participants to different experimental conditions on each testing day. The sequence generation and allocation were conducted by an independent researcher not involved in data collection to ensure allocation concealment.

Evaluation and Measurements

The evaluation of Proprioception

Shoulder proprioceptive accuracy was assessed using the Joint Position Sense Error (JPSE) test, a validated method for evaluating joint position sense, particularly in upper limb neuromuscular assessments [11]. The test quantifies the angular deviation between a passively imposed reference position and the participant's attempt to actively replicate it.

Participants were seated with the tested shoulder joint unrestrained to allow movement in the sagittal plane. A reference position was established by passively positioning the dominant arm at 60° and 100° of shoulder flexion. After memorizing this position with eyes open, participants closed their eyes and attempted to replicate the same angle with the contralateral limb. The deviation between the replicated and target angles was measured using a digital goniometer. Each measurement was repeated three times, and the average of the trials was used for analysis. Standardized instructions were given, and no tactile or verbal cues were provided during the test to ensure procedural consistency [11].

Brace Condition and Compression Rationale

In the brace condition, participants wore a commercially available elbow support brace (Nike NKS09-010 NBA Elite), which provides moderate circumferential compression and proprioceptive feedback through mechanoreceptor stimulation around the elbow joint. The brace was worn on the dominant arm throughout both the proprioception test and the shooting trials. The rationale behind brace application was based on evidence suggesting that external joint compression can enhance sensorimotor feedback, improve joint awareness, and mitigate the negative effects of fatigue on proprioceptive acuity.

By applying gentle pressure to the soft tissue around the elbow, the brace may increase afferent input from cutaneous and joint receptors, thereby facilitating central nervous system integration of joint position information. This potential enhancement in joint sense was systematically evaluated by repeating the JPSE protocol while the brace was worn, using the same procedure and angles as in the non-braced condition.

Modified Borg Scale (Borg)

Borg was used to evaluate fatigue levels. This scale ranges from 0 to 10, with each number corresponding to a specific level of perceived fatigue. A score of "0" indicates no fatigue, while "10" represents maximum fatigue. The Borg provides a

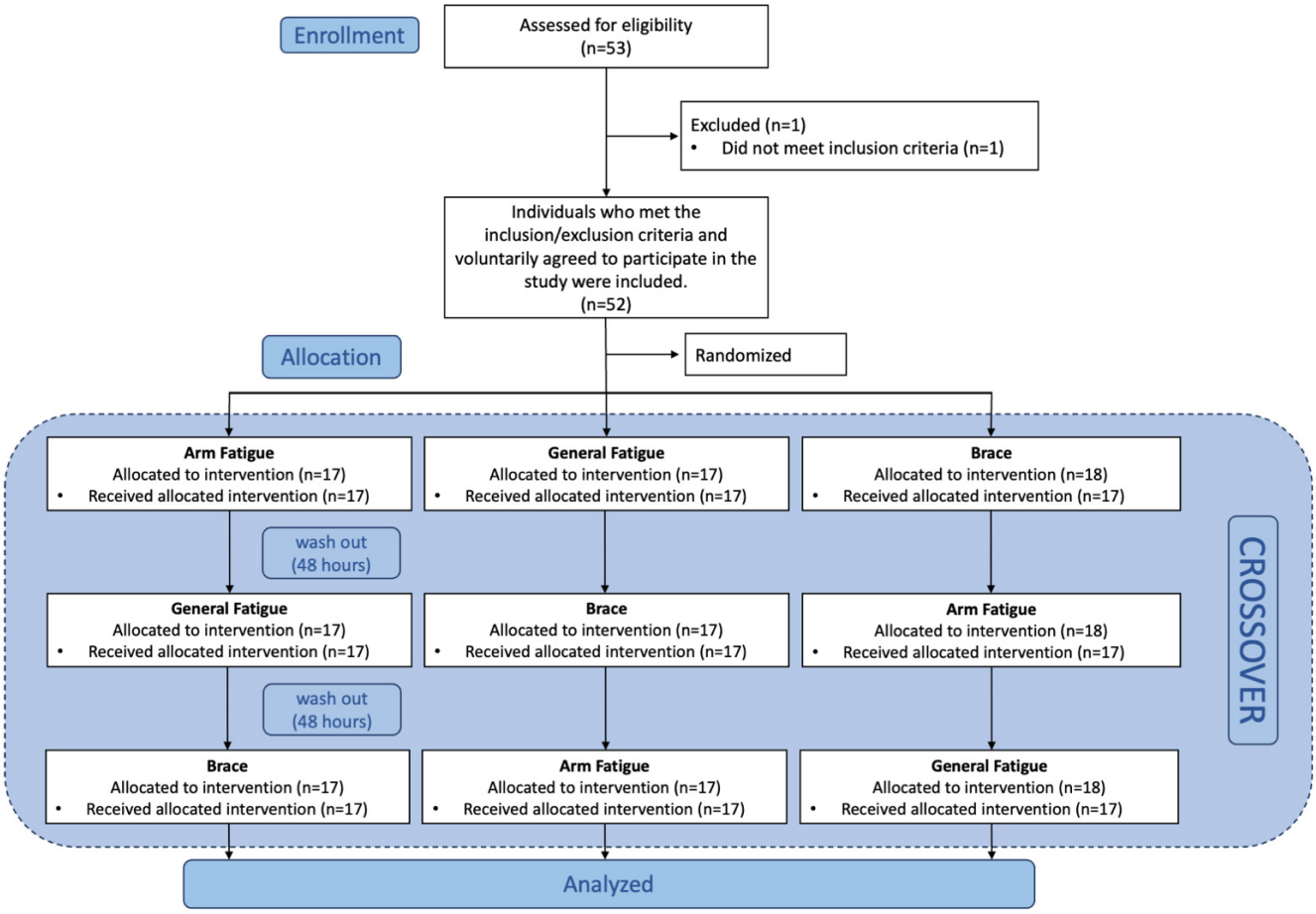

FIGURE 1. **Flow chart.**

standardized and reliable measure for assessing both arm and general fatigue levels [12].

Exercise to Fatigue the Arm and General

To induce localized arm fatigue, each participant's one-repetition maximum (1RM) was estimated using a submaximal strength testing protocol based on the number of repetitions completed with a given load. This approach allowed for individualized fatigue calculations for both the bench press and shoulder flexion exercises [13].

Based on these estimations, participants performed five sets of 15 repetitions of bench press and shoulder flexion exercises at 60% of their calculated 1RM, with 60 seconds of rest between sets. Exercises were conducted using standard gym equipment under supervision. During the final set, most participants were unable to complete all 15 repetitions independently. At this point, the supervising physiotherapist provided minimal support to ensure participants reached volitional fatigue, defined as the point where the participant could no longer perform repetitions with correct technique without external assistance.

To induce general fatigue, participants engaged in a standardized 60-minute basketball conditioning session supervised by a strength and conditioning coach. This session included aerobic drills such as shuttle runs and agility ladder exercises, plyometric activities including jump squats and lateral bounds, and basketball-specific skill work such as fast break drills and full-court scrimmage play. This combination was designed to simulate the physical and neuromuscular demands typically experienced during actual competitive basketball games.

Fatigue levels were validated through a combination of methods: participants' subjective ratings on the Modified Borg Scale (0-10), verbal confirmation of exhaustion, and observational

assessment of decreased movement quality or inability to complete exercises. All sessions were monitored by licensed physiotherapists to ensure participant safety and protocol consistency.

Goal Percentage

To determine the goal percentages, each player was instructed to attempt fifty free throws, with the number of successful attempts recorded. The percentage of accurate shots was subsequently calculated based on the total attempts.

Experimental Procedure

The study was conducted over three non-consecutive days, and participants were randomly assigned to different experimental conditions each day. On each testing day, all three experimental conditions - localized arm fatigue, general fatigue, and brace use - were administered simultaneously, but to different randomly assigned subgroups of participants (Figure 1).

Day 1: Participants were randomized into three groups, with each group undergoing one of the experimental conditions (localized arm fatigue, general fatigue, or brace use). Baseline assessments (shoulder proprioception, shooting accuracy, and Borg fatigue score) were performed, followed by the assigned intervention and post-condition assessments.

Day 2: After a 48-hour washout period, participants rotated to a different condition according to a counterbalanced schedule, ensuring that no participant repeated the same condition.

Day 3: After a 48-hour washout period, participants completed the remaining third condition with corresponding baseline and post-intervention measurements.

This design ensured that each participant experienced all three interventions across the study period while minimizing order and learning effects. Each participant served as her own control for comparisons among the three conditions, consistent with the principles of a randomized crossover study.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 27.0 (IBM Corp., Armonk, NY, USA). Data normality was assessed using the Shapiro-Wilk test. As the data were not normally distributed, nonparametric statistical tests were applied. Overall differences across experimental conditions (rest, after arm exercise, and after routine training) were evaluated using the Friedman test. When significant differences were detected, pairwise comparisons were conducted using the Wilcoxon Signed-Rank Test with Bonferroni adjustment to control for multiple comparisons (adjusted significance threshold: $P<0.0167$). The Bonferroni method was chosen to reduce the risk of Type I error by dividing the standard alpha level (0.05) by the number of pairwise comparisons (3), yielding a corrected significance level for each comparison. Changes in shooting performance, proprioceptive accuracy, and fatigue perception were compared between conditions. Effect sizes (r) were calculated for Wilcoxon tests. Spearman rank correlation analyses were performed to assess the relationships between changes in proprioceptive deflection angles, fatigue perception scores, and shooting accuracy. Statistical significance was set at $P<0.05$, except for pairwise comparisons where the Bonferroni-adjusted threshold was applied.

RESULTS

Participant characteristics are summarized in Table 1. A total of 52 female basketball players participated in this study. The mean age of the participants was 23.08±2.02 years, and the mean height was 176±12.05 cm. The mean weight was recorded as 68.75±7.58 kilograms, and the mean BMI was calculated to be 22.49±1.21 kg/m². On average, participants reported

TABLE 1. Participant Characteristics

Variables	Data (n=52)
Age (years)	23.08±2.02
Height (cm)	176±12.05
Weight (kg)	68.75±7.58
Body mass index (kg/m^2)	22.49±1.21
Years of basketball experience	9.2±3.0
Weekly training frequency	4.2±0.6

Data are shown as mean±standard deviation.

TABLE 2. The Comparisons of Arm Fatigue and General Fatigue After Arm Exercise and Routine Training

	Rest	After arm exercise	After routine training	Friedman Test χ^2	P-value	Effect size (Kendall's W)	95% CI (Arm exercise vs. Rest)	95% CI (Routine training vs. Rest)	Pairwise Comparisons (Wilcoxon Signed-Rank Test, Bonferroni corrected P<0.0167)
Arm fatigue (Borg)	1.00±0.87	7.35±0.96	4.15±0.82	104.00	**<0.001**	1.00	6.00-6.70	2.83-3.47	Rest vs Arm exercise: **P<0.001*** Rest vs Routine training: **P<0.001*** Arm exercise vs Routine training P=0.018 (NS)
General fatigue (Borg)	1.29±0.86	6.37±1.08	4.39±0.85	100.57	**<0.001**	0.97	4.70-5.46	2.77-3.43	Rest vs Arm exercise **P<0.001*** Rest vs Routine training: **P<0.001*** Arm exercise vs Routine training P=0.022 (NS)

Data are shown as mean±standard deviation. CI, confidence interval. Friedman tests were used to assess overall differences among conditions. Pairwise comparisons were conducted using Wilcoxon Signed-Rank Tests with Bonferroni adjustment.
*Statistically significant after Bonferroni correction (adjusted significance threshold: P<0.0167).
NS, not significant after Bonferroni correction. Statistically significant P-value is shown in bold.

9.2±3.0 years of basketball experience and trained 4.2±0.6 days per week.

Descriptive and comparative data on perceived arm and general fatigue across the three experimental conditions (rest, after arm exercise, and after routine training) are presented in Table 2. Fatigue levels were assessed at rest, after localized arm exercise, and after routine training. The analysis revealed statistically significant differences in both perceived arm fatigue and general fatigue scores across the three conditions. For arm fatigue, the mean Borg score at rest was 1.00±0.87, which increased to 7.35±0.96 after arm exercise and to 4.15±0.82 following routine training. The Friedman test indicated a significant overall difference (χ^2=104.00, Kendall`s W=1.00; P<0.001). Post-hoc pairwise comparisons using the Wilcoxon Signed-Rank Test with Bonferroni correction (adjusted significance threshold P<0.0167) showed that arm fatigue scores were significantly higher after arm exercise compared to rest (95% confidence interval [CI]: 6.00-6.70, P<0.001) and after routine training compared to rest (95% CI: 2.83-3.47, P<0.001). However, the comparison between post-arm exercise and post-routine training was not statistically significant after correction (P>0.05). Similarly, for general fatigue, the mean Borg score was 1.29±0.86 at rest, increasing to 6.37±1.08 after arm exercise and 4.39±0.85 after routine training (Table 2). The Friedman test again revealed a significant overall difference (χ^2=100.57, Kendall`s W=0.97; P<0.001). Pairwise comparisons showed that general fatigue scores were significantly higher after arm exercise compared to rest (95% CI: 4.70-5.46, P<0.001) and after routine training compared to rest (95% CI: 2.77-3.43, P<0.001). The difference between post-arm exercise and post-routine training, however, did not reach statistical significance after Bonferroni correction (P>0.05).

The effects of arm fatigue, general fatigue, and brace use on shooting accuracy and shoulder proprioception were analyzed and are presented in Table 3. Following localized arm fatigue, a significant decrease in shooting accuracy was observed. The median shooting percentage declined from 53.16% (range: 18-82%) before arm fatigue to 38.83% (range: 16-68%) after arm fatigue, with the Wilcoxon Signed-Rank Test yielding a statistically significant result (z= –2.447, r=0.34, 95% CI: 0.07-0.56; P=0.014). This

TABLE 3. Comparison of Goal Percentage and Proprioception Deflection Angle According to Arm Fatigue, General Fatigue and Brace Use

	Before arm fatigue	After arm fatigue	Z value	r	95% CI	P-value
Goal percentages (%)	53.16 (18-82)	38.83 (16-68)	-2.447	0.34	0.07-0.56	**0.014***
Proprioception (Deflection angle)	5.40 (3.17-8.83)	8.65 (4.50-13.50)	-2.981	0.41	0.18-0.61	**0.003***
	With braces	**Without braces**				
Goal percentages (%)	54.83 (40-70)	48.33 (30-66)	-2.208	0.31	0.04-0.53	**0.027***
Proprioception (Deflection angle)	3 (0.30-6.14)	5.30 (3.07-8.34)	-2.005	0.28	0.01-0.51	**0.035***
	Before general fatigue	**After general fatigue**				
Goal percentages (%)	54.33 (24-74)	49.16 (20-70)	-1.694	0.23	-0.05-0.47	0.090
Proprioception (Deflection angle)	4.05 (0.50-7.40)	5.35 (3.42-7.43)	-2.275	0.32	0.04-0.54	**0.049***

Data are shown as median (minimum-maximum). Negative z-values indicate a decline in shooting performance or proprioceptive accuracy after intervention.

*P<0.05, Wilcoxon Signed-Rank Test. Statistically significant P-values are shown in bold.

represents a moderate effect size, suggesting that arm fatigue has a meaningful impact on shooting accuracy. Additionally, shoulder proprioceptive accuracy deteriorated, as evidenced by an increase in the median deflection angle from 5.40° (range: 3.17°-8.83°) to 8.65° (range: 4.50°-13.50°) after arm fatigue (z= –2.981, r = 0.41, 95% CI: 0.15-0.61; P=0.003), corresponding to a moderate-to-large effect size. In the brace condition, the use of an elbow brace positively influenced both shooting accuracy and proprioceptive control. The median shooting percentage improved from 48.33% (range: 30-66%) without the brace to 54.83% (range: 40-70%) with the brace (z=–2.208, r=0.31, 95% CI: 0.04-0.54; P=0.027), indicating a moderate effect size. Similarly, shoulder proprioceptive accuracy was enhanced, with the median deflection angle decreasing from 5.30° (range: 3.07°-8.34°) without the brace to 3.00° (range: 0.30°-6.14°) with the brace (z= –2.005, r=0.28, 95% CI: 0.01-0.51; P=0.035), also reflecting a small-to-moderate effect. Regarding general fatigue, no statistically significant change was found in shooting accuracy. The median shooting percentage decreased slightly from 54.33% (range: 24-74%) to 49.16% (range: 20-70%) following general fatigue (z= –1.694, r=0.23, 95% CI: –0.05-0.47; P=0.090), corresponding

TABLE 4. Relationship Between Arm Fatigue, General Fatigue, and Proprioceptive Deflection Angle with Goal Percentage

	Goal percentages (%)			Goal percentages (%)	
After arm exercise	**r**	**P-value**	**After routine training**	**r**	**P-value**
Arm fatigue (Borg)	-0.469	**0.033***	**Arm fatigue (Borg)**	-0.344	**0.046***
General fatigue (Borg)	-0.245	0.063	**General fatigue (Borg)**	-0.076	0.123
Proprioception (Deflection range difference[a])	-0.787	**<0.001***	**Proprioception (Deflection range difference[b])**	-0.566	**0.045***

[a]Difference in shoulder proprioception deflection angle before and after arm exercise.

[b]Difference in shoulder proprioception deflection angle before and after routine training.

*Spearman correlation analysis.

Statistically significant P-value is shown in bold.

to a small effect size. However, a statistically significant impairment in shoulder proprioceptive control was observed, with the median deflection angle increasing from 4.05° (range: 0.50°-7.40°) to 5.35° (range: 3.42°-7.43°) after routine training (z= –2.275, r=0.32, 95% CI: 0.05-0.55; P=0.049), which reflects a moderate effect size.

Correlation analyses revealed a moderate, statistically significant negative association between perceived arm fatigue and shooting performance following arm-specific fatigue (r= –0.469, P=0.033, Table 4). Moreover, changes in proprioceptive deflection angles before and after arm fatigue demonstrated a strong negative correlation with shooting accuracy (r= –0.787, P<0.001), suggesting that greater proprioceptive degradation is closely linked with poorer shooting performance. In contrast, general fatigue exhibited a weaker and non-significant relationship with shooting accuracy (r= –0.245, P=0.063). Nevertheless, the changes in proprioceptive deflection after general fatigue were moderately and negatively correlated with shooting performance (r= –0.566, P=0.045).

DISCUSSION

This study aimed to investigate the effects of localized arm fatigue, general fatigue, and elbow brace use on shooting accuracy and shoulder proprioception in amateur female basketball players. The key findings indicated that localized arm fatigue significantly impaired both shooting accuracy and shoulder joint position sense, whereas general fatigue had a lesser impact, affecting proprioception but not shooting accuracy. In contrast, wearing an elbow brace during performance trials appeared to enhance both shooting accuracy and proprioceptive control. These results suggest that different types of fatigue may elicit distinct neuromuscular effects, and that external joint support - such as an elbow brace - can provide both proprioceptive and performance-related advantages.

Muscle fatigue has been widely reported to compromise motor control and coordination, particularly in movements requiring fine neuromuscular precision, such as shooting. Enoka and Duchateau [1] demonstrated that fatigue alters motor unit recruitment patterns and reduces execution accuracy during skilled tasks. Additionally, Gandevia [14] highlighted that fatigue not only reduces muscle force production but also impairs proprioceptive feedback mechanisms that are critical for fine motor adjustments. These disruptions can collectively undermine athletic performance during precision tasks. In line with these findings, the present study demonstrated that localized arm fatigue significantly impaired both shooting accuracy and shoulder proprioception in amateur female basketball players. The very high Kendall's W values obtained for fatigue induction (W=1.00 for localized and W=0.97 for general fatigue) support the robustness and reliability of the applied fatigue protocols. Moreover, a strong negative correlation was found between arm fatigue levels and shooting performance, indicating that fatigue affecting specific muscle groups responsible for technical execution can substantially reduce task efficiency. The observed increase in proprioceptive deflection angles under fatigue conditions further supports the notion that proprioceptive degradation is a key contributor to performance deterioration during fatigue.

Previous research has shown that the type and severity of fatigue can have varying effects on motor performance. For example, Uygur *et al*. [3] reported that elite male basketball players were able to maintain consistent free throw kinematics despite fatigue, suggesting that this may be due to the development of automatic motor patterns and stabilization strategies through training. Similarly, a meta-analysis by Li *et al*. [4] demonstrated that both severe physical fatigue and moderate mental fatigue can negatively affect basketball performance, though the extent of the impact may vary depending on the task type and athlete profile. Zhang *et al*. [15] also emphasized that tasks involving isolated and precision-based movements are more sensitive to localized fatigue rather than systemic fatigue. The findings of the present study are generally consistent with this literature. Following general fatigue induced by routine basketball training, a significant deterioration in shoulder proprioception was observed, whereas shooting accuracy was not significantly affected. This suggests that systemic fatigue may primarily impair sensorimotor control, leading to proprioceptive decline, but this degradation may not immediately translate into measurable performance deficits in

amateur-level technical tasks. Compared to studies highlighting the ability of elite athletes to maintain performance under fatigue, the current study's focus on amateur female players is a notable distinction. These results support the idea that training level and motor control capacity may influence the development of adaptive responses to fatigue.

Another key observation was the potential role of external joint support in maintaining proprioceptive and motor function. Kazemi *et al*. [9] and Cao *et al*. [16] reported that supportive devices like elbow braces can improve joint stability and increase afferent feedback from cutaneous and joint mechanoreceptors, thereby promoting more accurate joint position sense and facilitating smoother motor output. Mechanoreceptors such as muscle spindles, Golgi tendon organs, and Ruffini endings respond to joint movement and pressure changes, sending sensory information to the central nervous system. This process enables refined joint position sense and contributes to enhanced sensorimotor integration [17]. In line with these findings, the present study observed that the use of an elbow brace was associated with improved shoulder proprioceptive accuracy and marginal enhancements in shooting accuracy. Although the improvement in shooting percentage did not reach strict significance levels following multiple comparison corrections, the enhanced proprioceptive control demonstrated a small-to-moderate effect size (Cohen's d = 0.46), indicating potential practical significance. These findings reinforce the idea that external stabilization can contribute meaningfully to performance maintenance by enhancing afferent feedback pathways, even in the absence of statistically significant performance changes.

Correlation analyses further reinforced the central role of proprioceptive integrity in performance maintenance. Changes in proprioceptive accuracy were strongly and negatively correlated with free throw success rates after both localized and general fatigue, whereas perceived fatigue levels showed weaker and non-significant associations. This pattern supports the theoretical framework proposed by Gandevia [14], who argued that proprioceptive degradation plays a primary role in fatigue-induced impairments of motor tasks.

From an applied perspective, these findings underscore the importance of incorporating proprioceptive training into athletic preparation and rehabilitation programs. Training interventions focused on improving joint position sense and sensorimotor control could mitigate the adverse effects of fatigue, enhance performance consistency, and reduce injury risks [9, 16]. Coaches and practitioners are encouraged to integrate fatigue management strategies and proprioceptive exercises into regular basketball training routines.

A major strength of the present study is its randomized crossover design, which minimized inter-individual variability by allowing each participant to serve as her own control across different conditions. This methodological approach enhanced statistical precision and reduced confounding effects due to individual differences. However, despite the implementation of washout periods between experimental sessions, the possibility of residual carryover effects cannot be entirely excluded. Future studies should consider longer washout intervals or counterbalanced session orders to further mitigate such limitations.

Strengths and Limitations

A major strength of this study is its randomized crossover design, which allowed each participant to serve as her own control, thereby minimizing inter-individual variability and enhancing internal validity. The simultaneous evaluation of localized arm fatigue, general fatigue, and elbow bracing within the same cohort provides a comprehensive and comparative framework that is rarely addressed within a single experimental study. The inclusion of a standardized washout period and a counterbalanced condition order further reduced potential carryover and sequence effects. Moreover, the use of validated and standardized proprioceptive and performance-based outcome measures strengthened the methodological rigor and robustness of the findings.

Despite these strengths, several limitations should be acknowledged. First, the study sample consisted exclusively of amateur female basketball players, which may limit the generalizability of the results to other athletic populations, including male athletes, elite-level competitors, or different age groups. Second, the fatigue protocols were implemented under controlled experimental conditions, which may not fully reflect the complex and dynamic demands of

real-game environments characterized by psychological stress, tactical variability, and competitive pressure. Third, the investigation focused solely on the acute effects of fatigue, and therefore does not address the potential long-term impact of repeated fatigue exposure on proprioceptive function or technical performance.

Future Directions

Future research should include broader and more heterogeneous athletic populations and adopt longitudinal study designs to examine the sustained effects of fatigue on motor control and performance. The integration of objective assessment tools, such as motion analysis systems and electromyography (EMG), may provide more detailed insights into fatigue-related biomechanical and neuromuscular adaptations. In addition, experimental protocols that more closely replicate game-like conditions—including opponent interaction, decision-making demands, and mental fatigue—could enhance ecological validity. Finally, intervention-based studies evaluating structured proprioceptive training programs and advanced joint support strategies may facilitate the translation of laboratory-based findings into applied sports training and rehabilitation settings.

CONCLUSION

This study offers novel insight into how different types of fatigue influence proprioception and performance in basketball. The findings demonstrate that localized arm fatigue significantly impairs both shoulder proprioception and shooting accuracy, while general fatigue predominantly affects proprioceptive accuracy without causing immediate shooting deficits. Furthermore, the use of an elbow brace partially mitigated proprioceptive degradation, indicating that external support may provide a protective effect under fatigue conditions.

These findings carry practical implications for sports scientists, coaches, and rehabilitation specialists. Conditioning programs that include proprioceptive training - such as balance, joint position sense, and reflex-enhancing exercises - may help maintain sensorimotor control during fatigue states. Moreover, strategic use of bracing, especially during high-intensity sessions or post-injury return-to-play protocols, could enhance performance consistency and reduce injury risk. Integrating these approaches into routine training may be particularly beneficial for amateur-level athletes, who may lack the neuromuscular adaptations found in elite performers.

Ethics Approval and Consent to Participate

This study was approved by the Nuh Naci Yazgan University Scientific Research and Publication Ethics Committee (Decision no.: 2024/002-01, date: 12.02.2024). All procedures were conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from all individual participants included in the study.

Data Availability

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

Authors' Contribution

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

Conflict of interest

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

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Biomechanical Determinants of Shooting Performance: The Role of Wrist and Elbow Joint Range of Motion in Male Basketball Players

Yusuf Gözaçık[1]

[1]*Department of Sports Management, Dicle University, School of Physical Education and Sports, Diyarbakır, Türkiye*

ABSTRACT

Objectives: The aim of this study is to examine the relationship between wrist and elbow joint range of motion and shooting performance in young male basketball players.

Methods: The research was conducted as a cross-sectional correlational study. The study sample consisted of 23 male basketball players residing in Diyarbakır, selected through purposive sampling. The wrist and elbow joint range of motion values, along with shooting performance scores of the athletes, were measured. Frequency analyses were performed, and after confirming the normality of the data distribution, Pearson correlation and multiple regression analyses were conducted.

Results: According to the results, strong and significant positive correlations were observed between elbow flexion and pronation and shooting performance. In addition, elbow supination, as well as wrist flexion and extension, also showed statistically significant positive relationships with shooting performance. However, no significant relationship was found between shooting performance and radial deviation, ulnar deviation, or elbow extension.

Conclusions: In conclusion, elbow joint range of motion appears to have a significant and determining effect on shooting performance in young male basketball players. In contrast, wrist mobility contributes to shooting performance to a more limited extent and therefore cannot be considered a direct determining parameter.

Keywords: Basketball, Shooting Performance, Joint Range of Motion

Basketball is a dynamic sport that requires multifaceted physical competence. This discipline encompasses not only fundamental motor skills such as speed, agility, strength, endurance, and coordination but also technical and tactical knowledge. One of the core components of the game, the jump shot, serves not only as a means of scoring but also as a critical indicator of an athlete's individual skill and biomechanical efficiency. The shooting action is performed through a complex kinetic chain based on the coordinated movement of both the upper and lower body. Each joint and muscle group involved in this chain directly influences the quality and effectiveness of the motion [1].

The shooting motion primarily relies on the functional integration of the upper extremity. Sequential and controlled movements among the shoulder, elbow, and wrist joints are fundamental

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Corresponding author: Yusuf Gözaçık, PhD., Phone: +90 412 241 10 00, E-mail: yusuf.gozacik@dicle.edu.tr

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determinants of shooting accuracy. Within this process, the elbow and wrist joints play a critical role in both the mechanical generation of force and the control of the ball's direction, velocity, and rotation. The elbow joint contributes to the production of forward-propelling force, while the wrist joint is essential for determining the direction of the shot and for executing the final control at the moment the ball is released from the fingertips. Therefore, the functionality and range of motion of these two joints are directly associated with shooting technique [2].

Recent studies have highlighted the importance of upper extremity range of motion (ROM) as a key determinant of shooting performance. For example, an investigation into the effects of full and partial ROM training of the triceps muscle on three-point shooting accuracy reported that full ROM training significantly improved shooting performance [3]. Similarly, research evaluating the influence of shooting distance and player skill level on upper extremity muscle activity and joint energy production demonstrated that joint movements play a critical role in shooting mechanics [4]. Another study conducted on young basketball players revealed a direct relationship between shooting mechanics, joint angles, and shooting accuracy [5].

Range of motion refers to the maximum extent of movement a joint can perform within its anatomical limits. This capacity can be assessed either passively or actively and constitutes a significant parameter influencing athletic performance. Range of motion varies depending on several factors, including the structural characteristics of joint surfaces, the elasticity of connective tissues, the strength of surrounding muscles, the viscoelastic properties of muscle-tendon units, and individual factors such as age, sex, and training history. In joints that require fine motor control and are closely related to technical skills—such as the elbow and wrist—limitations in range of motion may restrict technical execution, reduce performance, and increase the risk of injury [6].

In basketball, the development of shooting performance is not limited to strength and technical training alone. An athlete's biomechanical capacities, flexibility level, and joint range of motion should also be considered integral components of performance. Therefore, in basketball - a sport where shooting skills are heavily utilized - the evaluation of upper extremity joints, particularly the elbow and wrist, in terms of their range of motion is essential. Such assessments can serve as valuable tools for individualizing training programs, monitoring technical development, and promoting athlete health and injury prevention [7].

For athletes, the age range of 16 to 18 represents a critical period during which physical and biomechanical development continues rapidly. During this stage, while the musculoskeletal system progresses toward full maturity, motor skills and technical proficiencies become more stable. The development of fundamental motor abilities - such as strength, speed, endurance, and coordination - plays a decisive role in the acquisition and execution of technical skills. Additionally, musculoskeletal development during this period is a key factor that directly influences athletic performance capacity [8]. Therefore, assessments of range of motion conducted during this stage can serve a guiding role in both tracking developmental progress and enhancing technical capacity.

In this context, the present study aims to systematically examine the relationship between wrist and elbow joint ROM and shooting performance in adolescent male basketball players. Although some prior studies have investigated the association between upper extremity ROM and shooting performance, most have been limited to specific joints or muscle groups and have not provided a comprehensive evaluation. This study is therefore unique in assessing both elbow and wrist ROM simultaneously and exploring their combined influence on shooting outcomes. By doing so, it seeks to offer a valuable foundation for developing technical skills and designing individualized training programs for young athletes.

METHODS

Research Design

This study was designed as a cross-sectional correlational research. Cross-sectional correlational studies are observational in nature and involve collecting data from individuals at a specific point in time to examine the relationship between two or more variables. Such studies analyze the strength and direction of relationships rather than causal effects.

Typically, data collection in cross-sectional research is conducted through surveys, observations, or measurement methods, with subsequent evaluation via statistical analyses [9]. In this study, the relationship between wrist and elbow joint range of motion and shooting performance was investigated in male basketball players aged 16 to 18. The study was observational, with no interventions or experimental manipulations applied.

Participants

In the present study, an effect size of 0.6 was selected based on the assumption that wrist and elbow joint range of motion could significantly influence shooting performance. In the literature, most similar studies have reported medium effect sizes (r = 0.3–0.5) [3, 10]. A stronger relationship was expected in this study, and accordingly, a higher effect size was chosen. Accordingly, a priori power analysis using G*Power was conducted to determine the required sample size for a bivariate normal model correlation with an effect size of d = 0.6, an alpha error probability (α) of 0.05, and a power (1–β) of 0.85. The analysis indicated that a minimum total sample size of 21 participants would be sufficient [11]. The study included 23 voluntary male basketball players from Diyarbakır aged 16 to 18 years, each with at least two years of basketball experience and no chronic musculoskeletal disorders or injuries. Participants were selected using purposive sampling and participated on a voluntary basis.

The fact that the participants consisted solely of males from a single city and a specific age group is considered a significant limitation in terms of external validity. However, the conducted power analysis (α = 0.05, 1-β = 0.85, effect size d = 0.6) indicates that the current sample size is sufficient to achieve the intended statistical power of the study. Therefore, despite these limitations, conducting the study with this number of participants is methodologically appropriate and possesses the capacity to detect the specified effect size [11].

Prior to the study, participants, their coaches, and families were provided with detailed explanations regarding the purpose, scope, and measurement protocols of the research. Informed voluntary consent was obtained from all participants before their inclusion in the study.

Data Collection Methods and Measurements

All measurements were performed by a single experienced examiner to ensure consistency and reliability throughout the data collection process. This approach minimized potential variability and maintained the accuracy of the assessments.

1. Measurement of Wrist and Elbow Joint Range of Motion

The range of motion of the participants' dominant wrist and elbow joints was measured using a manual (standard) goniometer. Measurements were conducted in accordance with the standardized protocols defined by the American Academy of Orthopaedic Surgeons (AAOS) [12].

Measurement of Wrist Range of Motion

Participants were seated with their forearm placed on a table and the wrist positioned freely [13]. Measurements were taken for the following movements:

Flexion: Measurement was recorded when the wrist was bent forward to its maximum degree.

Extension: Measurement was taken when the wrist was bent backward to its maximum degree.

Radial Deviation: The maximum angle achieved when the wrist was moved toward the thumb side was measured.

Ulnar Deviation: The maximum angle obtained when the wrist was moved toward the little finger side was recorded.

Measurement of Elbow Range of Motion

Participants were seated on a chair with back support, and measurements were conducted with the shoulders positioned in a neutral alignment [13].

Flexion: Measurement was taken when the elbow was bent to its maximum degree.

Extension: Measurement was recorded when the elbow was fully extended.

Supination: Measurement was conducted by externally rotating the forearm so that the palm faced upwards.

Pronation: The maximum angle obtained by internally rotating the forearm so that the palm faced downwards was recorded.

All measurements were taken three times by the

TABLE 1. Test-Retest Reliability of Wrist and Elbow ROM Measurements

Variables		ICC (95% CI)	P-value	Reliability level	Cronbach's alpha
Wrist joint	Wrist flexion	0.94	**<0.001**	Excellent	0.97
	Wrist extension	0.92	**<0.001**	Excellent	0.96
	Radial deviation	0.77	**<0.001**	High	0.87
	Ulnar deviation	0.87	**<0.001**	High	0.93
Elbow joint	Elbow flexion	0.98	**<0.001**	Excellent	0.99
	Elbow extension	0.96	**<0.001**	Excellent	0.98
	Elbow supination	0.91	**<0.000**	Excellent	0.98
	Elbow pronation	0.90	**<0.001**	High	0.94
Overall α					0.87

ICC, ıntraclass correlation coefficient; CI, confidence interval; ROM, range of motion.
Statistically significant P-values are shown in bold.

same researcher. The reliability of these measurements was assessed using the test–retest method, ensuring consistency and reproducibility of the recorded values.

Test–retest reliability analysis indicated that wrist and elbow joint range of motion measurements demonstrated high to excellent reliability (Intraclass Correlation Coefficient [ICC] = 0.77– 0.98, P<0.01), consistent with the ICC classification recommended in the literature [14]. Notably, wrist flexion and elbow flexion measurements exhibited the highest reliability. Internal consistency of the measurements was also high, with an overall Cronbach's α of 0.87 [14] (Table 1).

2. Shooting Performance

Prior to the measurements, a standard 5–10 minutes warm-up was performed, and no fatigue protocol was applied. Participants' shooting performances were subsequently evaluated using the American Alliance for Health, Physical Education, Recreation and Dance (AAHPERD) Basketball Shooting Test. During the test, it was assumed that potential confounding factors affecting performance (e.g., fatigue, motivation) were at similar levels across all athletes, ensuring the consistency and comparability of the measurements.

AAHPERD Basketball Shooting Test Protocol

In this study, the AAHPERD Shooting Test was administered to evaluate participants' shooting skills. The test is a widely used and validated performance assessment tool recognized for its reliability in measuring basketball players' shooting accuracy [15].

During the application, five distinct shooting positions were arranged at equal intervals, starting from a distance of 4.57 meters projected from the base of the basketball hoop onto the floor. These points were fixed and marked on the court, symmetrically positioned relative to the center of the hoop. The test duration was limited to one minute. Participants were instructed to shoot from the starting point towards the hoop, then retrieve the ball, dribble to the next position, and continue shooting. At least one shot was required from each position, and during each shot, participants had to keep at least one foot behind the shooting line [15].

Following missed shots, players were allowed to attempt layups (turnikes) with the conditions that no more than two consecutive layups were made and a maximum of four layups were permitted in total. Participants repeated this cycle within the rules until the allotted time elapsed. In the scoring system, each successful shot and successful layup attempt after a missed shot was awarded 2 points. However, only the first of two consecutive successful layups was scored. Attempts involving violations such as stepping over the shooting line, illegal ball handling, or dribbling infractions were not scored [15].

All measurements were conducted using a standard basketball and under consistent court conditions. Participants were given a standardized warm-up period before each test and a fixed rest interval between shots.

TABLE 2. Normality Test of Athletes' Joint Range of Motion and Shooting Scores

Variables		Skewness	Kurtosis	Shapiro-Wilk	P-value
Wrist joint	Wrist flexion	-0.249	-0.693	0.961	0.483
	Wrist extension	-0.051	-0.802	0.975	0.810
	Radial deviation	0.278	-0.725	0.933	0.129
	Ulnar deviation	-0.191	-0.409	0.954	0.357
Elbow joint	Elbow flexion	-0.977	0.774	0.925	0.087
	Elbow extension	0.177	-0.462	0.964	0.421
	Elbow supination	-0.245	-0.266	0.973	0.767
	Elbow pronation	-0.588	0.105	0.960	0.456
Shooting performance score		-0.735	-0.488	0.925	0.086

Ethical Principles

The study was conducted in accordance with scientific ethical principles, following the approval of the Social and Human Sciences Ethics Committee of Dicle University (Decision No.:2025/199, dated 24.03.2025). Data collection was carried out by the researcher. All relevant information regarding the study was communicated to participants prior to data collection. Participation was based on the principle of voluntariness, and the study was conducted in compliance with the Declaration of Helsinki [16].

Statistical Analysis

Data were analyzed using the SPSS 26.0 statistical software package. Descriptive statistics including mean, standard deviation, maximum, and minimum values were calculated for demographic information and parameters obtained from data collection tools. Skewness and kurtosis values were computed to assess the normality of the data distributions and the Shapiro–Wilk test was applied. After verifying the assumptions for parametric tests, relationships between variables were analyzed using Pearson correlation analysis. For regression analysis, similar

TABLE 3. PCA Factor Loadings and Explained Variance for Wrist and Elbow ROM

Variables		Factor 1 (Wrist ROM)	Factor 2 (Elbow ROM)
Wrist joint	Wrist flexion	0.89	0.12
	Wrist extension	0.85	0.15
	Radial deviation	0.78	0.20
	Ulnar deviation	0.80	0.18
Elbow joint	Elbow flexion	0.10	0.91
	Elbow extension	0.05	0.88
	Elbow Supination	0.12	0.85
	Elbow Pronation	0.08	0.83
Eigenvalue		3.05	2.80
Explained variance (%)		38	35
Cumulative variance (%)		73	—

PCA, principal component analysis; ROM, range of motion.

variables were combined into a single composite variable using Principal Component Analysis (PCA), followed by multiple regression analysis to examine the relationships between these components. A significance level of $P<0.05$ was adopted for all statistical analyses [17].

The normality of the data obtained from the athletes was assessed using skewness and kurtosis values alongside the Shapiro–Wilk test. According to Tabachnick and Fidell (2019), skewness and kurtosis values within the ±1.50 range indicate an acceptable normal distribution. In the present study, the data met these criteria, and the Shapiro–Wilk test results ($P>0.05$) further confirmed normality. The elbow extension variable did not follow a normal distribution in its original form ($W= 0.788$, $P<0.01$); however, after logarithmic transformation (Log10), it conformed to a normal distribution ($W= 0.964$, $P=0.421$). Variables that do not meet the normality assumption can be transformed (e.g., logarithmic, square root) to approximate normality, whereas variables already normally distributed should be retained in their original form [17, 18] (Table 2).

Principal Component Analysis (PCA) was conducted to construct two composite variables based on wrist and elbow ROM. Sampling adequacy for PCA was assessed using the Kaiser-Meyer-Olkin (KMO) test [19] and Bartlett's Test of Sphericity [20]. As a result, Composite Wrist ROM and Composite Elbow ROM variables were determined. The KMO value was 0.68, and Bartlett's test yielded $\chi^2= 163.12$, $P<0.01$, indicating the suitability of the sample for PCA. According to the eigenvalue >1 criterion, two factors were retained, explaining 73% of the total variance. Factor loadings ranged between 0.78 and 0.91, with highly loaded variables grouped into their respective composite variables (Table 3).

RESULTS

The mean age of the 23 male athletes included in the study was 17.00±0.79 years, mean height was 179.04±4.83 cm, mean weight was 75.08±6.08 kg, mean body mass index (BMI) was 23.37±0.76 kg/m^2, and mean sport age was 5.52±1.64 years (Table 4).

The mean values for the athletes' wrist joint were determined as follows: wrist flexion 80.73±2.83°, wrist extension 73.04±2.68°, radial deviation 20.47±1.37°, and ulnar deviation 28.65±1.94°. The mean values for the elbow joint were: elbow flexion 142.82±6.82°, elbow extension 4.13±3.25°, supination 46.26±2.00°, and pronation 41.26±2.19° (Table 5).

According to the descriptive statistics for the

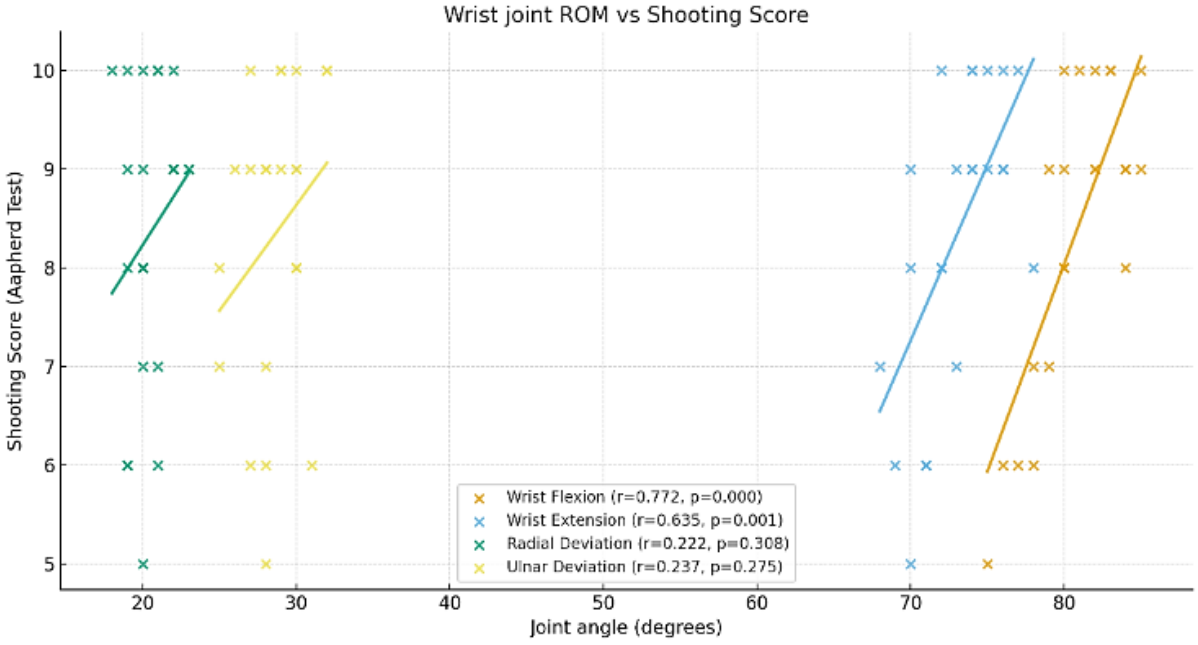

FIGURE 1. Correlation between athletes' wrist joint range of motion and shooting performance scores.

TABLE 4. Descriptive Statistics of the Athletes Participating in the Study

Variables	n	Mininimum.	Maximum.	Mean	Standard deviation
Age (years)	23	16.00	18.00	17.00	0.79
Height (cm)	23	174.00	192.00	179.04	4.83
Weight (kg)	23	63.00	88.00	75.08	6.08
BMI (kg/m²)	23	22.10	25.30	23.37	0.76
Sport age (years)	23	2.00	8.00	5.52	1.64

shooting performance scores of the 23 athletes participating in the study, the lowest score was 8.00 and the highest score was 14.00. The arithmetic mean of the participants' shooting scores was calculated as 11.39, with a standard deviation of 1.58 (Table 6).

The correlation analysis revealed several significant relationships between athletes' joint ROM and shooting performance scores. The highest positive correlations were observed for elbow flexion (r= 0.934; P<0.001) and elbow pronation (r= 0.919; P<0.001). Additionally, significant positive correlations were found for elbow supination (r= 0.852; P<0.001), wrist flexion (r= 0.772; P<0.001), and wrist extension (r= 0.635; P=0.001). In contrast, no statistically significant correlations were detected for radial deviation (r= 0.222; P=0.308), ulnar deviation (r =0.237; P=0.275), or elbow extension (r= -0.283; P=0.190) (Table 7).

Figure 1 illustrates the associations between athletes' wrist and elbow joint ROM and their shooting performance scores. Significant positive correlations were observed for elbow flexion, elbow supination, elbow pronation, wrist flexion, and wrist extension, whereas no statistically significant associations were detected for radial deviation, ulnar deviation, or elbow extension. The data correspond to the Pearson correlation coefficients (r) reported in Table 7.

Figure 2 illustrates the associations between athletes' elbow joint ROM and their shooting performance scores. Significant positive correlations were observed for elbow flexion, elbow supination, and elbow pronation, indicating that greater mobility in these movements is strongly associated with higher shooting performance. In contrast, elbow extension showed no statistically significant correlation with shooting performance. The data correspond to the Pearson correlation coefficients (r) reported in Table 7.

Examination of the regression analysis model summary revealed a correlation coefficient (R) of 0.929. The R^2 value was 0.864, and the adjusted R^2 was 0.850. These values indicate that approximately 85% of the variance in the dependent variable is explained by the model. The standard error of the estimate was calculated as 0.614. The Durbin-Watson statistic was found to be 1.773 (Table 8).

The Durbin-Watson statistic is interpreted as an indicator of whether there is autocorrelation among

TABLE 5. Descriptive Statistics of Joint Range of Motion for the Athletes

Variables		n	Minimum	Maximum	Mean	Standard deviation
Wrist joint	Wrist flexion	23	75.00	85.00	80.73	2.83
	Wrist extension	23	68.00	78.00	73.04	2.68
	Radial deviation	23	18.00	23.00	20.47	1.37
	Ulnar deviation	23	25.00	32.00	28.65	1.94
Elbow joint	Elbow flexion	23	125.00	152.00	142.82	6.82
	Elbow extension	23	0.00	10.00	4.13	3.25
	Elbow supination	23	42.00	50.00	46.26	2.00
	Elbow pronation	23	36.00	45.00	41.26	2.19

TABLE 6. Descriptive Statistics of Athletes' Shooting Performance

Variables	n	Minimum	Maximum	Mean	Standard deviation
Shooting performance score	23	8.00	14.00	11.39	1.58

TABLE 7. Correlation Analysis Between Athletes' Joint Range of Motion and Shooting Scores

Variables		Shooting performance score	
Wrist joint	Wrist flexion	**r**	0.772
		P-value	**<0.001**
	Wrist extension	**r**	0.635
		P-value	**<0.001**
	Radial deviation	**r**	0.222
		P-value	0.308
	Ulnar deviation	**r**	0.237
		P-value	0.275
Elbow joint	Elbow flexion	**r**	0.932
		P-value	**<0.001**
	Elbow extension	**r**	-0.257
		P-value	0.237
	Elbow supination	**r**	0.846
		P-value	**<0.001**
	Elbow pronation	**r**	0.920
		P-value	**<0.001**

r, Pearson correlation coefficient. Statistically significant P-values are shown in bold.

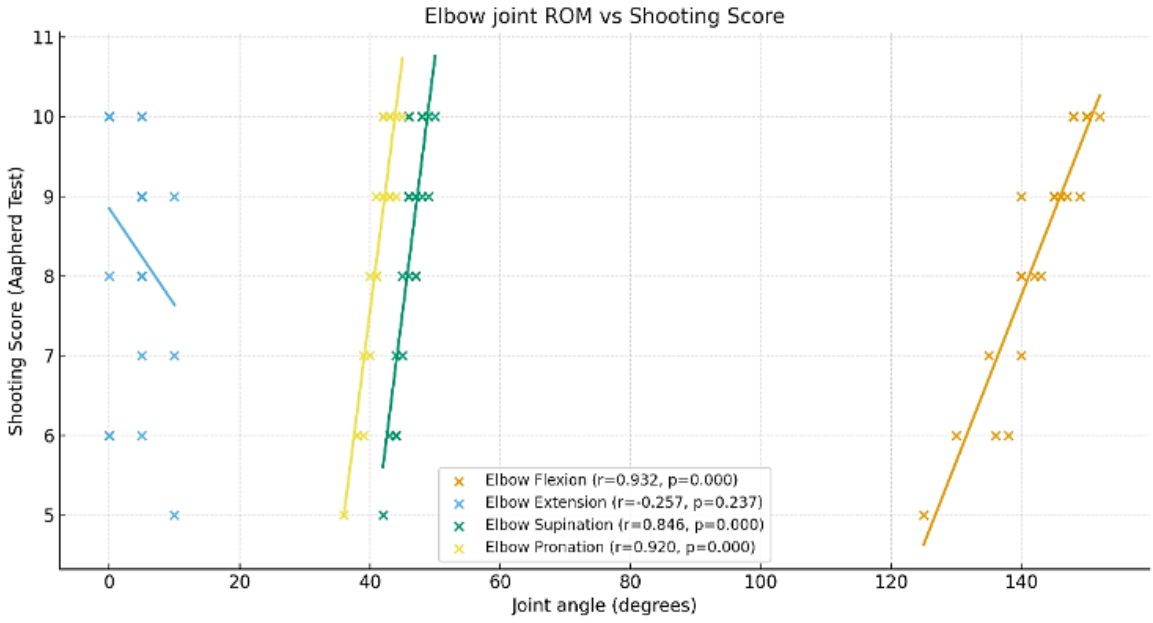

FIGURE 2. Correlation between athletes' elbow joint range of motion and shooting performance scores.

TABLE 8. Regression Analysis Model Summary Between Joint Range of Motion and Shooting Score

Model	R	R^2	Adjusted R^2	Standard error of the estimate	Durbin-Watson
	0.929[a]	0.864	0.850	0.614	1.773

[a]Independent Variables (Joint Range of Motion).

the error terms. In the present study, the error term value of 1.773 falls within the acceptable range of 1 to 3, indicating that the regression analysis can be appropriately conducted [17].

According to the analysis of variance (ANOVA) results for the regression model, the total sum of squares was determined as 55.47. Of this total, 47.92 was attributed to regression and 7.55 to error (residual) sources. The degrees of freedom were 2 for regression and 20 for residuals. The mean square for regression was calculated as 23.96, while the mean square for residuals was 0.37. The significance level of the model was found to be $F(2, 20)= 63.442$, $P<0.01$, indicating that the model is statistically significant (Table 9).

According to the regression coefficients table, the model's constant coefficient was found to be 11.39, with a significance level of $P<0.01$. The regression coefficient for wrist joint range of motion was calculated as -0.299 and was determined to be statistically non-significant ($P=0.252$). The regression coefficient for elbow joint range of motion was 1.724 and was found to have a significant effect on the dependent variable with a significance level of $P<0.01$. The standardized coefficients were determined as $\beta= 1.086$ for elbow range of motion and $\beta= -0.188$ for wrist range of motion (Table 10).

DISCUSSION

This study systematically demonstrates that wrist and elbow ROM play a determining role in shooting performance among male basketball players aged 16-18, quantitatively confirming the effects of joint mobility on performance and providing an original contribution to the literature by detailing this specific biomechanical relationship.

Although certain methodological limitations exist, several aspects of the research design mitigate their potential impact. While the small and homogeneous sample of young male players from a single city may initially appear restrictive, this controlled selection reduced variability among participants and thereby enhanced the internal validity of the findings. The absence of female participants limits the applicability of the results across genders; however, the study provides a valuable foundation for future research with more diverse samples.

Although the relatively small sample size may have reduced statistical power, the use of multivariate analyses and validity assessments strengthened the reliability of the results. The cross-sectional design, while not allowing causal inferences, was nonetheless appropriate for identifying preliminary associations and offers a basis for subsequent longitudinal or intervention-based studies. Potential uncontrolled confounders such as injury history, training volume, and player position could not be fully eliminated, yet the fact that participants were active basketball players with comparable competitive backgrounds likely reduced the risk of systematic bias.

Moreover, assessing shooting performance under standardized, non-competitive conditions, although not fully reflective of in-game dynamics, enabled a clearer examination of the biomechanical

TABLE 9. Analysis of Variance (ANOVA) Values for the Regression Analysis

Model	Sum of Squares	df	Mean square	F	P-value
Regression	47.92	2	23.96	63.44	**<0.001[b]**
Residual	7.55	20	0.37		
Total	55.47	22			

[b]Independent variables (Joint Range of Motion). Statistically significant P-value is shown in bold.

TABLE 10. Regression Coefficients and Significance Levels of Variables in the Regression Model

Model	B	SD	β	t	P-value
Constant	11.39	0.128		88,892	**<0.001**
Wrist joint range of motion	-0.299	0.254	-0.188	-1.179	0.252
Elbow joint range of motion	1.724	0.254	1.086	6.799	**<0.001**

Model=R^2=0.864 (P<0.05), β, standardized regression coefficient; SD, standard deviation.
Statistically significant P-values are shown in bold.

determinants of shooting performance independent of situational factors. Taken together, despite these limitations, careful data collection, adherence to scientific standards, and the application of rigorous statistical methods support the reliability of the findings and highlight their contribution to the existing body of knowledge.

In light of this methodological framework, the present results appear particularly noteworthy in demonstrating associations between shooting performance and upper extremity joint range of motion parameters.

Significant relationships were identified between shooting performance scores and certain joint range of motion parameters among the athletes. The results revealed strong positive and significant correlations particularly between elbow flexion and pronation movements and shooting performance.

Additionally, elbow supination, as well as wrist flexion and extension ranges of motion, showed statistically significant positive relationships with shooting performance. Conversely, no significant relationships were found between shooting performance and radial deviation, ulnar deviation, or elbow extension.

These results suggest that upper extremity joint mobility - especially specific movements at the elbow and wrist - may be key determinants of effective shooting performance in basketball. In this context, the results of the present study are discussed in relation to data from similar research reported in the literature.

Regression analysis indicated that elbow ROM was a significant predictor of shooting performance (β= 1.086, t= 6.799, P<0.01), whereas wrist ROM was not (β= -0.188, t= -1.179, P=0.252). The negative beta coefficient for wrist ROM suggests an inverse relationship in this sample. Theoretically, this may reflect that optimal shooting performance in young male basketball players is associated with a specific range of wrist motion, and excessive wrist flexion or extension could potentially impair accuracy. However, as this effect was not statistically significant, it should be interpreted cautiously. Nonetheless, they underscore the importance of supporting shooting skills not only with technical and tactical elements but also through enhancing relevant joint range of motion.

The possible mechanisms underlying the greater influence of elbow ROM compared to wrist ROM on shooting performance can be explained through the biomechanics of the shooting movement. The elbow joint generates the primary force that propels the ball forward, whereas the wrist primarily controls ball direction, release angle, and spin [1, 2]. Therefore, limitations in elbow range of motion can constrain the kinetic chain and reduce shooting effectiveness, while wrist range of motion has a more secondary effect. Additionally, elbow flexion and pronation optimize the coordinated activity of the upper limb muscles during the shot, allowing the ball to be released with greater accuracy and at a higher trajectory. This mechanical advantage may explain why elbow ROM is a more decisive factor for shooting performance compared to wrist ROM [21, 22].

In a study conducted by Pamuk and Kılınç [5], the shooting mechanics of young basketball players were analyzed based on their shooting performance. The findings revealed significant differences in wrist angles during the final phase of the shot among players with high shooting percentages (P<0.05). This result suggests that wrist joint range of motion may influence shooting accuracy and highlights the importance of considering wrist positioning in detailed analyses of shooting mechanics [5]. Consistent with our study, significant positive relationships were found between wrist flexion and extension range of motion and shooting performance. These findings support the

notion that fine-tuning wrist mechanics could be a key factor in enhancing shooting accuracy, although our study also suggests that other joints, such as the elbow, may play an even more influential role in overall performance.

Carr [23] examined the relationship between wrist flexion and extension range of motion and free-throw percentage in 45 male high school basketball players. The study found no statistically significant correlation between wrist range of motion and free-throw percentage (r= -0.12). This finding indicates that wrist range of motion may not be a decisive factor for free-throw performance, which might instead be more closely related to variables such as motor control, shooting technique, and individual shooting habits [23]. Similarly, in our study, no significant relationships were observed between radial and ulnar deviation variables of the wrist joint range of motion and shooting performance. These results suggest that while wrist flexibility is an important aspect of shooting mechanics, other factors such as motor control, technique, and habitual movement patterns may have a greater impact on free-throw success.

In a pilot study by Smajla *et al*. [21], the relationship between the strength capacity of elbow extensors and volar flexor muscle groups of the hand and shooting performance in basketball players was investigated. It was found that the strength capacity of the elbow extensor muscles was significantly associated with shooting performance [21]. These findings highlight the crucial role of elbow extensor strength in shooting performance, suggesting that targeted strengthening of these muscles could be an effective strategy to enhance players' accuracy.

In a study conducted by Cabarkapa *et al*. [22], the kinetic and kinematic characteristics of proficient and non-proficient shooters were compared during two- and three-point shots. The findings indicated that proficient shooters demonstrated greater elbow flexion during the preparatory phase of the shot, which increased shot height and positively influenced shooting accuracy. This suggests that kinematic characteristics play a significant role in executing accurate shots, and that elbow angles, in particular, are a key determinant of shooting performance [22]. In line with these findings, the present study also identified a strong positive and significant correlation between elbow flexion and shooting performance among the athletes. These results reinforce the importance of elbow mechanics in shooting, indicating that optimizing elbow flexion during the preparatory phase may be a critical factor for improving shooting accuracy.

Another study by Cabarkapa *et al*. [24], examined the shooting kinematics of professional male basketball players in relation to distance and shooting proficiency. The results showed that elite shooters performed their shots with lower elbow angles, which contributed to higher shooting accuracy [24]. Similarly, the findings of the present study indicated a positive relationship between elbow joint range of motion and shooting accuracy. This difference may be attributed to age-related anthropometric characteristics of the athletes. These findings suggest that while optimal elbow angles may vary across different populations, maintaining adequate elbow range of motion remains a consistent factor contributing to shooting accuracy.

Furthermore, in a separate study by Cabarkapa *et al*. [25], kinematic differences were analyzed based on shooting proficiency and distance in female basketball players. The results revealed that successful shooters exhibited lower elbow angles compared to less successful shooters, and that this kinematic distinction contributed to increased shooting accuracy. This finding underscores the importance of joint angles in shooting mechanics and suggests that joint positioning may have a decisive impact on shooting success [25]. Consistent with this, the current study found strong positive and significant correlations between elbow flexion and pronation movements and shooting performance. These results further emphasize the critical role of elbow joint positioning in shooting mechanics, suggesting that both flexion and pronation movements are important determinants of shooting success across genders.

In a pilot study conducted by Gür *et al*. [26], the independent contributions of upper extremity variables to free-throw accuracy were investigated. The findings indicated that elbow extensor strength, in particular, was significantly associated with free-throw success. In the present study, however, no significant relationship was found between elbow extension and shooting performance [26]. This discrepancy may be

attributed to differences in sample size, the athletes' physical characteristics, and their training levels. This discrepancy highlights that the influence of elbow extension on shooting performance may be context-dependent, varying with factors such as sample characteristics, training experience, and physical attributes of the athletes.

The findings of this study suggest that coaches and physiotherapists can place greater emphasis on elbow joint mobility and flexion-pronation mechanics when designing basketball-specific training programs. Targeted exercises to improve elbow flexion, pronation, and overall upper limb coordination may enhance shooting accuracy. Additionally, while wrist mechanics remain important, integrating multi-joint coordination drills could provide a more holistic approach to skill development.

Strengths and Limitations

A novel contribution of this study is the comprehensive examination of both wrist and elbow range of motion in relation to shooting performance, highlighting that elbow mechanics may play a more decisive role than previously recognized. This insight provides a clearer understanding of the biomechanical determinants of shooting success, offering practical guidance for individualized training interventions.
This study has several considerations that should be taken into account when interpreting the findings. The cross-sectional correlational design allows the identification of associations between joint range of motion and shooting performance; however, causal relationships cannot be inferred. In addition, the sample consisted of male basketball players aged 16–18 years from a single city, which may limit the generalizability of the results to other populations. Joint range of motion was assessed using a standard manual goniometer, which demonstrated high reliability, although more advanced biomechanical methods could provide additional detail. Furthermore, shooting performance was evaluated under standardized test conditions, which may not fully reflect the dynamic demands of competitive game situations. Despite these considerations, the sample size was determined through an a priori power analysis, supporting the methodological adequacy and reliability of the study.

Recommendations

•Specific flexibility and mobility exercises aimed at improving elbow flexion, pronation, and supination, as well as wrist flexion and extension, can be incorporated into training programs.

•Regular assessment of wrist and elbow joint range of motion in athletes may help guide the development of individualized exercise plans based on personal differences.

•When evaluating basketball shooting performance, joint mobility of the relevant segments should be considered alongside technical accuracy.

•During youth talent identification, specific joint range of motion parameters may be used as indicators of shooting potential.

•Further studies involving different age and performance groups are recommended to support and improve the generalizability of these findings.

•Basketball coaches and performance specialists should be educated on the importance of joint mobility contributing to shooting mechanics, and practical training workshops can be organized for this purpose.

CONCLUSION

In conclusion, this study revealed the effects of wrist and elbow joint range of motion on shooting performance in male basketball players aged 16-18. The strong positive and significant correlations observed particularly between elbow flexion and pronation and shooting performance suggest that these two movements are critically important for successful shooting in basketball. Additionally, elbow supination, as well as wrist flexion and extension, were also found to significantly influence shooting performance. On the other hand, no statistically significant relationship was found between shooting performance and wrist radial deviation, ulnar deviation, or elbow extension, although these movements may play a supportive role.

Ethics Approval and Consent to Participate

This study was approved by the Dicle University Social and Human Sciences Ethics Committee (Decision No: 2025/199; date: 24.03.2025). All procedures were conducted in accordance with the ethical standards of the institutional and national

research committee and with the 1964 Helsinki Declaration and its later amendments. Written informed voluntary consent was obtained from all individual participants included in the study.

Data Availability

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

Authors' Contribution

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

Conflict of Interest

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

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Generative Artificial Intelligence Statement

The author(s) hereby declare that the entire content of this study was conducted in accordance with scientific research methods and academic ethical principles. Artificial intelligence or similar applications were used solely to assist with grammar correction and English translation.

Editor's Note

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Chronobiology and Chronotherapy-Related Effects in Non-Traumatic Pain Presentations in the Emergency Department: A Retrospective Study

Jale Akgöl[1], Şerife Özdinç[2], Serra Karakoca[3], Bahar Karya Alkan[3], Melisa Baş[3], Berke Üzeyir Gündoğdu[3], Orhan Başaran[3], Ferit Suat Çetin[3], Esra Akkuş[3], Kaan Tanrıkulu[3], Zeynep Beyza Kaya[3], İsmail Şahin[3], Mert Aras[3]

[1]Department of Medical Pharmacology, Afyonkarahisar Health Sciences University, Faculty of Medicine, Afyonkarahisar, Türkiye; [2]Department of Emergency Medicine, Afyonkarahisar Health Sciences University, Faculty of Medicine, Afyonkarahisar, Türkiye; [3]Undergraduate Medical Students, Afyonkarahisar Health Sciences University, Faculty of Medicine, Afyonkarahisar, Türkiye

ABSTRACT

Objectives: Pain is one of the most frequent reasons for emergency department (ED) admissions, yet the chronobiological patterns of pain-related presentations remain insufficiently explored. This study aimed to retrospectively analyze the temporal and seasonal characteristics of non-traumatic pain-related ED visits, with a focus on daily and seasonal variations influenced by circadian rhythms.

Methods: This retrospective, descriptive, cross-sectional study included adult patients (≥18 years) who presented to the Afyonkarahisar Health Sciences University Adult ED with non-traumatic pain between January 1, 2023, and December 31, 2023. Cases were identified based on ICD-10 codes for headache (R51, G43, G44), chest pain (R07.1–R07.4), abdominal pain (R10.0–R10.4), and musculoskeletal pain (M54, M79, M25). Demographic data, admission times, diagnostic procedures, treatment initiation, and hospitalization rates were analyzed. Statistical analyses utilized appropriate parametric and non-parametric tests, with significance set at $P<0.05$.

Results: A total of 4524 patients were included (53.9% female; mean age 47.3±21.4 years). Chest pain was the most frequent diagnosis (40%), followed by headache (35.6%), musculoskeletal pain (19.6%), and abdominal pain (4.8%). ED visits peaked in the evening (mode: 21:09) and were least frequent after midnight. Headache admissions were significantly higher in autumn (30.7%), while chest pain was most common in winter (32%). Women more frequently presented with headache and abdominal pain, while men predominantly reported chest and musculoskeletal pain.

Conclusions: Non-traumatic pain-related ED visits appeared to follow certain chronobiological patterns with observable sex-related differences. These preliminary findings may contribute to understanding temporal trends in pain-related ED utilization and could serve as a basis for future studies exploring the potential role of chronotherapy in pain management. Validation through larger, multicenter, and prospective studies is needed to strengthen and generalize these findings.

Keywords: Chronobiology, Chronotherapy, Circadian Rhythm, Emergency Department, Non-Traumatic Pain

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Corresponding author: Jale Akgöl, MD., Assist. Prof., Phone: +90 272 246 33 35, E-mail: jaleakgol@gmail.com, jale.akgol@afsu.edu.tr

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Pain is one of the most common and significant reasons for admissions to the emergency department [1]. According to the definition of the International Association for the Study of Pain (IASP), pain is an unpleasant sensory experience that may or may not be associated with actual or potential tissue damage and is shaped by past experiences [2]. As a subjective sensation, pain is perceived differently depending on an individual's cultural background, educational level, psychosocial status, and past experiences, making its assessment and effective treatment challenging [3]. Pain management in the emergency department is a complex process aimed at alleviating the patient's complaints, supporting diagnostic procedures, and guiding treatment decisions. It operates under the influence of multiple factors such as time, patient volume, infrastructure, and available personnel. However, in practice, pain management is often delayed or carried out using non-standardized approaches due to the influence of these factors. In the large population of patients presenting to the emergency department with pain, the temporal patterns of pain experiences are often overlooked. These patterns are closely associated with biological rhythms. However, human physiology functions according to circadian rhythms in many systems, and these rhythms affect numerous parameters such as pain perception, response speed, and drug efficacy [4].

The circadian rhythm refers to the biological processes that repeat within a 24-hour cycle and is commonly described as the body's biological clock. These internal clocks regulate many biochemical processes, from controlling fundamental physiological functions such as sleep patterns, hormonal secretions, body temperature, and metabolism to DNA repair [5]. Chronobiology is the scientific discipline that studies biological rhythms in living organisms and their effects on physiological processes [6]. Many physiological events in the human body exhibit circadian rhythms. For example, it has long been known that the incidence of cerebrovascular events and acute myocardial infarction shows a distinct peak during the morning hours [7, 8]. Pain perception and onset may also vary at different times of the day [9]. Indeed, studies conducted in specific patient groups have demonstrated significant temporal distributions in emergency department presentations. For example, pain crises related to sickle cell anemia were found to peak particularly during the evening hours [10].

Studies conducted in warm climates have shown that admissions for acute renal colic are highest during the summer and lowest in the winter [11]. The literature has limited Data on the chronobiological patterns of pain-related emergency department visits. Understanding the temporal and seasonal distributions of admission times and pain types can improve clinical decision-making. It can also enhance the efficiency of resource planning.

The hypothesis of this study is that emergency department visits for non-traumatic pain exhibit a distinct temporal chronobiological pattern, which is influenced by demographic factors such as sex, age, pain type, and season, as well as by the timing of treatment and analgesic administration relevant to chronotherapy. The primary research question is whether such temporal patterns exist in non-traumatic pain-related emergency department visits and whether they vary according to demographic, clinical, and chronotherapy-relevant variables.

The aim of the study is to identify these temporal patterns and assess their associations with key demographic, clinical, and chronotherapy-related factors.

METHODS

This study was designed as a retrospective, descriptive, and cross-sectional analysis. Data were obtained from the Hospital Information Management System (HIMS) database of the Afyonkarahisar Health Sciences University (AFSU). The study commenced following the approval of the local ethics committee (Decision No: 2024/9, dated 01.11.2024).

Patients presenting with pain-related conditions potentially associated with asthma, myocardial infarction, migraine, cerebrovascular diseases, chest pain, headache, abdominal pain, or musculoskeletal pain were identified using the International Classification of Diseases (ICD) codes. Data on age, sex, presentation time, diagnostic tests and orders, consultation requests, medication initiation, and hospitalization status were analyzed. The inclusion and exclusion criteria are presented in Table 1. Patient presentation times were obtained directly from the Hospital Information Management System (HIMS),

TABLE 1. Inclusion and Exclusion Criteria for the Study Population

Inclusion criteria	Exclusion criteria
Age ≥18 years	Age <18 years
Presentation to AFSU adult emergency department (Jan 1-Dec 31, 2023)	Trauma-related cases
Non-traumatic pain with ICD-10 codes 1. Headache: R51, G43, G44 2. Chest pain: R07.1-R07.4 3. Abdominal pain: R10.0-R10.4 4. Musculoskeletal pain: M54, M79, M25	Pregnant women Forensic cases Incomplete records

AFSU, Afyonkarahisar Health Sciences University; ICD-10, ınternational classification of diseases, tenth revision; R51, headache; G43, migraine; G44, other headache syndromes; R07.1, chest pain on breathing; R07.2, precordial pain; R07.3, other chest pain; R07.4, chest pain_unspecified; R10.0, acute abdomen; R10.1, pain localized to upper abdomen; R10.2, pelvic and perineal pain; R10.3, pain localized to other parts of lower abdomen; R10.4, other and unspecified abdominal pain; M54, dorsalgia; M79, other soft tissue disorders, not elsewhere classified; M25, other joint disorders, not elsewhere classified.

which records admission data with second-level precision in real time. For the analyses, presentation times were categorized according to circadian rhythm–based time-of-day intervals as: Morning (06:00-11:59), Noon (12:00-15:59), Evening (16:00-20:59), and Night (21:00-05:59). For seasonal comparisons, months were grouped according to the standard meteorological definition for the Northern Hemisphere: Spring (March-May), Summer (June-August), Autumn (September-November), and Winter (December-February). In total, 4,524 patient records were evaluated, corresponding to 21.7% of all emergency department admissions in 2023.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics version 28.0 (or the latest version). Descriptive statistics were reported as mean, standard deviation, median, minimum–maximum values, and percentages. The normality of data distribution was assessed using the Shapiro-Wilk test. The Mann–Whitney U test (for two groups) and the Kruskal–Wallis test (for three or more groups) were applied for non-normally distributed variables. Post hoc analyses were conducted when significant differences were identified with the Kruskal–Wallis test. The homogeneity of variances was evaluated using Levene's test. For parametric data sets with unequal variances, group means were compared using Welch ANOVA and the Games–Howell post hoc test. Categorical variables were analyzed using the chi-square test, and Bonferroni correction was applied for multiple comparisons. Time of admission was considered the dependent variable in multivariate regression analysis, alongside age, sex, diagnosis group, and number of medications used. A P-value of <0.05 was considered statistically significant for all tests.

RESULTS

A total of 4,524 patients who presented to the emergency department with non-traumatic pain complaints during 2023 were included in the study. Of these patients, 53.9% were female and 46.1% were male. The mean age was 47.32±21.41 years, ranging from 18 to 103 years, and the median was 44 years. The most common presenting diagnoses were chest pain (40%), headache (35.6%), other musculoskeletal pain (19.6%), and abdominal pain (4.8%).

Regarding clinical interventions, 44.5% of patients underwent diagnostic testing, 12.9% required consultation, 72.6% received treatment in the emergency department, and 4.2% were hospitalized.

The distribution of emergency department admission times showed a median of 15:37, with a peak (mode) at 21:09. The interquartile range (Q1-Q3) was concentrated between 11:08 and 20:18 (Figure 1).

Seasonal distribution analysis revealed that 30.7%

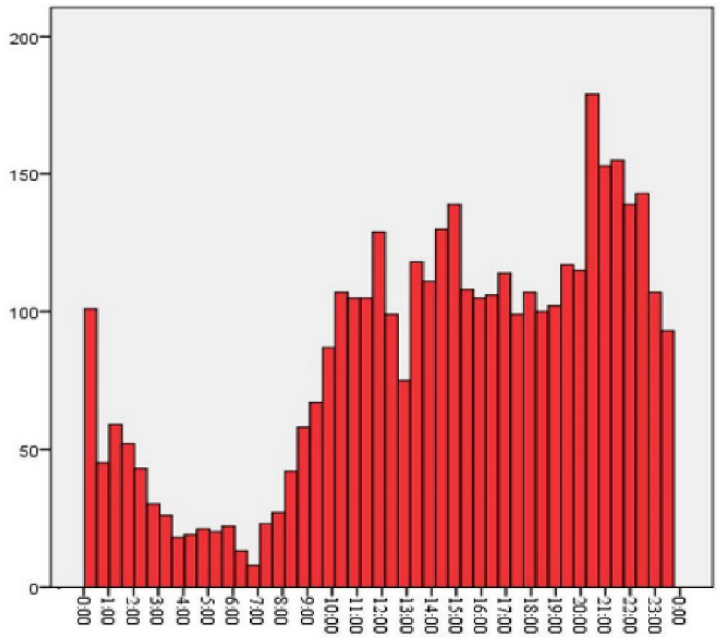

FIGURE 1. Admission times to the emergency department – frequency histogram.

of headache cases presented in autumn (P<0.0083), and 32% of chest pain cases presented in winter (P<0.0083) (Figure 2). No significant seasonal relationship was observed for abdominal pain or other pain groups. When analyzed by month, pain-related admissions exhibited a noticeable concentration during November and January (Figure 3).

The Kruskal-Wallis test showed a significant difference in admission times across seasons (P<0.001), with admission times being significantly later in autumn compared to other seasons (Kruskal-Wallis χ^2=1536.569, df=3, P<0.001

Regarding time-of-day differences, chest and abdominal pain patients presented significantly earlier than those in other pain groups (P<0.001 and P<0.015, respectively).

Treatment initiation rates, timing of diagnostic tests, order times, and hospitalization rates also varied

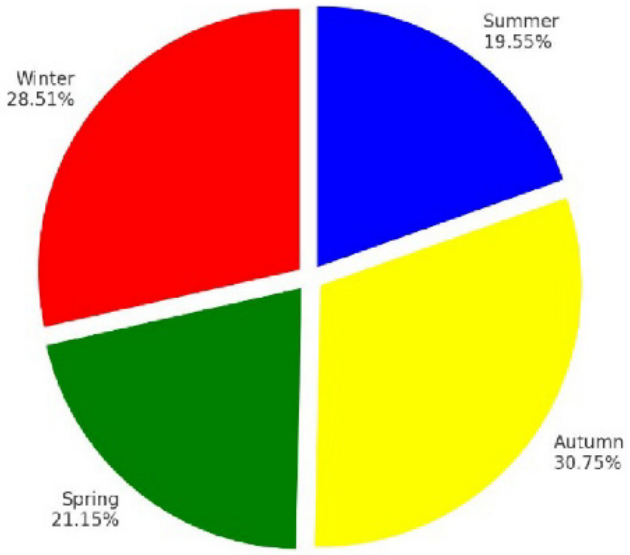

FIGURE 2. Seasonal distribution of emergency department admissions for all pain-related cases.

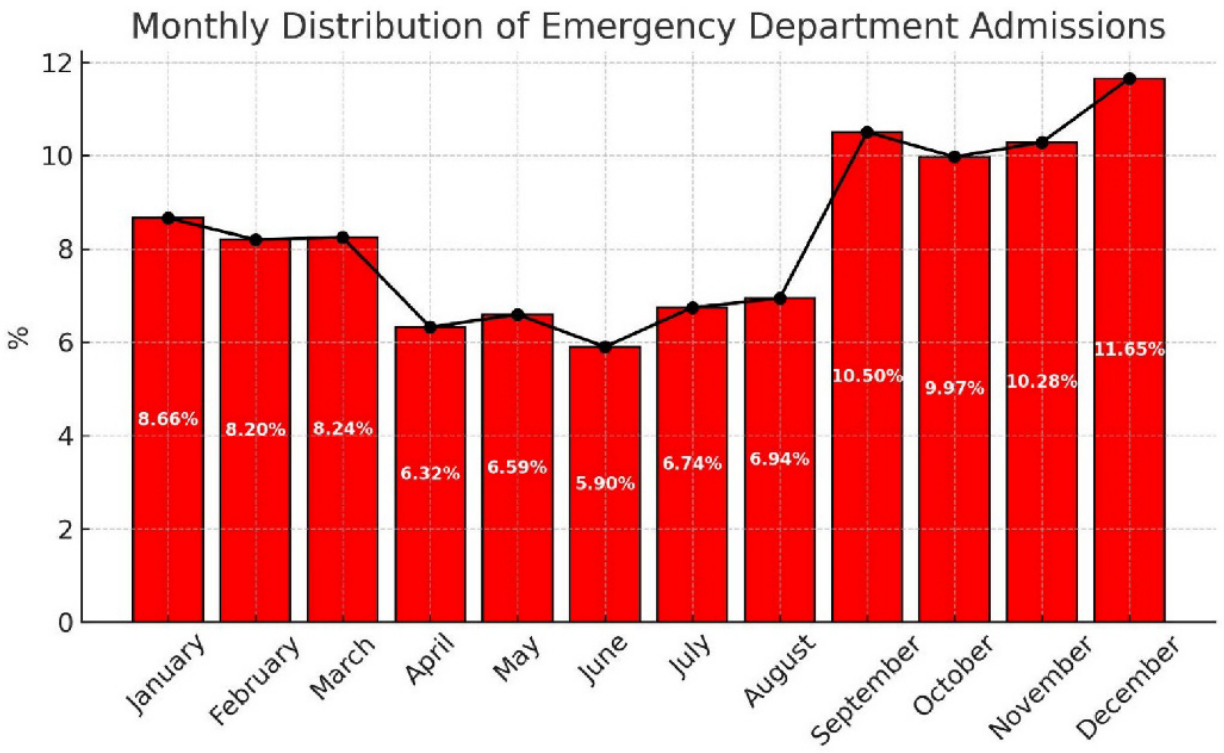

FIGURE 3. **Percentage frequency of emergency department visits for pain-related cases by month.**

TABLE 2. **Gender-Based Comparison of Demographic and Clinical Parameters in Patients with Non-Traumatic Pain**

Vaiable	Female	Male	Test (t /chi-square)	df	P-value
Age (years)	46.6 ± 20.9	48.1 ± 21.9	t = 2.513	4344	**0.012**
Time from admission to test (hours)	00:05:23	00:06:42	t = -2.144	1558	**0.016**
Time from test to order (hours)	00:04:00	00:04:00	t = -0.156	1489	0.876
Time from admission to consult (hours)	00:17:30	00:17:30	t = 0.548	581	0.584
Admission	2168 (54.3%)	1827 (45.7%)	χ^2 value	-	**0.006**
Diagnostic test performed	963 (48.9%)	1052 (52.2%)	χ^2 value	-	**<0.0001**
Consultation requested	230 (39.5%)	353 (60.5%)	χ^2 value	-	**<0.0001**
Treatment in the emergency department	1812 (55.2%)	1473 (44.8%)	χ^2 value	-	**<0.005**
Hospitalization	68 (36.0%)	121 (64.0%)	χ^2 value	-	**<0.0001**
Headache	1002 (62.2%)	609 (37.8%)	χ^2 value	-	**<0.0001**
Chest pain	793 (43.8%)	1017 (56.2%)	χ^2 value	-	**<0.0001**
Abdominal pain	113 (52.3%)	103 (47.7%)	χ^2 value	-	**<0.0001**
Other pain	530 (59.8%)	357 (40.2%)	χ^2 value	-	**<0.0001**

Data are mean±standard deviation or n (%) where appropriate. Times are presented in hour: minute: second format.
t-tests were used for age and duration variables, chi-square (χ^2) tests were used for proportions.
Statistically significant P-values are shown in bold.

TABLE 3. Seasonal Distribution of Clinical Processes and Diagnoses in Patients Presenting to the Emergency Department with Non-Traumatic Pain

Season-related emergency department processes		Winter n (%)	Spring n (%)	Autumn n (%)	Summer n (%)	P value
Medication administration	No	299 (24.1%)	294 (23.7%)	406 (32.8%)	240 (19.4%)	**<0.001**
	Yes	991 (30.2%)	663 (20.1%)	985 (30.0%)	646 (19.7%)	
Hospitalization	No	1233 (28.4%)	900 (20.8%)	1350 (31.1%)	852 (19.7%)	**0.005**
	Yes	57 (30.2%)	57 (30.1%)	41 (21.7%)	34 (18.0%)	
Diagnosis	Headache	451 (28.0%)	320 (19.9%)	495 (30.7%)	345 (21.4%)	**<0.001**
	Chest pain	580 (32.0%)	356 (19.7%)	566 (31.3%)	308 (17.0%)	
	Abdominal pain	64 (29.6%)	48 (22.2%)	69 (31.9%)	35 (16.2%)	
	Other pain	195 (22.0%)	233 (26.3%)	261 (29.4%)	198 (22.3%)	
Test time	Morning	154 (28.4%)	108 (19.9%)	155 (28.6%)	125 (23.1%)	**0.021**
	Noon	290 (34.2%)	157 (18.5%)	249 (29.3%)	153 (18.0%)	
	Evening	201 (30.0%)	123 (17.6%)	232 (36.4%)	114 (17.0%)	
	Night	350 (28.3%)	277 (22.3%)	350 (28.3%)	261 (21.1%)	
Order time	Morning	154 (28.4%)	108 (19.9%)	155 (28.6%)	125 (23.1%)	**0.002**
	Noon	290 (34.2%)	157 (18.5%)	249 (29.3%)	153 (18.0%)	
	Evening	201 (30.0%)	123 (17.6%)	232 (36.4%)	114 (17.0%)	
	Night	350 (28.3%)	277 (22.3%)	350 (28.3%)	261 (21.1%)	

P-values are based on Chi-square and Kruskal-Wallis tests.
Statistically significant P-values are shown in bold.

significantly by season (P<0.001; P<0.005; P<0.001; P<0.021; P<0.002; P<0.030, respectively).

Considering gender differences, women more frequently presented with headache (62.2%) and abdominal pain (52.3%), whereas men more commonly presented with chest pain (56.2%) and other musculoskeletal pain (59.8%). These differences were statistically significant (P<0.05) (Table 2). Gender differences were also observed in admission times, diagnostic test–order delays, medication initiation times, and hospitalization rates (Table 3). Detailed seasonal analyses, hourly distribution by type of intervention, and admission times by diagnosis group are shown in Figure 4.

Analgesic treatment was initiated in 28.7% (n=1,299) of patients presenting to the emergency department. Due to the low rate of opioid use, this group was excluded from statistical analysis. Regarding analgesic choice, 29.7% of treatments included paracetamol, while the remainder comprised non-steroidal anti-inflammatory drugs (NSAIDs). The most frequently used NSAID was dexketoprofen trometamol (55.4%), followed by diclofenac sodium (9.8%) and ibuprofen (3.5%).

Analgesic use was significantly higher in winter and autumn compared to spring (P<0.0001 and P<0.0001, respectively). However, no significant relationship was found between the pattern of analgesic use and the time of day or diagnostic test-order intervals.

DISCUSSION

This study revealed significant temporal and seasonal patterns in the presentations of patients admitted to the emergency department for non-traumatic reasons. These findings are consistent with previous research

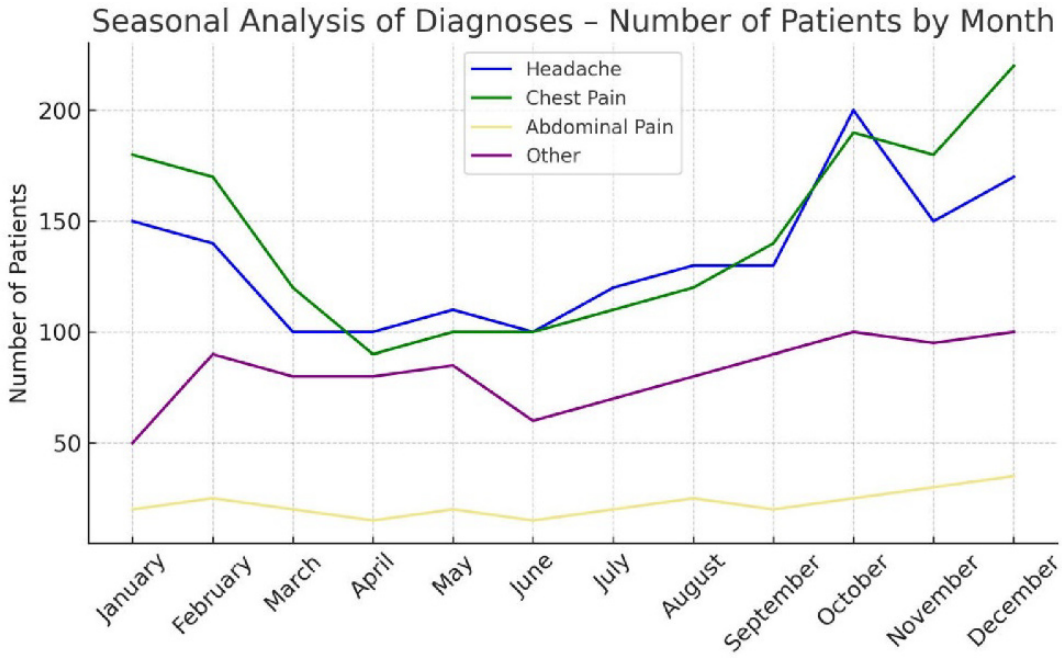

FIGURE 4. Seasonal distribution of non-traumatic pain diagnoses – number of patients.

that highlights how common pain is as a reason for emergency department visits [12]. Identifying the temporal distribution of pain, which is a common reason for emergency department visits, is important for clinical management and resource planning. According to our findings, pain-related admissions were at their lowest levels after midnight, began to increase in the morning hours, and reached their peak, particularly in the evening. The literature also indicates that general emergency department patient flow peaks around midday and is at its lowest in the early morning hours [13]. Indeed, various studies have demonstrated that pain thresholds and perception fluctuate throughout the day and that the pain system exhibits circadian characteristics [14]. Our findings, in this context, suggest that chronobiological rhythms may influence episodes of acute pain. When examining the seasonal distribution, headache-related admissions appear to increase during the autumn. Factors such as variable weather conditions in autumn and the return to a busy schedule after the summer holidays may affect stress levels, potentially leading to an increase in migraine, tension-type headaches, or musculoskeletal pain. Additionally, the onset of viral infections during autumn may increase emergency department visits for headaches or body pain. A combined effect of the seasonal cycles of different pain etiologies may be at play in this context [15]. Benkli *et al*. [16], in their meta-analysis comprising 1,513 systematic studies, reported that headaches exhibit a clear circadian peak between 21:00 and 03:00, with additional peak incidences observed during the spring and autumn seasons.

In chest pain admissions, a marked increase was observed during the winter, which is consistent with the higher frequency of cardiovascular emergencies, such as myocardial infarction, in this season. The meta-analysis by Kuzmenko *et al*. [17] reported that myocardial infarction is highest in the winter months and lowest during the summer. Similarly, it has also been reported that the onset of myocardial infarction occurs more frequently in the early morning hours [18]. Rabus *et al*. found that patients presenting with chest pain were more likely to arrive at the emergency department during the early hours. In contrast, patients presenting with other symptoms tended to seek emergency care at later times of the day [19]. Another study also found that the incidence of chest pain was significantly higher during the winter, peaking in January. Within the day, cases most commonly occurred in the early morning hours (6:00-11:00), with a particular peak observed at 11:00 [20]. Although the similarity with the literature supports the validity of our findings, it should be noted that these rhythms may

vary across different regions. In this study, although no circadian rhythm was observed in cases classified as abdominal pain and other types of pain, studies are reporting that emergency department admissions for abdominal pain are most common in February [21]. It is known that neuropathic pain, which may fall under the category of other types of pain, also exhibits a circadian rhythm and tends to worsen at night [22]. It is known that osteoarthritis pain tends to increase in the evening, whereas rheumatoid arthritis pain is more severe in the morning. Therefore, evaluating these diagnostic groups by subdividing them into their components may provide more detailed results. In our study, the rate of female patients presenting with pain complaints was higher compared to males. Except for chest pain, the number of female admissions was significantly higher in the other pain groups. The literature also shows that men are more likely to present with chest pain, whereas women are more frequently admitted with abdominal pain. It has been noted that women constitute a considerable proportion of patients presenting to the emergency department with pain-related diagnoses, which may partly be due to the higher prevalence of chronic pain disorders such as migraine and fibromyalgia in women. Women suffer from more than half of all chronic pain conditions and are more affected by these conditions than men [23-25]. On the other hand; it is known that gender differences are not merely an epidemiological reflection but also influence the processes of intervention and treatment in the emergency department. Our study observed some differences in managing female and male patients in the emergency setting. In particular, female patients were found to have longer evaluation and intervention times compared to male patients. This finding is consistent with data from the literature. In an extensive study conducted among young adults, it was reported that women presenting to the emergency department with chest pain underwent fewer ECGs for the same complaint, and only 19% of women with similar findings were categorized as "emergency" in triage, compared to 23% of men. The same study noted that the time it took for women to be seen by a physician was, on average, 11 minutes longer than for men [26]. Our finding also suggests that similar delays in managing female patients may occur. Furthermore, the lower hospitalization rates among female patients are also noteworthy. These differences may stem from biases such as interpreting women's pain as psychological or trivial or from healthcare professionals perceiving women's expressions of pain differently. Similarly, studies have shown that women presenting to the emergency department with abdominal pain are less likely to receive analgesics compared to men [27]. In the retrospective analysis by Hayes *et al*. [28], it was shown that the rate of administering combined opioid and non-opioid analgesia to female patients was significantly lower compared to male patients. In a study conducted in 2019 by Wilkinson *et al*. [29] involving 180,368 Swedish women who had experienced a myocardial infarction over the past decade, it was found that if women had received the same treatment as men, the number of women who died from myocardial infarction would have been lower. In light of all these data, it is evident that gender-related differences in care can exist in the emergency department. Our results, as emphasized in the literature, highlight the importance of ensuring gender equity in pain management in the emergency setting.

The rate of emergency department admissions due to pain is high in this study. A group of patients presenting with pain was evaluated, and it was observed that analgesic medication was administered to approximately one-third of the patients. Factors influencing this rate may include the deliberate avoidance of analgesic use to prevent the masking of diagnostic signs, the relatively mild severity of some cases, the deferral of analgesia administration to the admitting wards, the subjective nature of pain perception, and reduced pain sensitivity in elderly patients factors that are attributable to the patient, the physician, and the characteristics of the pain itself. Moreover, patients often seek emergency care without awaiting the outcomes of prior treatments.

The findings of this study may be linked to variations in pain-related emergency admissions and in the patterns of analgesic use. However, short-term circadian rhythms on an hourly basis may not directly impact analgesic preferences. From a chronopharmacological perspective, further exploration of the timing and type of analgesic administration in relation to chronobiological patterns constitutes a potentially valuable direction for future research aimed at improving analgesic efficacy and

minimizing adverse effects. [30].

Although we could not fully demonstrate the relationship between chronotherapy and the timing of analgesic administration in this study, previous research has highlighted distinct circadian patterns associated with various pain types. Specifically, hypnic and cluster headaches typically peak around 2 AM, migraine pain tends to reach its maximum intensity during the morning or midday hours, and neuropathic pain generally worsens in the evening. Such studies highlight the presence of these temporal associations; however, it is also evident that socioeconomic and sociocultural factors shape the frequency of emergency admissions. Future prospective studies that take into account pain severity and related variables may provide clearer insights into the temporal dynamics of pain presentations [31].

Strengths and Limitations

This study has certain limitations. As it was conducted in a single center and relied solely on hospital registry data, caution should be exercised when generalizing the results. The temporal distribution of pain complaints may vary across different geographical regions and healthcare institutions. Furthermore, due to the retrospective design, some subjective data, such as pain severity, could not be analyzed because they were not consistently recorded. Our study evaluated all pain complaints collectively, and detailed subgroup analyses by specific diagnoses were not performed. Therefore, chronobiological patterns specific to certain diagnostic groups may not have been fully identified. Future prospective studies that evaluate different pain etiologies separately may help better understand these conditions' unique rhythms.

This study adds to the limited number of investigations examining non-traumatic pain presentations from a chronobiological perspective in the emergency department context.

CONCLUSION

This study identified distinct chronobiological patterns in the temporal and seasonal distributions of non-traumatic pain presentations to the emergency department. Admissions tended to cluster particularly during the evening hours, whereas the lowest frequencies were observed after midnight. Seasonally, a notable increase in admissions was observed during the autumn months. These findings indicate that both circadian rhythms and environmental factors may contribute to variations in pain experience and healthcare-seeking behavior. Furthermore, sex-related differences in admission patterns were observed: women were more frequently admitted for headache and abdominal pain, whereas men were more frequently admitted for chest pain. The observed delays in diagnostic and treatment processes for female patients suggest the importance of considering gender-sensitive approaches in emergency pain management. The findings emphasize the potential value of incorporating temporal and biological rhythms into patient management strategies, workforce planning, and treatment protocols in emergency departments. Although preclinical data emphasize the impact of timing, the role of chronotherapy in emergency practice remains a subject of debate. Integrating chronotherapy principles into pain management could enhance treatment efficacy and optimize resource utilization. Future multicenter and prospective studies will provide a stronger scientific basis for clinical practice by more clearly elucidating chronobiological patterns specific to different pain etiologies.

Ethics Approval and Consent to Participate

This study was approved by the Afyonkarahisar Health Sciences University Non-Interventional Scientific Research Ethics Committee (Decision No.: 2024/9 and dated 01.11.2024). All procedures performed during data collection, review of patient records, and study implementation complied with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its subsequent amendments. Informed consent was waived because of the retrospective nature of the study and the analysis used anonymous clinical data.

Data Availability

All data generated or analyzed during this study

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

Authors' Contribution

Study Conception: JA; Study Design: JA, SK, BKA, MB, BÜG, OB, FSÇ, EA, KT, ZBK, İŞ, MA; Supervision: JA; Funding: N/A; Materials: N/A; Data Collection and/or Processing: ŞÖ, SK, BKA, MB, BÜG, OB, FSÇ, EA, KT, ZBK, İŞ, MA; Statistical Analysis and/or Data Interpretation: JA, ŞÖ; Literature Review: JA, SK, BKA, MB, BÜG, OB, FSÇ, EA, KT, ZBK, İŞ, MA; Manuscript Preparation: JA, ŞÖ; and Critical Review: JA, ŞÖ.

Conflict of Interest

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

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Generative Artificial Intelligence Statement

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

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The European Research Journal 2026;12(2):235-248 Original Article

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Biologic Therapies for Type 2 Inflammatory Diseases: A Bibliometric Analysis of Publication Trends and Intellectual Structure

Mehmet Emin Gerek[1], Tuğba Önalan[2]

[1]Department of Internal Medicine, Division of Clinical Immunology and Allergy, Necmettin Erbakan University, Faculty of Medicine Konya, Türkiye; [2]Division of Clinical Immunology and Allergy, Beyhekim Training and Research Hospital, Konya, Türkiye

ABSTRACT

Objectives: To comprehensively analyze the scientific literature (2005–2025) on biologic agents for Type 2 inflammatory diseases, including Severe Asthma (SA), Atopic Dermatitis (AD), and Chronic Rhinosinusitis with Nasal Polyps (CRSwNP). This study aimed to identify publication trends, thematic clusters, collaboration networks, and the field's intellectual foundation.

Methods: A bibliometric analysis was performed using data from the Web of Science (WoS) Core Collection (retrieved October 27, 2025). The search query targeted specific biologic agents (e.g., dupilumab, mepolizumab) and disease terms. Only original articles and reviews published in English were included. VOS viewer (v.1.6.20) software was used to analyze co-occurrence networks of author keywords and map inter-country and author collaborations.

Results: A total of 7,339 publications were analyzed. A significant acceleration in publishing occurred after 2017. The most productive countries were the USA (n=2,346), Italy (n=1,149), and the UK (n=936). Thematic analysis identified three distinct clusters: AD, SA, and CRSwNP. The intellectual foundation of the field is built upon highly cited phase 3 clinical trials (e.g., mepolizumab, dupilumab, benralizumab) published in top-tier journals (NEJM, The Lancet).

Conclusions: The research landscape for Type 2 biologics has grown rapidly since 2017, driven by clinical validation in landmark trials. The field's focus is evolving from demonstrating clinical efficacy toward mechanism-based personalized medicine and biomarker identification.

Keywords: Atopic Dermatitis, Bibliometrics, Biologic Therapy, CRSwNP, Severe Asthma, Type 2 Inflammation

Type 2 inflammatory diseases represent a group of chronic conditions. These include severe asthma (SA), atopic dermatitis (AD), and chronic rhinosinusitis with nasal polyps (CRSwNP). These diseases frequently overlap and impose a significant burden on quality of life [1].

These conditions were traditionally considered distinct clinical pathologies. However, recent translational research indicates they share a common pathophysiological basis. This foundation involves a complex inflammatory cascade. It is driven by eosinophils, mast cells, B cells, and type 2 helper T

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Corresponding author: Mehmet Emin Gerek, MD., Phone: +90 332 221 05 00, E-mail: drmegerek@gmail.com

Available Online at https://dergipark.org.tr/en/pub/eurj

lymphocytes. The cascade includes key cytokines such as Interleukin (IL)-4, IL-5, and IL-13 [2, 3].

The elucidation of this common inflammatory axis has revolutionized therapeutics, particularly in the last decade. For many years, the treatment of severe forms was limited to broad-spectrum immunosuppressive agents, such as systemic corticosteroids. This approach has radically transformed with the clinical introduction of targeted biological therapies.

Agents targeting specific pathways have been developed. These include anti-IL-5 (mepolizumab, reslizumab, benralizumab), anti-IL-4/IL-13 (dupilumab), and anti-IgE (omalizumab). This development has accelerated the shift from symptom-focused treatment to a mechanism-based "precision medicine" paradigm [4-6].

Consequently, treatment has become individualized, clinical response rates have improved, and the disease burden has significantly decreased [7].

This paradigm shift in clinical practice has also led to rapid growth in scientific literature. New indications for biologic agents, long-term follow-up results, and real-world data have substantially increased the publication volume in this field [8-10].

However, this rapid accumulation of knowledge creates a high volume of information. This density makes it difficult for clinicians and researchers to systematically evaluate current trends. Although numerous systematic reviews address specific drugs and some bibliometric studies focus on single pathologies like chronic rhinosinusitis, a holistic evaluation of the "Type 2 inflammation" concept as a unified research domain is currently lacking [11-13]. To date, a comprehensive analysis quantitatively mapping this field across multiple pathologies has not been conducted. Such an analysis would map the intellectual structure, thematic evolution, and collaboration networks for biologic therapy research in type 2 inflammatory diseases.

In this context, bibliometric analysis is a powerful and standard tool. It reveals the structure, evolution, and influential actors (authors, countries, institutions) of a research area. It also identifies the intellectual foundation, such as the most-cited works.

The aim of this study is to comprehensively analyze the literature on biologic agents for type 2 inflammatory diseases (SA, AD, and CRSwNP) published between 2005 and 2025. Using the Web of Science database, this study seeks to identify publication trends, thematic clusters, collaboration networks, and the intellectual foundation of the field.

METHODS

A bibliometric analysis and scientific mapping were performed in this study. The focus was the scientific literature concerning "Biologic Agents Used in Type 2 Inflammatory Diseases." The analysis process consisted of dataset definition, data collection, filtering, and bibliometric network analysis.

Data Source and Search Strategy

The research data were obtained from the Web of Science (WoS) Core Collection database. This database is considered the "gold standard" for scientific citation analyses and bibliometric studies. The data search was performed on October 27, 2025. A comprehensive Boolean search query was developed to capture the intersection of the specific disease spectrum and targeted therapies. This query included keywords for the "Disease Group" (Severe Asthma, Atopic Dermatitis, and Chronic Rhinosinusitis with Nasal Polyps) and the "Treatment Group" (generic terms such as 'biologic' and specific International Nonproprietary Names [INN] of key monoclonal antibodies). The data search was conducted using the "Topic" (TS) field in the WoS interface, which covers titles, abstracts, and keywords. The final search query used was as follows:

TS=(("severe asthma" OR "atopic dermatitis" OR "chronic rhinosinusitis with nasal polyps" OR "CRSwNP") AND ("biologic*" OR "monoclonal antibod*" OR omalizumab OR mepolizumab OR reslizumab OR benralizumab OR dupilumab OR tezepelumab))

Inclusion Criteria

The raw data obtained from the search query were filtered using the WoS interface to sharpen the focus of the analysis and ensure the inclusion of high-quality, peer-reviewed content. The filtering applied the following inclusion criteria:

•*Document Type:* Only publications categorized

as "Article" or "Review Article" were included. Editorial materials, meeting abstracts, letters to the editor, corrections, and book chapters were excluded to maintain the analysis's focus on original research and comprehensive reviews.

•*Year Range:* Studies published between 2005 and 2025 were selected to cover current trends and historical development. The year 2005 was chosen as the starting point to capture the early clinical development phases of key biologics prior to their widespread approval.

•*Language:* Only articles published in "English" were included in the analysis to avoid linguistic bias in text mining and keyword analysis.

Following these filtering steps, a final dataset of 7,339 publications was obtained for analysis.

Data Download and Analysis Methodology

Bibliographic data for 7,339 publications were exported from the WoS database. This included metadata, abstracts, keywords, and cited references. The "Full Record and Cited References" format and "Plain Text" (.txt) files were selected. Due to the database's bulk download limitations, the dataset was downloaded as 15 separates .txt files.

VOSviewer software (version 1.6.20) was used to create, map, and visualize the bibliometric networks.

During data processing, it was determined that the "Keywords Plus" and "All Keywords" options could not be used for thematic analysis. This was due to technical limitations (inability to read/inactive options) in the VOSviewer version used. This technical constraint necessitated a change in the analysis methodology. Consequently, it was decided to use "Author Keywords" data, which VOSviewer processed without issues. This approach provided a valid and robust alternative for analyzing the field's thematic structure based on concepts explicitly declared by the authors.

All performance analyses (productivity by year, country, institution, author) and scientific mapping analyses (thematic clustering, collaboration networks) were performed using this final dataset and methodology.

Statistical Analysis

Since this study was designed as a descriptive bibliometric analysis, conventional inferential

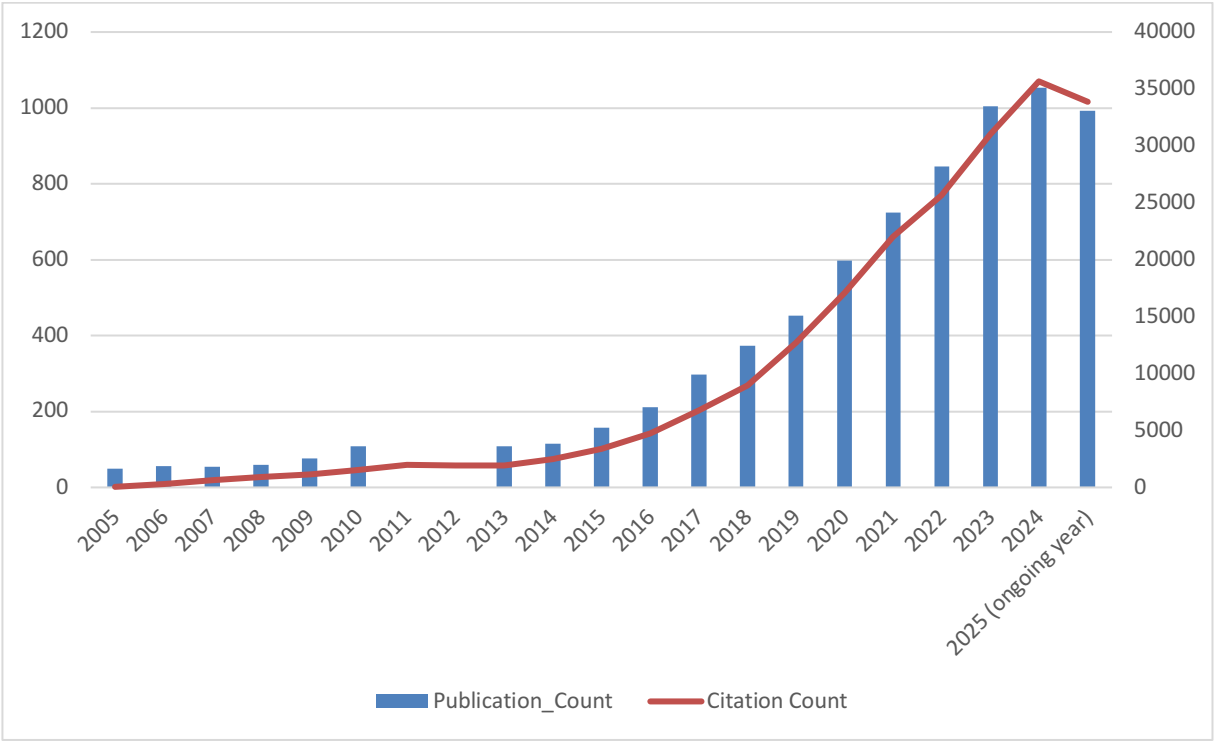

FIGURE 1. Evolution of the annual publication count (columns) and cumulative citation count (line) in the field from 2005–2025.

statistical methods (e.g., hypothesis testing) were not applied. Data analysis was based on descriptive statistics, presenting absolute frequencies (n) and percentages (%) to characterize publication trends, country productivity, and institutional contributions. Key bibliometric performance indicators, including the h-index, total citation counts, and average citations per item, were calculated using the analysis tools provided by the Web of Science Core Collection database. Additionally, network characteristics such as link strength and cluster density were computed using the algorithms embedded in VOSviewer software to visualize the field's thematic and collaborative structure.

RESULTS

Evolution of Publication and Citation Trends

A total of 7,339 publications (articles and reviews) were identified within the research scope during the analyzed period of 2005–2025. These publications received 214,816 total citations (147,402 excluding self-citations). The h-index, indicating the field's impact, was calculated as 185. The average number of citations per publication was 29.27.

The temporal development of the field, including annual publication counts and cumulative citation trends, is shown in Figure 1. The annual publication count followed a relatively modest trajectory until 2017. It began a significant increase after that date. Annual production reached 1,005 in 2023 and peaked in 2024 with 1,054 publications.

In parallel with this increase in publication numbers, the cumulative citation count (Figure 1, orange line) also exhibited a sharp acceleration, particularly after 2017. (Note: The data for 2025 are partial, as they reflect the incomplete year at the time of analysis.)

A similar trend was observed when examining the total annual citations for the research field (Figure 2). The annual citation count increased rapidly after 2017. This increase paralleled the major therapeutic

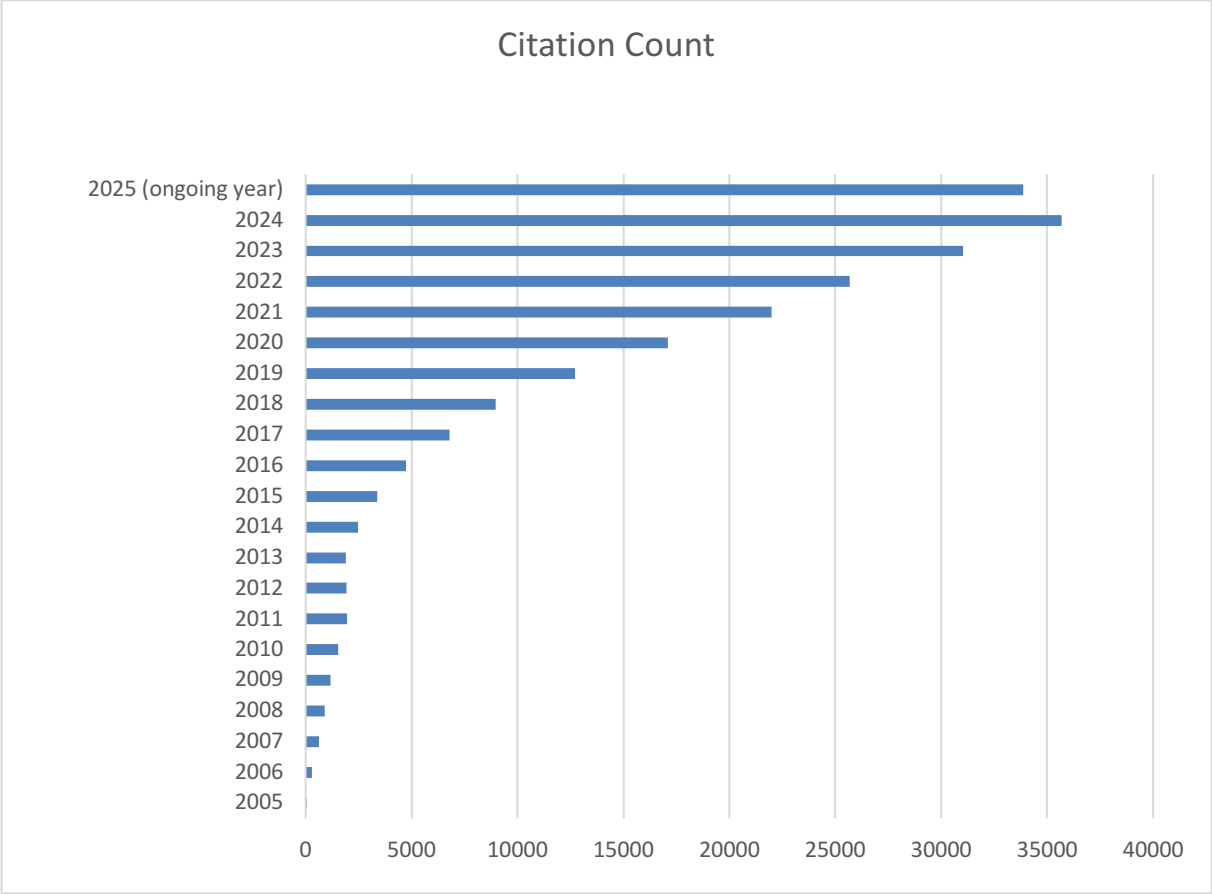

FIGURE 2. Total annual citations received by publications in the research field (2005–2025).

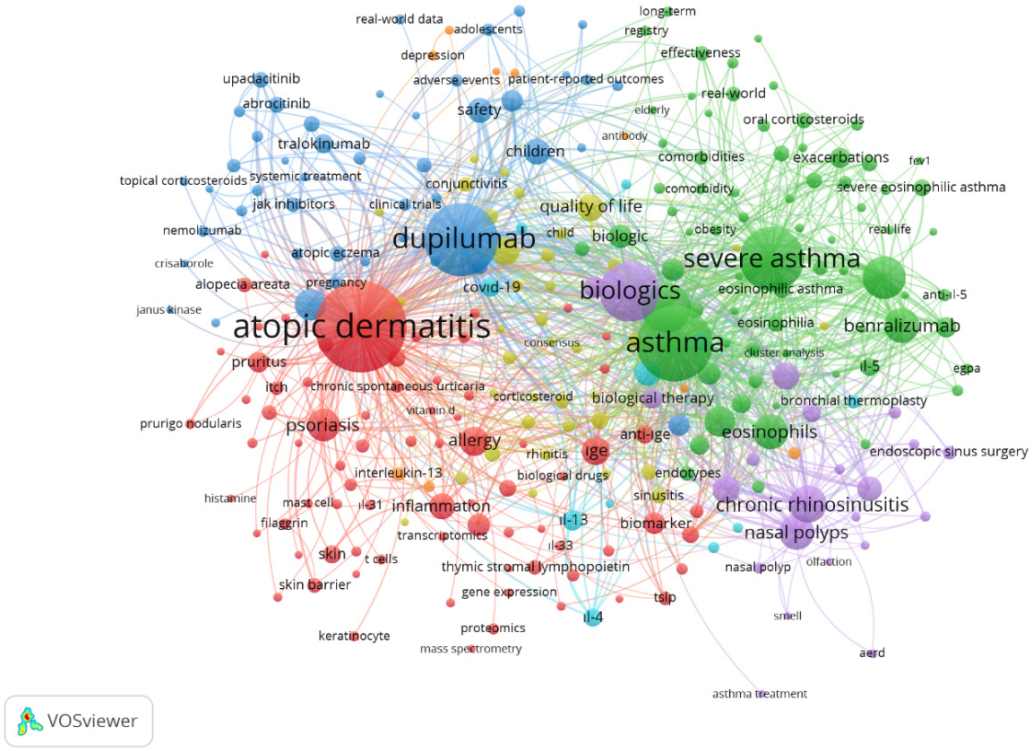

FIGURE 3. **Author keywords co-occurrence network (Minimum 15 occurrences). Colors represent different thematic research clusters identified by VOSviewer.**

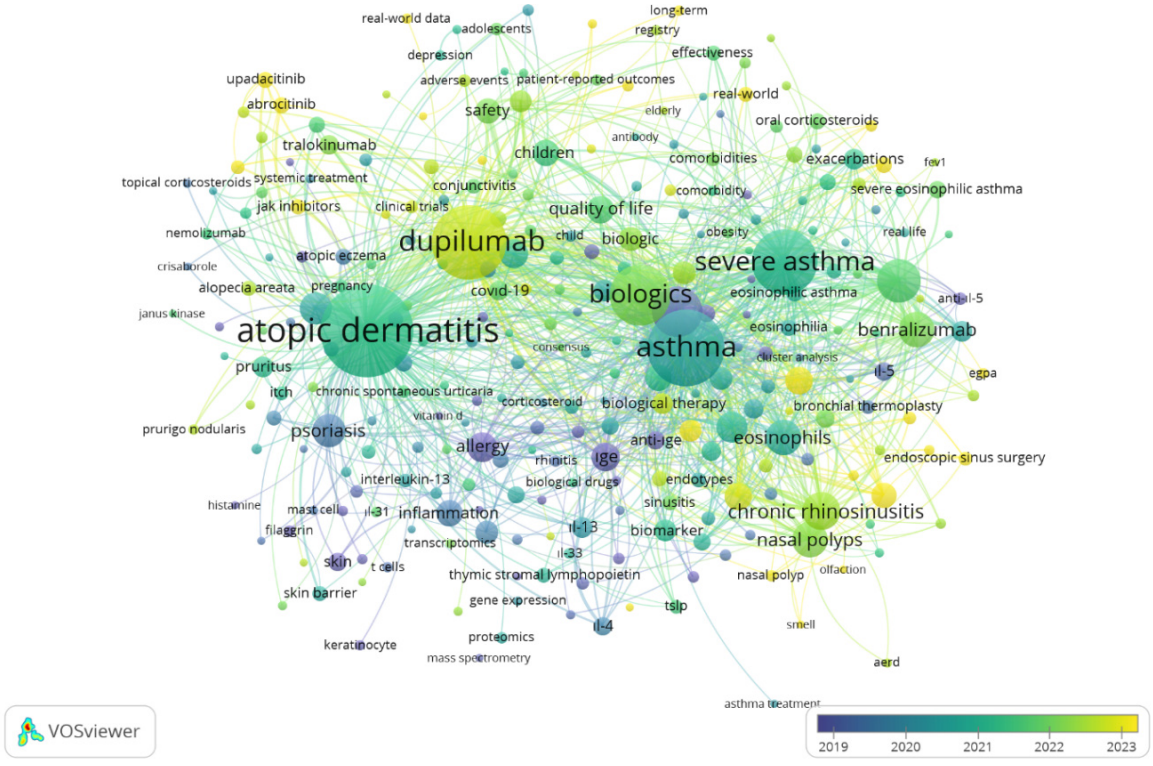

FIGURE 4. **Overlay visualization of the author keywords network. Colors indicate the average publication year (blue/purple, older topics; yellow, newer/emerging topics), highlighting current trends in the field.**

approvals for biologic agents in Type 2 inflammation. The count reached its highest levels in 2023–2024.

Thematic Structure and Emerging Topics of the Research Field

A co-occurrence analysis of author keywords was performed to identify the intellectual structure and thematic foci of the research field. This analysis utilized the keywords from 7,339 publications.

The analysis revealed 275 keywords that were used at least 15 times. The network mapping of these keywords indicated three main thematic clusters (Figure 3). These clusters were closely related but distinctly separate:

•*The Atopic Dermatitis Cluster (Red):* This cluster, centered on "atopic dermatitis," included concepts such as "dupilumab," "IL-4/IL-13 signaling," "pruritus," and "skin barrier."

•*The Severe Asthma Cluster (Green):* This cluster was centered on "severe asthma" and "asthma." It encompassed treatments such as "biologics," "eosinophils," "omalizumab," "mepolizumab," and "benralizumab," along with the "IL-5 inhibition" mechanism.

•*The Nasal Polyps Cluster (Blue/Purple):* This cluster, centered on "chronic rhinosinusitis with nasal polyps" (CRSwNP) or "nasal polyps," included "sinusitis," "endoscopic sinus surgery," and associated biologic therapies.

The overlay visualization of the network revealed the evolution of research trends in the field (Figure 4). Blue tones represented older topics, while yellow tones represented new (emerging) topics.

According to the map, the concepts "dupilumab," "severe asthma," "eosinophils," and "atopic dermatitis" stood out as the most intensely studied and most recent topics (seen in yellow) in recent years. This finding indicated that the research focus shifted toward the common pathophysiology and shared biologic therapies among these three diseases (SA, AD, and CRSwNP). This shift coincided with the approval of targeted therapies for Type 2 inflammation.

Country-Level Productivity and International Collaboration

A productivity analysis at the country level was conducted to examine geographical contributions to the research field. The analysis determined that 74 different countries contributed to the dataset of 7,339 publications.

Table 1 shows the 10 most productive countries in the field. The United States (USA) was the clear leader, having produced 2,346 publications. This figure accounted for 31.9% of the total production. The USA was followed by Italy (n=1,149, 15.7%), the United Kingdom (n=936, 12.8%), China (n=728, 9.9%), and Germany (n=694, 9.4%), respectively.

Scientific collaboration networks among countries were mapped using VOSviewer software (Figure 5).

TABLE 1. Top 10 Most Productive Countries in the Field of Type 2 Biologic Therapy Research (2005–2025)

Rank	Country	Publications	Contribution (%)
1	USA	2,346	31.9%
2	Italy	1,149	15.7%
3	England	936	12.8%
4	China	728	9.9%
5	Germany	694	9.4%
6	Japan	686	9.3%
7	France	531	7.2%
8	Canada	528	7.2%
9	Spain	500	6.8%
10	Netherlands	387	5.3%

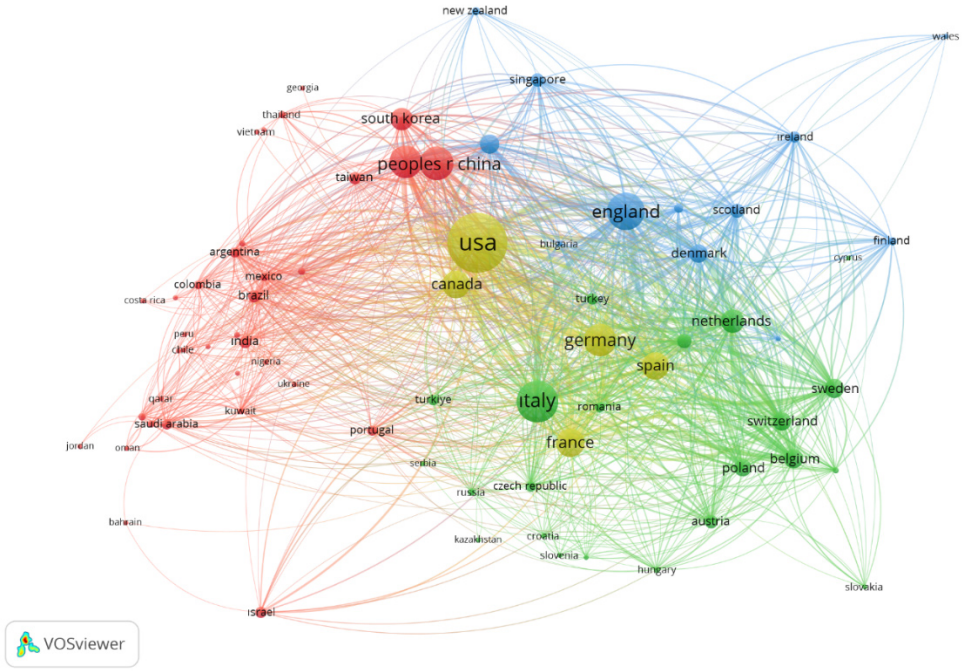

FIGURE 5. International co-authorship collaboration network among countries. The size of the nodes represents the country's total publication count, and the thickness of the lines represents the collaboration strength between countries.

In the map, the size of the nodes represented the country's total publication count. The thickness of the lines represented the strength of the collaboration.

The analysis revealed distinct international collaboration structures. The USA (yellow cluster) functioned as a global "hub," with strong ties to both European and Asian countries. European countries showed dense regional collaboration clustering (e.g., the red and green clusters, which included the UK, Germany, Italy, and France).

It was observed that the participation and international collaboration of Asian countries increased rapidly in recent years. This was particularly true for China (blue cluster), Japan, and South Korea.

Most Productive Institutions

An institutional-level analysis was conducted to identify the most influential and productive research institutions in the field. Table 2 lists the 10 most productive institutions.

The analysis revealed that European-based academic institutions had a notable dominance in the field. King's College London (n=120) ranked first as the most productive institution. It was closely followed by Imperial College London (n=111) and the University of Verona (n=103).

The list of the top 10 most productive institutions included institutions from Italy (4 institutions), the United Kingdom (3 institutions), and the USA (2 institutions).

It was noted that the WoS database indexed institutions separately under different faculty/department names (e.g., multiple entries for the University of Verona and King's College London). Therefore, the actual contribution of these institutions may be higher than the figures presented in the table.

The presence of these productive institutions in the UK and Italy indicated that these countries possessed dense collaboration structures. This was consistent with their leadership in clinical trials.

Most Productive Authors and Collaboration Networks

An author-level analysis was conducted to identify key researchers and their collaboration structures in the field. The 10 most productive authors are listed in Table 3.

Canonica GW (n=128, 1.74%) emerged as the most productive author, with foundational studies in Severe Asthma and Biologics. Guttman-Yassky E

TABLE 2. Top 10 Most Productive Research Institutions in the Field of Type 2 Biologic Therapy Research (2005–2025)

Rank	Institution	Country	Publications	% of Total (7,339)
1	King's College London – Faculty of Life Sciences & Medicine	England	120	1.64%
2	Imperial College London – Faculty of Medicine	England	111	1.51%
3	University of Verona – School of Medicine & Surgery	Italy	103	1.40%
4	Magna Graecia University of Catanzaro – Health Sciences Department	Italy	100	1.36%
5	University of Verona – Department of Medicine	Italy	99	1.35%
6	National Heart and Lung Institute	England	98	1.34%
7	University of Naples Federico II – School of Medicine & Surgery	Italy	98	1.34%
8	University of Colorado Anschutz – School of Medicine	USA	96	1.31%
9	University of Milan – Faculty of Medicine & Surgery	Italy	90	1.23%
10	Oregon Health & Science University – Department of Dermatology	USA	87	1.19%

(n=123, 1.68%) closely followed; she was recognized for pioneering research, particularly on Atopic Dermatitis and skin barrier dysfunction. Silverberg JI (n=100) focused on the epidemiology and comorbidities of Atopic Dermatitis.

The list included key opinion leaders who specialized in the field's three main diseases (Asthma, AD, CRSwNP).

The co-authorship network map visualized the "research ecosystems" formed around these key authors (Figure 6). The network analysis included 123 researchers with 225 total publications. It revealed five main groups clustered around disease-based collaboration (shown by cluster colors).

It was clearly observed in the network map that Canonica GW (red cluster) assumed a central "bridge" role among different groups. He was a key figure connecting Asthma, AD, and CRSwNP research areas.

TABLE 3. Top 10 Most Productive Authors in the Field of Type 2 Biologic Therapy Research (2005–2025)

Rank	Author	Publications	% of Total (7,339)	Primary Expertise
1	Canonica GW	128	1.74%	Severe asthma & biologics
2	Guttman-Yassky E	123	1.68%	Atopic dermatitis, barrier dysfunction
3	Silverberg JI	100	1.36%	AD epidemiology & QoL
4	Heffler E	96	1.31%	Asthma phenotypes
5	Simpson EL	91	1.24%	Dupilumab clinical trials
6	Bachert C	79	1.08%	CRSwNP & endotypes
7	Pelaia G	70	0.95%	Asthma biologics
8	Jackson DJ	67	0.91%	Airway inflammation
8	Patruno C	67	0.91%	AD & immunodermatology
10	Chen Z	66	0.90%	Emerging translational research

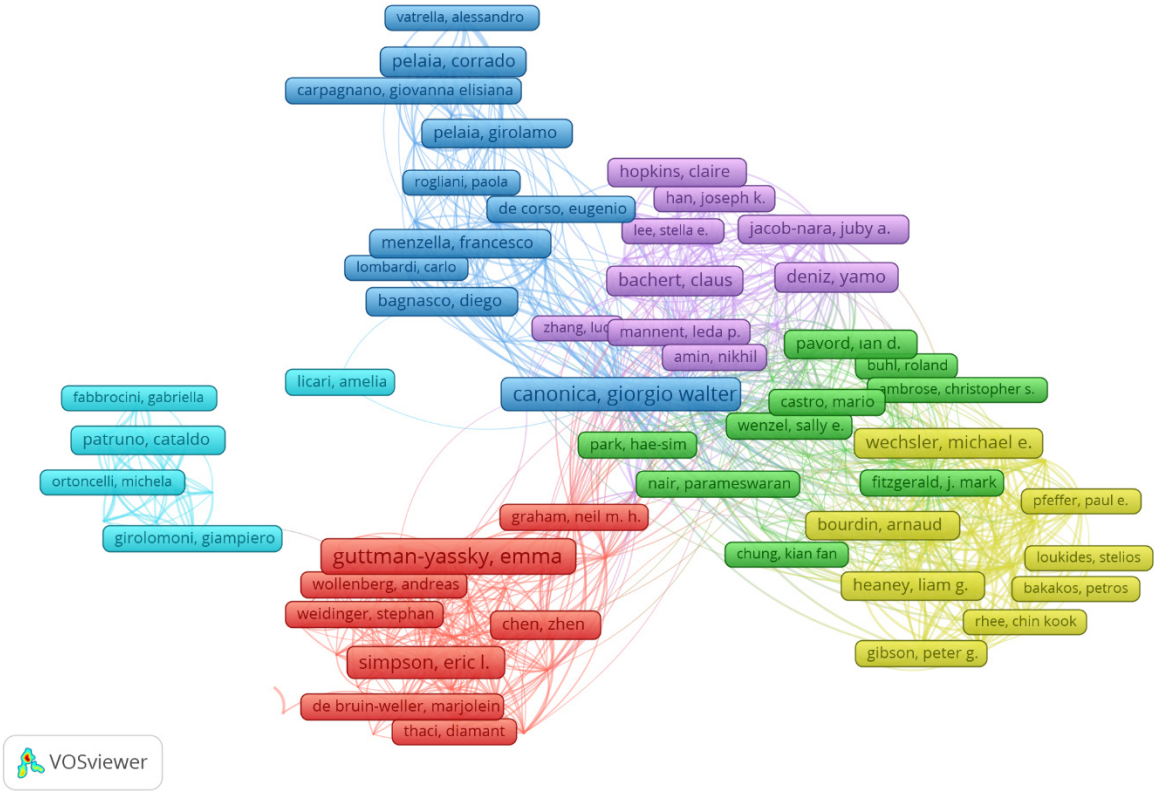

FIGURE 6. Co-authorship network is among the most productive authors. Colors indicate different collaboration clusters (research ecosystems) identified by VOSviewer.

Most Cited (Landmark) Publications

The top 10 most cited publications were analyzed to determine the intellectual foundation and most influential studies of the research field. These publications were listed in Table 4.

The analysis showed that the field's most influential publications were overwhelmingly published in top-tier medical journals. These included the New England Journal of Medicine (NEJM) (n=6) and The Lancet (n=4).

The entire list consisted of results from large phase 3 clinical trials. These trials demonstrated the efficacy and safety of key biologic agents used in Type 2 inflammatory diseases. These agents included mepolizumab, dupilumab, and benralizumab.

The most cited publication was the study by Ortega *et al*. [14] (2014), published in NEJM (1,785 citations). This study examined mepolizumab treatment in severe eosinophilic asthma. A review article ranked second (Lancet, 2018; 1,636 citations), which summarized the role of biologics in the field [15]. The phase 3 study of dupilumab in atopic dermatitis (NEJM, 2016; 1,555 citations) was third [16].

This finding confirmed that the field's development was directly linked to these landmark clinical trials. These trials led to the clinical approval of these specific biologic agents.

DISCUSSION

This study aims to bibliometrically map publications on biologic agent use in Type 2 inflammation-associated diseases. These diseases include severe asthma, atopic dermatitis, and chronic rhinosinusitis with nasal polyps (CRSwNP) from 2005 to 2025. Our analysis of 7,339 publications reveals that research production shows a significant increase since 2017. Studies cluster around three main disease areas. Knowledge production is largely driven by institutions and researchers based in the USA and Europe (particularly Italy and the UK). Furthermore, the field's intellectual foundation is primarily based on phase 3 clinical trials published in high-impact

TABLE 4. Top 10 Most Cited Publications in the Field of Type 2 Biologic Therapy Research (2005–2025)

Rank	Article Title	Year	Journal	Citations
1	Mepolizumab Treatment in Patients with Severe Eosinophilic Asthma	2014	NEJM	**1,785**
2	Asthma (Review)	2018	Lancet	**1,636**
3	Two Phase 3 Trials of Dupilumab vs Placebo in Atopic Dermatitis	2016	NEJM	**1,555**
4	Mepolizumab and Exacerbations of Refractory Eosinophilic Asthma	2009	NEJM	**1,515**
5	Dupilumab Efficacy and Safety in Moderate-to-Severe Uncontrolled Asthma	2018	NEJM	**1,479**
6	Oral Glucocorticoid-Sparing Effect of Mepolizumab in Eosinophilic Asthma	2014	NEJM	**1,289**
7	Dupilumab Treatment in Adults with Moderate-to-Severe AD	2014	NEJM	**1,117**
8	Dupilumab in Severe CRSwNP: LIBERTY NP SINUS-24/52	2019	Lancet	**1,112**
9	Benralizumab for Severe Uncontrolled Eosinophilic Asthma (CALIMA)	2016	Lancet	**1,090**
10	Benralizumab in Severe Asthma (SIROCCO)	2016	Lancet	**1,080**

medical journals. This suggests that Type 2 inflammation research is evolving toward clinical validation and treatment optimization rather than basic science [17, 18].

The bibliometric analysis shows that the significant acceleration in the literature began in 2017. This date coincides with the period when targeted biologic agents (mepolizumab, benralizumab, dupilumab) received the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) approval for major indications. Specifically, phase 3 clinical trials published between 2014 and 2017 strongly demonstrated the efficacy of these drugs in treating severe eosinophilic asthma and atopic dermatitis [14, 16, 19]. The rapid translation of evidence obtained during this period into clinical practice was the primary determinant of the publication explosion. Therefore, the production increase after 2017 is a direct reflection not only of scientific interest but also of the introduction of biologics into clinical practice and the resulting paradigm shift [11, 20].

The second key finding shows that the research field is organized around three main thematic clusters: severe asthma, atopic dermatitis, and CRSwNP. This clustering reveals that these diseases are no longer evaluated as separate pathologies. Instead, they are considered within a spectrum of "Type 2 inflammatory diseases" developing on a common immunological axis. Recent translational and clinical research has shown that cytokines such as IL-4, IL-5, and IL-13 play a central role in the shared pathogenesis of these diseases. This occurs through eosinophilic inflammation, IgE-mediated sensitization, and epithelial barrier dysfunction [1]. This biological commonality has also determined the direction of modern therapeutic approaches. Systematic reviews evaluating biologic efficacy have revealed that agents targeting Type 2 cytokines provide similar clinical benefits across different disease phenotypes [13]. In parallel, it is reported that a new paradigm is emerging. This paradigm emphasizes "remission" and "endotype-based treatment" and addresses diseases holistically through shared pathomechanisms [21]. The European. Academy of Allergy and Clinical Immunology (EAACI) and the European Forum For Research And Education in Allergy and Airway Diseases (EUFOREA) guidelines support this approach. They emphasize the need for mechanism-based integrated strategies in the diagnosis and treatment of Type 2 inflammatory diseases [11, 22]. The central placement of mechanism-focused terms such as "dupilumab," "eosinophils," "IL-4/IL-13," and "IL-5" in the keyword network is the clearest bibliometric reflection of this thematic shift.

While our bibliometric analysis highlights the dominance of downstream effectors such as "eosinophils," "IL-4," and "IL-13" in the keyword

network, the underlying immunological landscape is more complex. Current research increasingly links these distinct clinical phenotypes to a shared epithelial barrier dysfunction driven by upstream "alarmins" (TSLP, IL-33, IL-25) and innate lymphoid cells type 2 (ILC2s) [1]. These epithelial-derived cytokines act as broad orchestrators of Type 2 inflammation, bridging the gap between innate and adaptive immunity across the respiratory and cutaneous tracts [2]. The rising academic interest in these upstream mechanisms suggests that the field is moving beyond cytokine inhibition toward restoring barrier integrity and targeting early inflammatory triggers [3]. Therefore, the tight interconnection of thematic clusters in our map represents more than just a conceptual link; it mirrors a tangible evolution in clinical practice, marking a definitive shift from organ-specific symptom management toward endotype-driven therapeutic strategies targeting the root cause of the inflammatory cascade.

Our analysis also reveals remarkable patterns in geographical productivity and international collaboration. The USA traditionally stands out with large research budgets and scientific infrastructure. The bibliometric data place it in the leading position. In contrast, institutions in Europe, particularly the UK and Italy, are making an impact with high productivity and strong collaboration networks. This European bloc, where "multicenter phase 3 clinical trial" networks are concentrated, is a significant actor in research production. The "research ecosystems" formed around key researchers and institutions show that research is conducted at a systemic level. For example, in bibliometric analyses of chronic rhinosinusitis, it has been observed that publication markers concentrate not only on disease focus but also on institutional diversity and inter-institutional connections related to the "Type 2 inflammation spectrum" [12]. These findings show that institutional collaboration and co-author networks play a critical role in the field's rapid growth, not just individual publication counts [23]. Thus, the direction of research is evolving toward a "global collaboration paradigm," not just mechanism-based content.

Bibliometric network analysis allows for an assessment beyond mere publication or citation counts. It permits evaluation of the interaction intensity and structural positions of actors in knowledge production. The high inter-institutional link strength and author centrality indices in this study show that Type 2 inflammation research possesses a strong, sustainable collaboration ecosystem formed around specific opinion leaders. It has been previously shown that such high-density networks increase scientific innovation performance and accelerate research outputs [24, 25]. Indeed, multi-layered network analyses reveal that embeddedness among researchers and knowledge transfer pathways are primary dynamics directly affecting innovation and productivity. In this context, it can be said that the current field is maturing not only thematically but also at a network-structural level.

One of the most striking findings is the nature of the field's intellectual foundation. The most cited studies are all large-sample phase 3 clinical trials that present clinical efficacy and safety data, rather than basic scientific discoveries. This situation demonstrates the decisive role of the evidence-based medicine paradigm in literature's development. Studies on mepolizumab (Ortega *et al*. [14], 2014), dupilumab (Simpson *et al*. [16], 2016; Castro *et al*. [19], 2018), and benralizumab (FitzGerald *et al*. [27], 2016; Bleecker *et al*. [26], 2016) published in journals like NEJM and The Lancet not only transformed clinical practice but also determined the direction of scientific production. As a direct result, international guidelines have incorporated biologic agents into treatment algorithms. Thus, these drugs have become fundamental components of the modern "standard of care" [11, 20]. This trend clearly reveals that clinical validation is a primary driving force and that knowledge production is shaped on a translational axis.

Our time-overlay analysis shows that scientific production is shifting toward mechanism-based and personalized treatment strategies. The current literature indicates that future studies will focus on identifying predictive biomarkers, expanding long-term safety data, and integrating real-world evidence with clinical trial results [28]. Beyond biological mechanisms, future research must also address the "implementation gap," focusing on the cost-effectiveness of these high-cost therapies and strategies to improve patient access in diverse healthcare systems. This trend is supported by efforts to standardize parameters such as FeNO, serum IgE levels, and peripheral eosinophil counts as response

biomarkers. Furthermore, Bayesian network meta-analyses partially compensate for the lack of head-to-head data comparing agents like tezepelumab, mepolizumab, benralizumab, and dupilumab [29]. Additionally, dual or sequential biologic strategies are increasingly being evaluated in select refractory cases. The safety profiles of these approaches require prospective validation [30, 31]. These trends demonstrate that Type 2 inflammation research is maturing beyond pharmacological treatment to include translational biology, patient stratification, and long-term monitoring.

Strengths and Limitations

One of the main strengths of this study is that it is one of the first comprehensive bibliometric analyses to map this literature. The research process was based on the Web of Science Core Collection database, the "gold standard" in bibliometric studies. All analyses were conducted using VOSviewer software. This approach provides a strong methodological foundation for data reliability and visualization quality.

However, the study has some limitations. First, relying solely on the WoS database introduces a coverage limitation. While WoS favors high-impact international journals, it may underrepresent regional clinical studies or newer niche journals that are often indexed in Scopus or Embase. Consequently, our analysis might present a slightly conservative estimate of the total global volume, particularly regarding local clinical practices not published in top-tier journals. Furthermore, including only English-language articles limited the bibliometric reflections of literature published in other languages. Another critical limitation of the field itself, as reflected in our geographical analysis, is the disproportionate contribution of high-income countries. This dominance suggests that the current literature may not fully represent the specific phenotypes, environmental exposures, or treatment accessibility challenges found in low- and middle-income regions. Additionally, the overwhelming prevalence of industry-sponsored trials in the citation network highlights a potential bias toward pharmaceutical efficacy data over independent, real-world cost-effectiveness studies. Finally, the necessity of using only "Author Keywords" for thematic analysis, while ensuring explicit author intent, may have limited the capture of broader concepts that citation-based algorithms (e.g., KeyWords Plus) might have revealed. While these limitations do not affect the study's overall orientation, they provide a methodological roadmap for future, broader, multilingual analyses.

CONCLUSIONS

This bibliometric analysis has comprehensively mapped the literature published on biologic therapies for Type 2 inflammatory diseases from 2005 to 2025. The findings show that scientific production has increased rapidly since 2017, paralleling landmark phase 3 clinical trials. Research is thematically clustered around severe asthma, atopic dermatitis, and chronic rhinosinusitis. Knowledge production is largely guided by US and European-based institutions. The intellectual foundation is built upon high-evidence-level clinical studies published in journals such as NEJM and The Lancet.

The results reveal that Type 2 inflammation research is evolving from pure clinical efficacy toward mechanism-based and biomarker-focused personalized therapies. In the coming period, the rational use of biologics and patient stratification-based approaches will determine the research agenda. This will be driven by an increase in real-world data and network meta-analyses.

Ethics Approval and Consent to Participate

This study was approved by the Chief Physician's Office of Necmettin Erbakan University Faculty of Medicine Hospital (date: 11.11.2025). This study was conducted using a publicly available and anonymized dataset, such as the Web of Science. As the data is publicly accessible and does not contain any patient-identifying information, institutional review board (IRB) approval was not required for this study. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Data Availability

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available on

request from the corresponding author, upon reasonable request.

Authors' Contribution

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

Conflict of Interest

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

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Generative Artificial Intelligence Statement

The authors affirm that they exclusively used Artificial Intelligence tools for language editing and proofreading this manuscript. Following the AI-assisted language refinement, the authors conducted a thorough review of all suggested changes. It is hereby confirmed that these revisions did not alter the intellectual, scientific, or conceptual content, and that the original meaning of the text remains fully intact and representative of the authors' work.

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Febrile Seizures: Clinical Factors Influencing the Length of Hospital Stay

Yusuf Deniz[1]

[1]*Department of Pediatrics, Karabük University Faculty of Medicine, Karabük, Türkiye*

ABSTRACT

Objectives: This study aimed to identify the clinical factors influencing the length of hospital stay (LOS) in children aged 0–60 months hospitalized with febrile seizures (FS), and to explore potential immunological and psychosocial modulators that may affect hospitalization decisions.

Methods: A retrospective observational study was conducted at a tertiary hospital in Karabük, Türkiye, between September 2023 and September 2025. Medical records of patients diagnosed with FS (ICD-10 R56.0) were reviewed using the FONET and e-Nabız systems. Demographic and clinical variables - including age, sex, fever, seizure duration, type (simple/complex), recurrence, infection focus, and presence of seasonal allergic rhinitis (SAR) - were analyzed. Factors associated with LOS were evaluated using multivariable linear regression ($P<0.05$).

Results: A total of 93 children (53.3% male, mean age: 24.6±13.7 months) were included. The mean LOS was 48.2±44.7 hours. Regression analysis showed that recurrence and SAR were associated with shorter LOS ($P=0.062$ and $P=0.065$, respectively), while seizure duration and prematurity had no significant effect. Bronchitis, otitis, and tonsillitis were linked to longer stays, whereas acute gastroenteritis and mild upper respiratory infections were linked to shorter ones. Family history was strongly associated with recurrence but not with LOS.

Conclusions: Hospital stay in FS is influenced more by immunological and psychosocial dynamics than by traditional demographic factors. The presence of SAR may act as a modulatory condition reducing LOS, possibly through pre-activated immune regulation. Family experience appears to facilitate earlier discharge. Integrating these factors into clinical decision-making may improve resource use and standardize hospitalization criteria.

Keywords: Febrile Seizures, Length of Hospital Stay, Seasonal Allergic Rhinitis, Immune Modulation

Febrile seizure (FS) is defined as a convulsion accompanied by fever in the absence of central nervous system infection, metabolic disorder, or a previous history of afebrile seizures [1]. It represents one of the most common true pediatric emergencies encountered by general pediatricians in daily practice [1–4].

Since its first description, the long-term neurodevelopmental outcomes and risk of subsequent epilepsy have been among the most widely studied

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Corresponding author: Yusuf Deniz, MD., Assist. Prof.; Phone: +90 370 444 04 78, E-mail: ydeniz123@hotmail.com

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aspects of FS [3, 4]. Pioneering population-based studies in the 1990s, particularly those by Berg *et al.* [1], marked a turning point in understanding the natural history of febrile seizures [1–4]. These studies showed that most FS cases are benign, but seizures lasting longer than 15 minutes, those with focal features, or recurrent attacks carry a higher risk [3, 4]. This distinction between "simple" and "complex" febrile seizures has since formed the foundation of clinical and epidemiological research over the past three decades.

Modern clinical guidelines - including those from the American Academy of Pediatrics (AAP), the American Academy of Family Physicians (AAFP), and the Italian Society of Pediatrics - have built upon this framework to standardize management practices [5–12]. Current evidence indicates that approximately 70% of FS are simple, and that advanced imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI), as well as urgent electroencephalography (EEG), are unnecessary in most cases [5, 7, 9, 11]. Younger age, higher fever, and a positive family history are recognized as risk factors for a first episode, while antipyretic or antiepileptic prophylaxis has no preventive benefit [6, 8, 10, 12].

In recent years, the research focus has shifted from long-term epilepsy risk to short-term clinical management and efficient resource utilization. Recent analyses show that hospitalization criteria remain broad and are often based on physician experience [7, 9, 11]. Particularly in the 2020s, multivariate studies have revealed that factors influencing hospital stay in FS are still not systematically evaluated [13–16]. Variables such as seizure duration >5 minutes, complex features, high fever, and specific infection foci - especially pneumonia and urinary tract infections - are associated with longer hospital stays, yet these factors remain insufficiently standardized in clinical decision-making [13–16].

These findings suggest a paradigm shift in the management of FS: the question has moved from "Will this child develop epilepsy?" to "Which patient truly requires hospitalization, and for how long?"
The present study aimed to identify the clinical determinants of hospital stay duration in children aged 0–60 months after febrile seizures using multivariable analysis. Defining these factors is crucial for optimizing hospital resource use and reducing unnecessary admissions.

METHODS

Study Location and Period

This retrospective observational study was conducted in the Department of Pediatrics at a tertiary care hospital in Karabük, Türkiye. The study covered a two-year period between September 1, 2023, and September 1, 2025. Data were obtained from the hospital's electronic medical record system (FONET).

Identification of Participants and Study Groups

Children aged 0–60 months (28 days–5 years) diagnosed with febrile seizure (ICD-10 code R56.0) were included. The total number of cases was determined by retrospective record screening. All patients were categorized according to their length of hospital stay (LOS):

•*Group 1 (Short stay):* LOS ≤ 2 days
•*Group 2 (Long stay):* LOS > 2 days

Data Collection and Validation

All data were collected retrospectively from electronic archives. Data accuracy was verified by checking for duplicate or incomplete entries entered by different users. Laboratory parameters were not analyzed; only clinical and demographic variables were evaluated. The collected variables included:

•Age (months)
•Sex
•Presence of chronic illness
•Family history of febrile seizure
•Body temperature (°C)
•Seizure duration (minutes)
•Seizure type (simple / complex)
•Seizure pattern (first / recurrent)
•Primary infection focus (upper respiratory tract infection, tonsillitis, otitis, pneumonia, urinary tract infection, bronchitis, acute gastroenteritis, etc.)
•Length of hospital stay (days)

Variable Definitions / Clinical Parameters

•*Simple Febrile Seizure:* generalized, lasting <15

minutes, and occurring only once within 24 hours.

•*Complex Febrile Seizure:* lasting ≥15 minutes, with focal features or >1 episode within 24 hours.

•*LOS:* LOS > 2 days.

•*Primary Infection Focus:* determined according to the physician's diagnosis based on clinical and laboratory findings.

Inclusion Criteria

•Age 0–60 months

•Diagnosis of febrile seizure (ICD-10 R56.0)

•Available data on fever, seizure duration, and hospital stay

Exclusion Criteria

•Central nervous system infections (meningitis, encephalitis)

•Afebrile or metabolic seizures

•Incomplete or erroneous medical records

•History of chronic neurological or metabolic disease

Acute or Chronic Disease Control

Patients with missing clinical data were excluded to ensure data transparency. The national e-Nabız health information system (https://enabiz.gov.tr/) was used to identify and exclude children with chronic diseases. Nutritional deficiencies were not classified as chronic illnesses.

Statistical Analysis

All analyses were performed using SPSS for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA). The distribution of continuous variables was assessed using skewness and kurtosis. Age and LOS were normally distributed. Descriptive statistics were presented as mean ± standard deviation (SD) for continuous variables and as frequency (n) and percentage (%) for categorical variables. Categorical comparisons (e.g., seizure type, recurrence, family history, sex) were tested using the chi-square (χ^2) test. Continuous variables across two groups (e.g., age, LOS) were compared with the independent-samples t-test. Independent predictors of hospital stay were identified through multivariable linear regression analysis. Statistical significance was set at $P<0.05$.

RESULTS

A total of 93 children were included in the study, of whom 53.3% (n=50) were male and 46.7% (n=43) were female. The mean age was 24.6±13.7 months. The mean body temperature during febrile seizures was 38.6±0.7°C, the mean seizure duration was 3.8±2.6 minutes, and the mean LOS was 48.2±44.7 hours. Simple febrile seizures accounted for the majority of cases (94.4%, n=88), while 5.6% (n=5) were classified as complex. First-time febrile seizures were observed in 54.8% (n=51) of children, whereas 45.2% (n=42) had recurrent episodes.

The most common primary infection focus was upper respiratory tract infection (49.5%, n=46), followed by tonsillitis (15.1%, n=14), while 16.1% (n=15) of children had normal physical examination

TABLE 1. Clinical Factors Influencing the Length of Hospital Stay (Explained by Linear Regression Analysis)

Variable	B	SE	95% CI	β	t	P-value
(Intercept)	65.71	10.66	[44.04, 87.37]	0.00	6.16	**<0.001**
FS episode = Recurrent	-21.31	11.04	[-43.76, 1.14]	-0.31	-1.93	0.062
Prematurity	-18.86	13.15	[-45.59, 7.87]	-0.22	-1.43	0.161
FS duration	-2.48	2.06	[-6.67, 1.70]	-0.19	-1.21	0.236
SAR	-29.35	15.38	[-60.61, 1.91]	-0.29	-1.91	0.065

SE, standard error; CI, confidence interval; SAR, seasonal allergic rhinitis, FS, febrile seizures.
$F(5.34) = 3.04$, $P= 0.023$, $R^2 =0.31$; Reference Categories: FS episode = First, Prematurity = Term, Chronic disease = None.
Statistically significant P-value is shown in bold.

findings at presentation. Most children were born at term (87.1%, n=81), whereas 12.9% (n=12) were preterm. Seasonal allergic rhinitis (SAR) was the only chronic condition identified and was present in 19.4% (n=18) of patients. A positive family history of febrile seizures was noted in 12.9% (n=12) of first-degree relatives and 5.4% (n=5) of second-degree relatives.

No significant correlations were observed between LOS and age ($r = -0.054$, $P=0.60$), body temperature ($r = -0.079$, $P=0.65$), or seizure duration ($r = -0.216$, $P=0.18$). LOS did not differ significantly according to sex, prematurity, or family history ($P=0.161$). However, the primary infection focus showed borderline significance ($P=0.06$). The longest hospital stays were observed in cases with bronchitis and combined otitis-tonsillitis, whereas the shortest stays occurred in children with acute gastroenteritis or normal physical examination findings (Table 1).

Multivariable linear regression analysis demonstrated that seizure recurrence and the presence of SAR were associated with a trend toward shorter hospital stays ($P=0.062$ and $P=0.065$, respectively), while prematurity and seizure duration had no significant effect on LOS. The regression model was statistically significant and explained approximately 31% of the variance in hospital stay duration (Table 1).

DISCUSSION

In our study, demographic variables such as age, sex, and prematurity were not significantly associated with the length of hospital stay after febrile FS. This finding is consistent with the pioneering works of Berg *et al*. [1, 2, 4] and Shinnar *et al*. [3] and the multicenter analysis by Kannikeswaran *et al*. [13]. In the literature, these variables are more often linked to seizure recurrence or the later development of epilepsy rather than to LOS [3, 6].

The most noteworthy finding of this study was the trend toward shorter LOS in children with an atopic constitution, particularly those with seasonal SAR. The relationship between atopy and FS has been only marginally explored in previous studies [17, 18]. Our results suggest that an atopic predisposition might play a modulatory role in the pathophysiology of FS. In children with SAR, immune elements that remain "primed for SAR" may become activated earlier and suppressed more quickly during a febrile event, leading to a more stable clinical course. A similar immunological mechanism has been observed in atypical hemolytic uremic syndrome (aHUS), where treatment with fresh frozen plasma (FFP) remains effective despite high serum complement C5 levels [19, 20].

In summary, immune elements that have not undergone a specific "set-up" process for a certain condition may, in some circumstances, promote a more stable immune response. Thus, immune mediators initially "prepared" for SAR but not "set up" for FS could help moderate the inflammatory response during seizures, possibly explaining the shorter LOS observed in these patients. The well-known association of the proinflammatory cytokine IL-1β with both SAR and FS supports the idea of a potential mechanistic link between these conditions [21, 22]. To our knowledge, this hypothesis has not been previously discussed in the literature and may provide a basis for future research exploring neuroimmune modulation in febrile seizures. Atopic conditions such as SAR may, therefore, be viewed not as aggravating factors but as modulatory processes in febrile seizure dynamics.

Another distinctive finding of our study was the shorter hospital stay observed in recurrent FS cases compared with non-recurrent ones. Previous studies have suggested that complex or frequently recurrent seizures prolong hospitalization [13, 16]; however, our results indicate the opposite trend. This may be explained by parental experience - families of children with recurrent FS are often more familiar with the benign nature of these events, which may reduce anxiety and support earlier discharge decisions. Similarly, physicians may act with greater confidence when not influenced by social or emotional pressure [14, 15]. This interpretation aligns with the "social indications" concept described by Green and MacFaul in 1985 [15]. Nevertheless, this observation should not be interpreted as evidence of "biological stability" in recurrent FS.

We also found that infections such as bronchitis, otitis, and tonsillitis were associated with longer hospital stays, whereas cases of acute gastroenteritis (AGE) and mild upper respiratory infections were discharged earlier. This suggests that the course of FS is influenced by the severity and nature of the infection

focus, consistent with the findings of Padonou *et al*. [7] and Ferretti *et al*. [8].

Finally, a positive first- or second-degree family history was strongly associated with the recurrence of FS, consistent with the classical cohorts of Berg *et al*. [1, 2, 4] and Shinnar *et al*. [3] and the national study by Dreier *et al*. [16]. However, this familial predisposition did not correlate with hospital stay duration - an important nuance suggesting that genetic susceptibility may lower seizure threshold but does not necessarily affect clinical severity. This distinction has not been adequately emphasized in previous studies.

Strengths and Limitations

This study has some important strengths. The analysis focuses on factors affecting length of hospital stay, which is a clinically relevant outcome for both physicians and healthcare systems. In addition, variables such as seizure characteristics and recurrence were evaluated together, allowing a more comprehensive assessment. The use of multivariable analysis also helped reduce the effect of confounding factors.

Due to its retrospective nature, this study allows for associative rather than causal interpretations. The findings reflect the patient profile of a tertiary care center in Karabük, and results may vary across different populations or clinical settings. Although this limits the generalizability of the findings, internal validity was maintained through the use of a homogeneous electronic medical record system.

Only clinical and demographic parameters were analyzed, while laboratory and biochemical markers (e.g., CRP, IL-1β, electrolytes) were excluded. This decision was made to minimize data heterogeneity inherent to retrospective studies and to focus on clinical predictors. However, this approach inevitably limits the direct interpretation of immunological mechanisms, leaving such inferences indirect.

What's Known on the Subject

Febrile seizures are one of the most common true pediatric emergencies encountered by general pediatricians in daily practice. Previous studies have mainly focused on recurrence and long-term epilepsy risk, while factors influencing hospitalization duration remain insufficiently explored.

What This Study Adds

This study identifies clinical and immunological factors associated with the length of hospital stay in children with febrile seizures. It highlights the potential modulatory role of atopy, particularly seasonal allergic rhinitis, and emphasizes the influence of family experience on hospitalization decisions. These findings offer a new perspective for optimizing clinical management and resource utilization.

CONCLUSIONS

This study suggests that the length of hospital stay in febrile seizures cannot be fully explained by demographic or conventional clinical variables alone. Instead, it appears to be influenced by a dynamic interaction among immunological, psychosocial, and clinical factors. The following conclusions and recommendations can be drawn:

•Inclusion of atopic status in clinical assessment

The finding that atopic conditions such as seasonal allergic rhinitis (SAR) are associated with shorter hospital stays indicates that atopy may act not as a comorbidity but as a modulatory marker. Therefore, considering atopic constitution in febrile seizure risk classification may improve clinical evaluation.

•Integrating family experience into clinical management

The shorter hospital stays observed in recurrent cases highlight the role of family experience and effective physician–family communication. Structured educational programs for families after the first seizure may help reduce anxiety-driven hospitalizations.

•Exploring the immunological dimension of febrile seizures

The hypothesis that "pre-activated" immune components in atopic backgrounds such as SAR may play a regulatory role in the pathophysiology of febrile seizures introduces a new paradigm. Joint evaluation of cytokines like IL-1β, which are associated with both atopy and febrile seizures, could further elucidate neuroimmune interactions.

Ethics Approval and Consent to Participate

This study was approved by the Karabük University Non-Interventional Clinical Research Ethics Committee (Decision No: 2025/2505; date: 02.10.2025). All procedures were conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Because the study was retrospective and no additional intervention was performed on the participants, the informed consent form was waived.

Data Availability

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

Authors' Contribution

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

Conflict of Interest

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The authors declare that no generative artificial intelligence tools were used for data analysis, interpretation of results, or generation of scientific content in this manuscript. All scientific content was produced by the authors in accordance with accepted scientific research methods and academic ethical principles. Artificial intelligence–based tools were used only for limited language editing and grammatical corrections in some sections of the text.

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Three Decades of SSRI/SNRI Withdrawal Research: A Bibliometric and Science-Mapping Study

Erdoğdu Akça[1]

[1]*Department of Psychiatry, School of Medicine, Marmara University, İstanbul, Türkiye*

ABSTRACT

Objectives: Selective serotonin reuptake inhibitors (SSRIs) and serotonin–norepinephrine reuptake inhibitors (SNRIs) are widely prescribed, and growing evidence highlights the clinical and methodological challenges of withdrawal during discontinuation. This study aimed to provide a comprehensive bibliometric map of SSRI/SNRI withdrawal research to clarify production patterns, citation structures, thematic evolution, and global collaboration.

Methods: A comprehensive search of the Web of Science Core Collection (1995–2025) identified 652 original articles on SSRI/SNRI withdrawal, discontinuation, or tapering. Records were analyzed using the bibliometrix package in R for performance metrics, co-citation and keyword networks, and country–institution collaboration structures.

Results: The corpus comprised 652 documents published in 308 journals, citing 18,957 references, with an annual growth rate of 7.79%. Articles received a mean of 41.6 citations. The Journal of Clinical Psychiatry (22 articles; h=20) and Journal of Clinical Psychopharmacology (18; h=14) were the most influential sources. Highly cited documents ranged from 270 to 730 citations, predominantly from Lancet, Pediatrics, The New England Journal of Medicine, and American Journal of Psychiatry. Keyword frequency was led by "depression" (n=172), "fluoxetine" (n=153), and "antidepressants" (n=152). The United States produced 240 articles (36.8%; 12,904 citations), forming the central hub of the collaboration network.

Conclusions: SSRI/SNRI withdrawal research shows concentrated authorship, journal anchoring, and geographically centralized knowledge production, with emerging themes in tapering, pregnancy outcomes, and pharmacogenetics.

Keywords: Selective Serotonin Reuptake Inhibitor (SSRI), Serotonin-Norepinephrine Reuptake Inhibitor (SNRI), Withdrawal, Discontinuation, Tapering, Bibliometric

Selective serotonin reuptake inhibitors (SSRIs) and serotonin–norepinephrine reuptake inhibitors (SNRIs) have become the central pharmacologic options for depressive and anxiety disorders, yet discontinuation frequently elicits symptoms whose prevalence, severity, and duration vary across studies [1–4]. This variability makes it difficult to distinguish withdrawal phenomena from true relapse, and the distinction directly influences clinical choices about reinstating medication, slowing the taper, or introducing alternative treatments [5, 6]. Reviews describe common symptom profiles such as

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Corresponding author: Erdoğdu Akça, MD., Assist. Prof., Phone: +90 216 627 62 72, E-mail: erdogduakca@gmail.com

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dizziness, sensory disturbances, insomnia, irritability, and anxiety, but they also underline inconsistent timing and trajectories, which in turn limit the field's capacity to provide clear guidance for practice [6, 7]. Diagnostic uncertainty during discontinuation may prolong antidepressant exposure and discourage deprescribing. Clarifying how the literature has evolved, which methodological conventions framed reporting, and which topics gained or lost prominence is therefore important. A structured mapping of the SSRI/SNRI withdrawal evidence base serves a dual purpose: it supports more reliable clinical decisions at the point of tapering, and it frames a research agenda that targets the mechanisms, risks, and outcomes most relevant to patients and clinicians.

The research landscape on SSRI and SNRI withdrawal has expanded unevenly over three decades, lacking a coherent map of its intellectual structure. A bibliometric analysis is therefore warranted to map how this literature has grown, which journals serve as core publication venues, and where citations concentrate, since narrative reviews cannot easily quantify these structural features. Science-mapping lets us identify core topics and their evolution, reveal the journals and authors that drive agenda-setting, and locate foundational references, while performance indicators benchmark productivity and impact. Concretely, this approach can answer which journals constitute the clinical "core," how themes such as discontinuation and tapering have shifted over time, which countries and institutions act as collaboration hubs, and which papers form the field's citation network [8]. Established methods were used and tools were for co-citation and co-occurrence mapping and for network centrality assessment, following best-practice guidance in bibliometric science-mapping [9–11].

Although early narrative discussions of SSRI and SNRI withdrawal existed, methodologically rigorous syntheses did not gain momentum until the 2010s, leaving a prolonged period during which evidence remained fragmented and difficult to integrate [1, 2, 7, 12]. This fragmentation limited the field's ability to generate consistent guidance on discontinuation and tapering, particularly in routine clinical practice where individualized risk assessment is required [13]. In parallel, several qualitative and survey-based studies have reported patient-reported withdrawal experiences that differ in duration and severity from those emphasized in early clinical trial reports and guideline summaries [7, 13–15]. This discrepancy between guideline-oriented assumptions and reported patient outcomes contributed to ongoing clinical uncertainty around deprescribing, including challenges in distinguishing withdrawal phenomena from relapse and in determining appropriate tapering strategies. Together, these developments highlight the need to address how SSRI/SNRI withdrawal research has evolved, which methodological and conceptual traditions have influenced reporting practices, and where gaps persist within the indexed literature.

Clarifying how the SSRI/SNRI withdrawal literature is organized is clinically salient because evidence gaps shape everyday prescribing and deprescribing. The persistent challenge is to distinguish withdrawal from relapse, a decision that alters whether clinicians reinstate treatment, slow the taper, or consider adjunctive supports. By outlining where findings converge and where they diverge, a field-level map can guide the use of hyperbolic tapering principles, improve shared decision-making, and highlight areas that warrant pragmatic, real-world trials.

The aim of this study is to provide a comprehensive bibliometric map of the SSRI/SNRI withdrawal literature by integrating production trends, citation trajectories, co-citation structures, thematic evolution, and cross-national collaboration patterns. Using the original articles indexed in the Web of Science Core Collection, this study examines which journals and authors anchor the field, how conceptual clusters have shifted over time, and how countries and institutions form the underlying collaboration network. The study addresses four central questions: How has the field grown? Which works constitute its intellectual backbone? How have themes evolved? And which regions drive or remain peripheral to knowledge production?

METHODS

Data Source and Search Strategy

The choice of bibliographic database is a recognized methodological determinant in bibliometric research. In this study, the Web of Science Core Collection (WoSCC) was selected because of its

standardized indexing practices, long citation history, and strong suitability for citation-based network analyses [16]. Prior methodological work has shown that WoSCC provides more stable and comparable citation links for co-citation and collaboration network mapping, whereas broader databases may increase coverage at the expense of structural consistency. Nevertheless, reliance on a single database may underrepresent publications from journals or regions not indexed in WoSCC, particularly those from emerging research economies or non-English-language outlets [17]. Accordingly, the observed productivity, collaboration patterns, and citation structures should be interpreted as reflecting the WoS-indexed core of the SSRI/SNRI withdrawal literature rather than the entire global research output. To identify studies specifically addressing SSRI/SNRI withdrawal, discontinuation, and tapering, it was applied a structured Topic Search. Because the terms "withdrawal," "discontinuation," and "tapering" are widely used across pharmacologically distinct drug classes, exclusion filters were applied to remove non-antidepressant withdrawal literatures (e.g., benzodiazepines, opioids, antipsychotics, corticosteroids). This step was guided by pilot searches showing that inclusion of these domains substantially altered thematic and citation network structures without contributing conceptually to SSRI/SNRI withdrawal research. Exclusion terms were applied at the level of drug class rather than clinical outcomes, and the full search syntax is reported to ensure reproducibility. The search was executed on September 23, 2025, and the final search syntax used in WoSCC was as follows:

TS=(((SSRI OR "selective serotonin reuptake inhibitor") AND (withdrawal OR discontinuation OR

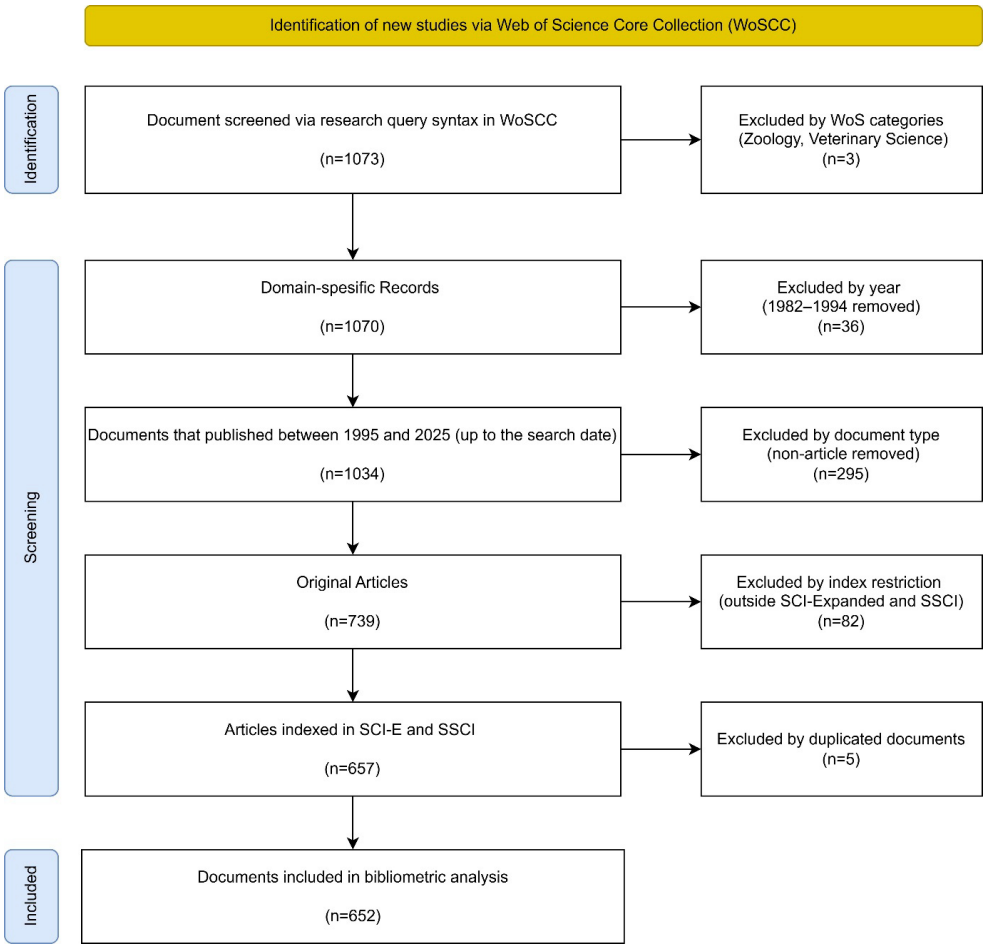


FIGURE 1. PRISMA-style flow diagram illustrating the identification, screening, eligibility, and inclusion of SSRI/SNRI withdrawal studies from the Web of Science Core Collection (1995–2025).

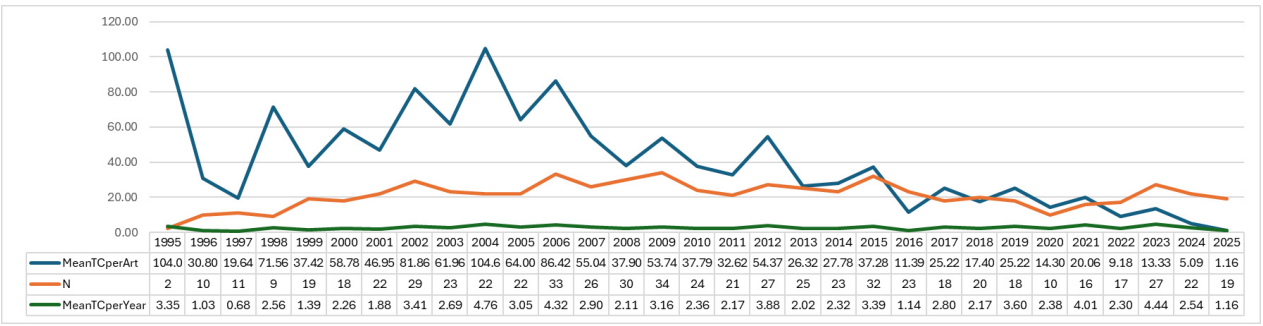
taper OR "dose reduction" OR "hyperbolic taper")) OR ((SNRI OR "serotonin norepinephrine reuptake inhibitor") AND (withdrawal OR discontinuation OR taper OR "dose reduction" OR "hyperbolic taper")) OR ("discontinuation syndrome" AND (SSRI OR SNRI OR "selective serotonin reuptake inhibitor" OR "serotonin norepinephrine reuptake inhibitor"))) NOT TS=("benzodiazepine withdrawal" OR "benzodiazepine discontinuation" OR "benzodiazepine taper*" OR "opioid withdrawal" OR "opioid discontinuation" OR "opioid taper*" OR "morphine withdrawal" OR "methadone withdrawal" OR "buprenorphine withdrawal" OR "antipsychotic withdrawal" OR "antipsychotic discontinuation" OR "antipsychotic taper*" OR "pregabalin withdrawal" OR "gabapentin withdrawal" OR "propranolol withdrawal" OR "propranolol discontinuation" OR "propranolol taper*" OR "propranolol dose reduction" OR "stimulant withdrawal" OR "amphetamine withdrawal" OR "methylphenidate withdrawal" OR "antiepileptic withdrawal" OR "anticonvulsant withdrawal" OR "psilocybin withdrawal" OR "ketamine withdrawal" OR "LSD withdrawal" OR "corticosteroid withdrawal" OR "steroid withdrawal" OR "cannabis withdrawal" OR "cannabinoid withdrawal" OR "TCA withdrawal" OR "tricyclic antidepressant withdrawal" OR "MAOI withdrawal" OR "monoamine oxidase inhibitor withdrawal")

The initial search returned 1,073 records. Refinements were then applied to restrict the dataset to the years 1995–2025 (search date: September 23, 2025), include only original research articles, and limit indexing to SCI-Expanded and SSCI. Records assigned to the Zoology or Veterinary Science categories were excluded. After deduplication, the final analytic dataset consisted of 652 documents (Figure 1).

This study used publicly available bibliographic data and involved no human participants or identifiable information; therefore, ethical approval was not required.

Statistical Analysis

All records were exported in BibTeX format and analyzed with the bibliometrix package in R (version 4.4.2) for performance and science-mapping analyses [18]. Author Keywords (DE) and Keywords Plus (ID; algorithmically generated from the titles of cited references to capture broader, latent themes) were examined. Standard bibliometric indices were calculated: h-index (largest h such that h papers have ≥ h citations), g-index (emphasizing highly cited work; top g papers receive ≥ g^2 citations), and m-index (time-normalized impact: h-index divided by years since first publication). At the document level, computed total citations (TC), citations per year (TCpY), local citations within the corpus (LC), and global citations across the database (GC) were computed. Network analyses included co-citation, keyword co-occurrence, and co-authorship; node importance was quantified with betweenness centrality (bridging role), closeness centrality (proximity to all nodes), and PageRank (relative structural influence). Visualizations were generated with "biblioshiny", using normalized association strengths and modularity-based clustering to identify substructures in the SSRI/SNRI withdrawal literature.



	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025
MeanTCperArt	104.0	30.80	19.64	71.56	37.42	58.78	46.95	81.86	61.96	104.6	64.00	86.42	55.04	37.90	53.74	37.79	32.62	54.37	26.32	27.78	37.28	11.39	25.22	17.40	25.22	14.30	20.06	9.18	13.33	5.09	1.16
N	2	10	11	9	19	18	22	29	23	22	22	33	26	30	34	24	21	27	25	23	32	23	18	20	18	10	16	17	27	22	19
MeanTCperYear	3.35	1.03	0.68	2.56	1.39	2.26	1.88	3.41	2.69	4.76	3.05	4.32	2.90	2.11	3.16	2.36	2.17	3.88	2.02	2.32	3.39	1.14	2.80	2.17	3.60	2.38	4.01	2.30	4.44	2.54	1.16

FIGURE 2. Annual publication counts (N), mean total citations per article (MeanTCperArt), and mean citations per year (MeanTCperYear) for SSRI/SNRI withdrawal studies (1995–2025).

RESULTS

Descriptive Overview of the Dataset

The dataset comprised 652 original articles on SSRI/SNRI withdrawal published between 1995 and 2025 in 308 sources. The annual growth was 7.79%, with a mean article age of 14.3 years and 41.59 citations per paper, based on 18,957 cited references. 1,735 Keywords Plus and 1,358 author keywords were identified. In total, 3,405 authors contributed; 37 papers were single-authored, and articles had on average 5.79 co-authors. International coauthorship accounted for 19.48% (n=127) of publications.

Annual Scientific Production and Citation Trends

Annual publication output increased from 1995 into the late 2000s, rising from 2 papers in 1995 to a peak of 34 in 2009, then stabilized between 16 and 32 papers per year from 2010 to 2025. The 2020 decline likely reflects pandemic-related disruptions, with output recovering to 27 papers in 2023. Citation trends show that early papers received substantially more citations, with peaks in 1995 with 104, 2004 with 104.64, and 2006 with 86.42, reflecting longer citation windows and foundational impact. Recent years show lower averages due to limited time for citation accrual. Mean citation per year (TC/year) also peaked in 2004, 2006, 2021, and 2023, indicating that several publications remained influential over time. Overall, the field expanded rapidly in the 2000s and has remained stable and impactful in the past decade. Figure 2 provides detailed annual patterns and citation metrics.

Source Impact and Journal-Level Contribution

Analysis of source distribution showed that a small group of journals dominates this literature. The

TABLE 1. Top 15 Journals Ranked by Number of Publications, Showing Publication Output, Citation Impact, and Publication Timeline

Source	NP	TC	h index	g index	m index	PY (start)
1. JOURNAL OF CLINICAL PSYCHIATRY	22	1999	20	22	0.741	1999
2. INTERNATIONAL CLINICAL PSYCHOPHARMACOLOGY	17	970	14	17	0.467	1996
3. JOURNAL OF CLINICAL PSYCHOPHARMACOLOGY	18	886	14	18	0.538	2000
4. JOURNAL OF AFFECTIVE DISORDERS	16	551	12	16	0.4	1996
5. JOURNAL OF PSYCHOPHARMACOLOGY	13	396	11	13	0.407	1999
6. NEUROPSYCHOPHARMACOLOGY	12	872	11	12	0.458	2002
7. EUROPEAN NEUROPSYCHOPHARMACOLOGY	9	219	8	9	0.421	2007
8. JOURNAL OF THE AMERICAN ACADEMY OF CHILD AND ADOLESCENT PSYCHIATRY	8	1001	8	8	0.296	1999
9. ANNALS OF PHARMACOTHERAPY	8	255	7	8	0.28	2001
10. ENCEPHALE-REVUE DE PSYCHIATRIE CLINIQUE BIOLOGIQUE ET THERAPEUTIQUE	12	202	7	12	0.233	1996
11. JOURNAL OF SEXUAL MEDICINE	8	319	7	8	0.389	2008
12. PSYCHOPHARMACOLOGY	9	308	7	9	0.241	1997
13. THERAPEUTIC DRUG MONITORING	7	285	7	7	0.259	1999
14. BRITISH JOURNAL OF CLINICAL PHARMACOLOGY	6	348	6	6	0.2	1996
15. CNS DRUGS	6	300	6	6	0.222	1999

NP, number of publication; TC, total citations; PY (start), year of first publication.

ten most productive sources with total publication count were Journal of Clinical Psychiatry (n=22), Journal of Clinical Psychopharmacology (n=18), International Clinical Psychopharmacology (n=17), Journal of Affective Disorders (n=16), Journal of Psychopharmacology (n=13), Encephale (n=12), Neuropsychopharmacology (n=12), European Neuropsychopharmacology (n=9), Psychopharmacology (n=9), and Annals of Pharmacotherapy (n=8), together forming the core dissemination channels.

Impact indicators reinforced this pattern. Journal of Clinical Psychiatry had the highest h-index (h=20), followed by International Clinical Psychopharmacology and Journal of Clinical Psychopharmacology (each h=14), with total citations between 779 and 970. Their long publication spans in this area (from the late 1990s and early 2000s onward) likely contribute to their strong cumulative influence. More detailed trajectories and values for these outlets, along with the broader journal set, are presented in the source-by-year visualizations (Table 1 and Figure 3).

Author Productivity and Impact

Most Prolific Contributors

Based on full counting, the most productive authors were Fava M (14 articles), Papakostas GI (9 articles), Strawn JR (8 articles), Baldwin DS (6 articles), Bose A (5 articles), Clayton AH (5 articles), Emslie GJ (5 articles), and four-article contributors are Collins HM, Cosci F, and Den Boer JA. Fractionalized productivity largely preserves this hierarchy while down-weighting large multi-author papers (e.g., Fava M=3.39, Papakostas GI=2.74, Strawn JR=1.66, Baldwin DS=3.00, Devane CL=1.39, Montgomery SA=1.58). Detailed author frequencies and fractional counts are shown in Table 2.

Citation Influence and Temporal Bursts

Impact metrics highlight several prominent authors: Fava M (h=13; TC=884; NP=14), Papakostas GI (h=9; TC=667; NP=9), Strawn JR (h=7; TC=425; NP=8), Baldwin DS (h=5; TC=113; NP=6), Bose A (h=5; TC=419; NP=5), Clayton AH (h=5; TC=271; NP=5), Emslie GJ (h=5; TC=689; NP=5), Collins HM (h=3; TC=27; NP=4), Cosci F (h=4; TC=48; NP=4), and Den Boer JA (h=4; TC=107; NP=4). Citation "bursts" appear for Emslie GJ (2002; TC=419), Bose A (2002; TC=287), Fava M (2007–2008; TC=256–273), Papakostas GI (2007–2008; TC=256–301), Clayton AH (2014–2015; TC=83–79), and Strawn JR (2015; TC=191; 2023; TC=97). Within the corpus, the most locally cited works cluster around Bate A, De-

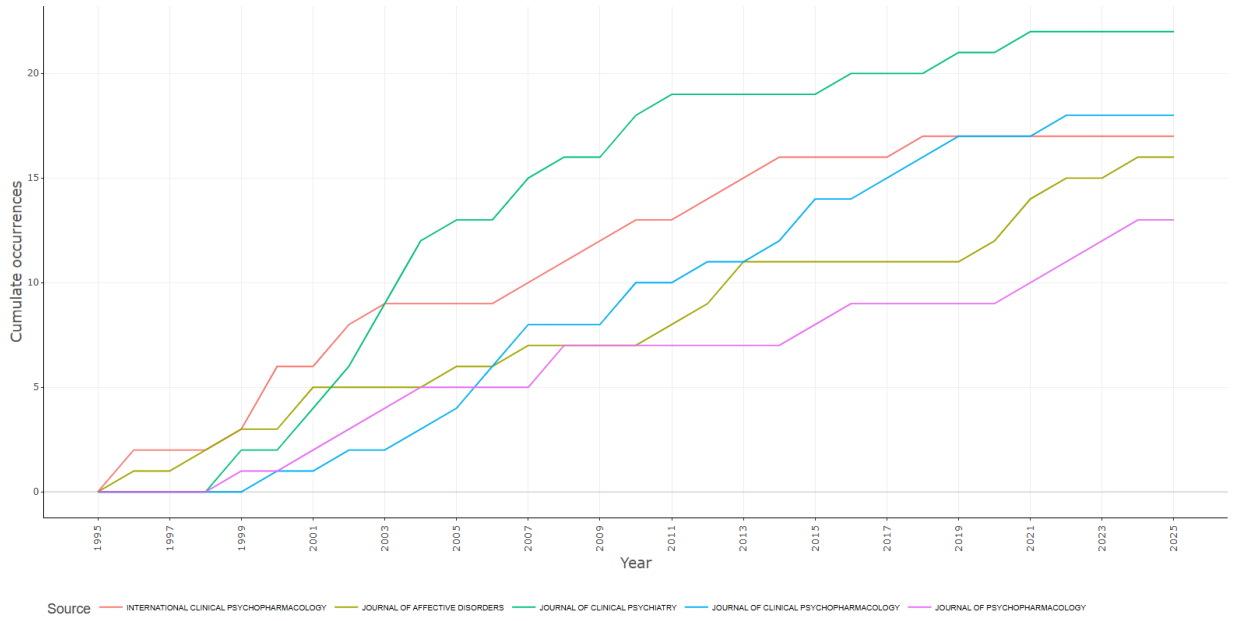


FIGURE 3. ACumulative publication counts of the five most productive journals in SSRI/SNRI withdrawal research (1995–2025).

TABLE 2. Top 15 Contributing Authors Ranked by Number of Publication, Showing Publication Output, Citation Impact, Fractional Contribution, and Career Timeline

Author	NP	TC	Articles fractionalized	h index	g index	m index	PY (start)
1. FAVA M	14	884	3.39	13	14	0.5	2000
2. PAPAKOSTAS GI	9	667	2.74	9	9	0.39	2003
3. STRAWN JR	8	425	1.66	7	8	0.58	2014
4. BALDWIN DS	6	113	3.00	5	6	0.20	2001
5. BOSE A	5	419	1.39	5	5	0.21	2002
6. CLAYTON AH	5	271	1.18	5	5	0.20	2001
7. EMSLIE GJ	5	689	0.87	5	5	0.21	2002
8. COSCI F	4	48	0.81	4	4	0.17	2003
9. DEN BOER JA	4	107	0.45	4	4	0.22	2008
10. MICHELSON D	4	214	0.47	4	4	0.15	2000
11. RUSH AJ	4	143	0.60	4	4	0.17	2002
12. THASE ME	4	395	0.93	4	4	0.22	2008
13. AMSTERDAM J	3	206	0.37	3	3	0.11	2000
14. CHOUINARD VA	3	31	0.65	3	3	0.37	2018
15. CIOFFI L	3	29	0.45	3	3	0.60	2021

NP, number of publication; TC, total citations; PY (start), year of first publication.

las-Cuevas C, Edwards R, Kiuru A, and Sanz EJ (LocalCitations=25). Full temporal and local-citation profiles appear in Figure 4 (author–year panels) and Table 2.

Highly Cited Documents, Foundational References, and Words

Top-Cited Documents And Within-Corpus Influence

The ten most globally cited documents were: Whittington CJ, 2004, Lancet (TC=730), Hudak ML, 2012, Pediatrics (634), Chambers CD, 2006, NEJM (584), Emslie GJ, 2002, J Am Acad Child Adolesc Psychiatry (419), Yankelevitch-Yahav R, 2015, J Vis Exp (410), Olfson M, 2006, Am J Psychiatry (332), Bull SA, 2002, JAMA (296), Parsey RV, 2006, Biol Psychiatry (285), Burke WJ, 2002, J Clin Psychiatry (282), and Goldstein DJ, 2004, J Clin Psychopharmacol (270). Within-corpus impact (local citations) similarly concentrates on a small set: Sanz EJ, 2005, Lancet (LC=25), Nordeng H, 2001, Acta Paediatr (23), Bull SA, 2002, JAMA (16), Zeskind PS, 2004, Pediatrics (9), Olfson M, 2006, Am J Psychiatry (8), followed by Fabre LF, 1995, Biol Psychiatry (7), Olver JS, 1999, CNS Drugs (7), Simon GE, 2002, Am J Psychiatry (7), Van Geffen ECG, 2005, Eur J Clin Pharmacol (7), and Csoka A, 2008, J Sex Med (6). Full distributions are presented in Table 3.

References

Cited-reference concentration highlights seminal methodological and clinical works: Hamilton, 1960 (JNNP; LC=58), Montgomery, 1979 (BJP; 44), Rosenbaum, 1998 (Biol Psychiatry; 45), Mittal, 2011 (Psychiatry Res; 37), Coupland, 1996 (J Clin Psychopharmacol; 32), Price, 1996 (Br J Clin Pharmacol; 29), Hamilton, 1959 (Br J Med Psychol; 25), Sanz, 2005 (Lancet; 25), Chambers, 1996 (NEJM; 23), and Nordeng, 2001 (Acta Paediatr; 23) (Figure 5).

Temporal Evolution of Research Topics

The most frequent keywords in the corpus were “depression (n=172)”, “fluoxetine (n=153)”, “antidepressants (n=152)”, “serotonin reuptake inhibitors (n=136)”, and “SSRI (n=110)”. Keyword timing shows three phases. In the late 1990s–early 2000s, keywords

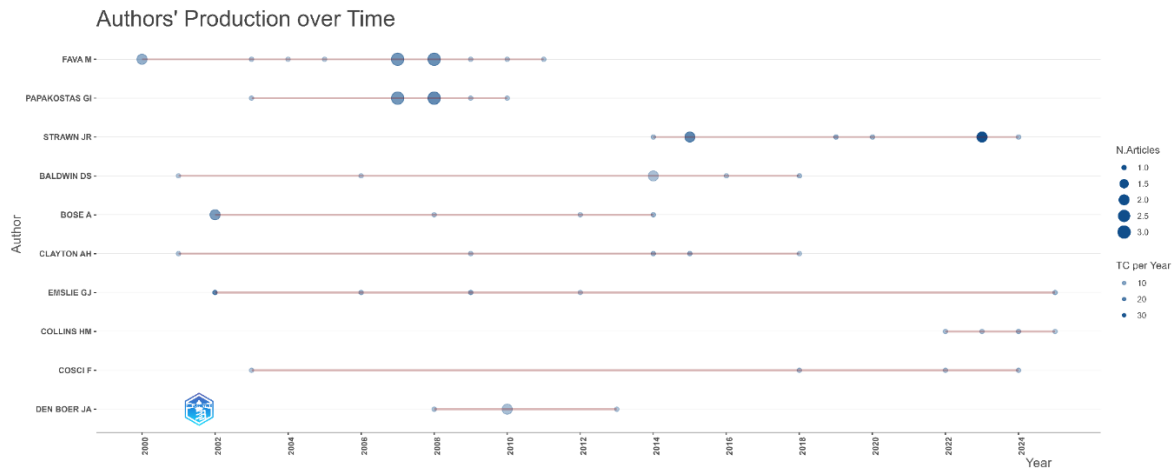


FIGURE 4. Publication timelines of the most prolific authors in SSRI/SNRI withdrawal research, with bubble size indicating article count and shading indicating citations per year.

related to classical pharmacology and trial design cluster around 1998–2003 (Q1 to median), including "alprazolam", "benzodiazepines", "clomipramine", "tricyclic antidepressants", "agoraphobia", "obsessive-compulsive disorder", and "controlled trial". From the mid-2000s, high-frequency terms include "fluoxetine", "paroxetine", "sertraline", "SSRI" , "double-blind", "efficacy", "tolerability", "placebo", "withdrawal", "discontinuation", and "relapse". Diagnostic descriptors such as "major depression" and "major depressive disorder" also become more frequent, together with women, prevalence, and anxiety. After

TABLE 3. Top 15 Documents Ranked by Local Citation Impact, Showing Publication Year, Local And Global Citations, Their Ratios

Document	Year	LC	GC	LC/GC Ratio (%)
1. SANZ EJ, 2005, LANCET	2005	25	260	9.62
2. NORDENG H, 2001, ACTA PAEDIATR	2001	23	131	17.56
3. BULL SA, 2002, JAMA-J AM MED ASSOC	2002	16	296	5.41
4. ZESKIND PS, 2004, PEDIATRICS	2004	9	196	4.59
5. OLFSON M, 2006, AM J PSYCHIAT	2006	8	332	2.41
6. FABRE LF, 1995, BIOL PSYCHIAT	1995	7	167	4.19
7. OLVER JS, 1999, CNS DRUGS	1999	7	23	30.43
8. SIMON GE, 2002, AM J PSYCHIAT	2002	7	265	2.64
9. VAN GEFFEN ECG, 2005, EUR J CLIN PHARMACOL	2005	7	47	14.89
10. CSOKA A, 2008, J SEX MED	2008	6	93	6.45
11. BUVAT J, 2009, EUR UROL	2009	6	148	4.05
12. TRINDADE E, 1998, CAN MED ASSOC J	1998	5	205	2.44
13. KROENKE K, 2001, JAMA-J AM MED ASSOC	2001	5	243	2.06
14. BOLTON JM, 2006, J SEX MARITAL THER	2006	5	31	16.13
15. HADDAD PM, 2001, J PSYCHOPHARMACOL	2001	4	18	22.22

LC, local citations within the corpus; GC, global citations across the database; PY (start), year of first publication.

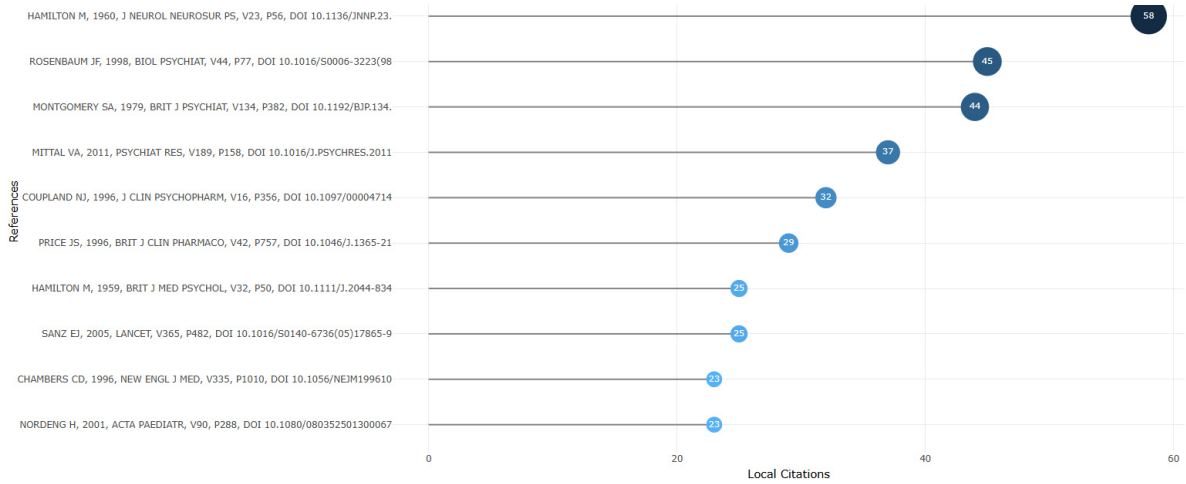


FIGURE 5. Most locally cited references within the SSRI/SNRI withdrawal corpus, ranked by local citation counts.

2016, median years cluster around "risk", "pregnancy", "management", "cohort", and biological/pharmacogenetic terms (prefrontal cortex, activation, CYP2D6, polymorphisms). Emerging keywords include "treatment response", "abrupt discontinuation", "validation", and "adults" (Figure 6).

Network of Citations and Keywords

Co-citation Structure of the Literature

The co-citation network split into four clusters. Cluster 1 (safety/pharmacovigilance) bridges adverse-event work with discontinuation: Sanz 2005, Chambers 1996, Sternbach 1991, Costei 2002. Cluster 2 is the methodological core with rating-scale anchors: Hamilton 1960 and Montgomery & Åsberg 1979, plus Sheehan 1998 and Trivedi 2006. Cluster 3 covers early effectiveness/service work: Bull 2002 set 1, Kessler 1994, Lin 1995. Cluster 4 centers SSRI discontinuation/tolerability: Rosenbaum 1998, Price 1996, Schatzberg 1997.

Connectivity concentrates in few nodes. Hamilton 1960 and Montgomery & Åsberg 1979 lead betweenness and closeness; Rosenbaum 1998 and

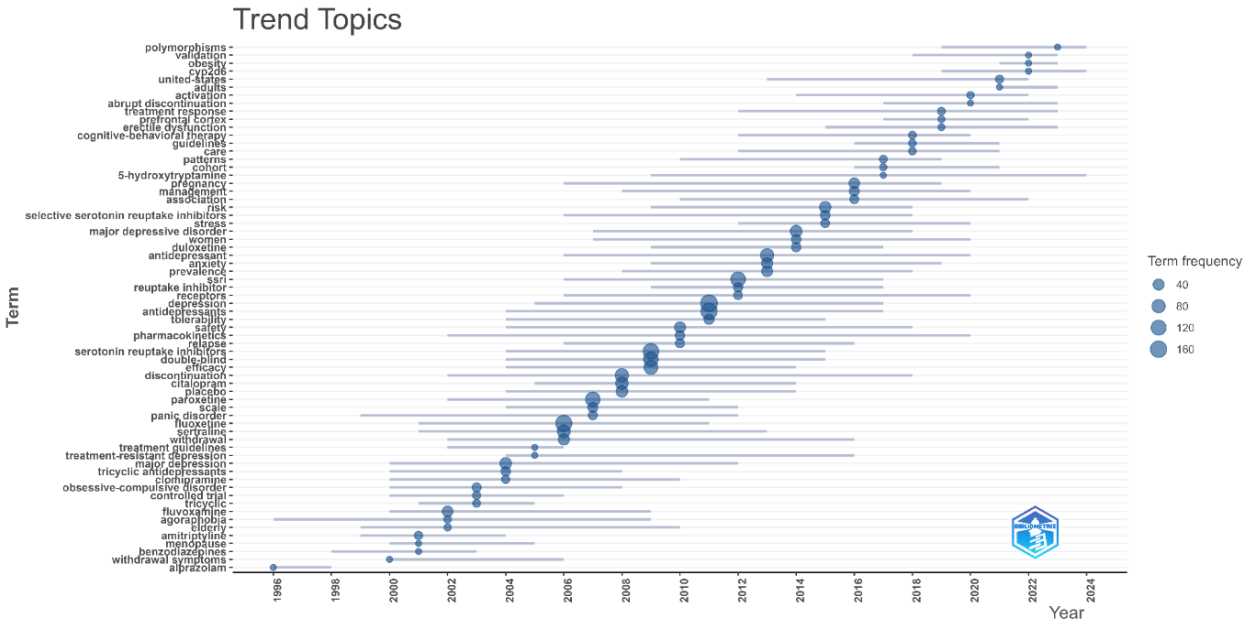


FIGURE 6. Trend topics over time, showing the temporal emergence and frequency of key terms.

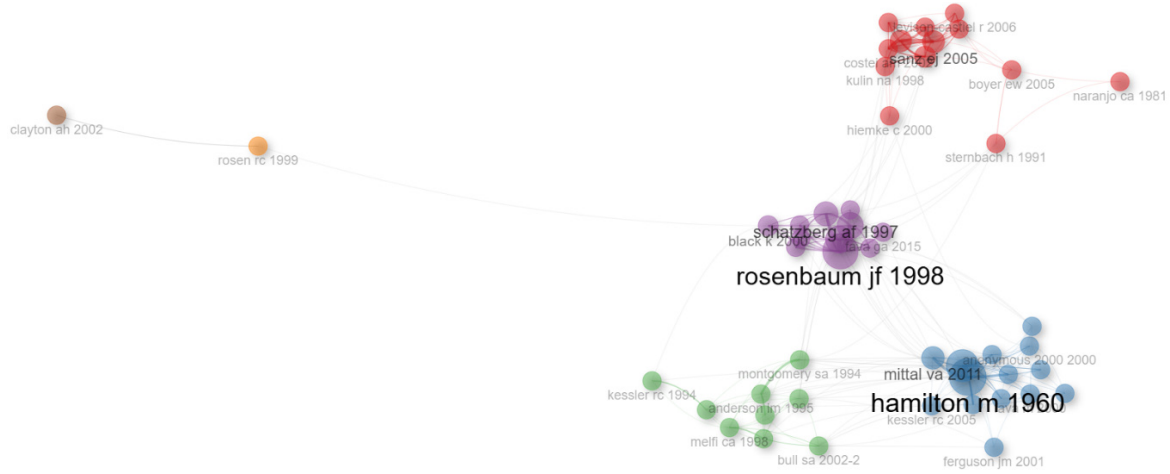


FIGURE 7. Co-citation network showing four major clusters representing safety/pharmacovigilance, methodological foundations, early effectiveness studies, and SSRI discontinuation/tolerability. Node size reflects citation prominence, and edge density indicates co-citation strength.

Price 1996 show high PageRank within discontinuation; Sanz 2005 and Costei 2002 act as central connectors in safety-related citations. By contrast, Rosen 1999 and Clayton 2002 remain peripheral. The network map appears in Figure 7.

Keyword Co-occurrence Network and Thematic Structure

The co-occurrence analysis reveals two tightly connected thematic clusters: a pharmacotherapy/SSRI core and a trial-methods/efficacy core. Within the pharmacotherapy core, depression is the principal hub (betweenness=75.74; closeness=0.020; PageRank =0.062), followed by fluoxetine, antidepressants, serotonin reuptake inhibitors, and paroxetine. Discontinuation-related concepts are strongly integrated, with "discontinuation" and "withdrawal" linking drug names (e.g., citalopram, venlafaxine) to safety- and risk-related terms. The methods/efficacy cluster is centered on "double-blind" and "efficacy", which form bridging nodes to specific agents such as sertraline, escitalopram, and duloxetine. Collectively, the network indicates that discontinuation/withdrawal discourse is embedded in the broader SSRI treatment literature while methodological rigor (double-blind, placebo) forms a parallel axis that connects drug-

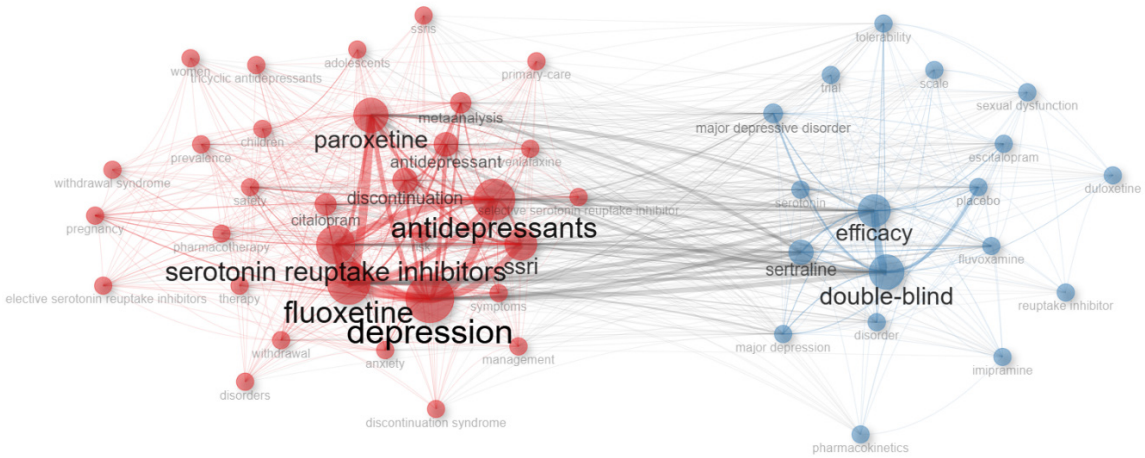


FIGURE 8. Keyword co-occurrence network showing two main thematic clusters: a pharmacotherapy-focused cluster (red) and a trial-methods/efficacy cluster (blue), with node size reflecting term frequency.

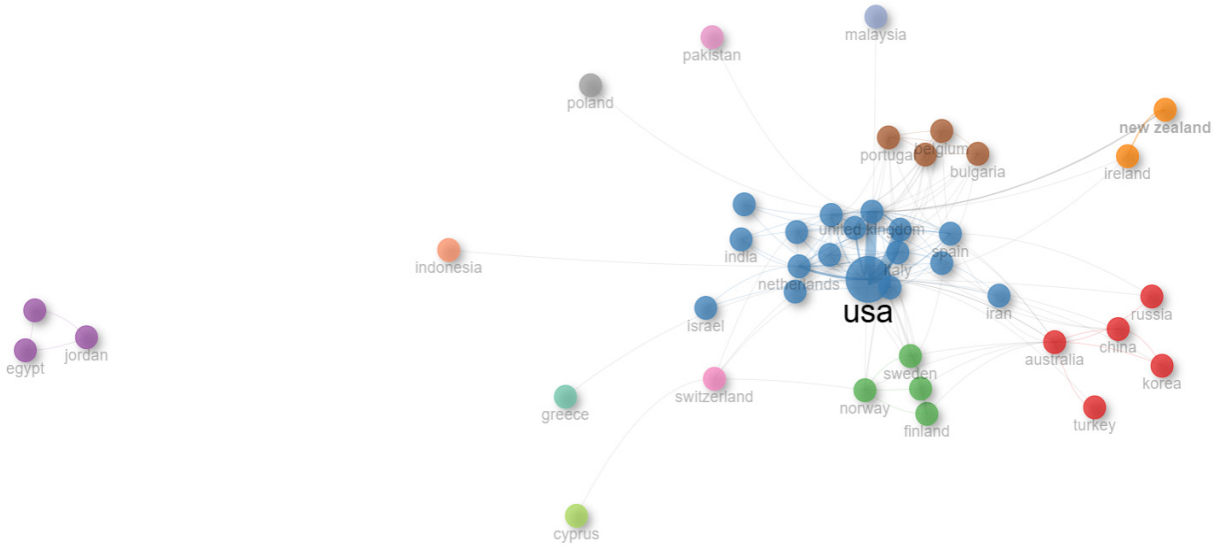


FIGURE 9. Country collaboration network showing the USA and UK as central hubs, with regional clusters forming around Europe, Asia-Pacific, and the Middle East.

specific lines of evidence to outcomes in major depressive disorder (Figure 8).

Geographic and Institutional Contributions

The United States produced 240 articles (36.8%; TC=12904; 53.8 citations/article), followed by the United Kingdom with 48 articles (7.4; 2370, 49.4) and Canada with 31 articles (4.8; 1529, 49.3).

Netherlands, Germany, France, Japan, and China also contributed notable article counts. Several countries had high citation density, including Norway (97/article) Finland (83.2/article), Israel (66.8/article), New Zealand (61.2/article), and Denmark (54.8/article). International coauthorship rates were highest in Denmark (MCP=50%), Australia and Sweden (MCP=42.9%), Canada (MCP=38.7%), and the UK (MCP=31.3%); the USA showed lower MCP (12.5%). Turkey produced 25 articles (3.8%; TC=420; 16.8/article), with predominantly national collaborations (SCP=24; MCP=4%). In the collaboration network, the USA had the highest centrality, followed by the UK. Other countries with measurable centrality included the Netherlands, Italy, and Australia. Turkey showed minimal centrality. The collaboration network is shown in Figure 9. Institution-level analysis showed that a small group of universities produced most of the literature. The most prolific were Harvard University (42 articles), University of Cincinnati (32 articles), Karolinska Institute (23 articles), University of Pennsylvania (21 articles), and Massachusetts General Hospital (20 articles). Among Turkish institutions, Istanbul University had the highest output (7 articles).

DISCUSSION

The overall publication trajectory demonstrates that SSRI/SNRI withdrawal research has evolved into a mature and consistently productive field. Output expanded rapidly during the early 2000s and then stabilized at a steady level, suggesting consolidation of core concepts rather than decline in scholarly attention. High citation averages for early publications indicate that foundational work established durable conceptual anchors that continue to guide current inquiry. The persistence of an annual publication baseline after 2010, despite changing diagnostic frameworks and shifts in antidepressant prescribing, reflects the ongoing clinical relevance of discontinuation phenomena. Together, these patterns portray a stable yet incrementally diversifying research ecosystem.

The temporal phases discussed below were not imposed a priori but were derived inductively from multiple bibliometric indicators. Specifically, phase boundaries were informed by the convergence of (a) peaks and inflection points in annual publication and

citation-per-year trends (Figure 2), (b) shifts in the median and interquartile distribution of high-frequency keywords over time (Figure 6), and (c) changes in the dominant clusters observed in co-citation and keyword co-occurrence networks (Figures 7 and 8). The alignment of these independent outputs supported the identification of three broad periods characterized by distinct thematic and citation profiles.

Early publications receive high citation counts because they coincide with the first peak in citation-per-year values and the initial concentration of withdrawal-related keywords (Figures 2 and 6). Three phases explain this pattern. In 1995–2003, widely used rating scales such as the Hamilton Depression Rating Scale and MADRS became standard citations in trials [19, 20], and early reports offered the first systematic accounts of withdrawal versus relapse [21]. Dependence on these tools and definitions produced high totals for foundational papers. The 2004–2006 peaks align with major debates on pediatric SSRI safety. Influential meta-analyses and regulatory evaluations, including the Lancet re-evaluation of pediatric trials and suicidality reviews informing FDA warnings, became cornerstone references for risk–benefit discussions [22, 23]. At the same time, research on neonatal adaptation syndrome and pregnancy-related exposure risks expanded the field's visibility and clinical relevance [24, 25]. These studies triggered policy shifts, guideline updates, and extensive scholarly engagement, explaining why the mid-2000s cluster consistently dominates global citation counts. Since 2010, focus has moved to tapering strategies, hyperbolic dose reductions, neurobiological mechanisms, and pharmacogenetics [26, 27]. This era has witnessed concerted efforts, led principally by Fava's work, to cultivate deeper understanding and bring attention to the clinically underappreciated phenomenon of SSRI withdrawal [6]. Although these studies underpin practice, shorter citation windows limit cumulative counts. Real-world discontinuation research has broadened evidence by charting symptom trajectories and patient-reported outcomes [7]. Overall, early citation dominance reflects methodological centrality, safety debates, and regulatory impacts, while newer work shows rising annual impact despite lower cumulative totals.

The concentration of highly cited work in the Journal of Clinical Psychiatry, Journal of Clinical Psychopharmacology, Journal of Affective Disorders, and Psychopharmacology indicates that SSRI/SNRI-withdrawal research remains centered in clinical psychopharmacology rather than basic neuroscience or public health [28, 29]. These outlets have steereed debates on tolerability, relapse, and discontinuation syndrome, indicating a field oriented to medication management rather than purely mechanistic inquiry. Strong citation counts in pediatric and perinatal venues, including Pediatrics and NEJM cohorts on neonatal adaptation and prenatal SSRI exposure, mark a parallel stream on pregnancy safety and infant outcomes [30, 31]. Such papers attract attention because they inform regulatory guidance and risk-benefit decisions, complementing the adult-focused psychopharmacology core. Taken together, these journal patterns show a divided yet complementary landscape: one rooted in adult clinical psychopharmacology with a focus on discontinuation management, and another centered on perinatal safety with broad medical and societal implications.

Author-level patterns indicate that SSRI/SNRI withdrawal research is driven by a small, influential core. Mario Fava, George Papakostas, and Jeffrey Strawn combine high output, strong h-indices, and repeated citation bursts, signaling sustained field leadership. Their work covers discontinuation, relapse risk, and tapering strategies, and functions as key reference points for later studies [32, 33]. Child and adolescent psychiatry figures are also prominent. Emslie and colleagues produced highly cited early trials on antidepressant efficacy and withdrawal-related adverse events in youth [34]. This visibility reflects clinical and regulatory pressures on pediatric SSRI use following early-2000s safety debates. Citation "burst" years cluster around three periods, each corresponding to external shocks in the field. The 2002 burst aligns with rising concerns about pediatric SSRI risks and early warnings regarding suicidality. The intense 2007–2008 burst coincides with FDA black-box warnings and renewed scrutiny of paroxetine and other SSRIs for discontinuation effects [23, 35]. A later burst in 2014–2015 reflects a shift toward relapse-prevention and tapering research, including analyses of long-term outcomes after discontinuation [6]. Overall, authorship patterns reveal a field structured around a relatively small group of recurring contributors whose work governs both

scientific understanding and clinical practice.

Trend-topic and co-occurrence patterns indicate three phases in SSRI/SNRI withdrawal research. In 1995–2004, work was formed by anxiety/OCD frameworks and older agents (clomipramine, benzodiazepines). Frequent terms such as alprazolam, clomipramine, agoraphobia, and controlled trial reflect this orientation. Early clomipramine efficacy for obsessive-compulsive symptoms was documented in pivotal 1990s studies[36]. Similarly, and panic/agoraphobia-focused SSRI comparisons established standardized protocols that underpinned later trials [37]. As noted earlier in the citation-trend analysis, the steep rise in citations around 2004–2006 aligns closely with this thematic shift. Following the 2004 FDA black-box warning on pediatric antidepressant use, the field moved decisively toward questions of safety, withdrawal differentiation, and relapse risk. The most recent period prioritizes risk-stratified, mechanistic, and real-world questions. Large population-based studies emphasize pregnancy outcomes and perinatal risk [38], while pharmacogenetic work on CYP2D6 variation highlights metabolic and individual-difference mechanisms [39]. The emergence of personalized tapering, especially hyperbolic dose-reduction models, marks a significant redefinition of withdrawal research [27]. Consistent with the citation trends discussed earlier, newer keywords such as pregnancy, risk, CYP2D6, polymorphisms, abrupt discontinuation, and management cluster in the post-2016 period, showing strong alignment between thematic evolution and the field's most recent citation patterns.

The geographic layout of SSRI/SNRI withdrawal research is highly centralized. The United States and the United Kingdom serve as core hubs for global production and collaboration, with very high betweenness indicating that they bridge otherwise separate national clusters. This centralization aligns with clinical psychopharmacology, where regulators, major academic centers, and pharmaceutical infrastructures are concentrated in these countries. A secondary European cluster (Netherlands, Italy, Germany, France) maintains steady output but only moderate integrative capacity. Collaboration within this group is common, while links to distant regions are fewer. Asian contributors such as Japan, China, and Korea appear regularly but show low centrality, indicating participation without strong bridging roles. Turkey holds a peripheral yet developing position. With 25 publications and predominantly national collaboration (MCP 4%), its near-zero betweenness reflects limited integration with major international networks, likely constraining citation impact and visibility. Stronger cross-border collaboration is needed to move closer to the field's epistemic core.

Strengths and Limitations

To our knowledge, it is the first bibliometric and science-mapping study focused specifically on SSRI/SNRI discontinuation/withdrawal rather than antidepressants in general. A rigorous, highly specific search strategy with explicit exclusions for non-antidepressant withdrawals isolates the field's conceptual core. Several limitations warrant consideration. The analysis relies exclusively on the Web of Science Core Collection, which, although comprehensive, omits items indexed only in Scopus, PubMed, PsycINFO, or regional databases, potentially underrepresenting non-Anglophone and lower-income countries. Citation-based metrics privilege older publications and high-income research systems, which can inflate the apparent centrality of early US/UK studies. Bibliometric methods capture production, influence, and network structure but cannot assess study quality, clinical relevance, or the validity of withdrawal definitions across trials. Another limitation of this study is the inconsistent use of terms such as "discontinuation syndrome," "withdrawal," and "relapse" across the literature. These terms are often used interchangeably, which may play a role in outcome reporting, indexing, and thematic clustering. Accordingly, some observed patterns may partly reflect terminological heterogeneity rather than substantive differences between studies. A further limitation is that, to ensure methodological consistency, the analysis was restricted to original research articles, which may have excluded influential reviews, guidelines, and consensus statements, although their impact is partly reflected through citation and co-citation patterns. Finally, keyword- and co-citation–based clustering reflects reported terminology rather than full conceptual heterogeneity, so emerging themes may be obscured by inconsistent labels.

CONCLUSION

This study shows that research on SSRI/SNRI withdrawal has grown steadily over three decades yet remains concentrated within a small group of countries, institutions, and investigators. Early methodological and safety papers continue to shape the field, while more recent work points toward individualized tapering, pregnancy-related risk, and pharmacogenetic approaches. However, tapering strategies remain nonstandard, real-world deprescribing evidence is scarce, and definitions and measurement of withdrawal are inconsistent across studies, limiting comparability and meta-analytic synthesis. Addressing these gaps through standardized tapering protocols (including hyperbolic dose reductions tested in pragmatic settings), harmonized withdrawal criteria, and more globally distributed, patient-centered research will be essential for developing a more coherent and clinically actionable understanding of antidepressant discontinuation.

Ethics Approval and Consent to Participate

The study was conducted using only information obtained from previously published scientific publications and publicly available data sources. Live human participants, animal experiments, or identifiable personal data were not involved at any stage of the research; therefore, ethical approval was not required. Accordingly, the study fully complies with national and international research ethics standards. The researcher's study received written approval from the head of the department (Date: 18.11.2025).

Data Availability

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

Authors' Contribution

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

Conflict of Interest

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Generative Artificial Intelligence Statement

An AI-based language model (ChatGPT, OpenAI) was used to assist with grammar checking and language refinement during manuscript preparation. The tool was not used to generate scientific content, analyze data, or draw conclusions. All interpretations and final content are the sole responsibility of the author.

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The European Research Journal 2026;12(2):272-279 Original Article

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Analysis of Early Reoperation and Mortality Following Intertrochanteric Fracture Fixation in Elderly Patients: A Focus on Frailty, Bone Quality, and Technical Factors

Recep Karasu[1], Namık Şahin[2], Mustafa Dinç[1], Hünkar Çağdaş Bayrak[3]

[1]Department of Orthopedics and Traumatology, Bursa City Hospital, Bursa, Türkiye; [2]Department of Orthopedics and Traumatology, Acıbadem Bursa Hospital, Bursa, Türkiye; [3]Department of Orthopedics and Traumatology, Çekirge State Hospital, Bursa, Türkiye

ABSTRACT

Objectives: Intertrochanteric femur fractures in older adults carry high risks of early complications and mortality. This study aimed to identify independent predictors of early fixation failure and postoperative mortality by integrating patient-, fracture-, and technique-related factors.

Methods: This single-center retrospective cohort included 150 patients aged ≥65 years who underwent surgical fixation with a Dynamic Hip Screw (DHS) or Proximal Femoral Nail Antirotation (PFN-A) for the AO Foundation/Orthopaedic Trauma Association (AO/OTA) 31-A intertrochanteric fractures. Evaluated variables included comorbidity burden assessed using the Charlson Comorbidity Index, bone quality assessed using the Singh Index, pre-fracture mobility assessed using the Functional Ambulation Scale, fracture pattern, and reduction–fixation quality.

Results: Early reoperation occurred in 12.6% of patients, and 6-month mortality was 24%. Independent predictors of early reoperation included fracture instability (AO/OTA 31-A3; adjusted odds ratio [aOR]=1.41) and higher Charlson Comorbidity Index score (aOR=2.47). In contrast, better bone quality (higher Singh Index; aOR=0.16), superior reduction–fixation quality (aOR=0.12), and higher pre-fracture mobility (aOR=0.75) were protective against reoperation. Early mortality was independently associated with a higher Charlson Comorbidity Index score (aOR=1.74), poorer bone quality (aOR=0.42), and lower pre-fracture mobility (aOR=0.24). Implant type did not significantly influence either outcome.

Conclusions: Early fixation failure is primarily influenced by fracture instability, osteoporosis severity, and fixation quality, whereas early mortality reflects comorbidity burden and frailty. Incorporating comorbidity burden, bone quality, and pre-fracture mobility into preoperative assessment may improve risk stratification and guide personalized perioperative management in elderly patients with intertrochanteric femur fractures.

Keywords: Intertrochanteric Femur Fracture, Early Reoperation, Mortality Predictors, Geriatric Trauma, Fixation Stability

Proximal femur fractures are a major cause of morbidity and mortality in older adults and represent a growing public health concern with aging populations worldwide. Intertrochanteric fractures account for approximately half of all proximal femur fractures and predominantly occur

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Corresponding author: Recep Karasu, MD., Phone: +90 224 975 00 00, E-mail: recepkarasu@hotmail.com

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after low-energy falls in elderly individuals with compromised bone quality and reduced physiological reserve [1-3].

Despite advances in surgical techniques and perioperative care, intertrochanteric fractures remain associated with substantial early complications. Reported rates of fixation failure and reoperation range from 8% to 20%, while early postoperative mortality remains considerable, reflecting the combined effects of fracture instability, osteoporosis, comorbidity burden, and impaired functional capacity [4-7]. Early functional decline is common, and many patients fail to regain their pre-fracture level of mobility, further contributing to adverse outcomes [8, 9].

Surgical fixation aims to restore stability and allow early mobilization; however, mechanical failure - such as implant cut-out, varus collapse, or fixation loss - continues to pose a significant clinical challenge. Previous studies have identified various patient-related, fracture-related, and technical risk factors for fixation failure and mortality, but the reported findings are heterogeneous and often based on descriptive or univariate analyses [10-12]. Such approaches may not adequately account for the complex interplay between comorbidity burden, bone quality, fracture pattern, and quality of reduction and fixation.

However, there remains limited evidence from analytical studies that integrate these factors within multivariable models to identify independent predictors of early reoperation and mortality in elderly patients with intertrochanteric fractures. A clearer understanding of these predictors is essential for improving preoperative risk stratification, optimizing surgical decision-making, and guiding perioperative management in this vulnerable population.

Therefore, the present study aimed to identify independent predictors of early fixation failure requiring reoperation and early postoperative mortality in patients aged 65 years and older who underwent surgical fixation for intertrochanteric femur fractures. By evaluating patient-related, fracture-related, and technical factors within a multivariable framework, this study seeks to provide clinically relevant insights into determinants of early outcomes following intertrochanteric fracture fixation.

METHODS

Study Design and Ethical Approval

This was a single-center, retrospective cohort study conducted at the tertiary academic training and research hospital. The study was reported in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines. The local Institutional Review Board granted ethical approval before the initiation of the study (Approval No: 2015/11-08). The study was conducted in accordance with the Declaration of Helsinki. The requirement for individual informed consent was waived due to the retrospective and anonymized nature of the data analysis.

Study Population and Eligibility

A total of 318 patients aged 65 years or older who presented with a proximal femur fracture between January 2013 and December 2014 were screened using electronic medical records and radiographic archives. Inclusion criteria were: (1) age ≥65 years; (2) an acute, low-energy intertrochanteric femur fracture classified as the AO Foundation/Orthopaedic Trauma Association (AO/OTA) 31A; (3) surgical treatment with either a Dynamic Hip Screw (DHS) or Proximal Femoral Nail Antirotation (PFN-A); and (4) availability of complete clinical and radiological data with a minimum follow-up of 6 months. Exclusion criteria included: treatment with arthroplasty or alternative implants, pathological or periprosthetic fractures, polytrauma, preoperative transfer or refusal of surgery, death prior to surgery, requirement for immediate intensive care, and incomplete documentation. After stepwise application of these criteria, 150 patients m*et all* eligibility requirements and were included in the final analysis.

Data Collection and Variables

All preoperative, intraoperative, and postoperative variables were systematically retrieved and recorded using a standardized, predefined case report form to ensure consistency and minimize information bias. Baseline demographic characteristics - including age, sex, fracture side, and mechanism of injury - were documented for all patients. Comorbidity burden was

quantified using the Charlson Comorbidity Index (CCI), which provides a validated cumulative score based on the presence and severity of chronic medical conditions [13].

Preoperative anesthetic risk was classified according to the American Society of Anesthesiologists (ASA) Physical Status system [14].

Bone Quality Evaluation: The degree of osteoporosis was assessed on preoperative antero-posterior (AP) hip radiographs using the Singh Index [15].

Functional Status: Preoperative ambulatory capacity was determined using the Functional Ambulation Scale (FAS) [16].

Laboratory Parameters: Preoperative hematological (hemoglobin, hematocrit, white blood cell count, platelet count, lymphocyte percentage) and biochemical profiles (blood urea nitrogen, creatinine, AST, ALT, serum calcium, fasting blood glucose) were collected.

Surgical Data: The time from hospital admission to surgery (days), type of anesthesia (general or spinal), and the implanted fixation device (DHS or PFN-A) were documented.

Radiographic Assessment

All fractures were classified using the AO/OTA 31A system. Preoperative, immediate postoperative, and follow-up AP and lateral hip radiographs were evaluated independently by two fellowship-trained orthopedic surgeons who were blinded to patient outcomes.

The Total Stability Score used in this study is a novel composite metric created by integrating four radiographic parameters - tip–apex distance [8], neck–shaft angle, lag screw position based on Cleveland zones, and medial cortical continuity [9]. Each parameter has been independently validated in previous biomechanical and clinical research as a predictor of mechanical failure and implant cut-out [8, 9]. By systematically combining these well-established predictors into a single structured scoring system, the Total Stability Score provides a more comprehensive and clinically practical assessment of reduction–fixation stability than any individual parameter alone. The primary outcomes of the study were:

Early Reoperation: Defined as any unplanned secondary surgical intervention related to the index fracture (e.g., due to implant cut-out, mechanical failure, nonunion, or deep infection) within the first 6 postoperative months.

Early Mortality: Defined as all-cause mortality occurring within the first 6 postoperative months, verified against the national death registry.

Statistical Analysis

All statistical analyses were performed using SPSS version 27.0 (IBM Corp., Armonk, NY, USA) and Stata version 16.0 (StataCorp LLC, TX, USA). Continuous variables were tested for normality using the Shapiro–Wilk test and presented as mean ± standard deviation (SD) or median with interquartile range (IQR), as appropriate. Categorical variables were summarized as frequencies and percentages. Two primary binary outcomes were examined: early reoperation and early mortality within the first 6 postoperative months. Given the dichotomous structure of both outcomes, multivariable binary logistic regression (logit model) was used to identify independent predictors among clinical, functional, laboratory, and radiographic variables.

Univariate analyses were first performed to screen candidate predictors. Variables with a P-value <0.10, together with clinically relevant covariates, were entered into multivariable logistic regression using a backward stepwise selection procedure. Regression coefficients (β) were exponentiated to obtain adjusted Odds Ratios (ORs) with 95% Confidence Intervals (CIs). An OR >1 indicated an increased likelihood of the adverse outcome, whereas OR <1 reflected a protective effect. Model performance was assessed using the Hosmer–Lemeshow goodness-of-fit test, which evaluates calibration by comparing predicted and observed event probabilities across deciles of risk. A non-significant Hosmer–Lemeshow P-value (>0.05) was interpreted as good model calibration. All statistical tests were two-tailed, and a P-value <0.05 was considered statistically significant.

RESULTS

Patient Demographics and Baseline Characteristics

A total of 150 patients who fulfilled the inclusion criteria were evaluated. The mean age of the cohort

was 80.7±8.1 years, and 59.4% were female. The majority of fractures resulted from low-energy simple falls (86.7%). The right hip was involved in 56% of cases. The median time to surgery was 4 days (IQR: 2–7). Comorbidity burden was substantial, with a median Charlson Comorbidity Index (CCI) of 5 (IQR: 4–6), and 61.3% of patients classified as ASA III–IV. Bone quality assessment showed that 78.7% of patients had a Singh Index of 1–3, indicating pronounced osteoporosis. Fractures were classified as AO/OTA 31A1 (27.3%), 31A2 (52.0%), and 31A3 (20.7%). DHS was used in 65.3% of cases and PFN-A in 34.7%. Postoperative reduction quality, assessed using the Total Stability Score, was good or excellent in 64% of patients. Baseline characteristics are presented in Table 1.

TABLE 1. Baseline Characteristics of the Study Cohort (n=150)

Characteristic	Data
Age (years)	80.7±8.1
Sex, n (%)	
Male	61 (40.6)
Female	89 (59.4)
Fracture side, n (%)	
Right	84 (56.0)
Left	66 (44.0)
Time to surgery (days)	4 [2–7]
ASA score, n (%)	
I–II	58 (38.7)
–IV	92 (61.3)
Charlson comorbidity index	5 [4–6]
Functional ambulation scale	3 [2–4]
Singh index, n (%)	
Grade 1–3	118 (78.7)
Grade 4–6	32 (21.3)
AO/OTA Classification, n (%)	
31-A1	41 (27.3)
31-A2	78 (52.0)
31-A3	31 (20.7)
Fixation type, n (%)	
DHS	98 (65.3)
PFN-A	52 (34.7)
Total stability score, n (%)	
Good/Excellent (3–4)	96 (64.0)
Poor/Moderate (0–2)	54 (36.0)

Data are shown as mean±standard deviation or median (interquartile range) or n (%) where appropriate. AO/OTA, AO Foundation/Orthopaedic Trauma Association; ASA, American Society of Anesthesiologists; DHS, dynamic hip screw; PFN-A, proximal femoral nail antirotation.

Primary Outcomes and Univariate Associations

Within the first six postoperative months, 19 (12.6%) patients underwent a second surgical procedure. The reasons for reoperation were implant failure (n=14, 73.7%), fixation loss (n=3, 15.8%), and deep infection (n=2, 10.5%). Early all-cause mortality occurred in 36 (24.0%) patients.

Univariate comparisons of key variables between patients with and without early reoperation, and between survivors and non-survivors, are presented in Table 2. Variables with a univariate association ($P<0.10$) were selected for inclusion in the multivariable models.

Multivariable Logistic Regression for Early Reoperation

The results of the multivariable logistic regression model for early reoperation are shown in Table 3. The model was statistically significant and demonstrated excellent calibration (Hosmer-Lemeshow P=0.997), explaining approximately 47% of the variance (Pseudo $R^2 = 0.47$). Five factors were identified as independent predictors. A one-level increase in fracture instability (e.g., from A2 to A3) increased the odds of reoperation by 41% (aOR=1.41). A one-point increase in the CCI nearly doubled the odds (aOR=2.47). Conversely, a one-point increase in the Singh Index (better bone quality) and the Total Stability Score (better reduction/fixation) reduced the odds of reoperation by 84% (aOR=0.16) and 88% (aOR=0.12), respectively. A higher preoperative functional level (FAS) was also protective, with each one-point increase reducing the odds by 25% (aOR= 0.75).

Multivariable Models for Early Mortality

When all potential predictors were included in a single model for early mortality, significant

TABLE 2. Univariate Analysis of Predictors for Primary Outcomes

Variable	Reoperation (n=19)	No Reoperation (n=131)	P-value	Mortality (n=36)	Survivors (n=114)	P-value
Age (years)	82.1±7.5	80.5±8.2	0.401	83.2±7.0	79.9±8.3	**0.021**
CCI	6 [5–7]	5 [4–6]	**0.035**	6 [5–7]	5 [4–6]	**0.003**
Singh index	2 [1–2]	3 [2–3]	**0.018**	2 [1–3]	3 [2–3]	**0.009**
FAS	2 [1–3]	3 [2–4]	**0.007**	2 [1–2]	3 [2–4]	**<0.001**
Total stability score	2 [1–2]	3 [2–4]	**0.001**	3 [2–3]	3 [2–4]	0.890
AO/OTA 31-A3	7 (36.8%)	24 (18.3%)	0.058	8 (22.2%)	23 (20.2%)	0.788

Data are shown as mean±standard deviation or median (interquartile range) or n (%) where appropriate. AO/OTA, AO Foundation/Orthopaedic Trauma Association; CCI, Charlson comorbidity index; FAS, functional ambulation scale.

Statistically significant P-values are shown in bold.

multicollinearity was detected (Hosmer-Lemeshow P<0.05). Therefore, separate, parsimonious models were constructed for the most clinically relevant predictors (Table 4). A one-point increase in the CCI was associated with a 74% increase in the odds of early mortality (aOR 1.74). Better bone quality (Singh Index) and higher preoperative ambulatory function (FAS) were strong protective factors, reducing the odds of mortality by 58% (aOR=0.42) and 76% (aOR=0.24) per one-point increase, respectively.

Model Performance

The final multivariable model for early reoperation demonstrated strong explanatory capacity, achieving a Pseudo R^2 of 0.47 and excellent calibration (Hosmer-Lemeshow P=0.997). In contrast, a comprehensive multivariable model for early mortality could not be reliably interpreted due to marked multicollinearity and poor model fit (Hosmer–Lemeshow P<0.05). Consequently, separate parsimonious models were constructed for key predictors. Although these individual models yielded modest Pseudo R^2 values (CCI: 0.075; Singh Index: 0.054; FAS: 0.101), each demonstrated adequate calibration with non-significant Hosmer-Lemeshow statistics (P>0.05), indicating good agreement between predicted probabilities and observed event rates.

DISCUSSION

This retrospective cohort study suggests that early fixation failure and early postoperative mortality after intertrochanteric femur fractures in elderly patients are influenced by distinct but overlapping clinical domains. Early reoperation was primarily associated with fracture instability, bone quality, reduction–fixation stability, and pre-fracture ambulatory status,

TABLE 3. Multivariable Logistic Regression for Early Reoperation

Predictor	Adjusted odds ratio (aOR)	95% CI	P-value
Fracture type (A3)	1.41	1.01 – 1.98	**0.047**
Charlson comorbidity index	2.47	1.06 – 5.75	**0.036**
Singh index	0.16	0.04 – 0.70	**0.016**
Total stability score	0.12	0.04 – 0.33	**0.001**
Functional ambulation scale	0.75	0.56 – 0.93	**0.008**

aOR, adjusted odds ratio; CI, confidence interval. Multivariable logistic regression model adjusted for all variables with P<0.10 in univariate analysis.

Statistically significant P-values are shown in bold.

TABLE 4. Parsimonious Logistic Regression Models for Early Mortality

Model/Predictor	Adjusted odds ratio (aOR)	95% CI	P-value
Model 1–CCI	1.74	1.23–2.46	**0.002**
Model 2–Singh index	0.42	0.23–0.78	**0.007**
Model 3–FAS	0.24	0.11–0.52	**<0.001**

CCI, Charlson comorbidity index; FAS, functional ambulation scale.
Statistically significant P-values are shown in bold.

whereas early mortality was predominantly determined by systemic factors reflecting frailty, including comorbidity burden, bone quality, and functional capacity. These findings underscore that outcomes in geriatric intertrochanteric fractures are not solely dependent on surgical technique but are strongly shaped by patient-related biological reserve. The observed reoperation rate (12.6%) and early mortality rate (24%) are consistent with ranges reported in contemporary clinical series of elderly hip fracture patients, which document reoperation rates between 4–20% and 6-month mortality rates between 10–30% [6, 7]. Mechanical failure remained the dominant cause of reoperation, supporting the concept that fixation instability in osteoporotic bone is multifactorial and cannot be fully mitigated by implant selection alone.

In this study, fracture instability (AO/OTA 31-A3) emerged as a significant predictor of early reoperation, in agreement with established biomechanical principles and previous clinical studies demonstrating increased risks of varus collapse and implant cut-out in unstable patterns [8-10, 17-19]. These findings reinforce the importance of accurate fracture classification and meticulous intraoperative reduction to minimize mechanical complications.

Bone quality, assessed using the Singh Index, was independently associated with both mechanical failure and early mortality. This observation aligns with prior reports indicating that poor trabecular architecture compromises implant anchorage and increases susceptibility to fixation failure, regardless of implant type [6, 7, 15, 20].

Comorbidity burden and pre-fracture mobility, assessed using the CCI and FAS, respectively, were among the strongest predictors of early mortality. These variables represent integrated markers of physiological reserve and frailty and have been consistently associated with mortality risk following hip fracture surgery [7, 21, 22]. Our findings further support the growing recognition that functional status may be as important as chronological age in predicting early postoperative outcomes.

Regarding implant selection, no significant association was observed between implant type and early reoperation or mortality. This finding is consistent with existing evidence suggesting that, while implant choice may influence outcomes in selected fracture patterns, biological factors such as osteoporosis and frailty often outweigh implant-related considerations in determining early prognosis [23-25].

Strengths and Limitations

The major strengths of this study include its comprehensive evaluation of patient-related, fracture-related, and technical factors within a unified multivariable analytical framework. By simultaneously incorporating comorbidity burden, functional status, bone quality, fracture pattern, and reduction–fixation characteristics, this study provides a more holistic assessment of early outcomes than analyses based solely on isolated or univariate predictors. Another important strength is the use of clearly defined and clinically relevant endpoints - early reoperation and early postoperative mortality - which represent meaningful outcomes in geriatric hip fracture care. Furthermore, the Total Stability Score integrates multiple well-established radiographic parameters into a single composite measure, enhancing the clinical practicality of fixation stability assessment.

Several limitations should be acknowledged. First, the retrospective design inherently limits causal inference and may be subject to residual confounding

despite multivariable adjustment. Second, the relatively modest sample size and single-center nature of the study may restrict the external generalizability of the findings, particularly to institutions with different surgical protocols or patient demographics. Third, bone quality was evaluated using a radiographic index rather than dual-energy X-ray absorptiometry, which may not fully capture systemic osteoporosis severity. Fourth, although functional status was assessed preoperatively, comprehensive multidimensional frailty indices and cognitive or nutritional parameters were not available, potentially underrepresenting the full spectrum of geriatric vulnerability. Finally, the analysis focused on early outcomes within six months, and long-term functional recovery, late mechanical failure, and survival beyond this period could not be assessed.

Despite these limitations, the study provides clinically relevant insights into early risk stratification by highlighting the combined impact of biological reserve, fracture characteristics, and fixation quality on early reoperation and mortality after intertrochanteric fracture fixation in elderly patients.

CONCLUSION

Early fixation failure and early postoperative mortality after intertrochanteric femur fractures in elderly patients are primarily driven by patient-related factors, including comorbidity burden, bone quality, and pre-fracture functional status, while fracture instability and reduction–fixation quality remain the key technical determinants of mechanical failure. Implant type alone did not significantly influence early outcomes. These findings suggest that successful management of geriatric intertrochanteric fractures requires not only appropriate surgical technique but also careful preoperative assessment of frailty and physiological reserve. Incorporating simple and readily available measures of comorbidity, bone quality, and functional status into routine evaluation may improve early risk stratification and support more individualized perioperative decision-making in this high-risk population.

Ethics Approval and Consent to Participate

This study was approved by the Şevket Yılmaz Training and Research Hospital Clinical Research Ethics Committee (Decision No: 2011-KAEK-25 2015/11-08; date: 06.03.2015). All procedures were conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Because the study was retrospective and no additional intervention was performed on the participants, the informed consent form was waived.

Data Availability

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

Authors' Contribution

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

Conflict of Interest

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Generative Artificial Intelligence Statement

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

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The European Research Journal 2026;12(2):280-288 Original Article

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Craniofacial Morphological Features in Childhood Age with Autism Spectrum Disorder

Fatih Çetinkaya[1], Hürriyet Çetinok[2]

[1]Department of Speech and Language Therapy, İstanbul Atlas University, Faculty of Health Sciences, İstanbul, Türkiye; [2]Department of Anatomy, İstanbul Atlas University, Faculty of Medicine, İstanbul, Türkiye

ABSTRACT

Objectives: Autism spectrum disorder often exhibits unusual craniofacial morphology, which may indicate underlying neurodevelopmental abnormalities in childhood. This study aimed to investigate the relationship between craniofacial anthropometric measurements, the severity of autism, and language development in children with autism spectrum disorder.

Methods: This study included 66 children (50 boys, 16 girls) aged 6–10 years who were diagnosed with autism spectrum disorder. Three-dimensional facial scanning was performed after the removal of glasses, and anthropometric measurements were analyzed in relation to scores on the Gilliam Autism Rating Scale and the Turkish Communication Development Inventory.

Results: A significant association was found between increased head circumference and higher autism severity (P=0.035), with larger head size correlating with decreased forehead height, may suggest greater symptom severity in boys. No significant associations were observed in girls. Craniofacial metrics showed no association with language development in either gender.

Conclusions: Head circumference and forehead height have been identified as potential craniofacial indicators of autism severity in boys with autism spectrum disorder. These findings highlight the effectiveness of craniofacial phenotyping in clarifying the neurodevelopmental characteristics associated with autism. Future research, utilizing larger and more sex-balanced cohorts, is imperative to clarify these relationships and their potential diagnostic relevance.

Keywords: Autism Spectrum Disorders, Craniofacial Morphology, Facial Asymmetry, Head Circumference, Three-Dimensional Facial Scanning

Autism spectrum disorder (ASD) is a neurodevelopmental condition marked by enduring variations in social communication, limited interests, and repetitive behaviors [1]. This condition impacts individuals globally, irrespective of race, culture, or socioeconomic status, with symptoms generally appearing in early childhood [2–4]. The disorder is characterized by two primary domains: deficits in social communication and the presence of repetitive or restrictive behaviors [3, 5, 6]. Symptom presentation and severity differ across developmental stages; however, most affected children exhibit early delays in language and social engagement [3, 7–9].

Craniofacial anomalies frequently occur in

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Corresponding author: Hürriyet Çetinok, MD., PhD., Assist. Prof., Phone: +90 850 450 34 39 ext. 1206, E-mail: hurriyet.cetinok@atlas.edu.tr

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neurodevelopmental disorders. This is due to the shared embryonic ectodermal origin of the craniofacial complex and the brain, which develop in close coordination during gestation [1]. Severe dysmorphology is generally linked to syndromic conditions like Crouzon or Treacher Collins syndromes; however, subtle craniofacial variations have been noted in children with autism [10]. The observed subtle differences may indicate atypical neurodevelopmental processes instead of merely isolated facial anomalies.

The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) and standardized rating scales are the main tools used for behavioral evaluations in the autism diagnostic process [11]. Nevertheless, these instruments are not objective and depend on observable actions, which could differ among assessors and phases of development. Better early detection and prognosis accuracy may be possible if objective morphological or biological correlations could be identified.

Gender dimorphism in craniofacial morphology is extensively documented, with males typically displaying larger cranial dimensions and distinctive facial proportions [12–15]. The significant male predominance in autism suggests that gender-linked developmental pathways may affect craniofacial characteristics, potentially acting as quantifiable markers of neurodevelopmental differences. Empirical evidence, including the chromodomain helicase DNA-binding 8 (CHD8) haploinsufficiency models, reinforces the notion that gender-specific genetic mechanisms can influence craniofacial and cerebral development in autism [16].

Despite an increasing number of findings, little research has rigorously addressed the differences in craniofacial morphology between boys and girls with autism and the correlation of these characteristics with clinical severity or language development. This study examines craniofacial features in boys and girls with autism and explores how they relate to autism severity and linguistic abilities. We anticipated that craniofacial features could be associated with the severity of autism and that gender-related morphological patterns might enlighten the neurodevelopmental origins of these relationships.

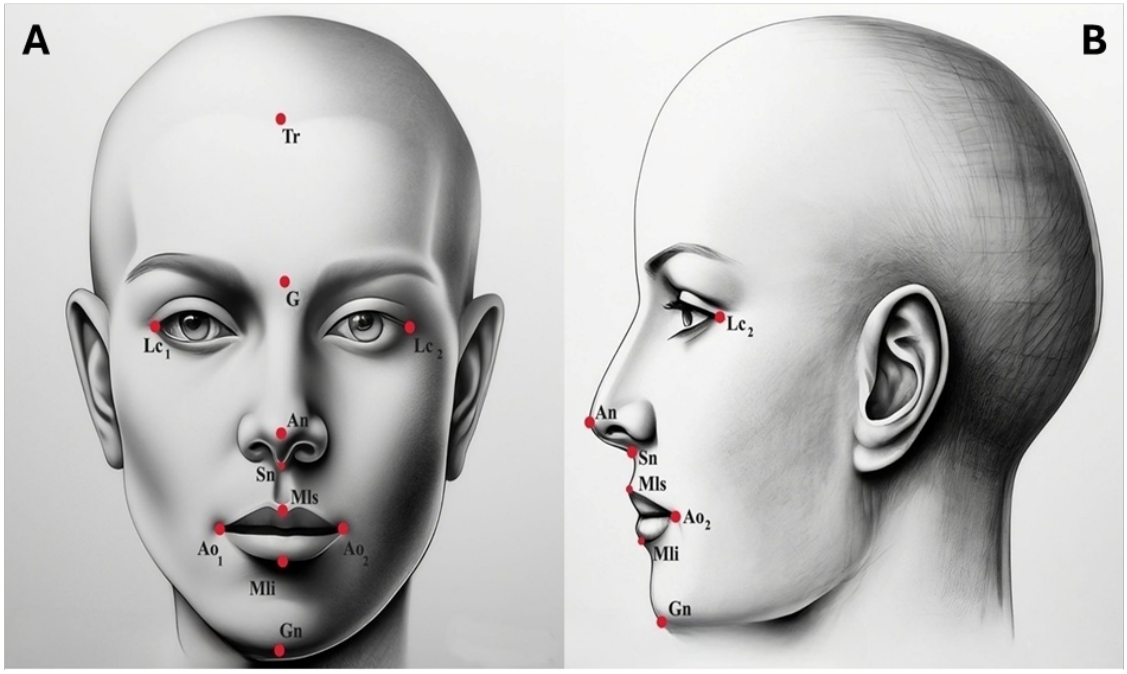

FIGURE 1. Three-dimensional facial views illustrate the placement of key anthropometric landmarks used for craniofacial measurements. (A) Profile view showing the overall facial contour and reference points for vertical dimensions. (B) Lateral view demonstrates the localization of measurement landmarks. Tr, trichion; G, glabella; Lc1–Lc2; lateral canthus; An; apex nasi; Sn, subnasale; Mls, middle point of labium superius; Ao1–Ao2, angulus oris; Mli, middle point of labium inferius; Gn, gnathion.

METHODS

Subjects

Our study included 66 children with ASD, comprising 50 boys and 16 girls, all aged between 6 and 10 years. The study was carried out in accordance with the Declaration of Helsinki. Approval from the Ethics Committee of Istabul University-Cerrahpaşa was obtained (approval date and number: 12.12.2024 – 56267), and written informed consent was obtained from all parents. The sample consisted of children residing in Turkey, diagnosed according to the criteria outlined in the DSM-5.

Children were excluded from the study if they had: (i) a maternal history of drug use during pregnancy known to cause morphologic or morphometric craniofacial anomalies (e.g., isotretinoin-containing medications); (ii) any diagnosed syndromes associated with craniofacial anomalies; (iii) metabolic diseases such as Fabry disease that can cause facial asymmetry; or (iv) a history of facial trauma resulting in structural abnormalities. Based on these criteria, 3 boys and 1 girl were excluded from the initial recruitment pool. Participants meeting the exclusion criteria were identified through review of medical records and parental reports during enrollment.

The data were evaluated separately for boys and girls, and means and standard deviations were applied. We used the Gilliam Autism Rating Scale (GARS-2) to determine the degree of autism and the Turkish Communication Development Inventory-II (TIGE-II) to measure communication and grammar skills.

The degree of ASD symptoms was determined using the GARS-2 [17]. GARS-2 consists of 42 items distributed across three subscales, each including 14 items. The scoring of the GARS-2 is done by summing the raw scores, converting them into percentages and standard scores, and calculating the Autistic Disorder Index (ADI).

The TIGE-II) [18] is a tool that measures early communication and grammar skills. It has been prepared according to the structure of the Turkish language, and its reliability and validity have been proven.

Age and body mass index (BMI) were examined as potential covariates. Since no significant correlations were found between these variables and craniofacial measurements, they were not included in the final regression analyses.

Measurements

3D scanning was performed using the 3DeVOK iReal M3 Color 3D Scanner after the children who wore glasses had removed them. Landmarks were placed according to established anatomical guidelines. Scans were obtained in a well-lit environment, with children standing motionless, relaxed, maintaining a neutral facial expression (lips slightly closed, eyes open), at 50 cm from the device. Each child was scanned multiple times to ensure that all relevant facial regions were accurately captured. A trained research assistant and clinician used Microsoft 3D Builder to set anthropometric landmarks on the 3D models, which were then verified by a second clinician. Any differences were resolved through consensus to ensure accuracy. To ensure measurement consistency, intra- and inter-observer reliability were assessed in a subset of 15 randomly selected scans. Intraclass correlation coefficients (ICCs) were calculated using a two-way random-effects model, with values ranging from 0.92 to 0.97, indicating excellent reliability.

To make the necessary measurements from the 3D models obtained, measurements were made using 3D modeling programs. The necessary anthropometric points (head circumference, lateral canthus distance, philtrum height, nasal tip height, mandibula height [distance from the lower lip middle line to the gnathion point], forehead height [the distance from the hairline to the glabella point], angulus oris distance) were marked on the model and measured (Figure 1). Head circumference was measured directly on each child using a non-stretchable tape at the widest part of the head, from the glabella to the occiput, following standard anatomical landmarks, with the child in a neutral position. Each measurement was repeated twice, and the average value was recorded. Measurement reliability was evaluated, yielding an ICC of 0.95, indicating excellent consistency.

Statistical Analysis

Analyses were conducted using SPSS 26.0 (SPSS Inc., Chicago, IL, USA). Data distribution was assessed using the Kolmogorov–Smirnov test. Variables with normal distribution are presented as

mean ± SD and analyzed with independent samples t-tests, with Levene's test applied to assess equality of variances; Cohen's d was reported as the effect size. Variables not following a normal distribution are presented as median [IQR] and analyzed using the Mann–Whitney U test, with rank-biserial correlation as the effect size.

Relationships between anthropometric measures (head circumference, lateral canthus distance, philtrum height, nasal tip height, mandibular height, forehead height, and angulus oris distance) and participant characteristics (gender, TIGE-II, GARS-2, and autism severity) were evaluated using Pearson's r for normally distributed data and Spearman's ρ for non-normally distributed data.

A two-tailed P<0.05 was considered statistically significant. Post-hoc power analysis indicated that detecting medium effect sizes (Cohen's d = 0.5 or r = 0.3) with 80% power would require ≥64 participants per group or ≥84 total. Given the current sample size (boys n = 50, girls n = 16), the study is underpowered for medium effects, and results should be interpreted with caution.

RESULTS

The mean and median ages of boys and girls were similar (boys: mean 7.46, median 7.00; girls: mean 7.50, median 7.50). Group comparisons for craniofacial measurements are presented in Table 1. Numerical values were not repeated in the text to avoid redundancy. According to these analyses, no significant gender differences were observed in most craniofacial parameters, whereas head circumference and forehead height differed significantly between boys and girls (Table 1).

Correlation analyses between GARS-2 scores and craniofacial measurements are shown in Table 2. In boys, a weak positive correlation was found between head circumference and GARS-2 (r =0.299, P=0.035). This effect size was interpreted as small, not strong. No other craniofacial parameter showed a significant association with GARS-2 scores in boys or girls. The relationship between head circumference and GARS-2 is illustrated in Figure 2.

Associations between TIGE-II scores and craniofacial measurements are presented in Table 3. No statistically significant correlations were observed in either boys or girls. In girls, lateral canthus distance showed a moderate negative correlation (r = –0.443), but it was not statistically significant (P=0.086).

Group differences in GARS-2 scores were examined using the independent samples t-test (Table 4). Variances were equal according to Levene's test, and boys and girls differed significantly in GARS-2 scores (P=0.020).

Nonparametric comparisons using the Mann–

TABLE 1. Results of the Kolmogorov–Smirnov test for Normality in Boys and Girls

Variable	Boys (n=50)	P-value (Boys)	Girls (n=16)	P-value (Girls)
Head circumference (cm)	53.60±2.14	0.187	52.13±2.17	0.166
Forehead height (mm)	38.95±5.75	0.200	45.7±2.04	0.176
Nasal tip height (mm)	12.60±1.92	**0.044**	12.80±2.04	0.200
Lateral canthus distance (mm)	87.22±5.52	0.200	86.26±5.29	0.200
Angulus oris distance (mm)	36.55±5.57	0.158	35.60±5.65	0.116
Mandibula height (mm)	27.44±3.87	0.200	25.96±4.84	0.144
Philtrum height (mm)	12.61±1.97	0.171	12.25±1.66	0.082
TIGE-II (score)	423.72±316.63	**0.001**	463.31±322.54	**0.001**
GARS-2 (score)	90.26±12.23	0.135	89.50±12.86	0.200

Data are presented as mean±standard deviation. TIGE-II, Turkish Communication Development Inventory-II; GARS-2, Gilliam Autism Rating Scale. Statistically significant P-values are shown in bold.

TABLE 2. Pearson Correlation Coefficients (r) and P-values for the Relationships Between Craniofacial Measurements in Boys

	Boys (n=50)		Girls (n=16)	
	r	P-value	r	P-value
Head circumference (cm)	**0.299**	**0.035**	0.190	0.689
Forehead height (mm)	-0.089	0.537	0.052	0.849
Nasal tip height (mm)	-0.129	0.372	-0.016	0.954
Lateral canthus distance (mm)	0.092	0.527	0.262	0.328
Angulus oris distance (mm)	0.191	0.184	0.015	0.957
Mandibula height (mm)	0.145	0.315	-0.007	0.978
Philtrum height (mm)	0.074	0.607	-0.090	0.740

Statistically significant P-value is shown in bold.

Whitney U test showed significant gender differences in head circumference (P=0.008) and forehead height (P=0.002), while other variables did not differ significantly between groups (Table 5). Distribution test P-values were removed to avoid confusion, as recommended.

DISCUSSION

In this study, we examined the relationship between anthropometric measurements and both autism severity and language development in children diagnosed with ASD. Our findings indicate that head circumference in boys showed a meaningful association with higher GARS-2 scores, suggesting that cranial growth patterns may be linked to symptom severity in males. In contrast, this relationship was not observed in girls, which may point to sex-specific neurodevelopmental trajectories influenced by hormonal, genetic, or structural factors. The comparable age distribution across genders suggests that these

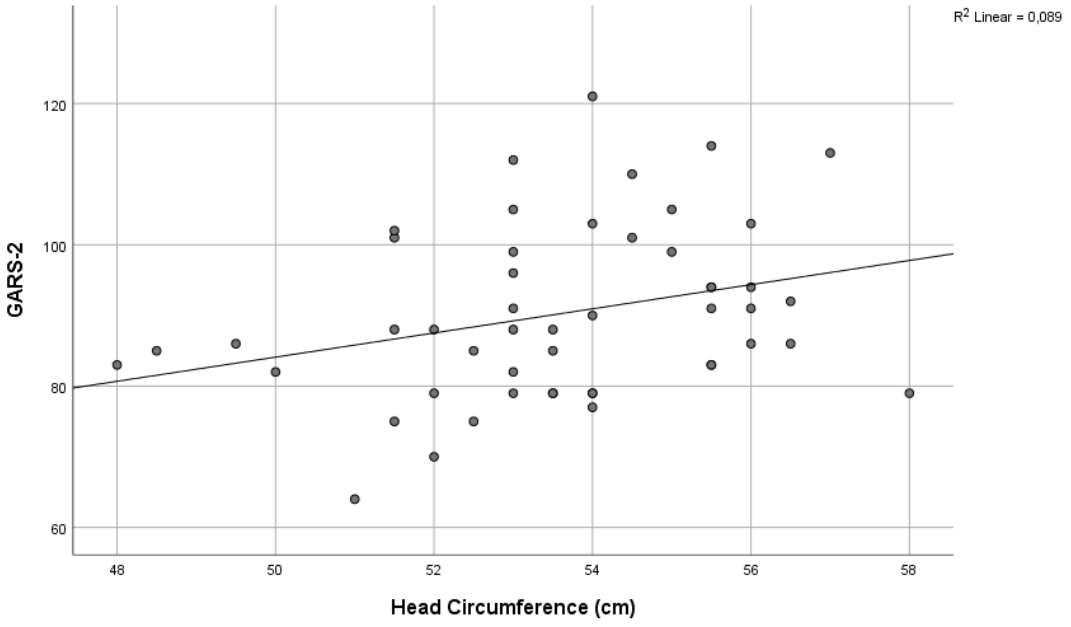

FIGURE 2. Relationship between head circumference and GARS-2 scores, as demonstrated by a scatter plot with fitted regression line. GARS-2, Gilliam Autism Rating Scale-2.

TABLE 3. Spearman's rho Correlation Coefficients (r) and P-values for the Relationships Between Craniofacial Measurements in Boys and Girls

	Boys (n=50)		Girls (n=16)	
	r	P-value	r	P-value
Head circumference (cm)	0.219	0.126	-0.161	0.553
Forehead height (mm)	0.116	0.422	-0.335	0.205
Nasal tip height (mm)	0.235	0.101	-0.035	0.899
Lateral canthus distance (mm)	0.126	0.385	-0.443	0.086
Angulus oris distance (mm)	0.178	0.216	0.068	0.804
Mandibula height (mm)	0.044	0.764	-0.050	0.853
Philtrum height (mm)	0.030	0.837	-0.201	0.455

differences are unlikely to be attributable to age-related craniofacial changes alone. However, considering that craniofacial morphology continues to change throughout development, longitudinal studies may provide deeper insight into these developmental mechanisms.

The gender-based differences observed in our sample support the notion that boys may exhibit earlier divergence in craniofacial and neurological development compared to girls. Boys tended to show larger head circumferences and lower forehead heights,

TABLE 4. Comparison of GARS-2 Scores Between Boys and Girls According to Craniofacial Measurements

Variables	Gender	Data	F	P-value	t	P-value
Head circumference (cm)	Girls	52.13±2.17	0.143	0.706	-2.395	**0.020**
	Boys	53.60±2.14				
Forehead height (mm)	Girls	45.71±6.88	**0.002**	0.967	3.895	**<0.001**
	Boys	38.95±5.75				
Nasal tip height (mm)	Girls	12.80±2.04	**<0.001**	0.986	0.192	0.849
	Boys	12.69±1.91				
Lateral canthus distance (mm)	Girls	86.26±5.29	0.227	0.636	-0.616	0.540
	Boys	87.22±5.60				
Angulus oris distance (mm)	Girls	35.60±5.65	0.086	0.770	-0.592	0.556
	Boys	36.55±5.57				
Mandibula height (mm)	Girls	25.96± (4.84)	0.391	0.534	-.1.247	0.217
	Boys	27.44± (3.87)				
Philtrum height (mm)	Girls	12.25±1.66	0.055	0.815	-0.660	0.512
	Boys	12.61±1.96				
GARS-2 (score)	Girls	89.50±12.86	0.161	0.690	-0.214	0.831
	Boys	90.26±12.23				

Data are presented as mean±standard deviation. GARS-2, Gilliam Autism Rating Scale. Levene's test P-value assesses homogeneity of variances. Independent samples *t*-test P-values < 0.05 indicate statistically significant differences between genders. Statistically significant P-values are shown in bold.

TABLE 5. Analysis Results of TIGE-II Scores of Boys and Girls According to All Variables

	Mann-Whitney U	Z statistic	P-value
Head circumference (cm)	223.50	-2.650	**.008**
Forehead height (mm)	188.00	-3.172	**.002**
Nasal tip height (mm)	376.50	-.352	.725
Lateral canthus distance (mm)	343.50	-.845	.398
Angulus oris distance (mm)	361.00	-.584	.560
Mandibula height (mm)	286.00	-1.706	.088
Philtrum height (mm)	384.00	-.239	.811
TIGE-II	356.50	-.677	.468

TIGE-II, Turkish Communication Development Inventory-II. Statistically significant P-values are shown in bold.

and this forehead height difference has been further discussed in terms of neurodevelopmental mechanisms. Specifically, variations in forehead height may reflect altered frontal bone growth and atypical early frontal cortex overgrowth, which could contribute to craniofacial differences observed in ASD [19- 22]. Although no significant association emerged between facial measurements and language development (as measured by TIGE-II scores) in either gender, the weak negative trend between lateral canthus distance and language scores in girls may be noteworthy in future studies. These findings reinforce the idea that ASD-related craniofacial markers may present differently across genders, suggesting the presence of distinct neurodevelopmental pathways [23-25].

Our results are consistent with previous research documenting morphological variability in ASD. Tripi *et al*. [26] reported sex-related differences in head circumference and forehead height, similar to the patterns observed in our study. Tan *et al*. [27] identified broader facial dimensions in males with ASD and later demonstrated craniofacial asymmetry in parents of affected children [28], supporting the heritability of these features. Hammond *et al*. [29] linked nasal width and facial asymmetry to abnormal craniofacial co-development. Although we did not examine asymmetry, the craniofacial patterns identified in our sample may reflect comparable developmental deviations. Additionally, due to the absence of a neurotypical control group in our study, it remains unclear whether these craniofacial traits differ from normal population patterns or represent ASD-specific characteristics. Normative pediatric craniofacial studies report sexual dimorphism in head and facial dimensions, which may overlap with or differ from ASD-related patterns.

Methodological considerations must be acknowledged when interpreting these findings. The pronounced imbalance in gender distribution - 50 boys compared to 16 girls - combined with the lack of an a priori power analysis represents an important limitation. Post-hoc evaluations indicated insufficient power to detect moderate associations, especially within the female subgroup. The absence of a neurotypical control group substantially limits the interpretability of whether the identified craniofacial differences, particularly forehead height and head circumference, are characteristic of ASD or fall within typical developmental variation. These elements highlight the need for replication in larger, gender-balanced studies.

Strengths and Limitations

This study provides a sex-stratified analysis of craniofacial morphology in ASD, contributing to the understanding of biological heterogeneity and sex-specific neurodevelopmental trajectories. The use of standardized clinical instruments (GARS-2 and TIGE-II) ensures data reliability and comparability. Furthermore, by identifying specific correlations between head circumference and symptom severity in males, the study highlights potential physical markers for ASD phenotyping.

The primary limitation is the small and imbalanced sample size (50 boys vs. 16 girls), which reduces statistical power—particularly in detecting

associations within the female subgroup. The absence of a neurotypical control group precludes the ability to distinguish ASD-specific traits from normative sexual dimorphism. Additionally, the cross-sectional design prevents causal inferences regarding cranial growth and symptom progression. Finally, the age-limit of the TIGE-II scale may have affected the precision of language assessments in older participants, and the study did not account for potential genetic or environmental confounders.

CONCLUSION

The present study indicates that specific craniofacial characteristics particularly head circumference and forehead height—may be associated with autism severity in boys with ASD. These findings suggest that craniofacial morphology may reflect underlying sex-specific neurodevelopmental processes rather than being merely descriptive features. Given the absence of a neurotypical control group, it remains unclear whether these craniofacial patterns are unique to ASD or represent normal sexual dimorphism, highlighting the need for careful interpretation. Although preliminary, these results emphasize the potential value of craniofacial measurements as complementary markers in research exploring biological heterogeneity in ASD. Future studies should employ longitudinal designs, incorporate neuroimaging and genetic analyses, and include typically developing controls to determine whether these craniofacial traits have diagnostic, prognostic, or mechanistic relevance in early neurodevelopmental assessment.

Ethics Approval and Consent to Participate

This study was approved by the Istanbul University-Cerrahpaşa Clinical Research Ethics Committee (Decision No: 2024/56267; date: 12.12.2024). All procedures were conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from all parents.

Data Availability

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

Authors' Contribution

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

Conflict of Interest

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

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Generative Artificial Intelligence Statement

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

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