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Effectiveness and Safety of Suction-Assisted Technologies in Pediatric Endourological Stone Surgery: A Systematic Review by the EAU-YAU Pediatric Urology Working Group

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ABSTRACT

Objective: Although new suction/vacuum technologies have been tested in adults in recent years, they remain uncommon in pediatric practice. This systematic review aimed to analyze the existing literature on the effectiveness and safety of new suction technologies for pediatric endourological stone interventions.

Materials and Methods: We conducted a systematic review (PROSPERO CRD420261335417) following the PRISMA 2020 and Cochrane guidelines, searching PubMed, Embase, MEDLINE, Web of Science, and Scopus to identify all published reports on new suction/vacuum technologies for pediatric endourological stone interventions through February 2026. The primary outcomes were intraoperative and postoperative complications and stone-free rates (SFRs). Secondary outcomes included operative time, hospital stay, and the need for ancillary procedures. We used the MINORS tool to assess the risk of bias in nonrandomized cohort studies.

Results: The review included a total of 367 patients (370 renal units) aged 1–17 years who underwent endourological stone surgery. The vacuum-assisted mini-PCNL group had a higher SFR of 81.3–100% and lower complication and reintervention rates of 0–27.8% and



0–12.5%, respectively, compared with conventional mini-PCNL. Furthermore, a higher SFR of 85.7–100% was observed with RIRS using FANS-UAS compared with an SFR of 60–88% in children who underwent conventional RIRS. The former group also had a lower reintervention rate (0–9.5% vs. 0–40%) and fewer grade III–IV complications (0% vs. 8.7%).

Conclusion: Current evidence, although limited and mainly based on observational studies, suggests that suction-assisted technologies may improve stone clearance and maintain acceptable safety profiles in selected pediatric endourological stone interventions. However, the absence of randomized trials, heterogeneous outcome definitions, and limited long-term follow-up prevent definitive conclusions.

Keywords: Children, flexible and navigable ureteral access sheath, kidney stones, percutaneous nephrolithotomy, RIRS, suction, vacuum.

INTRODUCTION

The development of new suction techniques in endourological procedures, such as percutaneous nephrolithotomy (PCNL) and retrograde intrarenal surgery (RIRS), has made these procedures more efficient and safer, particularly for adult patients.^{1,2} These technological advances have improved stone fragment clearance by enabling greater control of intrarenal pressure and providing better visualization during the procedure. Consequently, these technologies can achieve higher stone-free rates (SFRs), lower complication rates, and shorter operative times.^{1,2} The integration of suction ureteral access sheaths into RIRS improves the surgeon's visibility during the fragmentation of small stone fragments by minimizing the "snow globe effect."^{3,4} This suggests an increasing trend toward the use of RIRS in the endoscopic treatment of larger stone burdens. On the other hand, recent pediatric series suggest that vacuum-assisted mini-PCNL is a feasible and safe procedure for treating stones larger than 2 cm, even in very young children.⁵ Therefore, technological advances in minimally invasive endourological instruments have led to a reduction in the use of shock wave lithotripsy (SWL) in children in recent years.⁴

Irrigation and suction technologies were initially developed for adult patients but have recently begun to be integrated into pediatric endourological equipment. Consequently, new surgical outcomes are being reported in the pediatric literature.^{5–7} These advantages may be particularly important in children, in whom the smaller collecting system volume, increased vulnerability to elevated intrarenal pressure, infection-related risks, and technical limitations of miniaturized instruments make efficient fragment evacuation challenging. Suction-assisted systems may therefore improve visibility and stone clearance while potentially reducing operative

time and fluoroscopy exposure. Thus, the primary aim of this systematic review is to investigate the effectiveness and safety of new suction technologies for endourological interventions in children and adolescents with kidney or ureteral stones in terms of SFRs and complications according to the Clavien–Dindo classification. Secondary outcomes relate to surgical efficiency, including operative time, length of hospital stay, and the need for ancillary procedures.

MATERIALS AND METHODS

Data Sources and Search

Following registration of the study protocol in PROSPERO (registration ID: CRD420261335417), a review of the English-language literature was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 recommendations.⁸ All published reports on new suction/vacuum technologies for pediatric endourological stone interventions (PCNL and RIRS) up to February 1, 2026, were identified by searching electronic databases, including PubMed, Embase, Ovid MEDLINE, Web of Science, and Scopus. All procedures performed in our study were in accordance with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Studies were assessed for inclusion or exclusion using a predefined PICO (population, intervention, comparison, and outcome) approach (Appendix 1).

Study Selection

Studies reporting on the effectiveness and safety of new suction technologies for pediatric endourological stone interventions were eligible for inclusion (Appendix 1). Thus, only full-text articles that met the inclusion criteria according to the predefined PICO framework were included in the systematic review.

The exclusion criteria were as follows:

- Articles involving only children or adolescents with accompanying congenital anatomical abnormalities (e.g., UPJ obstruction, horseshoe kidney, ectopic kidney, duplex system, ureterocele, or ectopic ureter), because these conditions are strong confounders of SFR and complication outcomes
- Other systemic abnormalities (e.g., vertebral anomalies or scoliosis)
- Planned staged surgery for urinary tract stones
- Inability to complete at least 1 month of follow-up after surgery to assess residual fragments following a previous intervention (follow-up <1 month)
- Previous ureteral injury, known ureteral anomalies, or previous ureteral reconstruction (e.g., augmented bladder or kidney transplantation)
- Any medical history that would prevent the use of contrast medium during surgery
- Studies with inadequate outcome reporting or insufficient patient data
- Case reports, case series with <10 patients, letters, editorials, author replies, meeting abstracts, systematic reviews, narrative reviews, and meta-analyses

Data Collection and Extraction

The following search strategy was used: ("suction" OR "vacuum" OR "aspiration" OR "ureteral access sheath" OR "ClearPetra" OR "FANS-UAS" OR "DISS") AND ("RIRS" OR "retrograde intrarenal surgery" OR "ureteroscopy" OR "flexible ureteroscopy" OR "percutaneous nephrolithotomy" OR "PCNL" OR "Endoscopic Combined Intrarenal Surgery" OR "ECIRS") AND ("kidney stones" OR "urolithiasis" OR "renal stones" OR "ureteral stones" OR "ureteric stones") AND ("pediatric" OR "children" OR "paediatric" OR "adolescents").

All members of the European Association of Urology (EAU) Young Academic Urology (YAU) Paediatric Urology Working Group used Covidence systematic review software (Veritas Health Innovation, Melbourne, Australia) to screen all retrieved articles. Initially, the titles and abstracts of all 281 identified studies were retrieved and assessed through double screening in accordance with the prespecified inclusion criteria. Subsequently, the full texts of potentially eligible studies were assessed by all members of the EAU-YAU Paediatric Urology Working Group. Two investigators (I.S. and N.B.) independently extracted data from the eight

included articles. Any inconsistencies arising during title and abstract screening, full-text review, or data extraction were resolved through discussion, with consultation from two independent members of the EAU-YAU Paediatric Urology Working Group (M.I.D. and A.K.). The PRISMA flowchart showing the included articles is presented in Appendix 2.

Data were extracted using a standardized form, including information on study identification (first author, year of publication, and country), participants (age, sex, and number of patients/renal units), study design (e.g., prospective or retrospective cohort or RCT), diagnostic imaging (e.g., KUB, US, CT, or IVP), stone characteristics (size, location, volume, and density [Hounsfield units]), patient characteristics (e.g., genetic etiology, cystinuria, obesity, or metabolic disorders), patient position (e.g., prone, supine, lithotomy, or modified), surgical modality (e.g., super-mini percutaneous nephrolithotomy [SMP], vacuum-assisted mini-PCNL, standard PCNL, RIRS, or ureteroscopy [URS]), access sheath size, if used, radiation guidance (e.g., fluoroscopy, ultrasound, combined guidance, or fluoroles), lithotripsy modality (e.g., Ho:YAG laser, thulium fiber laser, pneumatic, or ultrasonic), suction modality (e.g., flexible and navigable suction ureteral access sheath [FANS-UAS], direct in-scope suction [DISS] ureteroscopy, or suction-evacuation sheath), operative time, irrigation details (e.g., type, flow rate, and pressure, if available), postoperative drainage (DJ stent, nephrostomy, both, or tubeless), intraoperative complications according to the Clavien–Dindo classification (e.g., none, bleeding, ureteral injury, collecting system perforation, intraoperative sepsis, or other), postoperative complications according to the Clavien–Dindo classification (e.g., urinary tract infection, bleeding requiring transfusion, bleeding requiring embolization, urinary sepsis, need for reoperation, or other), definition of stone-free status, definition of clinically insignificant residual fragments (CIRFs), stone-free rate (SFR), follow-up period, ancillary procedures (e.g., SWL, RIRS, or second-look PCNL), follow-up imaging (e.g., KUB, US, or CT), crystallographic or metabolic analysis, and key findings.

We contacted the corresponding authors by email to request access to any missing or incomplete data from the included studies. Some missing data were obtained in this way. Based on the responses received, data not reported in the existing articles were also recorded as "N/A" in the tables.

This systematic review included eight studies that provided relevant data on the topic. Because the included articles exhibited methodological and clinical heterogeneity, a meta-analysis was deemed inappropriate. Instead, a synthesis of the data was performed.

Risk of Bias and Quality Assessment

The risk of bias in each included study was independently evaluated by two investigators (I.S. and N.B.). The quality of the included studies was assessed using the validated Methodological Index for Non-Randomized Studies (MINORS) tool for nonrandomized cohort studies (maximum score, 16 points) and comparative studies (maximum score, 24 points). Any disagreements between the reviewers were resolved through discussion and consensus with two independent members of the EAU-YAU Paediatric Urology Working Group (M.I.D. and A.K.).

RESULTS

Following a search of the PubMed, Embase, Ovid MEDLINE, Web of Science, and Scopus databases, eight of 281 articles were found to be eligible for inclusion in this review.^{5–13} The main demographic and clinical characteristics of the patients in these articles are presented in Table 1 and Appendix 3. The articles included two prospective cohort studies and six retrospective cohort studies. These eight articles reported on a total of 367 patients (370 renal units) who underwent endourological stone surgery. The included studies comprised both single-center and multicenter cohorts, with participating centers from China, Türkiye, Italy, Spain, India, Singapore, the United Kingdom, and Uzbekistan. The patients had an average age of 7 years, ranging from 1 to 17 years. The overall male-to-female ratio across all studies was 217:150 (59.1% male). These patients were treated using various methods, including vacuum-assisted mini-PCNL in 139 patients, RIRS with a flexible and navigable suction ureteral access sheath (FANS-UAS-RIRS) in 140 patients, and RIRS with a conventional ureteral access sheath in 88 patients.

Stone-Free Rate

The SFR 1 month after the first attempt at vacuum-assisted mini-PCNL in children with a median age of 4.7 years (range, 0.5–15 years) was reported as 81.3–100% for a median stone size of 16.9 mm (range, 8–48 mm).^{5,6,13} Three studies reported an initial SFR of 60–88% 1 month after the first RIRS attempt in children with a median stone size of 15.8 mm (range, 7–28 mm) and a median age of 8.9 years (range, 1.5–14 years), in whom RIRS with a conventional ureteral access sheath (UAS) was used.^{5,10,12} On the other hand, five studies showed a higher single-stage SFR of 85.7–100% with RIRS using FANS-UAS for a median stone size of 10.7 mm (range, 9–23 mm) in children with a median age of 7.5 years (range, 1–17 years), compared with conventional RIRS.^{7,9–12} Details are provided in Appendices 3 and 4.

Intraoperative and Postoperative Complications

No significant intraoperative complications were reported with vacuum-assisted mini-PCNL. However, postoperative Clavien–Dindo grade I–II complications, including fever (6.3–

27.8%) and hematuria (0–8.1%), and grade IIIa complications, including urinary extravasation requiring nephrostomy (2.7–5.6%), were observed.^{5,6,13}

Intraoperatively, patients who underwent RIRS with FANS-UAS experienced Traxer grade 1 ureteral injury (0–14.3%) and grade 1 calyceal neck or forniceal injury during sheath placement (0–4.3%), whereas Traxer grade 1 ureteral injury (0–8.6%) and grade 2 ureteral injury (0–4.3%) were observed in patients who underwent RIRS with a conventional UAS.^{7,9–12}

Postoperatively, patients who underwent RIRS with FANS-UAS experienced similar Clavien–Dindo grade I–II complications, such as fever, pain, UTI, nausea/vomiting, hematuria, and incontinence due to stent migration, at rates of 0–19%, compared with those who underwent RIRS with a conventional UAS, who experienced fever, UTI, persistent hematuria, and pain at rates of 0–17.4%. However, more serious grade III–IV complications were observed in the latter group, including urethral stricture at a rate of 8.7%.^{7,9–12}

Operative Time, Hospital Stay, and Reintervention Rate

In patients who underwent vacuum-assisted mini-PCNL, the median operative time was 34.2 minutes (range, 27–167 minutes), and the median hospital stay was 2.8 days (range, 1–7 days). The reintervention rate was 0–12.5%.^{5,6,13}

On the other hand, the studies found that patients who underwent RIRS with FANS-UAS had a shorter operative time (median, 55.5 minutes [range, 26–86 minutes] vs. 82.7 minutes [range, 46–167 minutes]), a shorter hospital stay (median, 1.9 days [range, 1–3.4 days] vs. 3.1 days [range, 1–4 days]), and a lower reintervention rate (0–9.5% vs. 0–40%) than those who underwent RIRS with a conventional UAS.^{7,9–12}

Risk of Bias Assessment

The risk-of-bias summary for the eight included articles is presented in Appendix 5. Based on the MINORS assessment, four studies achieved relatively higher methodological quality scores.^{5,10,12,13} The remaining four studies showed limitations mainly related to retrospective design, absence of prospective sample size calculation, nonstandardized outcome assessment, and limited follow-up.^{6,7,9,11} These studies also raised concerns primarily related to data collection, sample size calculation, and outcome measurement.^{6,7,9,11}

DISCUSSION

The aim of this review was to investigate the effectiveness and safety of new suction technologies for pediatric endourological stone interventions. As most articles in the literature have focused on adult patient populations, comparative studies involving pediatric patients are extremely rare. The two suction

Table 1. Demographic characteristics of patients in the included articles								
Author, year [ref.]	Study period	Country	Study design	Total number of patients/ renal units	Mean/median age at admission	Male/female ratio, n	Patient group	Diagnostic imaging
Collura, et al., 2024 ⁵	November 2018–September 2022	Italy	Retrospective cohort study	30 patients/30 renal units	Median, 6.6 years (range, 1–12)	8/7	Vacuum-assisted mini-PCNL (n=15)	Noncontrast CT
					Median, 7.7 years (range, 1.5–14)	8/7	RIRS (n=15)	Noncontrast CT
Gallioli et al., 2022 ⁶	January 2017–December 2018	Italy and Spain	Prospective cohort study	13 patients/16 renal units	Median, 119 months (IQR, 97–160)	8/5	Semi-closed-circuit vacuum-assisted mini-PCNL (n=13)	CT, US, or KUB
Gauhar et al., 2024 ⁷	September 2023–May 2024	Singapore, Spain, the UK, India, Türkiye, Italy, China, and Uzbekistan	Retrospective cohort study	50 patients/50 renal units	Mean, 6.6±3.38 years (min, 1; max, 15)	33/17	FANS-UAS RIRS (n=50)	Low-dose noncontrast CT
Peng et al., 2024 ⁹	January 2022–November 2023	China	Retrospective cohort study	21 patients/21 renal units	Mean, 9.6 years (min, 6; max, 17)	11/10	Tip-flexible pressure-controlling ureteral access sheath (TFPC-UAS) (n=21)	US, CT, or IVP
Turedi et al., 2024 ¹⁰	January 2021–August 2024	Türkiye	Retrospective cohort study	46 patients/46 renal units	10.2±6.1 years	12/11	FANS-UAS RIRS (n=23)	CT
					9.2±4.8 years	13/10	Conventional UAS RIRS (n=23)	CT
Vaddi et al., 2025 ¹¹	October 2023–November 2024	India and Türkiye	Prospective cohort study	21 patients/21 renal units	Median 12.00 months (IQR, 8.00-21.25)	11/10	FANS-UAS RIRS (n=21)	Low-dose noncontrast CT and KUB
Wang et al., 2025 ¹²	January 2023–August 2025	China	Retrospective cohort study	75 patients/75 renal units	9.9±5.8 years (2–14)	13/12	FANS-UAS RIRS (n=25)	Low-dose noncontrast CT
					9.2±4.9 years (2–14)	29/21	Conventional UAS RIRS (n=50)	Low-dose noncontrast CT
Zhao et al., 2017 ¹³	September 2014–April 2016	China	Retrospective cohort study	111 patients/111 renal units	3.9±3.5 years (0.5–15)	71/40	Super-mini-PCNL (n=111)	N/A

CT: Computed tomography; FANS-UAS: Flexible and navigable suction ureteral access sheath; IQR: Interquartile range; IVP: Intravenous pyelography; KUB: Kidney–ureter–bladder radiography; Max: Maximum; Min: Minimum; N/A: Not applicable; PCNL: Percutaneous nephrolithotomy; Ref: Reference; RIRS: Retrograde intrarenal surgery; TFPC-UAS: Tip-flexible pressure-controlling ureteral access sheath; UAS: Ureteral access sheath; UK: United Kingdom; US: Ultrasonography.

CT: Computed tomography; FANS-UAS: Flexible and navigable suction ureteral access sheath; IQR: Interquartile range; IVP: Intravenous pyelography; KUB: Kidney–ureter–bladder radiography; Max: Maximum; Min: Minimum; N/A: Not applicable; PCNL: Percutaneous nephrolithotomy; Ref: Reference; RIRS: Retrograde intrarenal surgery; TFPC-UAS: Tip-flexible pressure-controlling ureteral access sheath; UAS: Ureteral access sheath; UK: United Kingdom; US: Ultrasonography.

modalities, vacuum-assisted PCNL and FANS-UAS RIRS, are used for different stone-burden scenarios and were therefore analyzed separately; no direct comparison between them was possible based on the available data. In addition, because of the methodological and clinical heterogeneity among the eight included articles, a narrative synthesis of the data was conducted rather than a meta-analysis.

Two main strategies have been developed for applying suction during PCNL: through sheaths or by integrating suction into an energy device. Both techniques have been found to decrease intrarenal pressure, which may prevent pyelorenal backflow, fluid reabsorption, and bacterial translocation.² Although sheath size may affect intrarenal pressure to some extent, suction sheaths and suction nephroscopes help provide continuous flow from the renal cavities to the outside by creating direct negative pressure that balances the inflow. This mechanism has been reported to reduce both intrarenal pressure and infectious complication rates.² For this purpose, several different suction techniques have been developed for PCNL. “Double-sheath vacuum suction” involves separating the inflow and outflow channels using two suction sheaths (16 Fr and 20 Fr). This creates a one-way flow channel that prevents the obstruction that may occur in single-sheath systems.¹⁴ Another suction technique described for PCNL is “SuperPerc.”¹⁵ This technique involves using a 10/12-Fr suction sheath together with a pediatric ureteroscope as a nephroscope. Although different techniques are available, the use of negative pressure during suction/aspiration has two fundamental common effects. First, it prevents renal backflow caused by overdistension and, consequently, pyelovenous and pyelolymphatic backflow, which can lead to bacteremia, sepsis, and fever in cases of infected stones.⁴ Second, it enhances fragment extraction through the whirlpool effect.⁴

Studies involving the use of different suction/aspiration sheaths during PCNL in adults have reported an SFR of 71.3–100%, an overall complication rate of 1.5–38.9%, an infection rate of 0–27.8%, and a bleeding rate of 0–8.9%.¹⁶ These techniques have become widespread in the pediatric population only in recent years; therefore, pediatric outcomes have only recently begun to be reported, with a higher SFR of 81.3–100% and lower complication rates, including an overall complication rate of 0–27.8%, fever in 6.3–27.8%, hematuria in 0–8.1%, and grade IIIa complications such as urinary extravasation requiring nephrostomy in 2.7–5.6%.^{5,6,13} Cross-modality SFR comparisons between vacuum-assisted PCNL (median stone size, 16.9–32 mm) and FANS-UAS RIRS (median stone size, 10.7–19.8 mm) should be interpreted cautiously because the two groups were not treated for equivalent stone burdens. More reliable evidence of efficacy comes from within-modality comparisons: both Turedi et al.¹⁰ and Wang et

al.¹² used propensity-matched or similarly distributed cohorts comparing FANS-UAS with conventional UAS for stones of comparable size, and both consistently demonstrated higher SFRs and lower reintervention rates with suction.

The widespread use of super-mini-PCNL (SMP) has led to the implementation of suction technology in pediatric PCNL cases.^{13,14} It has been demonstrated that simply switching from older- to newer-generation instruments and adding suction can extend the size limits for stone removal with SMP (a 7–8-Fr nephroscope with a 12–14-Fr sheath) to include large kidney stones measuring 2–4 cm.^{13,14} It has been reported that using the ClearPetra® suction sheath can reduce the PCNL access size to 14 Fr for ultra-mini-PCNL procedures. This enables a single-tract procedure for pediatric staghorn stones without complications.¹⁷ This suggests that suction could make PCNL less invasive without compromising effectiveness or safety. Of note, Zhao et al.¹³ used a metal suction-evacuation sheath rather than the ClearPetra® system used in other PCNL studies; therefore, the outcomes should be interpreted with this technical heterogeneity in mind.

The role of suction in RIRS for the pediatric population has also gained attention in recent years. A common method of achieving effective aspiration during RIRS is the direct in-scope suction (DISS) technique. The working channel of the scope allows alternating irrigation and aspiration through the flexible ureteroscope.¹⁸ Using smaller DISS scopes with this technique reduces intrapelvic pressure during stone dusting and improves visibility by eliminating the “snow globe effect.”¹³ Although DISS currently has limited capacity for removing larger stone fragments and may be affected by fragments becoming lodged in the working channel, it is anticipated that technological advances will make these procedures more efficient and may eliminate the need for a UAS.¹⁹

Another innovative technology is the use of flexible and navigable suction UASs. These devices can continuously aspirate dust and larger particles from both the renal pelvis and individual renal calyces because they are flexible and can reach every part of the collecting system.^{7,20} Owing to the flexibility of their tips, these suction UAS devices can closely follow the movement of the ureteroscope. This enables surgeons to aspirate dust and particles from all parts of the kidney.^{7,20} In particular, the thulium fiber laser (TFL) produces smaller dust particles than holmium lasers and causes substantially less retropulsion. This prevents the laser energy from continuously dispersing debris and allows the suction system to actively evacuate the dust. Therefore, combining TFL with active suction access sheaths may help eliminate the so-called “snow globe effect” and maintain clear visibility. Matching the super-pulsed, high-frequency dusting capabilities of TFL with continuous or intermittent vacuum

aspiration enables surgeons to continuously clear floating stone dust from the visual field.^{7,10,11} Additional advantages include a reduced need for basket extraction and improved removal of fine dust. A higher single-stage SFR of 85.7–100% was observed with RIRS using FANS-UAS compared with an SFR of 60–88% in children who underwent RIRS with a conventional UAS. The former group also had a lower reintervention rate (0–9.5% vs. 0–40%) and fewer grade III–IV complications (0% vs. 8.7%).^{7,9–12} The unusually high reintervention rate in the RIRS group may be attributed to a higher stone burden in one of the studies.⁵ Even in children younger than 5 years, a low incidence of high-grade complications has been reported during follow-up when more miniaturized equipment was used, including a 9.5/11-Fr UAS and a 7.5-Fr flexible URS.²¹

The ongoing miniaturization of endoscopic instruments, coupled with the adaptation of new suction systems initially designed for adults to pediatric use, is undoubtedly paving the way for innovations in pediatric endourology. Although suction techniques have been tested in adults, they remain uncommon in pediatric practice. Studies using suction- or vacuum-assisted mini-PCNL, SMP, and RIRS with FANS-UAS or DISS have demonstrated the safety and effectiveness of these techniques in children.^{5,10,12,22} Most included studies reported favorable SFRs with suction-assisted technologies; however, the limited number of comparative studies prevents firm conclusions regarding their superiority over conventional approaches. Furthermore, to our knowledge, no studies have specifically compared two suction techniques or technologies in children.

Despite our thorough approach, this review is not without limitations. First, because of our strict inclusion criteria, only a small number of studies were eligible. The included studies differed in methodology, patient demographics, and outcome measures, resulting in heterogeneity in the data. Thus, generalization of the results is difficult because of variability in the definition of SFR, the use of equipment of different sizes, differences in laser type and energy settings, and inconsistent reporting of outcomes by age group across studies. Second, the small number of patients treated with each technique makes comparisons difficult. It was not possible to directly compare mini-PCNL procedures performed with and without suction or procedures using laser or ultrasonic lithotripsy with and without suction. Therefore, the lack of comparable studies using similar techniques makes it difficult to draw firm conclusions. Third, although the included studies were conducted in different countries, the small number of studies highlights the need for additional multicenter studies to strengthen the global applicability of the clinical outcomes. In addition, the follow-up duration and imaging modalities used to determine stone-free status varied among studies. Another limitation of the included studies evaluating RIRS is the lack of

comparison with sheathless RIRS. None of the included studies reported systematic intrarenal pressure measurements; therefore, the pressure-reducing benefit of suction in children, which may differ from that in adults because of smaller collecting system volumes, remains to be quantified. Finally, the inclusion of only eight articles significantly limits our ability to draw definitive conclusions regarding the efficacy and safety of suction-assisted techniques in children. Although excluding children with congenital urinary tract anomalies may improve the internal validity of the study, it limits the generalizability of the findings to real-world pediatric stone populations.

CONCLUSION

This systematic review suggests that new suction technologies, such as vacuum-assisted mini-PCNL and RIRS with FANS-UAS, are promising and appear feasible in selected pediatric patients, with acceptable complication rates and satisfactory stone-free rates. These methods also show promise for larger stone burdens. However, larger multicenter comparative studies are needed to assess the potential of these technologies in pediatric stone management. The lack of standardization in outcome reporting also needs to be addressed.

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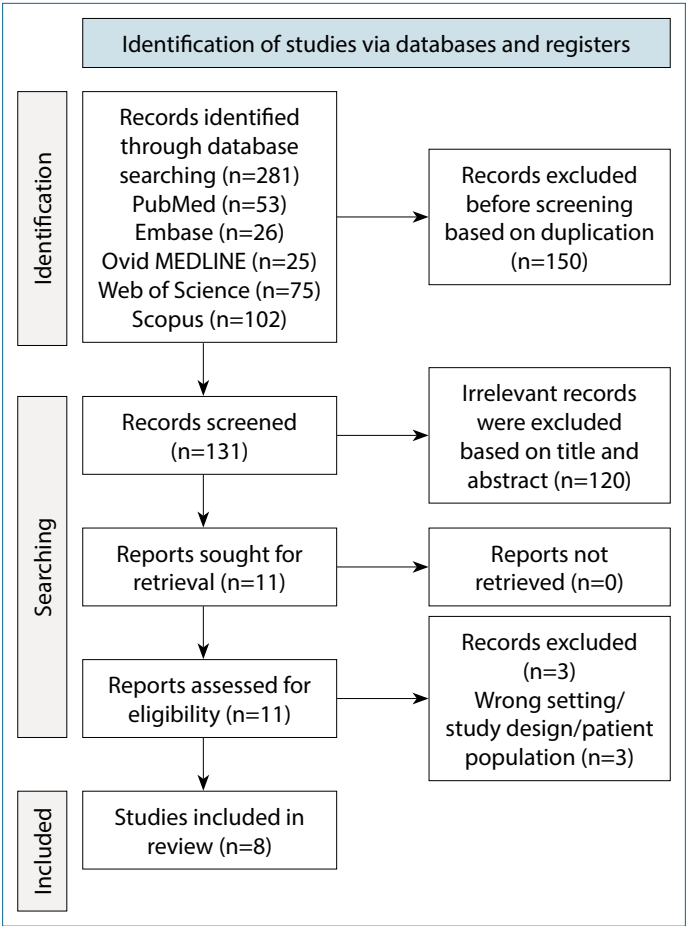
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Appendix 1. PICO criteria used to create the data extraction sheet

PICO component	Criteria
Patients (P)	Children and adolescents younger than 18 years with kidney or ureteral stones
Intervention (I)	Use of different suction/vacuum techniques during PCNL or RIRS
Comparators (C)	Suction vs. no suctionConventional endourological techniques without suction or nonsuction arms within comparative studies using the same surgical modalitySubgroup analysis, if available: comparison between different suction technologies (e.g., FANS-UAS vs. DISS)
Outcomes (O)	Primary outcomes: Complications and stone-free rates (1-month SFR or 3-month SFR, when available)Secondary outcomes: Operative time, hospital stay, and the need for ancillary procedures

DISS: Direct in-scope suction; FANS-UAS: Flexible and navigable suction ureteral access sheath; PCNL: Percutaneous nephrolithotomy; PICO: Population, intervention, comparator, and outcome; RIRS: Retrograde intrarenal surgery; SFR: Stone-free rate.



Appendix 2. The PRISMA flowchart.

Appendix 3. Stone and surgical characteristics in the included articles

[illegible]

Charrière; DJ: Double-J stent; FANS: Flexible and navigable suction; FANS-UAS: Flexible and navigable suction ureteral access sheath; Fr: French; FUAS: Flexible ureteral access sheath; Hz: Hertz; HU: Hounsfield unit; J: Joule; Max: Maximum; Min: Minimum; N/A: Not applicable; PCNL: Percutaneous nephrolithotomy; Ref: Reference; RIRS: Retrograde intrarenal surgery; TPFC-UAS: Tip-flexible pressure-controlling ureteral access sheath; UAS: Ureteral access sheath; W: Watt; YAG: Yttrium aluminum garnet.

Appendix 4. Surgical outcomes and complications in the included articles

Author, year [ref.]	Patient group	Irrigation details (e.g., type, flow rate, and pressure, if available)	Operative time (minutes)	Postoperative drainage	Intraoperative complications according to the clavien–dindo classification	Postoperative complications according to the clavien–dindo classification	Hospital stay (days)	Follow-up period (months)	Definition of SFR or CIRF	Stone-free rate	Ancillary procedures	Follow-up imaging	Stone or metabolic analysis
Collura et al., 2024 ⁵	Vacuum-assisted mini-PCNL (n=15)	N/A	Median, 85.3 min (range, 76–112)	Double-J stent or small-caliber nephrostomy	None	None	Median, 3.7 days (range, 3–5)	1 month	SFR: <2 mm; CIRF: 2–4 mm	1st month: 15/15 (100%), including 2/15 with CIRF	None	US	Cystine: 2/15 (13.3%); hypercalciuria: 1/15 (6.7%)
	RIRS (n=15)	Continuous irrigation of the collecting system while maintaining low intrarenal pressure to facilitate the passage of instruments and subsequent retrieval of stone fragments	Median, 150.6 min (range, 132–167)	Double-J stent	None	None	Median, 3.2 days (range, 3–4)	1 month	SFR: <2 mm; CIRF: 2–4 mm	1st month: 9/15 (60%)	Second RIRS: 6/15 (40%); residual fragments had a median diameter of 7 mm (range, 6–9)	US	Cystine: 3/15 (20%); hyperoxaluria: 3/15 (20%); hypocitraturia: 3/15 (20%)
Gallioli et al., 2022 ⁶	Semi-closed-circuit vacuum-assisted mini-PCNL (n=13)	Continuous inflow and suction-controlled outflow	Median, 128 min (IQR, 99–167)	Nephrostomy: 15/16 (93.7%); tubeless: 3/16 (18.7%); DJ stent: 3/16 (18.7%); single-J ureteral catheter: 5/16 (31.2%)	None	Grade I–II: 6/16 (37.5%), including fever in 5/16 (31.2%); grade IIIa: 1/16 (6.25%)	Median, 3 days (IQR, 1.8–4.8)	1 month	SFR: absence of residual fragments >4 mm	1st month: 81.3%	Second-look PCNL in 2 renal units: 2/16 (12.5%); SFR increased to 93.8%	US and KUB	Calcium oxalate monohydrate: 3/13 (23.1%); calcium oxalate monohydrate and calcium carbonate: 3/13 (23.1%); cystine: 2/13 (15.4%); phosphate-based infectious stones (e.g., struvite and carbonate apatite): 5/13 (38.5%)
Gauhar et al., 2024 ⁷	FANS-UAS RIRS (n=50)	Irrigation was performed using either a gravity-assisted saline bag with a 20-mL syringe flush at 5 centers or the TraxerFlow™ DUAL PORT at 3 centers. A continuous flow rate of 30–60 mL was maintained and actively adjusted based on real-time visualization of the pelvicalyceal system, with intermittent flushing using a 20-mL syringe.	Total time: 46.02±20.72 min; laser time: 12.78±11.95 min; ureteroscopy time: 25.39±20.26 min	DJ stent: 46/50 (92%); overnight ureteral catheter: 1/50 (2%); no stent or ureteral catheter: 3/50 (6%)	Traxer grade 1 distal ureteral injury: 2.4%; Traxer grade 1 forniceal injury during sheath placement: 2.4%	C–D grade II: 4/50 (8%), including fever >38°C within 24 h	2.08±0.72 days	1 month	No residual fragments (RFs) or a single RF measuring up to 2 mm	1st month: 100%	None	Low-dose noncontrast CT	N/A
Peng et al., 2024 ⁸	TFFC-UAS RIRS (n=21)	The infusion flow rate was set at 100–120 mL/min	56.5±17.1 min	4.6-Fr double-J stent for 4 weeks	None	C–D grade I: 14.3% (3/21), including fever in 1/21, pain in 1/21, and nausea/vomiting in 1/21; C–D grade II: 4.8% (1/21), including hematuria in 1/21	1.5±0.3 days	3 months	SFR: absence of stone fragments or radiologically detected residual stones <2 mm in size	1st day: 81.0% (17/21); 1st month: 85.7% (18/21)	SWL: 2/21	KUB, CT, or US	Calcium oxalate: 11/21 (52.4%); calcium phosphate: 2/21 (9.5%); uric acid: 3/21 (14.3%); mixed: 5/21 (23.8%)
Turedi et al., 2024 ¹⁰	FANS-UAS RIRS (n=23)	Aspiration was achieved by connecting the Y-shaped end of the UAS to a portable vacuum system, allowing manual control	50.4±21.1 min	DJ stent: 8/23 (34.8%); DJ stent with string: 11/23 (47.8%)	Grade I ureteral injury and calyceal neck injury: 1/23 (4.3%)	C–D grade I–II: 3/23 (13%), including incontinence due to stent migration in 1/23, pain in 1/23, and UTI in 1/23	1.6±1.8 days	11.2±3.9 months	Absence of residual stones (<2 mm)	1st month: 21/23 (91.3%); 3rd month: 22/23 (95.7%)	RIRS: 2/23	US and KUB	Calcium oxalate: 7/23 (30.4%); struvite: 1/23 (4.3%); cystine: 1/23 (4.3%); mixed type: 5/23 (21.7%)
	Conventional UAS RIRS (n=23)	N/A	67.9±21.0 min	DJ stent: 18/23 (78.3%); DJ stent with string: 5/23 (21.7%)	Grade 1 ureteral injury: 2/23 (8.7%); grade 2 ureteral injury: 1/23 (4.3%)	C–D grade I–II: 4/23 (17.4%), including UTI in 2/23, persistent hematuria in 1/23, and pain in 1/23; grade III–IV: 2/23 (8.7%), including urethral stricture requiring internal urethrotomy in 1/23 and laparoscopic uretero-ureterostomy in the 6th postoperative month in 1/23	1.8±2.0 days	24.4±6.4 months	Absence of residual stones (<2 mm)	1st month: 15/23 (65.2%); 3rd month: 20/23 (87%)	SWL: 1/23 (4.3%); mini-PCNL: 1/23 (4.3%); RIRS: 4/23 (17.4%)	US and KUB	Calcium oxalate: 6/23 (26.1%); struvite: 2/23 (8.7%); mixed type: 4/23 (17.4%)
Vaddi et al., 2025 ¹¹	FANS-UAS RIRS (n=21)	Suction pressure was regulated from a minimum of 10 cm H ₂ O to 100 cm H ₂ O. Gravity-based irrigation at 40 mL/min was used, and passive suction was maintained throughout the procedure.	Total time: median, 32.00 min (25.75–37.25); laser time: 784.00 sec (669.00–945.75); fluoroscopy time: 28 sec (22–34.5)	A 3.5-Fr DJ stent was placed in all cases for 2 weeks: 21/21 (100%)	Traxer grade 1 lower ureteral injury due to access sheath placement: 3/21 (14.3%)	C–D grade I fever: 2/21 (9.5%); C–D grade I hematuria: 4/21 (19%)	Median, 2 days (1–2)	Median, 11.00 months (8.75–12.00)	Absence of any fragment ≥2 mm on US or KUB at 4 weeks after surgery	1st month: 20/21 (95.2%)	None	US and KUB	Calcium oxalate monohydrate: 12/21 (57.1%); calcium oxalate dihydrate: 6/21 (28.5%); uric acid: 2/21 (9.5%); cystine: 1/21 (4.7%)
Wang et al., 2025 ¹²	FANS-UAS RIRS (n=25)	The flow rate was set at 0.3–0.4 L/min. Intermittent suction was applied through a Y-interface connected to a portable negative-pressure system, and the device pressure was set at 0.02–0.04 MPa.	48.6±19.8 min	5-Fr DJ stent: 25/25 (100%)	None	C–D grade I: 2/25 (8%); C–D grade II: 1/25 (4%), including fever in 1/25 (4%)	2.2±0.8 days	1 month	No fragments or residual fragments ≤2 mm	24/25 (96%)	None	CT	N/A
	Conventional UAS RIRS (n=50)	The flow rate was set at 0.3–0.4 L/min	69.2±22.1 min	5-Fr DJ stent: 50/50 (100%)	None	C–D grade I: 4/50 (8%); C–D grade II: 6/50 (12%), including fever in 6/50 (12%)	3.8±1.1 days	1 month	No fragments or residual fragments ≤2 mm	44/50 (88%)	None	CT	N/A
Zhao et al., 2017 ¹³	Super-mini-PCNL (n=111)	N/A	39.4±26.2 min (7–105)	DJ stent: 13/111 (11.7%)	None	Grade I fever: 7/111 (6.3%); grade I hematuria: 4/111 (8.1%); grade II extravasation requiring nephrostomy: 3/111 (2.7%)	2.7±1.5 days (1–7)	3 months	SFR: complete clearance or the presence of a stone fragment <4 mm without any clinical symptoms	1st month: 101/111 (90%); 3rd month: 106/111 (95.5%)	None	N/A	N/A

C-D: Clavien-Dindo classification; CIRF: Clinically insignificant residual fragment; CT: Computed tomography; DJ: Double-J stent; FANS-UAS: Flexible and navigable suction ureteral access sheath; Fr: French; h: Hour; H₂O: Water; IQR: Interquartile range; KUB: Kidney-ureter-bladder radiography; L: Liter; Min: Minute; mL: Milliliter; mm: Millimeter; MPa: Megapascal; N/A: Not applicable; PCNL: Percutaneous nephrolithotomy; Ref: Reference; RF: Residual fragment; RIRS: Retrograde intrarenal surgery; sec: Second; SFR: Stone-free rate; SWL: Shock wave lithotripsy; TFFC-UAS: Tip-flexible pressure-controlling ureteral access sheath; UAS: Ureteral access sheath; US: Ultrasonography; UTI: Urinary tract infection.

Appendix 5. MINORS tool for assessing the risk of bias in nonrandomized studies

Study ID	Clearly stated aim	Inclusion of consecutive patients	Prospective data collection	Endpoints appropriate to the study aim	Unbiased assessment of the study endpoint	Appropriate follow-up period	Loss to follow-up <5%	Prospective calculation of the study size	Adequate control group*	Contemporary groups*	Baseline equivalence of groups*	Adequate statistical analysis*	Total MINORS score
Collura et al., 2024 ⁵	2	2	1	2	2	1	2	1	1	2	2	2	20/24 ⁵
Gallili et al., 2022 ⁶	2	2	2	2	2	1	2	1	N/A	N/A	N/A	N/A	14/16 ⁶
Gauhar et al., 2024 ⁷	2	2	1	2	2	1	2	1	N/A	N/A	N/A	N/A	13/16 ⁷
Peng et al., 2024 ⁹	2	2	1	2	2	2	2	1	N/A	N/A	N/A	N/A	14/16 ⁹
Turedi et al., 2024 ¹⁰	2	2	1	2	2	2	2	1	2	2	2	2	22/24 ¹⁰
Vaddi et al., 2025 ¹¹	2	2	2	2	2	2	2	1	N/A	N/A	N/A	N/A	14/16 ¹¹
Wang et al., 2025 ¹²	2	2	1	2	2	1	2	1	2	2	2	2	21/24 ¹²
Zhao et al., 2017 ¹³	2	2	1	2	2	2	2	2	N/A	N/A	N/A	N/A	15/16 ¹³

*: Applicable only to comparative studies. MINORS: Methodological Index for Non-Randomized Studies; N/A: Not applicable; pts: Patients.

Histopathological Imaging and Artificial Intelligence for Cancer Diagnosis and Gleason Grading: A Brief Review of Prostate Cancer

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ABSTRACT

Objective: Prostate cancer, one of the most common cancers affecting men's health worldwide, ranks second among cancer-related deaths. Therefore, it represents a critical health issue, and early diagnosis is crucial. Several imaging and analytical methods are used to diagnose this condition.

Materials and Methods: This study focuses on research conducted between 2021 and 2025 that analyzes, diagnoses, and grades prostate cancer based on histopathological images using machine learning and deep learning algorithms. In this context, the reviewed studies also include an analysis of findings related to preprocessing and feature extraction methods that may aid in prostate cancer detection, as well as the classifiers used.

Results: According to the findings of the reviewed studies, artificial intelligence-supported prostate cancer detection systems demonstrate high clinical performance and significantly improve the diagnostic accuracy of pathologists by enabling rapid diagnosis. The presented research findings indicate a need for comprehensive studies focusing on prostate cancer using histopathological images.

Conclusion: The integration of artificial intelligence is transforming prostate cancer pathology from a traditional and subjective process into a data-driven and standardized framework. This systematic analysis of histopathological images will make significant contributions to future prostate cancer research, help achieve more reliable results in clinical decision-making processes, and lay the groundwork for personalized medicine.

Keywords: Deep learning, Gleason grading, histopathological imaging, machine learning, prostate cancer.



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INTRODUCTION

Prostate cancer (PCa), one of the most common cancers affecting men's health worldwide, is among the most frequently diagnosed malignant tumors and represents a critical health issue, ranking second among causes of cancer-related death. It shares many characteristics with other cancer types in terms of diagnostic and staging processes.^{1–3} Recently published data clearly demonstrate



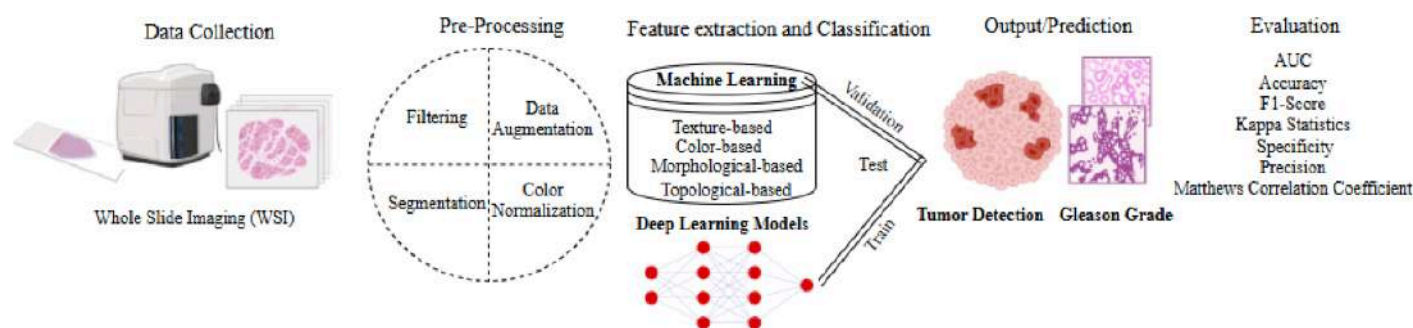


Figure 1. Artificial intelligence-based analysis of histopathological images.

the substantial burden of morbidity and mortality associated with this disease worldwide.^{3–5} The management of this disease, which poses a serious health threat, particularly to the elderly population, relies on accurate risk classification and the determination of appropriate treatment protocols based on precise pathological diagnosis and Gleason grading.^{5,6} Various imaging methods, including ultrasound, magnetic resonance imaging (MRI), positron emission tomography, and computed tomography (CT), are used in the diagnosis and staging of prostate cancer. However, histopathological examination is considered a reliable method because of its visual characteristics and is regarded as the gold standard.^{2,3,7}

In histopathological studies, tissue sections are examined in detail under a microscope by a histopathologist to evaluate cellular morphology, tissue organization, and pathological changes. While pathologists evaluate histological sections under a light microscope using morphological criteria, technological advances have enabled the integration of whole-slide imaging (WSI) systems into clinical practice. These systems enable the digitization of glass slides using high-resolution optical scanners, allowing images to be archived, analyzed, and shared in a digital environment and thereby facilitating the transition to the digital pathology paradigm in the diagnostic workflow.⁸ WSI systems have enabled the integration of artificial intelligence (AI)-based analytical approaches aimed at reducing the subjectivity and interobserver variability encountered in conventional histopathological evaluations. This digital transformation contributes to optimizing diagnostic accuracy and clinical consistency by providing a more objective, rapid, and reproducible decision-support infrastructure for prostate cancer diagnosis.⁹ AI algorithms show considerable potential for the histopathological grading of prostate cancer, particularly in radical prostatectomy specimens and needle biopsy samples.³ Growing evidence in the current literature confirms that AI integration has the potential to optimize every stage of imaging-based diagnostic processes for prostate cancer.^{10,11}

This article reviews recent studies on the analysis of histopathological images of prostate cancer and AI-based applications. The first section of the study provides information on prostate cancer and histopathological images. Subsequent sections first provide information on recently published studies involving AI-based analysis of histopathological images. The methodological approaches used in these studies and the AI algorithms applied to prostate cancer diagnosis are then discussed. Finally, the analysis of the studies discussed, their integration with decision-support systems, the limitations of machine learning- and deep learning-based studies for diagnosing prostate cancer from histopathological images, and potential directions for future research are addressed.

METHODS

Research Methodology and Analysis of Current Studies

The studies examined within the scope of this work were published in English between 2021 and 2025 in high-quality journals (Q1 or Q2 impact factor) included in the Science Citation Index Expanded (SCI-E). Therefore, journal articles and conference papers outside the scope of SCI-E were not included in the review. To identify the studies examined as part of this research, the keywords “histopathological image analysis, prostate cancer diagnosis, Gleason grading, artificial intelligence, machine learning, and deep learning” were searched in databases such as Google Scholar, Web of Science (WoS), PubMed, and Scopus. The literature reviewed within the scope of the study primarily focused on clinical diagnostic tasks such as the automatic detection of prostate cancer and disease staging through Gleason grading. Because the focus was strictly limited to prostate cancer diagnosis and Gleason grading based on histopathological images, studies involving other diagnostic methods, such as MRI, ultrasound, and CT, were not included. The detailed literature review, duplicate record removal, and article selection steps applied during the systematic review process are presented in Appendix 1 in accordance with PRISMA 2020 standards.¹² The methodological approaches, artificial intelligence architectures, datasets,

and performance results of the 12 original studies examined within the scope of this review are systematically summarized in Table 1 to provide a comparative analysis.^{5,8,11,13–21}

Analysis of Histopathological Images and Artificial Intelligence Approaches

Digital histopathology is a contemporary and innovative approach that represents a significant evolution in modern medicine and enables access to a large number of images for automated analysis. With the support of software tools, it enables the visualization and interpretation of cell and tissue samples in high-resolution images.^{22,23} This has ushered in a new era in the development of analytical techniques that assist clinicians and support the quantification of image features. With the development of WSI technology, advanced artificial intelligence systems are being developed to extract features from tumors and their surrounding microenvironments that can predict clinical outcomes. Because of differences in slide preparation processes and technical variability arising from the complex architecture of tumor tissues, integrating computer-aided diagnosis (CAD) systems into routine clinical diagnosis is not a simple process.¹⁰ The success of image analysis techniques depends directly on the quality of digital slide images. Various challenges exist in this process, including excessively large image sizes, insufficiently labeled data, image artifacts, heterogeneity among the images to be analyzed, color variations, and histological artifacts.^{2,23} CAD systems designed to overcome these obstacles can be used with high accuracy in the diagnosis of PCa and similar cancer types. The basic stages and design of the CAD system are presented in Figure 1.

Preprocessing in Histopathological Images

Methods commonly used during the preprocessing of histopathological images for noise reduction and detail enhancement include mean, median, adaptive mean, adaptive median, and Gaussian smoothing filters. These techniques eliminate unwanted noise from the images.^{2,24} The patch technique is used in studies to overcome difficulties arising from the large size of the images. This technique divides images into regions called patches, typically consisting of 1024×1024 pixels. This enables the identification of relevant areas.²⁵

Segmentation is another process applied to images. Through segmentation, digital images with similar color tones can be divided into meaningful subregions. Segmentation techniques are generally based on thresholding, region-based, edge-based, or machine learning methods.^{2,24} In histopathological images with complex tissue structures, such as those of prostate cancer, four basic tissue components (nucleus, stroma, lumen, and epithelium) have been segmented.²⁵ This

enables the transition from multiple nuclear components to independent components.²⁶

Image and color normalization is another applied step. This process is a fundamental preprocessing step used to eliminate color and contrast variations arising from different laboratories, scanners, or staining processes. Another process applied during image normalization is histogram equalization. In histopathological analyses, histogram equalization and histogram matching methods are widely used to eliminate color variations arising from lighting conditions and staining processes.^{24,27}

Data augmentation is another preprocessing step that must be performed before proceeding to feature extraction and classification. This method enables the creation of a more stable classifier model by reducing class imbalance. In recent years, AI-based methods such as generative adversarial networks (GANs) have also been proposed, enabling the automatic optimization of various training hyperparameters in data augmentation strategies. These approaches reduce the need for manual intervention by determining the most efficient augmentation configurations specific to the model and improve the overall performance of the system.²⁸ The stages and methods of image preprocessing in histopathological image analysis are presented in Table 2. Image examples illustrating the application of these methods are shown in Figure 2.

Feature Extraction

Feature extraction is a crucial step for the successful and appropriate application of the preprocessing stages. Feature extraction methods and their subtechniques for the histopathological image analysis of prostate cancer are presented in Table 3. Texture-based features can be obtained by analyzing statistical, structural, or spectral patterns that reflect density changes across the image surface and relationships between pixels. Studies have generally preferred texture-based methods such as the gray-level co-occurrence matrix (GLCM), gray-level run-length matrix (GLRLM), local binary pattern (LBP), and fractal dimension.^{24,29} Color-based features provide basic information about the intensity distributions and spectral characteristics of pixels within the region of interest (ROI). Feature extraction and selection processes in this category utilize different color spaces to distinguish tissue components. The pixel-based color moment descriptor (PCMD) feature is frequently preferred in studies.²⁹ Morphological features are derived from the shape and structure of histopathological images. To represent the cancerous region, features such as area, perimeter, maximum and minimum radius, equivalent diameter, circularity, and entropy are extracted.^{24,30}

Table 1. Comparative analysis of the studies examined within the scope of this review

Study	Dataset	Methodology	Clinical focus	Performance metrics	Contribution/result
Kott et al. ⁵	85 biopsies from 25 patients; 14,803 256x256-pixel image patches	Convolutional neural network (CNN) and 5-fold cross-validation	Cancer detection and Gleason grading (3, 4, and 5)	Accuracy: 91.50%; Precision: 0.93	Reduced interobserver variability and the risk of diagnostic error.
Singhal et al. ⁸	Thousands of biopsy slides (WSIs) from different centers	Deep learning model	Objective Gleason grading	Accuracy: 83%–89%	The objectivity and generalizability of the model were demonstrated using large multicenter datasets.
Bulten et al. ¹¹	160 biopsy samples	AI-based decision-support system	Gleason grading	Cohen's kappa: 0.82	The use of artificial intelligence improved the consistency of Gleason grading among pathologists.
Pinckaers et al. ¹³	4,712 biopsy images	ResNet-34	Cancer diagnosis using WSI	Area under the curve (AUC): 99%	Reduced GPU memory requirements by eliminating the need for manual pixel-level labeling.
Duenweg et al. ¹⁴	Digital histopathology images from 47 patients (31 training, 16 test)	ResNet-based deep learning and segmentation ensemble model	Detection of cancer areas and discrimination of Gleason patterns	Accuracy: 88%–89% for both models	Although both models were successful in cancer detection, ResNet demonstrated superior performance over the ensemble model in distinguishing Gleason patterns.
Raciti et al. ¹⁵	610 needle biopsy WSIs	Artificial intelligence-supported classification using hybrid models	Classification of all histological grades and tumor sizes	Classification accuracy: 100%	AI support achieved complete classification accuracy (100%) across all WSIs, regardless of tumor size.
Mun et al. ¹⁶	6,664 and 936 needle biopsy WSIs	Automated Gleason grading system (YAAGGS) using weakly supervised deep learning	Automated Gleason grading and classification	Accuracy: 77.50%	Automated end-to-end grading was performed on a large WSI dataset.
Jung et al. ¹⁷	593 independent WSI slides	DeepDx artificial intelligence system	Cancer detection and Gleason scoring analysis	Accuracy similar to pathology reports; higher agreement in Gleason scores	Independent datasets confirmed that the DeepDx system achieved greater grade-group agreement than pathologist reports.
Huang et al. ¹⁸	1,000 WSI samples obtained from 589 patients	Deep neural network-based artificial intelligence	High-efficiency prostate cancer detection	AUC: 92%	High diagnostic accuracy and efficiency were achieved in a large patient cohort.

Table 1 (cont). Comparative analysis of the studies examined within the scope of this review

Study	Dataset	Methodology	Clinical focus	Performance metrics	Contribution/result
Xiang et al. ¹⁹	6,174 WSIs	Graph convolutional network	Gleason grading	AUC: 99.20%	Cellular relationships were modeled by representing each whole-slide image as a graph structure.
Liang et al. ²⁰	Test dataset of 531 WSIs	Attention mechanism-based LSTM	Segment-based feature extraction and classification	Cohen's kappa: 0.93	Digital diagnostic pathology demonstrated strong generalizability in automatically extracting prostate cancer grading information and addressing a critical gap in pathologist expertise.
Kabir et al. ²¹	2,699 clinical cases	3-stage DL system: patch classification, segmentation, and random forest	Automated Gleason grading estimation	F1 score: 83.80%; Cohen's kappa: 0.92	Combined deep learning-based segmentation with the traditional random forest algorithm, providing a promising prognostic approach capable of efficiently and reliably generating clinically meaningful results.

Classification Process

Artificial intelligence is a field that mimics human intelligence to solve complex problems and relies on data-driven modeling. Machine learning, one of the cornerstones of this field, enables systems to recognize patterns in data without being explicitly programmed, while deep learning (DL) extracts information directly from data through multilayer neural networks.^{31,32} While the manual definition and extraction of features are prerequisites in studies based on traditional machine learning algorithms, feature extraction and classification processes in deep learning approaches are performed automatically through an end-to-end architecture that learns directly from the data. The cross-validation process applied during the classification stages, consisting of training, validation, and testing phases, is shown in Figure 3.

Traditional machine learning algorithms used in studies diagnosing PCa from histopathological images include support vector machines, artificial neural networks, k-nearest neighbors, random forests, and boosting-based methods.² Numerous studies have been conducted using different neural networks, such as convolutional neural networks (CNNs), deep neural networks, and GANs, for PCa diagnosis from histopathological images. However, CNNs are among the most frequently preferred algorithms in these studies.³³ Examination of the literature shows that CNN architectures with different structural depths and parameter characteristics, such as AlexNet, ResNet, VGGNet, SqueezeNet, and DenseNet, are commonly preferred.

Performance Evaluation Criteria

In machine learning- and deep learning-based classification studies, various performance metrics are used to measure the accuracy and reliability of models. These metrics enable the model's predictive ability to be evaluated from different perspectives. Expressing a model's performance using a single metric is insufficient to reflect all characteristics of the system. Therefore, to summarize the real-world performance of models that have completed the training process, multidimensional metrics should be reported using an independent test dataset.^{31,34} The key metrics used to evaluate classification performance, particularly in studies diagnosing prostate cancer from histopathological images, are accuracy, F1 score, area under the curve (AUC), and kappa statistics.

RESULTS

Analysis of the Studies

In recent years, interest in artificial intelligence-based systems and computer-aided diagnosis systems for prostate cancer diagnosis has increased significantly, particularly in pathology and various imaging modalities.³⁵ Artificial

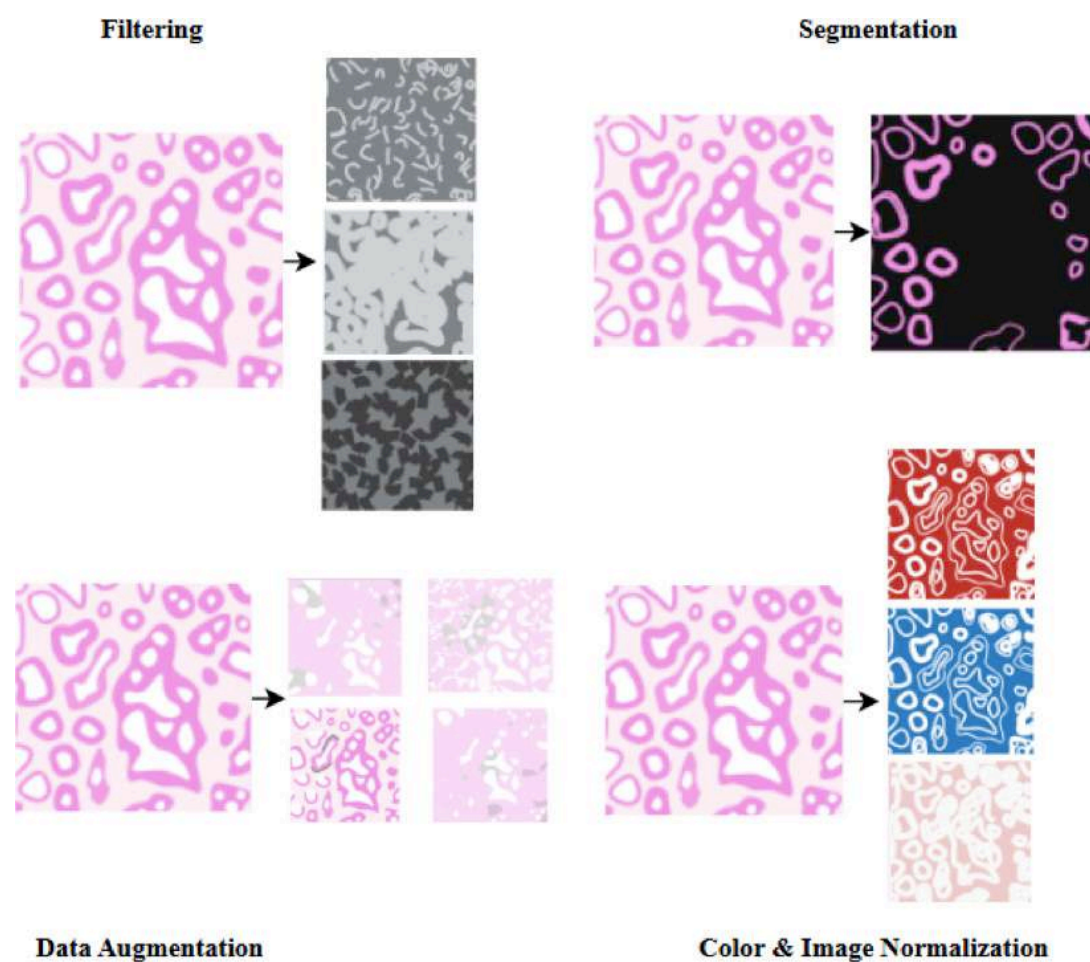


Figure 2. Example of image preprocessing steps applied to a histopathological image.

Table 2. Image preprocessing stages and methods in histopathological image analysis

Filtering	Segmentation	Data augmentation	Color and image normalization
Mean	Thresholding	Geometric transformations	Histogram equalization and matching
Median	Region-based	Photometric transformations	
Adaptive mean/median	Edge-based	Artificial intelligence-based methods	
Gaussian smoothing	Machine learning-based		

intelligence applications in prostate cancer pathology play an important role in digital pathology, diagnostic medicine, and precision oncology.³¹ The high-dimensional complexity and morphological variability inherent in histopathological images make this field particularly suitable for computational analysis. This review also focuses on studies investigating AI-based diagnosis and CAD systems for prostate cancer using histopathological images. With this focus, the architectures, dataset sizes, and diagnostic performance of studies conducted between 2021 and 2025, whose results are summarized in

Table 1, were evaluated. As a result of this evaluation, the methods applied in these studies were discussed in detail in the previous section. The high information density of digital histopathological images for diagnostic purposes makes this field an ideal area of research for machine learning.^{36,37} However, studies in the literature indicate a shift toward deep learning methods rather than traditional machine learning approaches. Among DL methods, CNN-based approaches have been preferred more frequently.^{13,14,19} Previous studies have also demonstrated that DL techniques can accurately

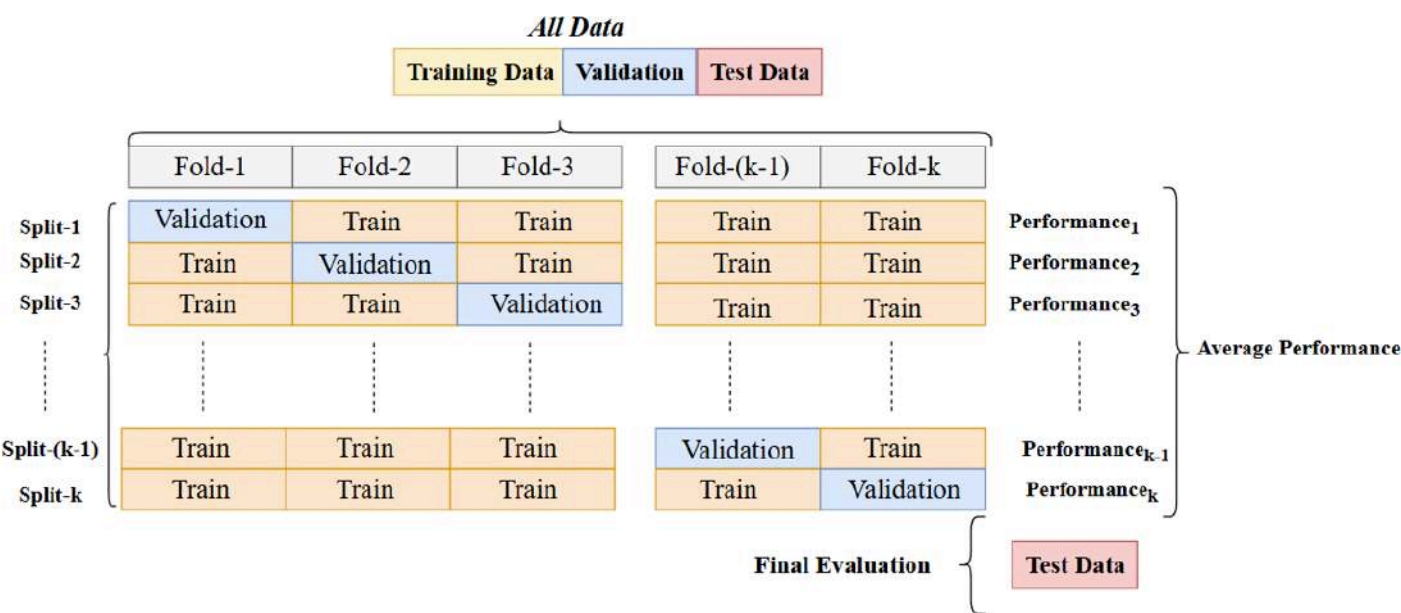


Figure 3. Cross-validation method used in the classification process.

Table 3. Feature extraction methods and subtechniques in histopathological image analysis of prostate cancer

Texture-based	Color-based	Morphological and topological
GLCM	PCMD	Size and area
GLRLM	Statistical features (mean, standard deviation, kurtosis, skewness)	Shape factors
LBP	Color space conversions	Boundary irregularity
Fractal dimension	Color histograms and moments	Cell graphs

GLCM: Gray-level co-occurrence matrix; GLRLM: Gray-level run-length matrix; LBP: Local binary pattern; PCMD: Pixel color moment descriptor.

detect cancer in histopathological images.³⁸ The main reason for this trend may be that, unlike traditional machine learning approaches, which are limited by their reliance on feature extraction processes, deep learning models can directly process latent features in complex tissue images and analyze them hierarchically.

When the performance evaluation processes of the reviewed studies are analyzed, accuracy, AUC, and kappa statistics are commonly used to assess model performance. In a field such as prostate cancer, where clinical decision-making is critical, focusing solely on accuracy rates can be misleading. In this context, the emphasis on AUC values in the reviewed studies demonstrates the balance between sensitivity and specificity and reflects the discriminatory power of the models across pathological classes. High AUC values indicate a strong ability of the models to discriminate between complex histopathological tissue patterns. Kappa

statistics, on the other hand, provide scientific evidence of the level of agreement between artificial intelligence and expert pathologists while accounting for agreement occurring by chance. The consistency of these metrics reinforces the reliability of the developed algorithms as clinical decision-support mechanisms. The algorithms that achieved the best performance in the reviewed studies, together with their accuracy, AUC, or kappa values, are presented in Figure 4.

The findings from the reviewed studies demonstrate that artificial intelligence applications in prostate cancer pathology provide strategic advantages in terms of speed, objectivity, and diagnostic consistency. The Gleason grading system, which is the focus of numerous studies, is being transformed from a traditional and subjective evaluation process into a data-driven and standardized framework through the integration of artificial intelligence. The results

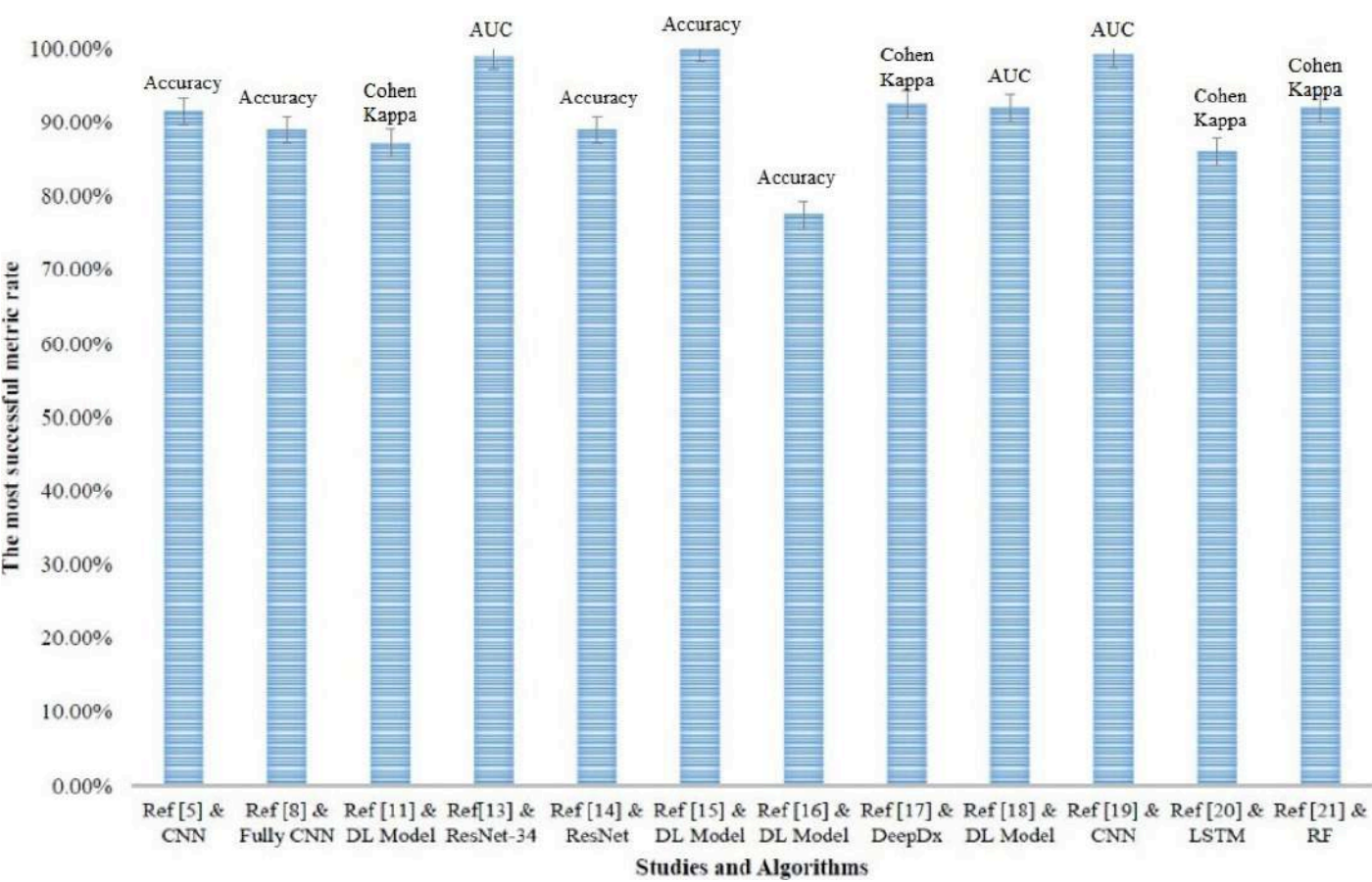


Figure 4. Distribution of algorithms used and their reported performance in the reviewed studies.

indicate that artificial intelligence is not merely a tool for accelerating workflow but also has the potential to reduce differences in interpretation among physicians and promote greater standardization in Gleason grading.

DISCUSSION

Despite the considerable potential offered by artificial intelligence in prostate cancer pathology, significant obstacles remain to the full integration of these systems into clinical practice. Legal and ethical concerns regarding patient data privacy are among the factors that hinder the establishment of clinical confidence. Although most of the current literature focuses on laboratory performance, logistical issues such as the robustness of these models across heterogeneous datasets, high storage costs, and practical integration into hospital workflows constitute major constraints on the widespread adoption of artificial intelligence as a diagnostic tool.^{23,39} Limitations arising from these fundamental constraints are also evident in the studies presented in Table 1. As shown by the comparative results in Table 1, the wide variability in model performance stems

directly from challenges associated with large multicenter datasets, as well as from the multifactorial balance between the sensitivity of pathologist annotations and the complexity of model architectures. Most studies employ deep learning models or hybrid approaches that combine multiple models. Standard machine learning algorithms are preferred less frequently, mainly because deep learning methods generally achieve higher and more stable performance. As shown in Table 1 and Figure 4, a substantial proportion of existing studies focus on only a single evaluation metric. However, for a clinically reliable decision-support system, it is essential to assess all performance metrics jointly and ensure that they yield consistent and coherent results. Reliance on a single metric may lead to misleading interpretations. Although the success of artificial intelligence models in prostate cancer pathology depends directly on the quality of the training data, data bias and insufficient data volume are among the most fundamental problems in the current literature.³ In a large proportion of studies, the limited size of the training set restricts the models’ ability to learn morphological variations across different populations and weakens the generalizability

of CAD systems. The enormous data burden resulting from dividing multiple biopsy samples from a single patient into millions of patches, together with the high computational cost required, is also considered a significant challenge. The use of WSI technology poses a substantial computational challenge because of the large volume of data. These images must be divided into smaller sections for analysis using current artificial intelligence methods.⁴⁰ In addition to the data burden, subjective differences in interpretation among experts in Gleason grading complicate the creation of the gold-standard datasets required for algorithm training. This critical need for high-quality, expert-annotated histopathological imaging data has led researchers to rely on established and more comprehensive open-access datasets in their studies. However, even these datasets have been reported to be limited and inconsistent. The most comprehensive histopathological dataset, frequently used and widely accepted in studies of Gleason grading and prostate cancer detection from histopathological images, was introduced through the Prostate Cancer Grade Assessment (PANDA) competition. This dataset consists of 12,625 prostate biopsy WSIs collected from six different centers.⁴¹ Another histopathological image dataset, recently introduced into the literature by Koziarski and colleagues and still used in relatively few studies, is DiagSet. This dataset consists of more than 2.6 million tissue sections.⁴² Other known publicly available datasets consisting of biopsy samples for PCa diagnosis include SICAP-MIL, Ibex, Gallo, and Tolkach.

A significant gap for future research is that most current deep learning approaches are trained on single-center datasets. This results in reduced model performance and limited generalizability when data from different centers are used, for example, data generated using different staining protocols, scanning devices, and clinical workflows. Therefore, there is a need for more robust models trained on multicenter datasets and validated using external datasets. In addition, the “black-box” nature of deep learning models constitutes another significant research gap in clinical applications. Particularly in critical pathological assessments such as Gleason scoring, the inability of pathologists to clearly identify the visual or histological features on which the model bases its decisions limits the reliability and acceptability of clinical decision-support systems. Considering these two fundamental problems together, the development and adaptation of explainable artificial intelligence (XAI) methods in pathology represent an important research need. XAI-based approaches can increase clinician confidence by making model decisions more transparent and can also facilitate the clinical integration of AI-powered diagnostic systems.

CONCLUSION

In conclusion, WSI technology serves as a critical and promising tool in diagnostic pathology by transforming traditional microscopic examination into a digital ecosystem, thereby enabling the development and clinical integration of high-performance artificial intelligence algorithms. The high-resolution digital data obtained through WSI preserve microscopic details of tissue architecture and allow deep learning models to perform hierarchical feature learning on large-scale datasets. The reviewed studies demonstrate that artificial intelligence has strong potential to support expert pathologists' decisions, particularly in Gleason grading and cancer detection. However, for these models to be fully integrated into routine clinical laboratories, methodological challenges such as dataset diversity, model interpretability, and high computational costs must be addressed. The adoption of these technologies within the digital pathology ecosystem will be a critical step toward advancing precision oncology in future research, provided that these clinical and technical bottlenecks are resolved.

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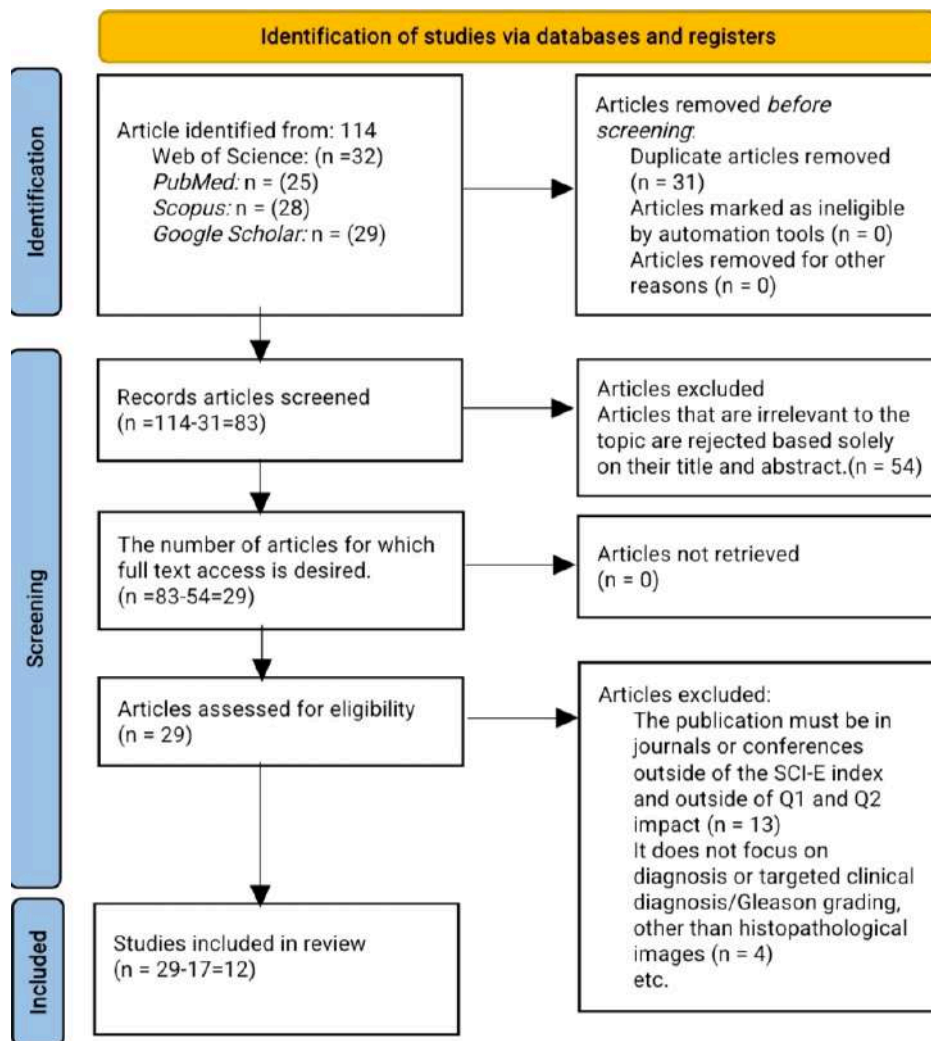
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Appendix 1.

Bicycle Handlebar–Related Blunt Abdominal and Pelvic Injuries in Children and Adults: A Radiological Perspective

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ABSTRACT

Objective: Bicycle handlebar–related abdominopelvic trauma can cause clinically significant injuries despite minimal external signs, potentially resulting in delayed diagnosis. Radiological evidence regarding CT injury patterns associated with this mechanism remains limited. This study aimed to evaluate and characterize the CT findings of bicycle handlebar–related blunt abdominal and pelvic injuries in pediatric and adult patients and to describe injury patterns, organ involvement, and clinical management.

Materials and Methods: This retrospective study included patients who presented with bicycle handlebar–related abdominopelvic injuries between July 2010 and November 2025. Patients who underwent contrast-enhanced abdominopelvic CT because of suspected abdominopelvic trauma were analyzed. CT findings were categorized and graded using the AAST injury scale.

Results: Twenty-three patients had CT findings indicative of abdominopelvic injury, with a total of 25 distinct injuries identified. Nineteen patients (82.6%) were pediatric patients aged 6–16 years, and 4 (17.4%) were adults aged 21–30 years; 86.9% were male. The most common organ injuries involved the spleen (24%) and liver (20%). CT was performed within 24 hours in 87.0% of patients, whereas delayed imaging occurred in 13.0%. Of the 19 intra-abdominal organ injuries, 89.5% were AAST grades 1–3, and 10.5% were grades 4–5. Most patients (82.6%) were managed conservatively, whereas 17.4% required surgical or interventional treatment.

Conclusion: Bicycle handlebar–related trauma is a distinctive mechanism of blunt abdominopelvic injury associated with a broad spectrum of CT findings, predominantly affecting school-aged boys but also occurring in adults. Awareness of characteristic CT patterns and standardized injury grading can facilitate timely diagnosis and appropriate management in both pediatric and adult populations.

Keywords: Bicycle, blunt trauma, children, computed tomography, handlebar.



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INTRODUCTION

Bicycle handlebar–related injuries constitute an important subgroup of traumatic injuries, particularly in the pediatric population, because of their potential for serious clinical consequences. The incidence of handlebar–related injuries in children has been reported to be approximately 1.15

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per 100,000.¹ Although less common in adults, several studies have demonstrated that bicycle handlebar injuries can also occur in the adult population.^{2,3} Owing to the small diameter of the handlebar end, a high-speed impact to the abdomen, or even a low-speed fall in which the rider lands on the handlebar, may result in severe intra-abdominal, abdominal wall, and abdominopelvic vascular injuries.^{4,5}

In bicycle falls, handlebar-related trauma is associated with a higher risk of abdominal and pelvic injuries than non-handlebar-related mechanisms. Moreover, because handlebar injuries are typically blunt rather than penetrating, skin inspection may reveal no significant external findings or only minimal signs representing the “tip of the iceberg.”⁶ Because of the nonspecific nature of the clinical symptoms and physical examination findings, previous studies have reported that the diagnosis and treatment of these injuries may be delayed, potentially leading to increased morbidity and mortality.^{7,8} Therefore, greater awareness among clinicians and radiologists is essential to prevent diagnostic and therapeutic delays.

Because of the relative rarity of this injury mechanism, most studies on bicycle handlebar-related injuries have focused primarily on clinical findings, patient management, and clinical outcomes. In these studies, radiological findings have either not been systematically described or have been addressed only briefly.

Computed tomography (CT) is the most frequently used cross-sectional imaging modality for the emergency evaluation of hemodynamically stable patients with abdominal and pelvic trauma because of its rapid acquisition, high sensitivity and specificity for detecting both solid- and hollow-organ abdominopelvic injuries, ability to guide appropriate patient management, relative cost-effectiveness, and widespread availability in most medical centers.^{9,10} Bicycle handlebar-related injuries can likewise be detected and accurately classified using CT, similar to other forms of blunt abdominal trauma.¹¹

The aim of the present study was to comprehensively evaluate the CT findings of bicycle handlebar-related blunt abdominal and pelvic injuries in pediatric and adult patients. By systematically analyzing injury patterns, organ involvement, and associated imaging features, this study sought to contribute to the limited radiological literature on handlebar-related trauma and improve diagnostic awareness among radiologists and clinicians, thereby facilitating timely and appropriate patient management.

METHODS

This retrospective study was conducted at Osmangazi University Hospital and included both pediatric and adult patients who presented to the emergency department with

KEY MESSAGES

- Bicycle handlebar trauma may present with minimal external findings despite a clinically significant spectrum of abdominopelvic injuries on CT, posing a risk of delayed diagnosis.
- Although most cases occur in school-aged boys, a notable proportion occur in adults, with the spleen and liver being the most frequently injured solid organs.
- Most injuries are low to moderate grade (AAST grades 1–3) and are managed conservatively, whereas a smaller subset requires surgical or interventional treatment.

bicycle handlebar-related injuries between July 2010 and November 2025. Ethical approval was obtained from Eskişehir Osmangazi University Non-Interventional Clinical Research Ethics Committee (Approval Number: 2025-435, Date: 04.11.2025). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patients were retrospectively identified through the electronic medical record system using the Turkish keywords “bisiklet” and “gidon,” which mean “bicycle” and “handlebar,” respectively. Among these patients, those who underwent CT because of suspected abdominopelvic trauma were included in the dataset. Electronic medical records were independently reviewed by at least two investigators. Demographic data, including age and sex, as well as the clinical course and medical or surgical management, were recorded for each patient.

Abdominopelvic injuries were diagnosed based on CT findings. All CT images were independently evaluated by two radiologists, one of whom had at least 10 years of radiology experience. In cases of disagreement, a third radiologist was consulted to reach a consensus. Patients who presented with bicycle handlebar-related trauma and underwent abdominopelvic CT because of suspected injury were included in the study. Patients without CT imaging and those with bicycle injuries unrelated to the handlebar were excluded. Clinical findings, treatment approaches (conservative or surgical), and patient outcomes were obtained from hospital records. CT findings were systematically categorized according to the involved organ systems and corresponding AAST injury grades.

Statistical Analysis

All data were analyzed using descriptive statistical methods. Continuous variables were summarized as the mean±standard deviation (SD) and range, whereas categorical variables were

Table 1. Demographic, radiological, and clinical features of bicycle handlebar-related injuries

Demographics		Number of patients, n=23
Mean age, years		12.47±6.23
Male, n (%)		20 (86.9%)
Mean length of hospital stay, days		5.5±3.47
Pediatric patients, n (%)		19 (82.6%)
Injuries, n (%)		Number of injuries, n=25 (100%)
Spleen		6 (24%)
Liver		5 (20%)
Kidney		3 (12%)
Pancreas		2 (8%)
Small bowel		3 (12%)
Abdominal wall hematoma		5 (20%)
Abdominal wall hernia		1 (4%)
Number of CT examinations, n (%)		23 (100%)
CT performed within 24 h		20 (87.0%)
CT performed after 24 h		3 (13.0%)
Intraperitoneal hemorrhage on CT		10 (43.5%)
AAST injury grade on CT, n (%)		Number of intra-abdominal organ injuries, n=19 (100%)
Low grade (1–3)		17 (89.5%)
High grade (4–5)		2 (10.5%)
Management, n (%)		23 (100%)
Conservative		19 (82.6%)
Surgical/interventional		4 (17.4%)

CT: Computed tomography; AAST: American Association for the Surgery of Trauma.

expressed as frequencies and percentages. Statistical analyses were performed using the Statistical Package for the Social Sciences, version 26.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The electronic medical record search initially yielded 4,698 entries based on the keywords “bicycle” and “handlebar.” Because of the broad nature of these terms and the extended study period, many records included nonspecific trauma cases. Therefore, all records were manually reviewed in detail. During this screening process, cases that did not meet the inclusion criteria—such as motorcycle accidents, collisions between bicycles and motor vehicles, pedestrians struck by bicycles, falls from bicycles without direct handlebar

impact, and cases involving patients who had not undergone CT—were excluded. Following this step, 114 patients with confirmed handlebar-related trauma who had undergone CT imaging were identified.

During the subsequent evaluation, 91 patients were excluded because the trauma involved regions outside the abdominopelvic area (e.g., the extremities, thorax, head, or neck) or because the CT findings were negative. Patients with only minimal findings, such as mild skin thickening or superficial soft tissue edema of the abdominal or pelvic wall, were considered CT-negative. Ultimately, 23 patients with isolated bicycle handlebar-related abdominopelvic trauma and positive CT findings were included in the final analysis.

All abdominopelvic CT examinations were performed with intravenous contrast and acquired during the portal venous phase. Of the 23 patients, 19 (82.6%) were in the pediatric group (aged 6–16 years), and 4 (17.4%) were in the adult group (aged 21–30 years). The mean age at the time of injury was 12.47 years (SD, 6.23). Of the 23 patients, 87.0% were male (n=20), and 13.0% were female (n=3) (Table 1).

A total of 25 abdominal and pelvic injuries were identified in 23 patients. The most commonly injured intra-abdominal organs were the spleen (n=6, 24%) and liver (n=5, 20%) (Fig. 1). Injuries involving the kidney (n=3, 12%), pancreas (n=2, 8%), small bowel (n=3, 12%), abdominal wall hematoma (n=5, 20%), and abdominal wall hernia (n=1, 4%) were also identified (Fig. 2, 3). Intraperitoneal hemorrhage accompanied other injuries in 10 of the 23 patients (43.5%). In 20 of the 23 patients (87.0%), CT imaging was performed within 24 hours of trauma. However, diagnosis was delayed in 3 patients; CT was performed within 24–48 hours in 2 patients and within 72–96 hours in 1 patient. Overall, 18 patients sustained 19 intra-abdominal organ injuries. Of these 19 injuries, 17 (89.5%) were classified as AAST grades 1–3 (mild to moderate), whereas 2 (10.5%) were classified as grades 4–5 (severe) on CT imaging (Table 1).

Among the 23 patients, 1 patient with a splenic injury underwent splenectomy, and 1 patient with a small bowel injury and perforation underwent surgical repair. One patient underwent diagnostic peritoneal lavage because of suspected intraperitoneal hemorrhage, and 1 patient with a pancreatic injury was treated with percutaneous pseudocyst drainage by interventional radiology. The remaining 19 patients (82.6%) were managed conservatively without surgical or interventional procedures. No deaths occurred following bicycle handlebar-related trauma in this cohort. The mean hospital length of stay was 5.5 days (SD, 3.47). Nineteen cases occurred between May and September, when weather conditions are generally favorable.

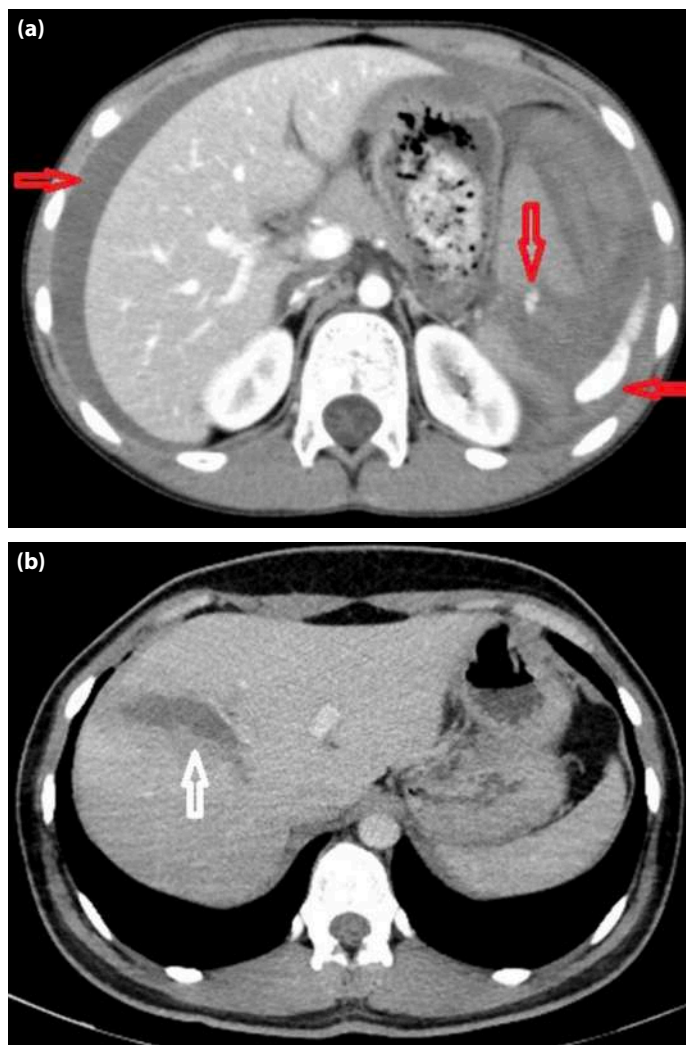


Figure 1. (a) A large splenic laceration with active intraparenchymal hemorrhage, contrast extravasation adjacent to the spleen, and hemorrhage extending toward the liver is observed (red arrows). The findings are consistent with an AAST grade 5 splenic injury. **(b)** Liver laceration in a 23-year-old woman with a handlebar injury to the right upper abdomen. Axial contrast-enhanced CT image shows an AAST grade 3 laceration (arrow) in the right hepatic lobe.

DISCUSSION

Although bicycle handlebar-related injuries are relatively uncommon, they can result in serious clinical consequences and therefore warrant careful attention. In our study, bicycle handlebar trauma was associated with a broad spectrum of injuries involving various abdominopelvic organs and tissues. Consistent with previous reports, injuries occurred most frequently in pediatric patients, particularly boys.^{12,13}

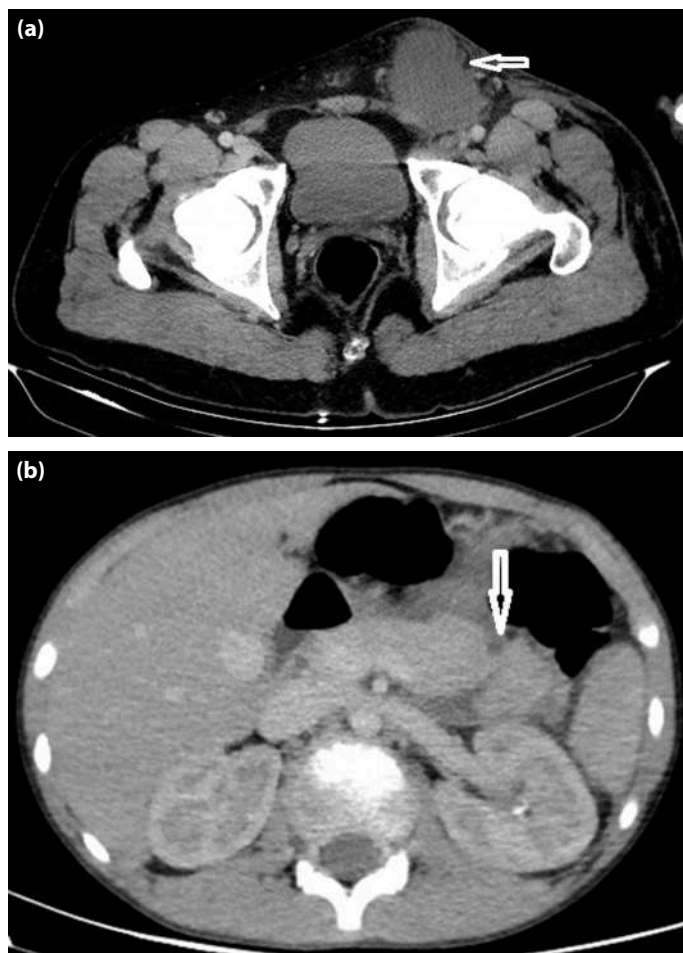


Figure 2. (a) Abdominal wall hematoma in a 30-year-old man with a handlebar injury. Axial contrast-enhanced CT image demonstrates an anterior pelvic wall hematoma (arrow) at the site of injury. **(b)** In a different patient, pancreatic transection consistent with an AAST grade 3 injury is present in the distal pancreas (white arrow).

In blunt abdominal trauma, the spleen is generally recognized as the most commonly injured organ.¹⁴ In our cohort, the most frequently injured solid organs were the spleen (24%) and liver (20%). Compared with other mechanisms of blunt abdominal trauma, pancreatic injuries have also been reported in association with bicycle handlebar trauma.^{15,16} In our cohort, 2 patients (8%) had AAST grade 3 pancreatic injuries.

Our study focused exclusively on bicycle handlebar-related injuries. However, handlebar-type trauma is not limited to bicycles; similar injury mechanisms may occur with motorcycles, kick scooters, and electric scooters. Injuries associated with these vehicles have been increasingly reported in the literature.¹⁷ Notably, the use of electric scooters has

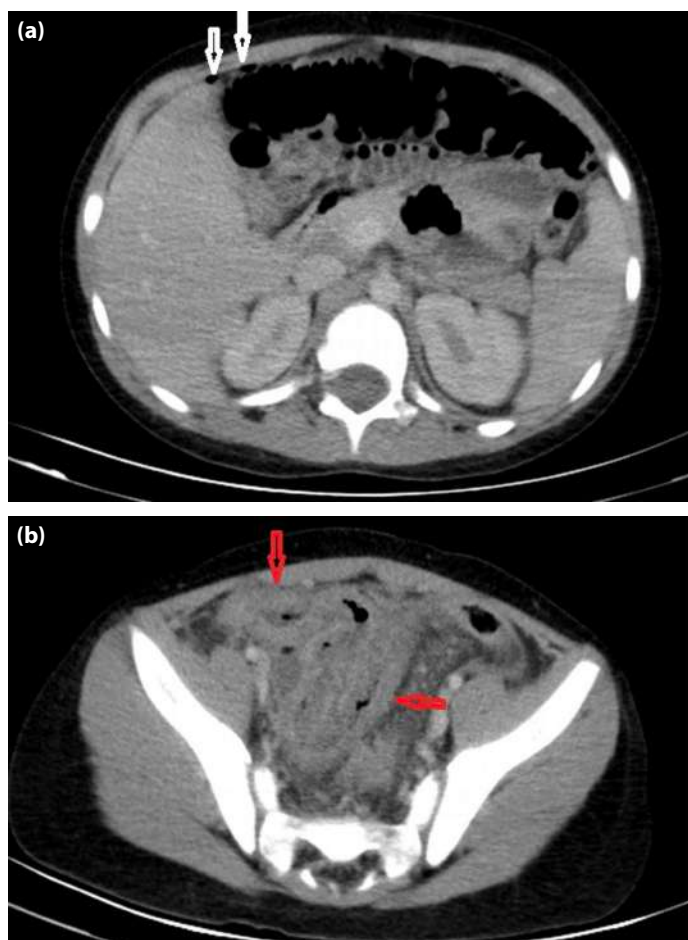


Figure 3. Small bowel perforation in a 6-year-old boy with a handlebar injury. **(a)** Axial contrast-enhanced CT image demonstrates free intraperitoneal air (white arrows). **(b)** Small bowel wall thickening is observed (red arrows). During surgical exploration, an ileal perforation was identified.

increased substantially among the pediatric population, and a corresponding rise in handlebar-related blunt abdominopelvic trauma may be anticipated.

Traumatic abdominal wall hernia has frequently been described in this patient group.^{18–20} We identified this condition in 1 patient. Given its potential to be overlooked during the initial post-trauma assessment, heightened awareness and careful evaluation by radiologists are important.

Handlebar-related abdominopelvic injuries represent a distinct trauma mechanism that may present in the emergency department with nonspecific and subtle clinical findings, potentially leading to delayed diagnosis and increased morbidity and mortality.^{6,7} In our study, diagnosis was delayed in 3 patients (13%), largely because symptoms and signs were

relatively mild during the initial clinical examination and CT was performed more than 24 hours after trauma. Therefore, more liberal use of imaging in this patient group may help prevent delayed diagnosis and reduce morbidity and mortality. In contrast, a study conducted in Türkiye reported that, among 293 patients presenting to the emergency department with non-handlebar-related bicycle injuries, 46.1% had lower extremity trauma, 22.5% had upper extremity trauma, 20.1% had head trauma, and 9.6% had thoracic trauma, whereas only 1.7% had abdominal trauma.²¹ This distribution highlights the relative rarity of abdominal involvement in general bicycle trauma and further supports the notion that handlebar-related mechanisms represent a distinct injury pattern with a disproportionately high risk of abdominopelvic injury.

Studies of bicycle handlebar-related injuries in the literature have predominantly focused on the pediatric population, whereas reported cases in adults have generally been rare and mostly limited to case reports.^{22,23} Although this specific trauma mechanism may be less common in adults, 18% of the patients in our cohort were adults, suggesting that it may not be as rare as previously assumed.

Cevik et al.²⁴ and other studies^{25,26} have reported high rates of surgical intervention following bicycle handlebar injuries. Consistent with the literature, our findings suggest that patients with bicycle handlebar trauma have relatively high rates of surgical or interventional management and hospitalization.

From a radiological perspective, bicycle handlebar-related abdominopelvic trauma poses a distinct diagnostic challenge because clinical findings may be subtle and external signs may be minimal despite clinically significant internal injuries. Notably, the radiology-focused literature on this mechanism remains limited, and imaging findings have often been reported only briefly or nonsystematically. Further imaging-based studies involving larger patient cohorts are needed to better characterize the full spectrum of CT findings, refine injury pattern recognition, and strengthen the evidence base for radiology-driven diagnostic pathways for this distinct trauma mechanism.

Limitations

This study has several limitations. First, its retrospective, single-center design and relatively small sample size, particularly the limited number of adult patients, may restrict the generalizability of our findings. Second, inclusion was limited to patients who underwent contrast-enhanced abdominopelvic CT, introducing selection bias toward hemodynamically stable patients and potentially underrepresenting milder injuries managed without CT. Third, case identification relied on electronic medical record keyword searches, which may have

missed eligible patients because of incomplete documentation or variability in coding. Finally, CT examinations were acquired only during the portal venous phase; therefore, some injury components, such as arterial bleeding, subtle bowel or mesenteric injuries, or evolving pancreatic injury, may have been underestimated.

CONCLUSION

Bicycle handlebar-related trauma is an important and distinctive mechanism of blunt abdominopelvic injury that predominantly affects school-aged boys and is associated with a broad spectrum of CT findings involving both solid organs and the abdominal wall. Because external signs may be minimal and symptoms may be nonspecific, delayed diagnosis remains a concern. Awareness of characteristic injury patterns and a low threshold for CT in appropriately selected patients may facilitate timely diagnosis, optimize management decisions, and reduce morbidity in both pediatric and adult populations.

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Informed Consent: Due to the retrospective nature of the study, the ethics committee waived the requirement for informed consent.

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Gene Expression Profiling of *URGCP* and *NTRK1-3*/MAPK Pathway Genes in Soft Tissue Sarcomas

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ABSTRACT

Objective: Sarcoma is a heterogeneous malignant cancer of mesenchymal origin, and approximately 80% of cases are soft tissue sarcomas (STSs). *NTRK* genes play roles in nerve cell development, while the MAPK signaling pathway contributes to tumor cell migration, invasion, and metastasis. *URGCP* is an oncogene involved in tumor development in many cancers; however, its role and molecular mechanism in STS remain unknown. This study aimed to investigate the expression and correlations of the *NTRK1*, *NTRK2*, and *NTRK3* genes, MAPK signaling pathway genes, and the *URGCP* oncogene in STS subtypes.

Materials and Methods: RNA was isolated from formalin-fixed, paraffin-embedded (FFPE) tissue samples obtained from 80 patients with STS. The expression levels of *NTRK1*, *NTRK2*, *NTRK3*, *MEK1*, *MEK2*, *ERK1*, and the *URGCP* oncogene were analyzed using quantitative real-time PCR (qPCR). Correlations among these genes and their associations with STS were evaluated.

Results: The analyses did not reveal statistically significant correlations between *NTRK* genes and genes involved in the MAPK signaling pathway. In addition, no statistically significant association was observed between *URGCP* expression and STS.

Conclusion: Although this study did not demonstrate a significant association between *URGCP* and soft tissue sarcoma, it reveals a link between *NTRK* genes and the MAPK signaling pathway in STS.

Keywords: MAPK signaling pathway, *NTRK1*, *NTRK2*, *NTRK3*, sarcoma, soft tissue sarcoma, *URGCP*.



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INTRODUCTION

Sarcoma is a rare type of cancer of mesenchymal origin, accounting for approximately 1% of all malignant cancers and arising in connective tissues such as bone, muscle, and adipose tissue. It has more than 100 subtypes, 20% of which originate from bone and 80% from soft tissue.¹⁻⁴ Soft tissue sarcomas (STSs) are malignant tumors of mesenchymal origin comprising numerous histological subtypes, particularly those arising from connective tissue, and account for only 1% of all malignant cancers.^{2,4} In our study, 12 subtypes of soft tissue sarcoma were investigated.

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Malignant peripheral nerve sheath tumors (MPNSTs) constitute approximately 10% of all soft tissue tumors and have a high tendency to metastasis.^{5–7} Inflammatory myofibroblastic tumor is a very rare tumor of intermediate grade because of its low recurrence risk and metastatic potential.^{8,9} Dedifferentiated liposarcoma is the most heterogeneous of all sarcomas, with a wide variety of histological patterns. It is a high-grade and aggressive disease, and high rates of local and metastatic recurrence are associated with very high mortality.^{10,11} Myxofibrosarcoma is a mesenchymal fibrous soft tissue sarcoma that can range from low to high grade, typically occurs in the extremities of older individuals, and is characterized by a high local recurrence rate, with metastases predominantly involving the lungs and bones.^{12–14} Leiomyosarcoma is one of the most common subtypes, accounting for approximately 20% of soft tissue sarcoma cases. It has substantial intrinsic aggressiveness and is one of the sarcoma subtypes with the highest risk of distant recurrence and reduced disease-specific survival.^{15,16} Synovial sarcoma is the best-defined “translocation-associated sarcoma,” characterized by the presence of a translocation involving the SS18 gene on chromosome 18 and one of several synovial sarcoma X genes on chromosome X. It is a high-grade sarcoma with an aggressive course and a high potential for early metastasis.^{17,18} Undifferentiated pleomorphic sarcoma is one of the most common soft tissue sarcomas. It is a high-grade, aggressive sarcoma previously known as malignant fibrous histiocytoma.¹⁹ Fibrosarcoma is a malignant neoplasm consisting of fibroblasts that may exhibit varying amounts of collagen production and a “herringbone” architecture. It is a rare, highly malignant tumor of mesenchymal cell origin.²⁰ Fibromatosis does not metastasize; however, particularly deep-seated lesions exhibit a destructive growth pattern by infiltrating the surrounding soft tissues.²¹ Extraskeletal mesenchymal chondrosarcoma is a rare malignant soft tissue tumor of chondroprogenitor cell origin. It is a high-grade malignancy with a high tendency for distant metastasis.²² Myofibroblastic sarcoma is a rare tumor composed of malignant myofibroblasts. In addition to having a high recurrence potential, metastasis is less common.²³

In particular, many chromosomal translocations are associated with malignant diseases.²⁴ Neurotrophic tropomyosin receptor kinases (NTRKs), arising from gene fusions belonging to the tropomyosin receptor kinase (TRK) family, are important factors in the development and differentiation of neural cells, as well as in maintaining neuronal homeostasis and regulating synapse formation and plasticity.²⁵

Mitogen-activated protein kinases (MAPKs), which comprise multiple modules, constitute one of the most highly conserved signaling pathways and have functions in cell proliferation, apoptosis, tumor growth, metastasis, and drug resistance.²⁶

KEY MESSAGES

- This study evaluated the expression of NTRK1/2/3, MAPK pathway genes (MEK1, MEK2, ERK1), and URGCP in soft tissue sarcoma (STS) subtypes.
- No statistically significant correlations were identified between NTRK genes and MAPK signaling components, and no molecular link suggesting MAPK pathway activation in STS could be demonstrated based on the present data.
- No statistically significant association was observed between URGCP expression and STS; therefore, no definitive evidence of its oncogenic relevance in soft tissue sarcoma could be established in this study.

Previous studies have revealed that ERK/MAPK, the most extensively studied signaling pathway, is more effective in proliferation and differentiation.^{27–29}

Cell proliferation upregulated gene 4 (*URGCP/URG4*), an oncogene, was identified by Dodurga et al.³⁰ as a novel gene induced by the X antigen encoded by hepatitis B virus. *URGCP* is located on chromosome 7 (7p13) and promotes cell proliferation through signaling pathways such as MAPK. It is involved in tumor development and formation in many cancer types, including hepatocellular carcinoma, osteosarcoma, and glioblastoma. Previous studies have shown that patients with high *URGCP* expression have shorter survival. Although *URGCP* is known to contribute to tumor formation by interacting with various signaling pathways, its effect on soft tissue sarcomas and its molecular mechanism remain unknown.³¹

The present study aimed to investigate the mRNA expression levels of the *NTRK1*, *NTRK2*, *NTRK3*, *MEK1*, *MEK2*, *ERK1*, and *URGCP* genes in formalin-fixed, paraffin-embedded sarcoma tissue samples using quantitative real-time PCR. Furthermore, the study sought to evaluate the potential associations between the expression profiles of these genes and the clinicopathological characteristics of sarcoma cases, thereby providing insight into the molecular mechanisms underlying sarcoma development and progression.

MATERIALS AND METHODS

Collection of Study Samples

The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Pamukkale University Non-Interventional Clinical Research Ethics Committee (Approval Number: 01, Date: 10.01.2023). A total of 80 cases diagnosed with sarcoma at the Pamukkale University Department of Pathology and archived as

formalin-fixed, paraffin-embedded (FFPE) tissue blocks were included in the study. In addition, five non-tumoral FFPE tissue samples were included as a control group and used as the reference group for gene expression normalization analyses. Patients aged 18–70 years with a histopathologically confirmed diagnosis of soft tissue sarcoma were included in the study. Due to the rarity of this tumor group, strict exclusion criteria were not extensively applied to ensure an adequate sample size. Tissue samples (FFPEs) embedded in paraffin blocks and fixed with 10% formaldehyde solution were used in the study. H&E-stained and different types of immunohistochemically stained sections from all cases were re-evaluated for diagnostic and prognostic parameters. The sections that best represented the tumor tissue and contained the least necrosis, hemorrhage, inflammatory cells, and stromal elements were selected. For RNA isolation from the paraffin blocks of these sections, consecutive serial sections were cut at a thickness of 20 µm using a microtome (Leica RM2125RT, Germany).

Total RNA Isolation, cDNA Synthesis, and mRNA Expression Detection by Real-Time PCR Assay

Prior to RNA isolation, FFPE sections were deparaffinized using xylene followed by ethanol washing, as recommended in the Qiagen miRNeasy FFPE Kit protocol. The Qiagen miRNeasy FFPE Kit (Cat. No. 217504) was used for total RNA isolation from paraffin-embedded samples. RNA isolation was performed by deparaffinization followed by spin-column purification according to the kit protocol. The concentration of the isolated RNA was determined, and all RNA samples were adjusted to 380 ng/µL to ensure standardization before cDNA synthesis. cDNA synthesis was performed using the WIZScript™ cDNA Synthesis Kit (REF: W2211) according to the procedure specified by the manufacturer. The mRNA expression changes of seven genes, including *NTRK1*, *NTRK2*, *NTRK3*, *MEK1*, *MEK2*, *ERK1*, and *URGCP*, were determined. GAPDH was used as the housekeeping gene for normalization. The reverse and forward sequences of the genes are given in Table 1. Reactions were prepared using SYBR® Green Master Mix and the Sugenomics qPCR SybrMaster Kit (Catalog No. PCR01C263), and quantitative real-time PCR was performed using a Qiagen Rotor-Gene device.

Statistical Analysis

mRNA expression levels were quantified using the $2^{-\Delta\Delta C_t}$ method. Relative gene expression analyses were performed using the RT² Profiler™ PCR Array Data Analysis software (Qiagen). (<https://www.qiagen.com/tr/shop/genes-and-pathways/data-analysis-centeroverview-page/>) Each qPCR reaction was performed in technical triplicate, and the mean Ct value was used for subsequent analyses. Patient demographic and

Table 1. Forward and reverse primer sequences of the genes used in the study

Gene name	Gene sequence
<i>MEK1_F</i>	5'-GGTGTCAAGGTCTCCACAAG-3'
<i>MEK1_R</i>	5'-CACGATGTACGGCGAGTTGCAT-3'
<i>MEK2_F</i>	5'-GTGGTCACCAAAGTCCAGCAC-3'
<i>MEK2_R</i>	5'-CCACGATGTACGGAGAGTTGCA-3'
<i>ERK1_F</i>	5'-TGGCAAGCACTACCTGGATCAG-3'
<i>ERK1_R</i>	5'-GCAGAGACTGTAGGTAGTTTCGG-3'
<i>URGCP_F</i>	5'-CTTCATCCTGAGTCCCTACCG-3'
<i>URGCP_R</i>	5'-GCCGTTCTGCTGCATTGCG-3'
<i>NTRK1_F</i>	5'-CACTAACAGCACATCTGGAGACC-3'
<i>NTRK1_R</i>	5'-TGAGCACAAGGAGCAGCGTAGA-3'
<i>NTRK2_F</i>	5'-ACAGTCAGCTCAAGCCAGACAC-3'
<i>NTRK2_R</i>	5'-GTCCTGCTCAGGACAGAGGTTA-3'
<i>NTRK3_F</i>	5'-CCGACACTGTGGTCATTGGCAT-3'
<i>NTRK3_R</i>	5'-CAGTTCTCGTTCAGCACGATG-3'
<i>GAPDH_F</i>	5'-GTCTCCTCTGACTTCAACAGCG-3'
<i>GAPDH_R</i>	5'-ACCACCCTGTTGCTGTAGCCAA-3'

clinicopathological characteristics were summarized using descriptive statistics. Categorical variables were presented as frequencies and percentages. Data are presented as fold-change values calculated using the $2^{-\Delta\Delta C_t}$ method. p-values >0.05 indicate no statistical significance. N/A: not applicable (statistical analysis could not be performed due to insufficient sample size in some subgroups).

RESULTS

The subtype data of the Sarcoma Patient Group were obtained from 80 paraffin-embedded block samples diagnosed as different types of "SARCOMA" by the Pamukkale University Department of Pathology and retained as archived material in paraffin blocks. Among 80 patients with soft tissue sarcoma, 12 different subtypes were identified in FFPE tissue samples. The subtypes and patient numbers are given in Table 2. In addition, a scatter plot is shown in Figure 1.

Of the 80 patient samples, 8 were malignant peripheral nerve sheath sarcoma (MPNSTs), 7 were dedifferentiated liposarcoma (DDLs), 9 were synovial sarcoma (SS), 7 were fibrosarcoma, 8 were undifferentiated pleomorphic sarcoma (UPS), 12 were inflammatory myofibroblastic tumor (IMT), 10 were myxofibrosarcoma (MFS), including low grade myxofibrosarcoma (2/10), 2 were extraskeletal mesenchymal chondrosarcoma, 1 was fibromatosis, 14 were leiomyosarcoma (LMS), 2 were myofibroblastic sarcoma.

When the distribution of patients with STS according to sex was analyzed, 57% (n=46) of the patients were male and 43% (n=34) were female (Fig. 2). Most of the patients were between the ages of 50–59 and 60–69 years (Fig. 2, 3).

mRNA Expression Changes in the Sarcoma Patient Group

The mRNA expression changes of seven genes in total, including *NTRK1*, *NTRK2*, *NTRK3*, *MEK1*, *MEK2*, *ERK1*, and the *URGCP* oncogene, which are downstream genes of the MAPK signaling pathway, were detected using real-time PCR. Gene expression changes among patients were evaluated using Ct (threshold cycle) values determined from the patient groups as a result of PCR reactions. *GAPDH* was used as the housekeeping gene for normalization purposes.

Expression changes were determined as shown in Table 3. Fold increases in the mRNA expression of the genes and p values obtained as a result of statistical evaluation are shown in Table 3.

According to our results, *NTRK1*, *NTRK2*, and *NTRK3* gene expression was increased in some soft tissue sarcoma patient groups. In synovial sarcoma, *NTRK1* expression increased 8.85-fold, *NTRK2* expression increased 3.07-fold, and *NTRK3* expression increased 7.11-fold; in fibrosarcoma, *NTRK1*

Table 2. Soft tissue sarcoma subtypes and number of patients

Soft tissue sarcoma subtype	Number of patients
Malignant peripheral nerve sheath tumor (MPNST)	8
Dedifferentiated liposarcoma (DDLs)	7
Synovial sarcoma (SS)	9
Fibrosarcoma	7
Undifferentiated pleomorphic sarcoma (UPS)	8
Inflammatory myofibroblastic tumor (IMT)	12
Myxofibrosarcoma (MFS)	8
Extraskeletal mesenchymal chondrosarcoma	2
Fibromatosis	1
Leiomyosarcoma (LMS)	14
Myofibrosarcoma	2
Low-grade myxofibrosarcoma	2

expression increased 10.15-fold and *NTRK3* expression increased 7.86-fold; in undifferentiated pleomorphic sarcoma, *NTRK2* expression increased 4.40-fold; in inflammatory myofibroblastic sarcoma, *NTRK2* expression increased 2.26-fold;

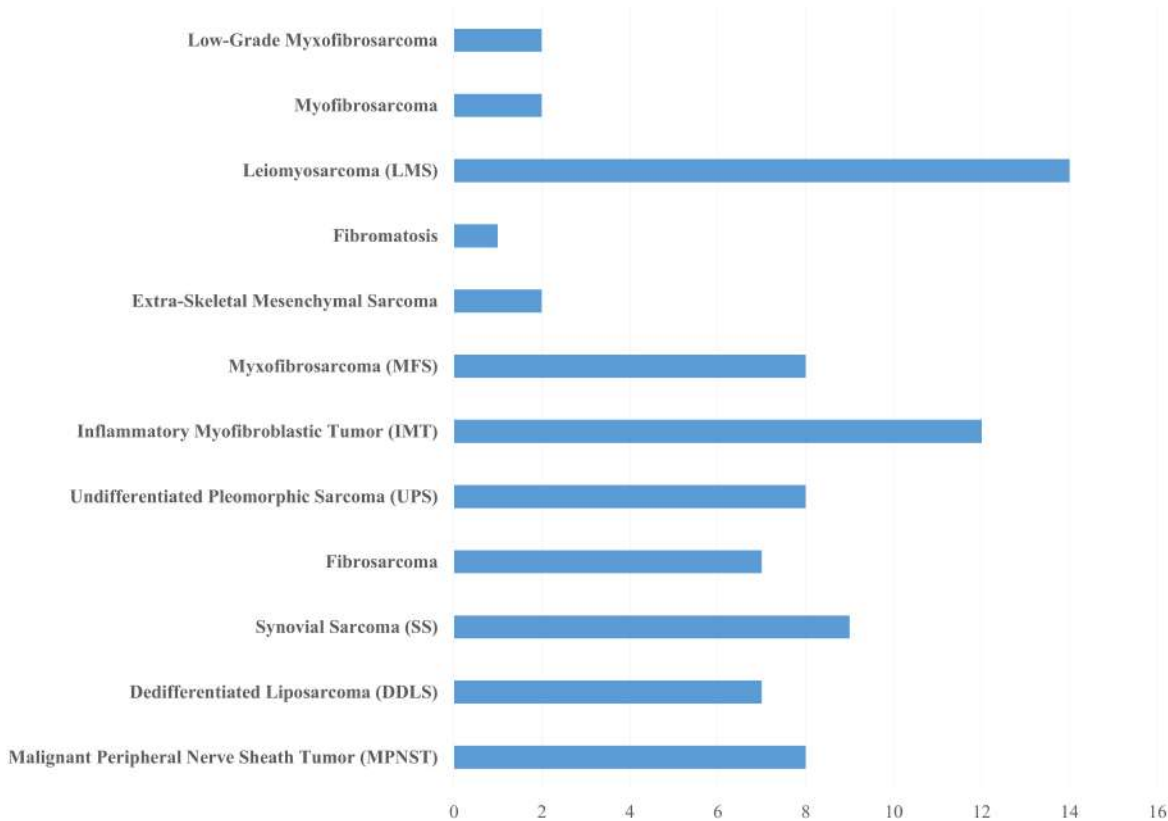


Figure 1. Distribution of soft tissue sarcoma subtypes.

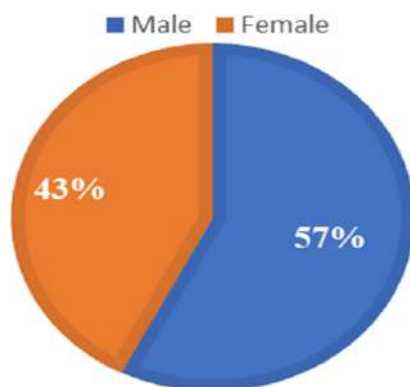


Figure 2. Distribution of soft tissue sarcoma cases according to sex.

in myxofibrosarcoma, *NTRK2* expression increased 7.47-fold; in extraskeletal mesenchymal chondrosarcoma, *NTRK2* expression increased 3.35-fold; in fibromatosis, *NTRK2* expression increased 199.81-fold and *NTRK3* expression increased 14.07-fold; and in leiomyosarcoma, *NTRK2* expression increased 3.58-fold. A trend toward increased mRNA expression of *NTRK1*, *NTRK2*, and *NTRK3* was observed; however, the differences were not statistically significant ($p > 0.05$). In the groups diagnosed with extraskeletal mesenchymal chondrosarcoma, fibromatosis, myofibroblastic sarcoma, and low-grade myxofibrosarcoma, p values were not provided because the number of patients was less than 3. In addition, in malignant peripheral nerve sheath sarcoma, *NTRK1* expression decreased 10.94-fold, *NTRK2* expression decreased 10.61-fold, and *NTRK3* expression decreased 6.06-fold; in undifferentiated pleomorphic sarcoma, *NTRK1* expression decreased 3.22-fold and *NTRK3* expression decreased 3.36-fold; in inflammatory myofibroblastic sarcoma, *NTRK1* expression decreased 9.44-fold and *NTRK3* expression decreased 7.16-fold; in myxofibrosarcoma, *NTRK1* expression decreased 2.30-fold and *NTRK3* expression decreased 2.33-fold; in extraskeletal mesenchymal sarcoma, *NTRK1* expression decreased 4.65-fold and *NTRK3* expression decreased 5.50-fold; in fibromatosis, *NTRK1* expression decreased 32.84-fold; in leiomyosarcoma, *NTRK1* expression decreased 4.91-fold and *NTRK3* expression decreased 7.13-fold; in myofibroblastic sarcoma, *NTRK1* expression decreased 179.46-fold, *NTRK2* expression decreased 4.22-fold, and *NTRK3* expression decreased 95.34-fold; and in low-grade myxofibrosarcoma, *NTRK1* expression decreased 51.89-fold, *NTRK2* expression decreased 3.44-fold, and *NTRK3* expression decreased 35.38-fold. These decreases were not statistically significant ($p > 0.05$). Since the number of patients in the groups diagnosed with extraskeletal mesenchymal chondrosarcoma, fibromatosis, myofibroblastic sarcoma, and low-grade myxofibrosarcoma was less than 3, no p value was provided.

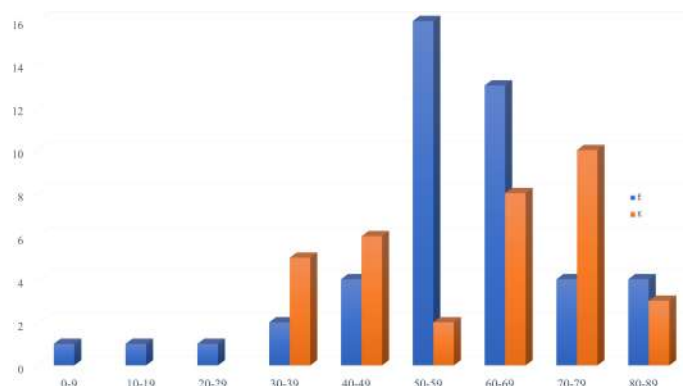


Figure 3. Age and sex distribution of soft tissue sarcoma cases.

When we evaluated the expression of *MEK1*, *MEK2*, and *ERK1*, genes involved in the MAPK signaling pathway that are thought to be related to soft tissue sarcomas, in our patient groups, *ERK1* expression was found to be 3.32-fold higher in dedifferentiated liposarcoma and 3.32-fold higher in synovial sarcoma; in synovial sarcoma, *MEK1* expression increased 6.24-fold, *MEK2* expression increased 7.20-fold, and *ERK1* expression increased 25.77-fold; in fibrosarcoma, *MEK1* expression increased 8.85-fold, *MEK2* expression increased 11.62-fold, and *ERK1* expression increased 24.11-fold; and in fibromatosis, *MEK1* expression increased 16.00-fold, *MEK2* expression increased 16.34-fold, and *ERK1* expression increased 8.74-fold. However, none of these changes were statistically significant ($p > 0.05$).

Regarding the mRNA expression changes of the *URGCP* gene, which is known as a novel oncogene, in soft tissue sarcoma patient groups, a 5.43-fold increase was observed in synovial sarcoma, a 9.09-fold increase in fibrosarcoma, and an 11.45-fold increase in fibromatosis; however, these changes were not statistically significant ($p > 0.05$).

DISCUSSION

Soft tissue sarcoma is a rare cancer with a prevalence of approximately 1% among all malignant tumor types. Soft tissue sarcomas occur in various age groups but are more common in middle-aged patients. There are more than 100 subtypes, including those occurring in adults, young adults, and pediatric patients. The incidence of soft tissue sarcomas, from most to least common, is highest in the lower extremities, followed by the upper extremities, retroperitoneum, and head and neck.^{2,31,32}

With the development of molecular diagnostic technologies for solid tumors, gene expression-based predictive approaches have increased considerably in recent years. The NTRK gene family is a biomarker that may be useful in the diagnosis and treatment of soft tissue sarcomas. The NTRK gene family

Table 3. mRNA expression changes in *NTRK1*, *NTRK2*, *NTRK3*, *MEK1*, *MEK2*, *ERK1*, and *URGCP* among soft tissue sarcoma subtypes

Soft tissue sarcoma subtype	Gene	Fold change	p	Soft tissue sarcoma subtype	Gene	Fold change	p
Malignant peripheral nerve sheath tumor	<i>NTRK1</i>	-10.94	0.281633	Myxofibrosarcoma	<i>NTRK1</i>	-2.30	0.206836
	<i>NTRK2</i>	-10.61	0.18192		<i>NTRK2</i>	7.47	0.605382
	<i>NTRK3</i>	-6.06	0.249364		<i>NTRK3</i>	-2.33	0.224951
	<i>MEK1</i>	-6.92	0.196281		<i>MEK1</i>	-3.95	0.199838
	<i>MEK2</i>	-3.66	0.683821		<i>MEK2</i>	-1.19	0.276038
	<i>ERK1</i>	-2.32	0.753324		<i>ERK1</i>	-1.38	0.504707
	<i>URGCP</i>	-6.94	0.34875		<i>URGCP</i>	-3.45	0.212101
Dedifferentiated liposarcoma	<i>NTRK1</i>	-1.42	0.333208	Extraskeletal mesenchymal chondrosarcoma	<i>NTRK1</i>	-4.65	N/A
	<i>NTRK2</i>	1.37	0.884828		<i>NTRK2</i>	3.35	N/A
	<i>NTRK3</i>	-1.28	0.397838		<i>NTRK3</i>	-5.50	N/A
	<i>MEK1</i>	-1.07	0.434377		<i>MEK1</i>	-7.29	N/A
	<i>MEK2</i>	-1.07	0.561547		<i>MEK2</i>	-3.10	N/A
	<i>ERK1</i>	3.32	0.500875		<i>ERK1</i>	-1.15	N/A
	<i>URGCP</i>	-1.19	0.397512		<i>URGCP</i>	-5.11	N/A
Synovial sarcoma	<i>NTRK1</i>	8.85	0.823855	Fibromatosis	<i>NTRK1</i>	-32.84	N/A
	<i>NTRK2</i>	3.07	0.676786		<i>NTRK2</i>	199.81	N/A
	<i>NTRK3</i>	7.11	0.872715		<i>NTRK3</i>	14.07	N/A
	<i>MEK1</i>	6.24	0.856598		<i>MEK1</i>	16.00	N/A
	<i>MEK2</i>	7.20	0.883655		<i>MEK2</i>	16.34	N/A
	<i>ERK1</i>	25.77	0.33401		<i>ERK1</i>	8.74	N/A
	<i>URGCP</i>	5.43	0.971169		<i>URGCP</i>	11.45	N/A
Fibrosarcoma	<i>NTRK1</i>	10.15	0.740549	Leiomyosarcoma	<i>NTRK1</i>	-4.91	N/A
	<i>NTRK2</i>	1.73	0.539956		<i>NTRK2</i>	3.58	N/A
	<i>NTRK3</i>	7.86	0.714319		<i>NTRK3</i>	-7.13	N/A
	<i>MEK1</i>	8.85	0.59401		<i>MEK1</i>	-5.14	N/A
	<i>MEK2</i>	11.62	0.523597		<i>MEK2</i>	-2.08	N/A
	<i>ERK1</i>	24.11	0.310417		<i>ERK1</i>	-5.35	N/A
	<i>URGCP</i>	9.09	0.673954		<i>URGCP</i>	-6.96	N/A
Undifferentiated pleomorphic sarcoma	<i>NTRK1</i>	-3.22	0.219797	Myofibroblastic sarcoma	<i>NTRK1</i>	-179.46	N/A
	<i>NTRK2</i>	4.40	0.623485		<i>NTRK2</i>	-4.22	N/A
	<i>NTRK3</i>	-3.36	0.257268		<i>NTRK3</i>	-95.34	N/A
	<i>MEK1</i>	-2.27	0.984675		<i>MEK1</i>	-47.18	N/A
	<i>MEK2</i>	-2.35	0.379809		<i>MEK2</i>	-36.63	N/A
	<i>ERK1</i>	-3.68	0.337059		<i>ERK1</i>	-61.71	N/A
	<i>URGCP</i>	-8.04	0.243411		<i>URGCP</i>	-104.51	N/A
Inflammatory myofibroblastic tumor	<i>NTRK1</i>	-9.44	0.100646	Low-grade myxofibrosarcoma	<i>NTRK1</i>	-51.89	N/A
	<i>NTRK2</i>	2.26	0.629345		<i>NTRK2</i>	-3.44	N/A
	<i>NTRK3</i>	-7.16	0.125581		<i>NTRK3</i>	-35.38	N/A
	<i>MEK1</i>	-13.09	0.095242		<i>MEK1</i>	-49.69	N/A
	<i>MEK2</i>	-4.21	0.152371		<i>MEK2</i>	-21.19	N/A
	<i>ERK1</i>	-3.49	0.450555		<i>ERK1</i>	-165.13	N/A
	<i>URGCP</i>	-11.95	0.10455		<i>URGCP</i>	-44.55	N/A

consists of three genes, *NTRK1*, *NTRK2*, and *NTRK3*. Each encodes a tropomyosin receptor kinase that interacts with downstream signaling pathways. They are important components of downstream signaling pathways involved in various processes such as cellular survival, differentiation, and growth.^{26,33}

The MAPK signaling pathway is a signaling cascade that plays an important role in cell proliferation, apoptosis, and differentiation. Abnormal activation of the MAPK signaling pathway caused by various mechanisms, such as receptor tyrosine kinases and gene mutations, is involved in tumor formation and progression by contributing to processes such as cell growth, survival, invasion, and metastasis.^{29,34}

Oncogenes are genes with important roles in apoptosis, proliferation, and the cell cycle. These genes, which play a role in carcinogenesis and cancer progression, are numerous, and increasing numbers of oncogenes are being discovered every year. One of these oncogenes is cell proliferation upregulated gene 4. The *URGCP* oncogene was first found to contribute to hepatocarcinogenesis. In later studies, it was found to be upregulated in cancer types such as gastric cancer and osteosarcoma. For this reason, the *URGCP* gene is likely to be an important molecular target for cancer treatment, considering its effects on cell cycle regulation.^{30,35,36}

Song et al.³⁷ investigated the relationship between various fusion genes and the clinicopathological features of 242 cases of seven types of soft tissue tumors using one-step RT-PCR. The study included patients from 1999 to 2021. The samples included cases of rhabdomyosarcoma (RMS), synovial sarcoma (SS), pediatric peripheral primitive neuroectodermal tumor (pPNET), alveolar soft part sarcoma (ASPS), dermatofibrosarcoma protuberans (DFSP), myxoid liposarcoma (MLPS), and soft tissue angiofibroma (AFST). By examining the relationship between fusion gene status and clinicopathological parameters, they revealed that fusion gene status was associated with age and location but not with sex or tumor diameter. Similarly, in our study, we think that fusion genes could not be associated with sex and tumor diameter but may be associated with age. In particular, an increased frequency was observed in patients aged 30 years and older.

NTRK fusions can be found in many different tumors, and on a molecular basis, *NTRK3* is the most common, followed by *NTRK1* and *NTRK2* expression. In a study by Siozopoulou et al.,³⁸ 70 FFPE-fixed soft tissue and bone tumor cases were evaluated. As a result of the study, the rate of sarcomas with expression was determined to be approximately 2.8% of the total population. In addition, the prognosis of *NTRK1*- and *NTRK3*-related sarcomas in relation to histomorphology was investigated, and it was found that sarcomas with *NTRK1*

fusion may exhibit low- or high-grade histomorphology. At the same time, morphologically high-grade tumors containing *NTRK1* may show an aggressive course, while low-grade tumors may exhibit a more favorable clinical course. On the other hand, sarcomas with *NTRK3* fusion are more aggressive neoplasms. However, contrary to this finding, a very low-grade tumor containing an *NTRK3* fusion was reported in the study. Likewise, in our study, particularly the *NTRK3* fusion gene was expected to be detected in high-grade tumors, but it did not reach statistical significance.

In a study conducted by Solomon et al.,³⁹ a total of 38,095 samples were obtained from 33,997 patients, and *NTRK1*, *NTRK2*, and *NTRK3* fusion gene detection was performed. Only 13 cases among 87 tumors and tumor types with 1,915 molecular diagnoses were associated with NTRK genes. In addition, only 3 of 17 inflammatory myofibroblastic tumors were associated with NTRK genes.

The MAPK signaling pathway plays an important role in various molecular mechanisms, such as proliferation, differentiation, and apoptosis, in normal cells. The signaling pathway can be overactivated due to mutations, amplifications, and translocations in genes responsible for the regulation of cell fate, survival, and genome integrity. This can lead to cancer and tumor formation. In a study conducted by Arconada-Luque et al.,⁴⁰ the relationship between the MAPK signaling pathway and sarcomagenesis was investigated. They compared the expression levels of different MAPKs using cell lines and mouse models and found no difference in ERK2 expression in tumor samples. Similarly, we did not find any difference in ERK expression levels in our study. In addition, Gu et al.²⁴ examined the relationship with the MAPK signaling pathway using a MEK inhibitor in malignant peripheral nerve sheath tumors. They confirmed the overactivation of the MAPK signaling pathway in malignant peripheral nerve sheath tumors. In our study, no difference was observed in the expression changes of the MAPK signaling pathway.

Cell proliferation upregulated gene 4 is an oncogene involved in various types of cancer. In leukemia and prostate cancer, overexpression of the *URGCP* gene is involved not only in tumor development but also in metastasis and recurrence. The molecular mechanism of the *URGCP* oncogene in soft tissue sarcomas is not yet known.

According to the data obtained in our study, the primary reason for the statistically nonsignificant mRNA expression changes is the insufficient number of patients in the study population. Since soft tissue sarcoma types are rare cancers, an equal and sufficient patient population could not be provided for each group. Therefore, no statistical significance

could be established in the study. We think that stress, inflammation, death receptor activation, oxidative stress, and therapeutic agents may influence the NTRK genes and MAPK signaling pathway during the clinical follow-up of patients included in the study and therefore contribute to changes in mRNA expression. According to the pathology results, *NTRK1*, *NTRK2*, and *NTRK3* gene expression was reported to be negative on immunohistochemical staining in our cases, which was similar to the findings of our study. After the *URGCP* oncogene, which we used in our study, was found to play a role in various cancer types, such as gastric cancer and hepatocellular cancer, it was thought that it might also have an effect on soft tissue sarcomas. However, no negative or positive effect was found in our results. For this reason, we think that it may serve as a basis for future research incorporating larger patient populations, disease prognosis, and a wider range of examinations. The discrepancy between mRNA expression levels and immunohistochemistry findings may be explained by post-transcriptional and post-translational regulatory mechanisms, including microRNA-mediated regulation, differences in protein stability, translational efficiency, and protein degradation. In addition, technical limitations of immunohistochemistry, particularly its sensitivity in detecting low-abundance proteins in formalin-fixed, paraffin-embedded (FFPE) tissues, may also contribute to this inconsistency.

Soft tissue sarcomas, which are rare among all cancer types, have more than 100 subtypes, and these subtypes are themselves rare malignant tumors. Therefore, the detection and prognosis of the disease have not been fully elucidated. Detection of NTRK genes is a promising marker, particularly for the detection of STS. In our study, we examined the expression levels of NTRK genes using real-time PCR. However, we think that real-time PCR should be supported by immunohistochemical staining. In addition, we anticipate that more accurate studies can be conducted with the support of next-generation sequencing (NGS), which has become widespread in recent years. We also believe that increasing the study cohort will improve the results and may provide a basis for further studies.

CONCLUSIONS

The correlation between the expression levels of the *NTRK1*, *NTRK2*, and *NTRK3* genes and the MAPK signaling pathway in STS was investigated for the first time, and their relationship with the *URGCP* oncogene was revealed for the first time. The molecular mechanism of the *URGCP* oncogene in STS has not been determined.

In this study, we observed increased *NTRK2* gene expression in synovial sarcoma, undifferentiated pleomorphic sarcoma,

inflammatory myofibroblastic tumor, myxofibrosarcoma (except low grade myxofibrosarcoma) extraskeletal mesenchymal chondrosarcoma, fibromatosis, and leiomyosarcoma; increased *NTRK3* gene expression in synovial sarcoma, fibrosarcoma, and fibromatosis; and increased *NTRK1* gene expression in synovial sarcoma and fibrosarcoma. In addition, decreased *NTRK1* gene fusion was observed in malignant peripheral nerve sheath sarcoma, undifferentiated pleomorphic sarcoma, inflammatory myofibroblastic tumor, myofibroblastic tumor, myxofibrosarcoma (including low grade myxofibrosarcoma), extraskeletal mesenchymal chondrosarcoma, fibromatosis, leiomyosarcoma, myofibroblastic sarcoma, decreased *NTRK3* gene fusion was observed in malignant peripheral nerve sheath sarcoma, undifferentiated pleomorphic sarcoma, inflammatory myofibroblastic tumor, myxofibrosarcoma (including low grade myxofibrosarcoma), extraskeletal mesenchymal chondrosarcoma, leiomyosarcoma, myofibroblastic sarcoma, and decreased *NTRK2* gene fusion was observed in malignant peripheral nerve sheath sarcoma, myofibroblastic sarcoma, and low-grade myxofibrosarcoma.

In dedifferentiated liposarcoma, synovial sarcoma, fibrosarcoma, and fibromatosis, *ERK1* of the MAPK signaling pathway was increased, while *MEK1* and *MEK2* were increased in synovial sarcoma, fibrosarcoma, and fibromatosis. The *URGCP* oncogene was also increased in synovial sarcoma, fibrosarcoma, and fibromatosis. The observed fluctuations in expression levels did not reach statistical significance ($p > 0.05$). In addition, p values could not be obtained because the numbers of patients with fibromatosis, myofibroblastic sarcoma, and low-grade myxofibrosarcoma were less than 3 and 3, respectively. A limitation of the present study is the heterogeneous distribution of soft tissue sarcoma subtypes and the small sample sizes in several rare subgroups, which may have reduced the statistical power of the analyses and limited the ability to detect statistically significant differences. Therefore, larger multicenter studies including greater numbers of patients are needed. Furthermore, additional investigations incorporating protein-level validation methods such as immunohistochemistry, Western blotting, and proteomic analyses would further strengthen the biological interpretation of the observed mRNA expression changes. Our results were similar to those reported in the literature and were consistent with the pathological findings.

Ethics Committee Approval: Ethics committee approval was obtained from Pamukkale University Non-Interventional Clinical Research Ethics Committee (Approval Number: 01, Date: 10.01.2023).

Informed Consent: Due to the retrospective design and the use of archived formalin-fixed paraffin-embedded (FFPE) tissue samples, the requirement for written informed consent was waived by the Ethics Committee.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Limited Public Health Utility of LCT Genotyping for Lactose Intolerance Screening in Adolescents: A Cross-Sectional Study from Türkiye

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ABSTRACT

Objective: Lactose intolerance (LI) is a common cause of gastrointestinal complaints in adolescents and young adults, often leading to unnecessary dietary restrictions and reduced calcium intake. Although lactase non-persistence (LNP) is genetically determined, its clinical expression varies considerably. Genetic variants associated with lactase persistence/non-persistence (LCT/MCM6 locus), particularly rs4988235 and rs3754686, are frequently used in genetic testing; however, their clinical utility remains uncertain, especially in populations with a high prevalence of LNP, such as Türkiye.

Materials and Methods: In this cross-sectional study, 209 individuals aged 12–25 years were evaluated. Lactose-related symptoms were assessed using a validated questionnaire (a score ≥ 7 defined symptomatic individuals). Consumption of raw milk and fermented dairy products was recorded using a standardized frequency scale. Genotyping of rs4988235 and rs3754686 was performed using TaqMan real-time PCR. Associations between genotype, symptom status, age at symptom onset, and dietary patterns were statistically analyzed.

Results: Among the participants, 116 (55.5%) were symptomatic and 93 (44.5%) were asymptomatic. Symptomatic individuals were significantly older ($p < 0.001$). The genotype distributions of rs4988235 and rs3754686 did not differ between symptomatic and asymptomatic individuals and were not associated with age at symptom onset. Dairy consumption patterns were not associated with symptom severity, although fermented dairy consumption was slightly higher among symptomatic individuals ($p = 0.046$).

Conclusion: Common LCT/MCM6 variants did not predict clinical LI in this adolescent and young adult population. Therefore, routine genetic testing appears unlikely to provide limited additional value in supporting the diagnosis of lactose intolerance, particularly in populations with a high prevalence of LNP. Symptom-based assessment and functional testing should remain central to diagnosis and management, and, supporting appropriate nutritional guidance and avoiding unnecessary dietary restriction.

Keywords: Adolescents, dairy consumption, lactose intolerance, LCT, MCM6.



INTRODUCTION

Lactose intolerance (LI) is a common clinical concern among adolescents and young adults and represents one of the most frequent causes of diet-related gastrointestinal symptoms worldwide. Primary lactase non-persistence (LNP), characterized by the genetically programmed decline in lactase-phlorizin hydrolase activity after childhood, is the predominant biological mechanism underlying lactose malabsorption.^{1–3} Although lactase activity is typically high during infancy, its expression progressively decreases during late childhood and adolescence, leading to the emergence of symptoms such as abdominal pain, bloating, diarrhea, and flatulence in susceptible individuals.^{4,5} Clinical recognition often occurs during adolescence, when the physiological decline in lactase activity coincides with greater autonomy in dietary choices.

The most extensively studied regulators of lactase expression are noncoding variants within the MCM6 gene, such as rs4988235 (–13910 C/T) and rs3754686 (–22018 G/A), which act as enhancer elements that modulate transcription of the adjacent LCT gene and thereby influence the persistence or downregulation of lactase activity into adulthood.^{6–8} In European and neighboring populations, the T allele of rs4988235 strongly correlates with lactase persistence, whereas the ancestral C allele is associated with LNP and adult-type hypolactasia.⁷ Türkiye is recognized as a population with a naturally high prevalence of genetically determined LNP, with previous phenotypic studies reporting low lactase activity in up to 70–80% of adults.^{9–11} Despite the high frequency of LNP genotypes, only a subset of individuals develop clinical intolerance, suggesting that symptom expression is influenced by multiple physiological, microbial, and environmental factors beyond genetic background.^{12–14}

Given the widespread availability of direct-to-consumer genetic testing and increasing clinician awareness of LCT polymorphisms, genotyping is sometimes requested as part of the diagnostic evaluation of LI. However, the clinical utility of genetic testing in populations with a high prevalence of LNP remains uncertain, particularly among adolescents, in whom symptom onset, dietary behavior, microbiota composition, and visceral sensitivity are highly variable.^{15–17} Furthermore, few studies have examined genotype–phenotype relationships specifically in adolescents or young adults, despite this being the developmental period during which symptoms most commonly manifest.

The present study aimed to evaluate the relationship among LI symptoms, dairy consumption patterns, and the two major LCT regulatory variants (rs4988235 and rs3754686)

KEY MESSAGES

- In populations with a high prevalence of lactase non-persistence, such as Türkiye, *LCT/MCM6* variants (rs4988235 and rs3754686) provide limited diagnostic value for clinical lactose intolerance.
- Genetic testing to support the diagnosis of lactase non-persistence offers minimal additional clinical benefit and should not be part of routine practice.
- Clinical evaluation based on symptoms is more informative than genotype. When necessary, functional testing should complement clinical assessment to ensure an accurate diagnosis and prevent unnecessary dietary restriction.

in a cohort of adolescents and young adults in Türkiye. By assessing genotype distributions, symptom severity, and dietary behavior in symptomatic and asymptomatic individuals, we sought to clarify whether these variants have diagnostic value in a population with a high baseline prevalence of LNP. Understanding this relationship is essential for guiding clinicians regarding the appropriate role of genetic testing and improving diagnostic pathways in routine practice.

MATERIALS AND METHODS

Study Design and Participants

This cross-sectional study included adolescents and young adults aged 12–25 years who presented to the Adolescent Health Clinic and the General Pediatrics Outpatient Clinic of Ankara University Faculty of Medicine, Ankara Children's Hospital, either with lactose-related gastrointestinal complaints or without such symptoms. Participants were recruited consecutively during routine outpatient visits and were eligible for inclusion if they were within the specified age range and able to complete the study questionnaires. Exclusion criteria included any documented chronic gastrointestinal disease, including celiac disease and inflammatory bowel disease, chronic use of medications that could affect gastrointestinal motility, and a history of abdominal surgery. Demographic characteristics, medical history, and dietary habits were obtained using structured questionnaires administered by trained researchers. The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Ankara University Faculty of Medicine Human Research Ethics Committee (Approval Number: İ09-671-24, Date: 10.10.2024). Written informed consent was obtained from all participants and from the parents or legal guardians of minors.

This was an exploratory cross-sectional study. Because reliable local data regarding the expected effect size of the association between LCT/MCM6 genotypes and clinical symptoms of LI were unavailable, a formal a priori sample size calculation was not performed. A total of 209 adolescents and young adults who met the eligibility criteria were enrolled during the study period.

Symptom Assessment

LI symptoms were evaluated using a validated symptom questionnaire assessing abdominal pain, bloating, diarrhea, flatulence, nausea, and urgency occurring within 30 minutes to 2 hours after consumption of milk or dairy products.¹⁸ Participants with scores ≥ 7 were classified as symptomatic, whereas those with scores < 7 were classified as asymptomatic.

Dairy Consumption Assessment

All participants completed a detailed frequency questionnaire assessing the consumption of raw milk and fermented dairy products (pasteurized yogurt, ayran, and kefir). Each item was scored on a 0–4 Likert scale, with higher scores indicating more frequent intake (0=never, 1=very rarely, 2=a few times per month, 3=a few times per week, and 4=every day). Raw milk and fermented dairy product consumption scores were analyzed separately.

Genotyping Procedures

Peripheral venous blood (minimum, 5 mL) was collected from all participants after informed consent was obtained. Genomic DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Germany) according to the manufacturer's instructions.

Genotyping of the rs4988235 (–13910 C/T) and rs3754686 (–22018 G/A) variants was performed using TaqMan® real-time PCR assays (Thermo Fisher Scientific).

Fluorogenic allele-specific probes were labeled with FAM for the minor allele and HEX for the major allele, with Black Hole Quencher™ 1 (BHQ1) at the 3' end. The primer concentration in each reaction was 36 μ M, and the probe concentration was 20 μ M. PCR reactions were performed using a LightCycler® 480 Real-Time PCR System (Roche Diagnostics). Genotypes were automatically assigned by the LightCycler software and manually confirmed. Any ambiguous or low-quality curves were classified as uninterpretable and excluded.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics (version XX; IBM Corp., Armonk, NY, USA). The normality of continuous variables was evaluated using the Shapiro–Wilk test and visual inspection of histograms. Continuous

variables are presented as mean $\pm$ standard deviation (SD) or median (Q1–Q3), depending on their distribution, and categorical variables as frequencies and percentages. Group comparisons were performed using independent-samples t-tests or Mann–Whitney U tests for continuous variables and chi-square or Fisher's exact tests for categorical variables. Correlations between symptom scores and dairy consumption were assessed using Spearman's rank correlation coefficients, and a two-sided p value < 0.05 was considered statistically significant.

RESULTS

Participant Characteristics

A total of 209 participants aged 12–25 years were enrolled. The mean age of the cohort was 17.9 ± 4.2 years, and 65.1% ($n=136$) were female. Based on the LI symptom score, 116 individuals (55.5%) were classified as symptomatic (score ≥ 7), and 93 (44.5%) were classified as asymptomatic (score < 7).

The symptomatic group was significantly older than the asymptomatic group (19.25 ± 4.26 vs. 16.31 ± 3.49 years, $p < 0.001$). Mean symptom scores were 20.34 ± 8.85 (range, 8–41) in symptomatic individuals and 1.69 ± 1.98 in the asymptomatic group.

Among the 105 symptomatic participants who reported the age at symptom onset, symptoms began at a mean age of 14.2 ± 4.7 years (range, 2–23 years).

Genotype Distributions

The rs4988235 (–13910 C/T) genotype distribution was similar between symptomatic and asymptomatic participants (Table 1). There was no significant difference between the groups ($p=0.842$). Similarly, rs3754686 (–22018 G/A) genotype frequencies did not differ between the groups. Furthermore, when rs4988235 and rs3754686 were considered jointly, 16 of 209 participants (7.7%) were homozygous for both non-persistence-associated genotypes (CC at rs4988235 and GG at rs3754686), whereas the remaining 193 (92.3%) carried at least one persistence-associated or heterozygous genotype.

Genotype and Symptom Onset

Neither rs4988235 nor rs3754686 genotype was associated with age at symptom onset. For rs4988235, both C/C and C/T genotypes showed symptom onset at approximately 13–14 years, with no significant difference. For rs3754686, ages at symptom onset were similar across T/T, T/C, and C/C genotypes (mean, 14.7–14.9 years), with no statistically significant differences. These results indicate that genetic variation did not influence the timing of symptom onset.

Table 1. Genotype distributions of rs4988235 (–13910 C/T) and rs3754686 (–22018 G/A) in symptomatic and asymptomatic groups, with estimated frequencies reported for the Turkish population

Variant/genotype	Symptomatic (%)	Asymptomatic (%)	Estimated frequency in Türkiye* (%)	p
rs4988235 (–13910 C/T)				0.842
C/C (G/G)	85.3	86.0	80–90	
C/T (G/A)	12.9	12.9	10–20	
T/T (A/A)	0.9	0.0	0–2	
rs3754686 (–22018 G/A)				0.596
T/T (A/A equivalent)	44.8	48.4	40–60	
T/C (A/G equivalent)	43.1	36.6	30–40	
C/C (G/G equivalent)	12.1	15.1	5–10	

*: Precise population-wide frequencies of these SNPs are not available for Türkiye; the available data represent estimates and ranges derived from the literature. References: Flatz et al.,¹ 1986; Özden et al.,² 1992; Enattah et al.,¹³ 2002; Itan et al.,³ 2010.

Table 2. Comparison of raw milk and fermented dairy product consumption between symptomatic and asymptomatic participants

Dairy product category	Symptomatic group (n=116), mean±SD	Asymptomatic group (n=93), mean±SD	Asymptomatic group, median (Q1–Q3)	Effect size (r)	p
Raw milk consumption	2.67±1.04	2.65±1.27	3.0 (2.0–4.0)	0.03	0.868
Fermented dairy product consumption	2.12±0.72	1.88±0.94	2.0 (1.3–2.3)	0.11	0.046

Higher scores indicate more frequent consumption on a 0–4 Likert-type scale (0=never, 4=every day). Values are presented as mean±standard deviation (SD) and median (Q1–Q3); p values and effect sizes (r) refer to between-group comparisons.

Dairy Consumption Patterns

Raw milk consumption was similar between symptomatic and asymptomatic participants (2.67 vs. 2.64, $p=0.868$, $r=0.03$). In contrast, fermented dairy product consumption was higher in the symptomatic group (2.12 vs. 1.88, $p=0.046$, $r=0.11$) (Table 2). Despite their generally better tolerance, fermented products were still consumed less frequently than raw milk in both groups.

When raw milk and fermented dairy product intake were analyzed within each genotype category (rs4988235 and rs3754686), no consistent or statistically significant patterns were identified. Consumption frequency did not vary meaningfully across genotypes.

Relationship Between Dairy Intake and Symptom Severity

In the symptomatic group ($n=116$), dairy consumption scores showed no significant linear correlation with symptom severity (Table 3). Although the correlation between kefir consumption and symptom severity was positive ($r=0.160$, $p=0.086$), its magnitude was very weak and did not reach statistical significance. Overall, increased fermented dairy product consumption did not reduce symptom scores, and higher milk intake did not predict greater symptom

Table 3. Spearman correlation coefficients between dairy product consumption scores and symptom severity among symptomatic participants ($n=116$)

Dairy product	Correlation coefficient (r)	p
Raw milk	-0.129	0.167
Ayran	-0.080	0.392
Yogurt	0.131	0.160
Kefir	0.160	0.086

severity (all $|r|<0.20$). These relationships are illustrated in the scatterplot matrix shown in Figure 1.

DISCUSSION

In this cross-sectional study of adolescents and young adults, more than half of the participants reported LI-related symptoms, with a mean age at symptom onset of approximately 14 years. This pattern is consistent with the physiological decline in lactase activity that begins in late childhood and becomes clinically apparent during adolescence.^{5,10} The age distribution of our cohort (12–25 years) was intentionally selected to reflect this

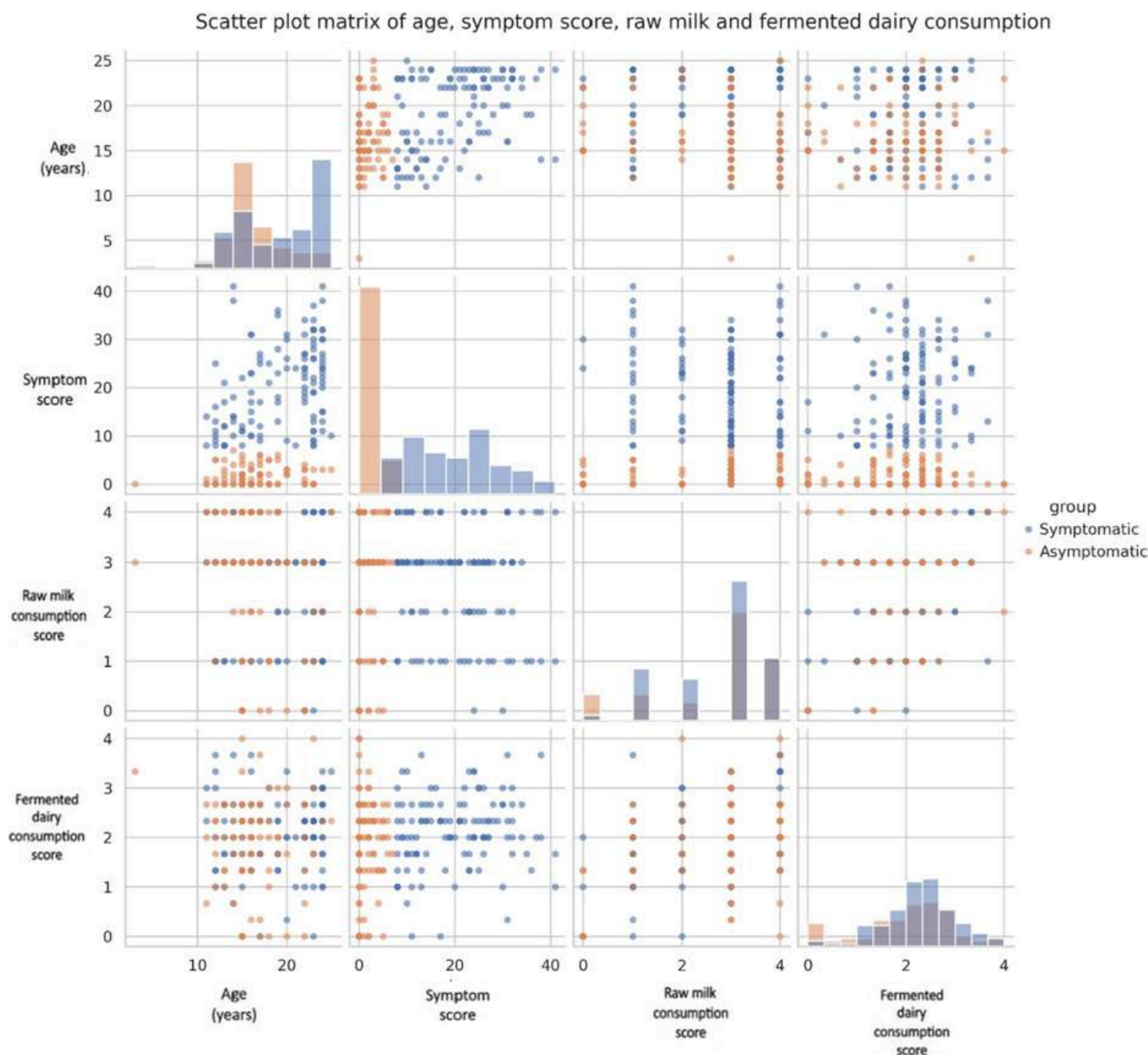


Figure 1. Scatterplot matrix of age, symptom severity, and dairy consumption scores in symptomatic and asymptomatic participants.

developmental period. According to both WHO definitions and the Lancet Adolescent Health Commission, adolescence extends up to age 24 because of prolonged neurobiological and psychosocial maturation. Including participants up to 25 years of age therefore allowed us to capture the full clinical spectrum of LI from late adolescence to young adulthood, the age range during which symptoms most commonly emerge.^{5,6}

A central finding of our study was the absence of a genotype–phenotype association for the two major LCT/MCM6 regulatory variants, rs4988235 (–13910 C/T) and rs3754686 (–22018 G/A). Genotype frequencies were nearly identical between symptomatic and asymptomatic individuals. These results are consistent with historical Turkish population studies demonstrating that the lactase non-persistence (LNP) phenotype and ancestral genotypes are highly prevalent,

with frequencies ranging from 70% to 90%.^{1,2} Our observed genotype rates (approximately 85% C/C for –13910 C/T) closely match the estimated frequencies for Türkiye and reflect expected population genetics rather than disease-specific biology.

These findings are biologically plausible because genetically determined lactase non-persistence does not universally translate into clinical LI. Many individuals with non-persistence-associated genotypes remain asymptomatic or report only mild symptoms, indicating that symptom expression is influenced by additional modifying factors beyond the LCT/MCM6 locus.^{5,7,9,16} In particular, gut microbiota composition, colonic fermentation capacity, habitual lactose exposure, variability in lactose tolerance thresholds, visceral hypersensitivity, and coexisting functional gastrointestinal disorders such as IBS are all likely to influence whether genetically predisposed individuals develop symptoms.^{5,9,16} Our results are consistent with this multifactorial model: LNP genotypes were frequent in the cohort, but their distribution did not differ between symptomatic and asymptomatic participants, and dairy consumption scores showed only very weak, nonsignificant correlations with symptom severity. Together, these observations support the view that LI in adolescents cannot be reliably predicted by LCT genotyping alone, particularly in populations in which LNP alleles are common.

Furthermore, asymptomatic adolescents with non-persistence genotypes may still develop intolerance later in life as lactase activity declines further, dietary habits change, or the intestinal environment shifts.^{5,16} Longitudinal data suggest that lactose tolerance can improve or worsen over time depending on continued lactose exposure, microbial adaptation, and the development of other gastrointestinal conditions, underscoring that clinically relevant LI represents a dynamic rather than a fixed genetic state.^{5,15} Our cross-sectional design cannot resolve these trajectories, but the coexistence of symptomatic and asymptomatic LNP carriers in our cohort highlights the need for individualized, symptom-guided counseling instead of lifelong dairy restriction based solely on genotype.

We also examined dairy consumption behaviors. Although fermented dairy intake was modestly higher among symptomatic individuals, raw milk consumption was similar between the groups, and raw milk remained the most frequently consumed dairy product overall. This suggests that cultural preferences and habitual consumption patterns may outweigh symptom-driven dietary modifications. Importantly, none of the dairy intake measures, including raw milk, yogurt, ayran, or kefir, showed significant correlations with symptom severity. This finding supports previous studies indicating that lactose

dose alone does not fully determine symptom generation and that sensitivity thresholds vary widely among individuals.^{17–19}

These findings have practical implications for clinical care. In regions such as Türkiye, where LNP genotypes are the norm rather than the exception, LCT genotyping has limited discriminatory value and cannot differentiate symptomatic from asymptomatic individuals. Routine genetic testing may therefore contribute to unnecessary costs without improving diagnostic accuracy. Current best-practice guidelines emphasize symptom-based clinical assessment and, when needed, hydrogen or methane breath testing as the diagnostic standards.^{5,19} Our study strongly supports the view that LI should remain a clinically driven diagnosis rather than a genetically defined one in populations with a high prevalence of LNP.

Limitations

Symptom assessment relied on self-reported questionnaires, which may introduce recall bias. Dairy intake scores were based on consumption frequency rather than precise lactose content, limiting dose–response interpretation. The sample size of 209 participants was primarily determined by feasibility within the study period rather than by a formal a priori power calculation for small genotype effects.¹² Given the high background prevalence of lactase non-persistence in Türkiye, the study was sufficiently powered to detect moderate-to-large differences in genotype distributions between symptomatic and asymptomatic groups but may have been underpowered to detect small effect sizes. As a result, subtle genotype–phenotype associations cannot be definitively excluded. Direct biochemical testing (breath hydrogen or enzyme assays) was not performed, preventing assessment of concordance among genetic, biochemical, and clinical measures.

CONCLUSION

In this cohort of adolescents and young adults, LI symptoms were common, yet the distribution of the major LCT/MCM6 variants (rs4988235 and rs3754686) was nearly identical between symptomatic and asymptomatic individuals. These findings indicate that genetically determined lactase non-persistence, although highly prevalent in Türkiye, does not reliably predict clinical intolerance. Symptom expression appears to be influenced more by physiological, microbial, and behavioral factors than by genotype alone.

Dairy consumption patterns and symptom severity showed no meaningful association, further supporting the concept that LI in this age group is multifactorial rather than genetically defined. Importantly, our results demonstrate that LCT genotyping provides limited diagnostic value in populations with a high prevalence of LNP and should not be routinely used to evaluate adolescents with suspected LI.

Clinical diagnosis should continue to rely primarily on symptom-based assessment, dietary history, and hydrogen–methane breath testing when needed. Recognizing the limited role of genetics in this context may help guide more cost-effective, accurate, and patient-centered diagnostic pathways in routine practice.

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Informed Consent: Written informed consent was obtained from all participants and from the parents or legal guardians of minors.

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Investigation of the Number of Falls and Fall Risk Factors in Geriatric Individuals Living in a Nursing Home

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ABSTRACT

Objective: Falls are a major cause of morbidity and mortality among older adults worldwide, highlighting the need to identify fall risk factors, particularly among nursing home residents. This study aimed to evaluate fall risk factors according to fall history in nursing home-dwelling geriatric individuals and to examine the association between these factors and the number of falls.

Materials and Methods: This cross-sectional study included geriatric individuals living in nursing homes who were classified as fallers (n=54) or non-fallers (n=66). Participants' demographic characteristics and fall-related risk factors were recorded. Comorbidity was assessed using the Charlson Comorbidity Index. Physical performance was evaluated using gait speed, the 5-Repetition Sit-to-Stand Test, and handgrip strength. Physical activity level was assessed using the Physical Activity Scale for the Elderly. Sarcopenia risk was evaluated using the SARC-F questionnaire. Nutritional status was assessed using the Mini Nutritional Assessment. Incontinence severity was evaluated using the Incontinence Severity Index, and activities of daily living (ADL) were assessed using the Katz Index of ADL.

Results: Fallers were older, used more medications, had a greater comorbidity burden, and demonstrated poorer physical performance and lower activity levels, a higher risk of sarcopenia, poorer nutritional status, greater incontinence severity, and reduced independence in ADL. Furthermore, fall frequency was significantly associated with assistive device use, gait speed, and nutritional status ($p<0.05$).

Conclusion: These findings demonstrate that multifactorial, targeted interventions addressing physical performance, mobility, and nutrition are essential for reducing fall risk and recurrent falls in geriatric individuals.

Keywords: Fall, geriatric individual, malnutrition, physical performance, sarcopenia.



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INTRODUCTION

Aging is characterized by various changes in sensory, cognitive, and neuromotor domains that can lead to balance and gait disturbances. Gait disturbances resulting from these changes are among the main causes of recurrent falls and significantly increase the risk of morbidity and mortality in geriatric individuals. Research shows that approximately 30% of individuals aged 65 years and older fall each year; this rate increases to 40% among individuals aged 85 years and older. The frequency of falls has been reported to be even higher among nursing home residents.^{1,2}

Reported fall risk factors among geriatric individuals living in different societies vary considerably. Geriatric individuals are more vulnerable to injury because of a greater burden of comorbid conditions, age-related physiological changes, and a reduced capacity for rapid functional recovery. This situation further increases the risk of falls. The etiology of falls is generally multifactorial and results from the interaction of predisposing and precipitating factors classified as intrinsic and extrinsic factors.^{3,4} Intrinsic factors include age-related deterioration in postural control systems, proprioceptive deficits, declines in vision and vestibular function, gait disorders, cognitive decline and dementia, polypharmacy, orthostatic hypotension, chronic diseases, sarcopenia, and frailty syndrome. These factors reduce an individual's ability to maintain or restore balance. Extrinsic factors, on the other hand, are related to the physical characteristics of the geriatric individual's environment and the use of assistive devices.^{4–6}

Geriatric individuals living in nursing homes have been shown to have a higher likelihood of falling because of cognitive decline, multiple illnesses, reduced self-care abilities, and weakened social support.^{2,6} Considering that falls cause hundreds of thousands of deaths worldwide each year, a detailed examination of the causes of falls in these settings is essential for the development of preventive strategies. Although many individual fall risk factors have been investigated previously, the present study provides a comprehensive evaluation of multiple fall-related factors among nursing home residents and examines their association with fall frequency. This integrated approach may improve clinical decision-making by helping clinicians identify the most relevant factors requiring intervention and supporting the development of individualized, multidimensional fall prevention programs. Accordingly, the aim of this study was to evaluate fall risk factors according to fall history in geriatric individuals living in nursing homes and to examine the association between these factors and the number of falls.

KEY MESSAGES

- Nursing home-dwelling geriatric individuals with a history of falls had poorer physical performance, higher comorbidity and medication use, poorer nutritional status, greater incontinence severity, higher sarcopenia risk, and lower independence in activities of daily living (ADL) than non-fallers.
- Fall frequency was significantly associated with assistive device use (walker), slower gait speed, and poorer nutritional status.
- Comprehensive, targeted interventions focusing on mobility, physical performance, and nutritional optimization are critical for reducing fall risk and preventing recurrent falls in geriatric individuals.

METHODS

Study Design

This cross-sectional study was conducted between January 2024 and August 2025 in four nursing homes located in Ankara province. Participants were classified into faller and non-faller groups based on their self-reported history of falls during the previous 12 months. Comparisons between the groups were performed to evaluate differences in fall-related risk factors, and correlation analyses were conducted to examine the association between these factors and the number of falls. The necessary ethics committee approval for conducting the study was obtained from Ethics Committee of Ankara Yıldırım Beyazıt University, Faculty of Health Sciences (Approval Number: 2022/19-1252, Date: 08.12.2022), and the study was conducted in accordance with the principles of the Declaration of Helsinki. Volunteers aged 65 years and older who were ambulatory with or without assistive devices, scored 24 points or higher on the Standardized Mini-Mental State Examination, and were able to communicate were included in the study. Individuals with dyslexia or aphasia and those who were illiterate were not included in the study. Before the study, participants were informed about the purpose and content of the research both verbally and in writing through an informed consent form.

Individuals who met the study criteria were divided into two groups, "Fallers" and "Non-fallers," based on their fall status during the previous 12 months. As part of the study, the sociodemographic characteristics of all individuals were recorded.

Sociodemographic Information

Individuals' age, sex, marital status, educational level, number of medications used, length of stay in the nursing home, number of falls, use of assistive devices, vertigo, orthostatic hypotension, and vision and hearing problems were assessed.

Comorbidities

Comorbidities were recorded using the Charlson Comorbidity Index. In this index, diseases are assigned 1, 2, 3, or 6 points according to their type. A total score >5 indicates a high mortality and morbidity rate.⁷

Physical Performance

Physical performance assessment included gait speed and lower- and upper-extremity muscle strength.

Gait Speed

A 10-meter gait test was used to assess individuals' gait speed. Individuals were asked to walk a predetermined distance of 10 meters at their usual walking speed. The time required to walk the distance was measured with a stopwatch. Gait speed was recorded in meters per second.⁸

Lower Extremity Muscle Strength

Lower extremity muscle strength was evaluated using the 5-Repetition Sit-to-Stand Test (5STS). For the test, individuals were asked to sit on a chair without armrests, with their backs against the chair, and to stand up and sit down 5 times. The total time required to complete the test was recorded in seconds.⁹

Grip Strength

Handgrip strength was measured using a Jamar hydraulic hand dynamometer. For the measurement, individuals were asked to remain seated, keep their elbows flexed at their sides, and squeeze the dynamometer with maximum force. The assessment was performed three times for each hand, and the mean value was recorded in kilograms.¹⁰

Physical Activity

The 12-item Physical Activity Scale for the Elderly (PASE), which assesses leisure-time, household, and work-related activities, was used to evaluate physical activity levels. The scale ranges from 0 to 400 points, with higher scores indicating higher physical activity levels.¹¹

Sarcopenia

The SARC-F scale, developed as a screening tool to assess the risk of sarcopenia, was used. The scale consists of 5 items assessing strength, assistance with walking, rising from a chair, climbing stairs, and falls. A total score ≥ 4 indicates a symptomatic condition for sarcopenia.¹²

Nutritional Status

The Mini Nutritional Assessment (MNA) tool was used to assess individuals' nutritional status. The questionnaire consists of 4 sections and 18 items, including anthropometric, general, dietary, and subjective assessments. Individuals' nutritional

status is classified as normal (≥ 24), at risk of malnutrition (17–23.5), or malnourished (<17) based on the total MNA score.¹³

Incontinence Severity

Individuals' incontinence status was assessed by classifying them according to the presence or absence of incontinence. The Incontinence Severity Scale (ISS), developed by Sandvik et al., was used to assess the severity of incontinence among individuals with incontinence.¹⁴ The scale consists of two questions assessing the frequency of urinary leakage and the amount leaked each time. Based on the total score obtained from the scale, incontinence severity was categorized as mild (1–2 points), moderate (3–6 points), severe (8–9 points), or very severe (12 points).

Activities of Daily Living

Activities of daily living (ADL) were assessed using the Katz ADL Index. The index assesses bathing, toileting, dressing, continence, transferring, and eating. Activities performed with assistance were scored as 0, whereas those performed independently were scored as 1. The total score ranges from 0 to 6, with a score of 6 representing full functional ability, 4 reflecting moderate functional capacity, and 2 indicating mild functional impairment.¹⁵

Sample Size

A pilot study was conducted to determine the sample size based on the gait speed variable. Based on the pilot study data, an effect size of $d=0.55$ was calculated. According to an a priori power analysis targeting 80% power with a significance level of $\alpha=0.05$, it was determined that each group should include at least 53 geriatric individuals.

Statistical Analysis

The Statistical Package for the Social Sciences, version 26 (IBM Corp., Armonk, NY, USA), was used for the statistical analysis of the data. The normality of the variables was examined using visual methods (histograms and probability plots) and analytical methods (Kolmogorov-Smirnov and Shapiro-Wilk tests). Variables that were not normally distributed were presented as median (IQR, 25th–75th percentile), and categorical variables were presented as frequencies and percentages. The Mann-Whitney U test was used to analyze nonnormally distributed variables in between-group comparisons. Spearman correlation analysis was used to examine associations between nonnormally distributed variables. All analyses were performed at a significance level of 0.05.

RESULTS

The study included 66 individuals (30 women and 36 men) with no history of falls, with a median age of 76 (69–80) years,

Table 1. Sociodemographic characteristics of the groups

Characteristic	Fallers (n=54)	Non-fallers (n=66)	p
Age, years, median (IQR)	80 (76–85.25)	76 (69–80)	<0.001*
Female sex, n (%)	31 (57.4)	30 (45.5)	0.193
Number of medications, median (IQR)	7 (5–9)	3 (0–5.25)	<0.001*
Marital status, n (%)			0.710
Married	10 (18.5)	5 (7.6)	
Widowed	44 (81.5)	61 (92.4)	
Education level, n (%)			0.842
Primary school	25 (46.3)	27 (40.9)	
Middle school	5 (9.3)	6 (9.1)	
High school	7 (13.0)	12 (18.2)	
Bachelor's degree	16 (29.6)	18 (27.3)	
Postgraduate	1 (1.9)	3 (4.5)	
Length of stay in the nursing home, years, median (IQR)	4 (1–15)	5 (1–36)	0.363
Assistive device use, n (%)			
None	27 (50.0)	58 (87.9)	<0.001
Walker	9 (16.7)	2 (3.0)	0.011
Single-point cane	17 (31.5)	5 (7.6)	0.001
Tripod cane	1 (1.9)	0	0.450
Rollator	0	1 (1.5)	0.550
Vertigo, present, n (%)	11 (20.4)	6 (9.1)	0.078
Hearing impairment, present, n (%)	15 (27.8)	17 (25.8)	0.773
Visual impairment, present, n (%)	33 (61.1)	32 (48.5)	0.167
Orthostatic hypotension, present, n (%)	8 (14.8)	4 (6.1)	0.080

n: Number; IQR: interquartile range; p: Probability value; p<0.05 indicates statistical significance.

and 54 individuals (31 women and 23 men) with a history of falls, with a median age of 80 (76–85.25) years. The median fall frequency was 0 (0–0) in the non-faller group and 1 (1–2) in the faller group. The length of stay in the nursing home was 3.5 (1.37–7) years for fallers and 5 (2–7) years for non-fallers. The sociodemographic characteristics of the groups are presented in Table 1.

Fall frequency and fall-related risk factors, including comorbidity, gait speed, lower extremity strength, grip strength, physical activity level, sarcopenia, nutritional status, incontinence severity, and level of independence, differed significantly between the groups (p<0.05) (Table 2).

The associations between fall frequency and fall risk factors in geriatric individuals are presented in Table 3. Fall frequency was significantly correlated with walker use, gait speed, and MNA scores ($\rho=-0.290$, $p=0.033$; $\rho=-0.352$, $p=0.009$; $\rho=-0.317$, $p=0.020$).

DISCUSSION

In our study, geriatric individuals living in nursing homes were divided into two groups according to fall status, and fall risk factors were identified; the faller group was characterized by older age, a greater number of medications used, and a higher comorbidity burden. In addition, compared with non-fallers, fallers exhibited poorer physical performance and lower activity levels, a greater risk of sarcopenia, poorer nutritional status, a higher prevalence of incontinence, and reduced independence in ADL. Furthermore, the significant associations between fall frequency and walker use, gait speed, and nutritional status among fallers indicate that mobility and nutrition play important roles in recurrent falls.

In this study, fallers were older, used a greater number of medications, had a higher mortality risk, and used assistive devices more frequently than non-fallers. In addition, among fallers, recurrent fall frequency was significantly associated

Table 2. Comparison of the groups in terms of fall frequency and fall risk factors

Variable	Fallers (n=54), median (IQR)	Non-fallers (n=66), median (IQR)	p
Charlson Comorbidity Index	1 (1–1)	0 (0–1)	<0.001*
Gait speed, m/s	0.49 (0.35–0.86)	0.91 (0.71–1.06)	<0.001*
5STS, s	15.73 (12.59–20.28)	13.41 (10.27–16.07)	0.002*
Right handgrip strength, kg	19.95 (13.26–24.42)	23.01 (17.13–30.0)	0.024*
Left handgrip strength, kg	17.53 (9.87–23.49)	19.80 (15.49–26.0)	0.038*
PASE score	8.60 (2.20–24.03)	13.94 (6.73–34.83)	0.039*
SARC-F score	3 (1–6)	1 (0–2)	<0.001*
MNA score	23 (19.50–26.62)	26.0 (23.50–27.0)	0.005*
Incontinence severity, n (%)			0.013*
None	28 (51.9)	49 (74.2)	
Mild	9 (16.7)	11 (16.7)	
Moderate	10 (18.5)	1 (1.5)	
Severe	1 (1.9)	1 (1.5)	
Very severe	6 (11.1)	4 (6.1)	
Katz ADL Index score	5 (5–6)	6 (6–6)	<0.001*

5STS: 5-Repetition Sit-to-Stand Test; ADL: Activities of daily living; MNA: Mini Nutritional Assessment; PASE: Physical Activity Scale for the Elderly; IQR: Interquartile range; m/s: Meters per second; s: Seconds; kg: kilograms; p<0.05 indicates statistical significance.

Table 3. Relationship between fall frequency and fall risk factors among fallers

Variable	rho	p
Number of medications	0.123	0.376
Assistive device use		
None	0.242	0.077
Walker	-0.290	0.033*
Single-point cane	-0.001	0.992
Tripod cane	-0.092	0.507
Rollator	–	–
Charlson Comorbidity Index	0.133	0.339
Age	0.171	0.217
Gait speed	-0.352	0.009*
5STS	0.246	0.073
Right handgrip strength	0.016	0.907
Left handgrip strength	0.018	0.889
PASE	0.076	0.584
SARC-F	0.210	0.128
MNA	-0.317	0.020*
Incontinence severity	0.007	0.962
Katz ADL Index	-0.264	0.054

5STS: 5-Repetition Sit-to-Stand Test; ADL: Activities of daily living; MNA: Mini Nutritional Assessment; PASE: Physical Activity Scale for the Elderly; rho: Spearman correlation coefficient; p<0.05 indicates statistical significance.

with walker use. These findings are consistent with previous evidence indicating that falls in geriatric individuals are multifactorial and influenced by intrinsic factors, such as age and health status, as well as extrinsic factors, including assistive device use and environmental conditions.⁵ Previous studies have also reported that recurrent falls are more common in geriatric individuals with impaired mobility, balance deficits, and greater functional limitations, all of which increase the likelihood of requiring walking aids.¹⁶ However, some studies have suggested that appropriately prescribed assistive devices may improve walking stability and reduce fall risk.¹⁷ This apparent discrepancy may be explained by the fact that our analysis focused exclusively on individuals with a history of falls and examined factors associated with recurrent fall frequency rather than factors associated with the occurrence of falls. Therefore, the observed association is more likely to indicate that the need for a walking aid reflects greater underlying mobility and balance impairments rather than an adverse effect of the devices themselves. In other words, the need for a walking aid may serve as a marker of greater functional impairment and susceptibility to recurrent falls. These findings highlight the importance of comprehensive mobility assessment and individualized fall prevention strategies for nursing home residents who require walking aids.

This study showed that fallers had significantly slower gait speed than non-fallers (0.50 vs. 0.92 m/s), and gait speed was significantly associated with recurrent fall frequency

among fallers. These findings are consistent with previous studies reporting that poor physical performance, particularly reduced gait speed, is associated with an increased risk of falls among nursing home residents.^{6,18} Similarly, previous studies have demonstrated that geriatric individuals with slower gait speed are more likely to experience recurrent falls.^{6,19} Bozkurt et al.²⁰ reported that a gait speed below 0.8 m/s is a risk factor for falls. However, another study found no significant association between gait speed and falls among nursing home residents.²¹ This difference may be explained by differences in study populations and outcome measures, as our analysis was restricted to fallers and examined factors associated with recurrent fall frequency rather than fall occurrence. These findings highlight the importance of gait speed assessment as a simple and practical clinical measure for identifying nursing home residents who may be at greater risk of recurrent falls.

This study showed that fallers had significantly lower handgrip strength in both the dominant and non-dominant hands than non-fallers. These findings are consistent with previous studies demonstrating that low handgrip strength is associated with an increased risk of falls among nursing home residents.^{6,22,23} Sürmeli et al.⁶ reported that low handgrip strength was independently associated with falls, while other studies have shown that fallers exhibit significantly weaker handgrip strength than non-fallers and that reduced grip strength is associated with falls before and after hospitalization.^{6,22,23} However, among individuals with a history of falls, handgrip strength was not associated with recurrent fall frequency in this study. The lack of an association between handgrip strength and recurrent fall frequency may be explained by the fact that the analysis was restricted to fallers, suggesting that although reduced handgrip strength may distinguish fallers from non-fallers, it may have limited value in explaining recurrent fall frequency. These findings suggest that handgrip strength may be useful for identifying geriatric individuals at risk of falls but should be interpreted together with other functional and clinical factors when evaluating the risk of recurrent falls.

In the present study, lower extremity muscle strength was assessed using the 5STS, and fallers demonstrated longer completion times (15.74 s) than non-fallers (13.42 s). However, no association was found between fall frequency and lower extremity muscle strength. Numerous studies using different measures of lower extremity muscle strength have reported that reduced muscle strength increases the risk of falls.^{24,25} Oya et al.²⁴ suggested knee extensor muscle strength as a potential tool for fall risk assessment and found lower muscle strength in fallers. Similarly, Aoyama et al.²⁵ reported that low muscle strength was associated with a higher fall frequency in geriatric women. Furthermore, a systematic review published in 2023 reported that a 5STS score >15 s, which reflects

poorer lower extremity muscle strength, may be associated with a high risk of falls.²⁶ Although our finding that fallers had poorer lower extremity muscle strength is consistent with previous evidence, muscle strength was not associated with fall frequency. This may be attributable to the relatively low number of falls among participants with a history of falls. Furthermore, fear of recurrent falls may lead geriatric individuals to restrict their daily activities and avoid high-risk situations, potentially attenuating the association between muscle strength and fall frequency.

In this study, fallers had lower physical activity levels than non-fallers. This finding is consistent with previous studies reporting that fallers generally demonstrate lower physical activity levels, likely because of declines in physical function following a fall.^{27,28} Trevisan et al.²⁸ found that nursing home residents with a history of falls exhibited lower physical activity levels and poorer physical function. In contrast, evidence regarding the association between physical activity and fall frequency remains inconsistent. One study reported no significant association between physical activity and a single fall event, whereas higher physical activity levels were associated with a lower risk of recurrent falls.²⁹ The absence of an association between physical activity level and fall frequency in our study may be explained by the relatively low number of recurrent falls among fallers and by activity restriction resulting from fear of falling.

In this study, SARC-F scores were significantly higher in fallers than in non-fallers; however, no association was found between SARC-F scores and recurrent fall frequency among fallers. Notably, the mean SARC-F score among fallers was 3, indicating that most individuals were at low risk of sarcopenia. Cruz-Jentoft et al.³⁰ reported that sarcopenia increases the risk of falls because reductions in muscle strength, muscle mass, gait speed, and physical performance contribute to higher fall rates among geriatric individuals with sarcopenia. In contrast, other studies have found no significant association between sarcopenia and falls.^{31,32} Cawthon et al.³² found no association between sarcopenia and a history of falls in community-dwelling geriatric men and suggested that falls may be more strongly influenced by impaired balance, neuromuscular coordination, reflex responses, and environmental factors than by muscle mass or strength alone. The lack of an association between SARC-F scores and recurrent fall frequency in our study may be explained by the low risk of sarcopenia among fallers, as reflected by their mean SARC-F score below the diagnostic cutoff, and by the multifactorial nature of recurrent falls.

In the present study, fallers had lower MNA scores than non-fallers, and among fallers, lower MNA scores were associated with a higher recurrent fall frequency. Consistent with our

findings, malnutrition has been reported to be significantly associated with an increased risk of falls in older adults because inadequate protein and energy intake may lead to reduced muscle mass, loss of muscle strength, and impaired postural stability.³³ Similarly, Eckert et al.³⁴ reported that malnutrition was associated with an increased risk of falls in community-dwelling older adults, while studies conducted among nursing home residents have also shown that lower MNA scores are associated with a higher fall frequency and that malnutrition predicts adverse outcomes, particularly falls. In contrast, Vivanti et al.³⁵ found that malnutrition was not directly associated with the number of falls during hospitalization, and Yücel et al. reported no difference in the number of falls between sarcopenic and non-sarcopenic individuals with respect to malnutrition risk.^{31,35} These inconsistent findings may be explained by differences in study populations, care settings, follow-up periods, and outcome measures, as our study specifically examined recurrent fall frequency among nursing home residents. These findings suggest that routine nutritional assessment and timely nutritional interventions may contribute to reducing recurrent falls and should be considered an integral component of fall prevention strategies for nursing home residents.

In this study, incontinence was more common in fallers than in non-fallers, and moderate-to-severe incontinence was also more prevalent among fallers. However, no association was found between incontinence severity and recurrent fall frequency among fallers. These findings are consistent with previous studies reporting that geriatric individuals with incontinence have an increased risk of falls because urinary urgency and nocturia may increase the likelihood of falling.^{1,36} Similarly, incontinence has been recognized as an important contributor to fall risk in geriatric individuals because age-related urinary symptoms often increase exposure to high-risk situations, particularly during nighttime mobility.³⁷ In contrast, Bernardes et al.³⁸ found no significant difference in fall frequency between individuals with and without urinary incontinence. The lack of an association between incontinence severity and recurrent fall frequency in our study may be explained by the absence of more detailed assessments of incontinence characteristics, such as incontinence type, frequency, and nocturnal symptoms. In addition, factors such as muscle strength, balance, environmental conditions, and medication use may limit the role of incontinence as an independent determinant of falls.

In this study, fallers were more dependent in ADL than non-fallers; however, ADL dependency was not associated with recurrent fall frequency among fallers. Consistent with our findings, Damar et al.³⁹ reported that nursing home residents with lower Katz ADL scores had a higher risk of falls. Similarly, Sürmeli et al.⁶ found that dependence in ADL was associated with fall risk in nursing home residents and that malnutrition,

muscle strength, gait speed, and age were also independent determinants of falls. However, Katz ADL scores primarily reflect functional dependence and may not fully capture the factors influencing recurrent fall frequency. The lack of an association between ADL dependency and recurrent fall frequency in our study may be explained by the generally mild level of dependency and relatively preserved cognitive function of the participants, allowing them to maintain most daily activities despite a history of falls.

Although several individual fall-related risk factors have been investigated previously, our study provides a comprehensive evaluation of multiple clinical, functional, nutritional, and geriatric parameters within the same population of nursing home residents. Unlike many previous studies that focused primarily on the occurrence of falls among community-dwelling geriatric individuals or evaluated a limited number of risk factors, our study simultaneously examined factors associated with both fall status and recurrent fall frequency in a nursing home population. This comprehensive approach enabled the identification of mobility- and nutrition-related factors specifically associated with recurrent falls, providing findings that may support more targeted fall prevention strategies in institutionalized geriatric individuals.

Limitations

Several limitations of this study should be acknowledged. First, although the study was multicenter, the inability to reach the entire target population and the relatively small sample size may limit the generalizability of the findings to geriatric individuals living in nursing homes. Second, fall history and fall frequency were assessed based on self-reports, and the possibility of recall bias cannot be excluded. Finally, some important fall risk factors, such as degenerative and other diseases (neurological, orthopedic, rheumatological, etc.), cognitive status, balance, depression, and environmental risks within nursing homes, were not comprehensively evaluated in this study.

CONCLUSION

This study demonstrated that geriatric individuals with a history of falls were more disadvantaged than non-fallers in terms of physical performance, physical activity, sarcopenia risk, nutritional status, incontinence, and ADL. In addition, the significant associations between fall frequency and walker use, gait speed, and nutritional status suggest that mobility limitations and undernutrition may be influential factors in recurrent falls. Effective fall prevention strategies should consider these multiple factors to reduce fall frequency and improve the quality of life of geriatric individuals. Furthermore, we believe that interventions aimed at reducing fall risk in geriatric individuals should focus on improving physical performance, supporting mobility, and optimizing nutritional status.

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FOXM1 Drives Subtype-Specific Dysregulation of Prognostic miRNAs in Breast Cancer Cells

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ABSTRACT

Objective: Breast cancer is a heterogeneous disease comprising distinct molecular subtypes, including estrogen receptor-positive (ER+) breast cancer and triple-negative breast cancer (TNBC). MicroRNAs (miRNAs) regulate post-transcriptional gene expression and play important roles in cancer progression by functioning as oncogenic or tumor-suppressive molecules. Forkhead box M1 (FOXM1) is an oncogenic transcription factor that is frequently upregulated in aggressive breast cancers, particularly TNBC, and is associated with proliferation, invasion, metastasis, therapy resistance, and poor prognosis.

Materials and Methods: Clinically relevant miRNAs associated with survival in patients with ER+ and ER– breast cancer were identified using publicly available datasets. The expression levels of miR-34a-5p, miR-873-5p, miR-193b-5p, miR-330-5p, miR-10b-5p, miR-155-5p, and miR-484 were evaluated in MDA-MB-231 TNBC cells and MCF-7 ER+ breast cancer cells following FOXM1 knockdown.

Results: FOXM1 knockdown was associated with increased expression of several tumor-suppressive miRNAs, including miR-34a-5p, miR-873-5p, miR-193b-5p, miR-330-5p, miR-10b-5p, and miR-484, in MDA-MB-231 cells. In contrast, most of these miRNAs were not markedly altered in MCF-7 cells, except for miR-10b-5p, whose expression decreased following FOXM1 knockdown. miR-155-5p expression remained largely unchanged in both cell lines.

Conclusion: Our findings demonstrate that FOXM1 knockdown was associated with differential expression of clinically relevant miRNAs in the two breast cancer cell models examined. These findings suggest that FOXM1-associated alterations in miRNA expression may contribute to the aggressive phenotype of TNBC and warrant further mechanistic investigation.

Keywords: Breast cancer, estrogen receptor-positive breast cancer, FOXM1, microRNA, miR-10b-5p, miR-193b-5p, miR-34a-5p, triple-negative breast cancer



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INTRODUCTION

Breast cancer (BC) is the most frequently diagnosed malignancy among women worldwide and remains one of the leading causes of cancer-related mortality, accounting for approximately 670,000 deaths annually, according to the World Health Organization (WHO).^{1,2} Despite considerable advances in early diagnosis and systemic therapies, BC remains a highly heterogeneous disease comprising distinct molecular subtypes that differ substantially in their biological behavior, prognosis, and therapeutic response. Based on the expression status of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), BC is classified into ER-positive (ER+), HER2-positive, and triple-negative breast cancer (TNBC) subtypes.³

ER+ breast cancer accounts for approximately 70% of all breast cancer cases and generally has a more favorable clinical outcome because of its responsiveness to endocrine therapies such as tamoxifen and fulvestrant.³ In contrast, TNBC lacks ER, PR, and HER2 expression and accounts for approximately 15% of invasive breast cancers. TNBC is characterized by aggressive tumor behavior, high metastatic potential, frequent recurrence, and poor overall survival. Owing to the absence of well-defined therapeutic targets, chemotherapy remains the primary systemic treatment; however, nearly half of patients with TNBC fail to achieve a pathological complete response following neoadjuvant chemotherapy.⁴ These clinical challenges highlight the urgent need to better understand the molecular mechanisms underlying TNBC progression and identify novel therapeutic targets.

Forkhead box M1 (FOXM1), a member of the Forkhead box family of transcription factors, plays a central role in regulating genes involved in cell-cycle progression, proliferation, migration, invasion, metastasis, and therapeutic resistance.^{5,6} FOXM1 is frequently overexpressed in TNBC and numerous aggressive solid tumors, in which elevated FOXM1 expression has consistently been associated with unfavorable clinical outcomes and reduced patient survival.⁵ Previous studies have demonstrated that FOXM1 contributes to multiple oncogenic processes, including tumor growth, epithelial–mesenchymal transition, stemness, and chemotherapy resistance. Nevertheless, the downstream molecular networks through which FOXM1 exerts these oncogenic effects have not yet been fully characterized.^{7,8}

MicroRNAs (miRNAs) are small noncoding RNA molecules that regulate post-transcriptional gene expression through sequence-specific binding to complementary regions within target messenger RNAs, thereby modulating mRNA stability and translation.^{9,10} Aberrant miRNA expression contributes to tumor initiation and progression by regulating diverse biological

KEY MESSAGES

- FOXM1 regulates subtype-specific expression of prognostic miRNAs in breast cancer cells.
- FOXM1 silencing differentially alters oncogenic and tumor-suppressive miRNAs across luminal and triple-negative breast cancer models.
- These findings identify FOXM1-associated miRNA networks as potential biomarkers and therapeutic targets in breast cancer.

processes, including proliferation, apoptosis, angiogenesis, metastasis, and therapeutic resistance. Depending on their target genes, miRNAs may function as either oncogenes or tumor suppressors.^{9,10}

Previous studies, including our own, have demonstrated that dysregulated miRNA expression is closely associated with breast cancer progression, therapeutic response, and patient survival.¹⁰ In particular, we previously identified FOXM1-associated alterations in miR-186 and miR-200b expression in TNBC cells.¹¹ Building on these findings, further characterization of additional clinically relevant miRNAs associated with FOXM1 signaling may provide a more comprehensive understanding of FOXM1-related regulatory networks in breast cancer.

Therefore, in the present study, we investigated the association between FOXM1 knockdown and the expression profiles of clinically relevant prognostic miRNAs in MDA-MB-231 and MCF-7 breast cancer cells. We further evaluated the prognostic significance of these miRNAs using publicly available patient datasets and examined whether FOXM1 knockdown was associated with differential miRNA expression patterns in these two breast cancer cell models. Our findings suggest that FOXM1 is associated with subtype-specific alterations in the expression of clinically relevant miRNAs and may contribute to the dysregulation of tumor-suppressive miRNAs linked to breast cancer prognosis. These observations provide further insight into the molecular mechanisms underlying TNBC progression and warrant additional mechanistic investigation.

MATERIALS AND METHODS

Cell Lines and Culture Conditions

Human triple-negative breast cancer (MDA-MB-231) and estrogen receptor-positive (ER+) breast cancer (MCF-7) cells were obtained from the American Type Culture Collection (Manassas, VA, USA). MDA-MB-231 and MCF-7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM)/F12 supplemented with 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO, USA). Nontumorigenic human breast epithelial

cells (MCF10A) were grown in DMEM/F12 supplemented with 5% horse serum, epidermal growth factor, hydrocortisone, insulin, and cholera toxin (Calbiochem). Cells were cultured at 37°C in a humidified incubator with 5% CO₂.^{11–15}

Transfection With siRNAs

FOXM1 expression was silenced using two independent small interfering RNAs (siRNAs) targeting different regions of the FOXM1 transcript (FOXM1 siRNA#1, SASI_Hs01_00052108; FOXM1 siRNA#2, SASI_Hs01_00243977; Sigma-Aldrich, St. Louis, MO, USA), which were previously validated by our group for efficient FOXM1 knockdown.^{11–15} A nontargeting siRNA (MISSION® siRNA Universal Negative Control, Sigma-Aldrich) was used as a negative control.

Exponentially growing MDA-MB-231 and MCF-7 cells were seeded 24 h before transfection and transfected with either FOXM1 siRNAs or control siRNA at a final concentration of 50 nM using HiPerFect Transfection Reagent (Qiagen, Valencia, CA, USA), according to the manufacturer's instructions. Cells were harvested 72 h after transfection for subsequent RNA isolation and gene expression analyses. All experiments were independently repeated at least three times.^{11–15}

RNA Isolation and Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR) for miRNA Profiling in BC Cells

Total RNA, including small RNAs, was isolated from untreated MCF10A cells and from MDA-MB-231 and MCF-7 cells transfected with FOXM1 siRNAs or control siRNA using the High Pure RNA Isolation Kit (Roche Diagnostics, Mannheim, Germany), according to the manufacturer's protocol. RNA concentration and purity were determined spectrophotometrically.

For miRNA expression analysis, 1 µg of total RNA was reverse transcribed using the Mir-X™ miRNA First-Strand Synthesis Kit (Takara Bio USA, Mountain View, CA, USA). Quantitative real-time PCR (RT-qPCR) was performed using TB Green® Premix Ex Taq™ II (Takara Bio USA) on a LightCycler® 480 Real-Time PCR System (Roche Applied Science). U6 small nuclear RNA (U6 snRNA) served as the endogenous control for miRNA normalization to maintain methodological consistency with our previous study using the same experimental approach.¹¹ The expression levels of miR-34a-5p, miR-873-5p, miR-193b-5p, miR-330-5p, miR-10b-5p, miR-484, and miR-155-5p were analyzed in triplicate.

FOXM1 mRNA expression was quantified using Platinum™ Taq DNA Polymerase (Life Technologies, Carlsbad, CA, USA) with gene-specific primers, while β-actin was used as the internal reference gene. Relative expression levels were calculated using the comparative 2^{-ΔΔCt} method. All reactions were performed

in triplicate, and the mean values from three independent biological experiments were used for statistical analysis.¹¹

Bioinformatic Analysis of miRNA Expression and Patient Survival

The expression profiles of the selected miRNAs in breast cancer were explored using the Database of Differentially Expressed miRNAs in Human Cancers (<https://www.biosino.org/dbDEMC/index>). To evaluate the prognostic significance of these miRNAs, Kaplan–Meier survival analyses were performed using publicly available breast cancer datasets from The Cancer Genome Atlas (TCGA) through the KM Plotter platform (<https://kmplot.com/analysis/>).

Statistical Analysis

Data are presented as the mean±SD from three independent biological experiments. Statistical analyses were performed using GraphPad Prism software (version 8.00; GraphPad Software Inc., San Diego, California, USA). Comparisons between two groups were performed using an unpaired two-tailed Student's t-test, whereas comparisons involving more than two groups were analyzed using one-way analysis of variance (ANOVA), followed by an appropriate post hoc multiple-comparison test. Each miRNA was analyzed individually, and no false discovery rate (FDR) correction was applied. Statistical significance was defined as p<0.05.

RESULTS

Expression of miR-10b-5p, miR-34a-5p, miR-330-5p, miR-873-5p, and miR-155-5p Is Associated With Favorable Prognosis and Longer Survival in Patients With BC

To determine the clinical relevance of the selected miRNAs, Kaplan–Meier survival analyses were performed using the TCGA breast cancer dataset (KM Plotter), separately for ER– and ER+ patient cohorts. High expression of miR-10b-5p (HR=0.39, p=0.013), miR-34a-5p (HR=0.31, p=0.0079), miR-330-5p (HR=0.37, p=0.014), miR-155-5p (HR=0.37, p=0.025), and miR-873-5p (HR=0.27, p=0.0031) was significantly associated with improved overall survival in patients with ER– breast cancer (Fig. 1). In contrast, the expression of miR-193b-5p (HR=0.57, p=0.16) and miR-484 (HR=0.68, p=0.32) was not significantly associated with survival.

In the ER+ cohort, elevated miR-10b-5p expression was also associated with favorable overall survival (HR=0.51, p=0.011). Conversely, increased expression of miR-34a-5p (HR=0.31, p=0.0079), miR-193b-5p (HR=1.94, p=0.04), miR-873-5p (HR=3.63, p=2.4×10⁻⁶), and miR-484 (HR=3.63, p=4×10⁻⁴) was associated with poorer overall survival, whereas miR-330-5p (HR=0.76, p=0.32) and miR-155-5p (HR=0.62, p=0.11) were not significantly associated with patient outcomes.

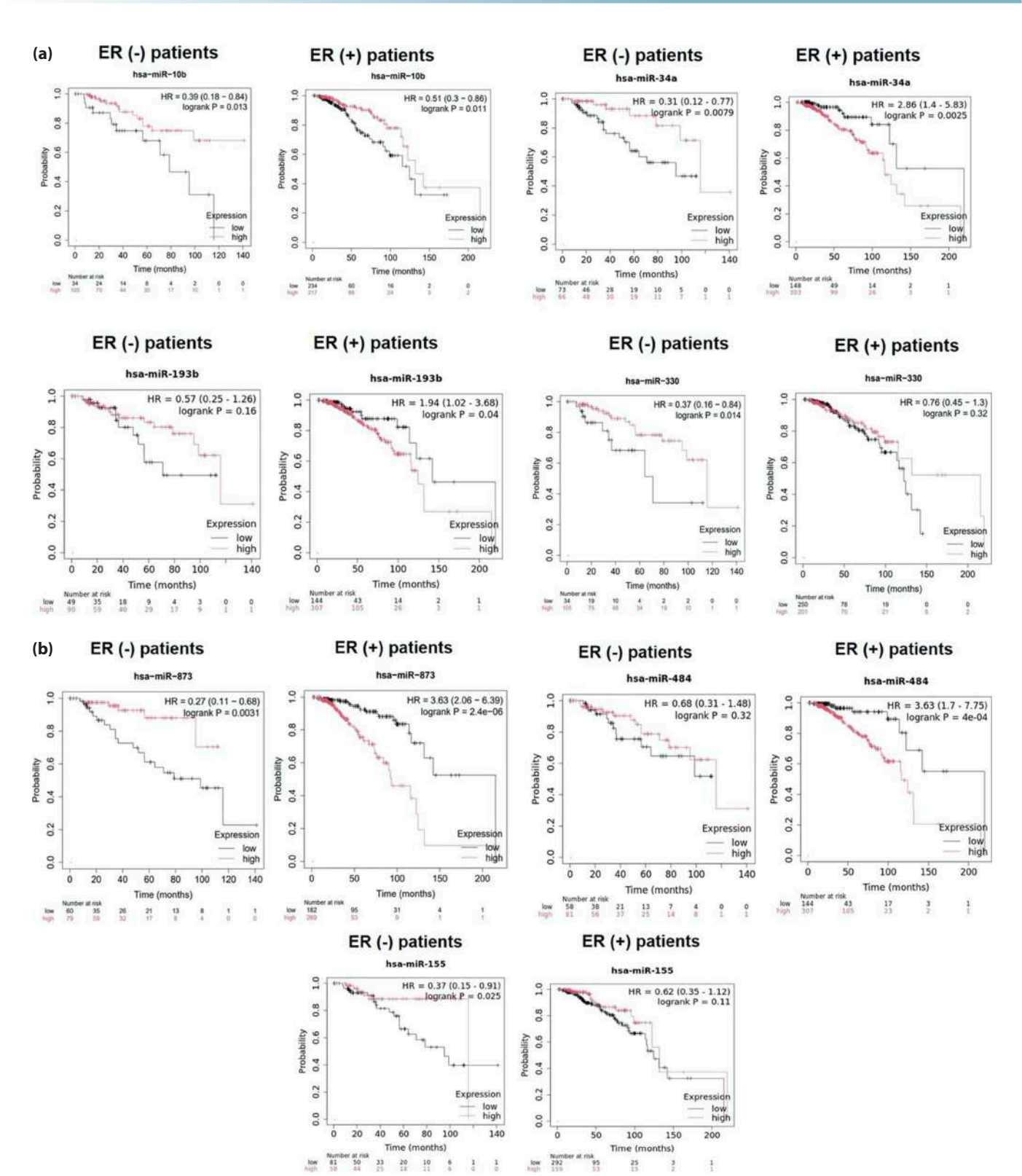


Figure 1. Kaplan–Meier survival analysis of miRNAs and their association with breast cancer survival. The clinical significance of the selected miRNAs was evaluated using the NCI-TCGA breast cancer database based on Kaplan–Meier survival analysis. Kaplan–Meier survival curves illustrate the associations between miRNA expression and patient survival in ER+ **(a)** and ER– **(b)** patients.

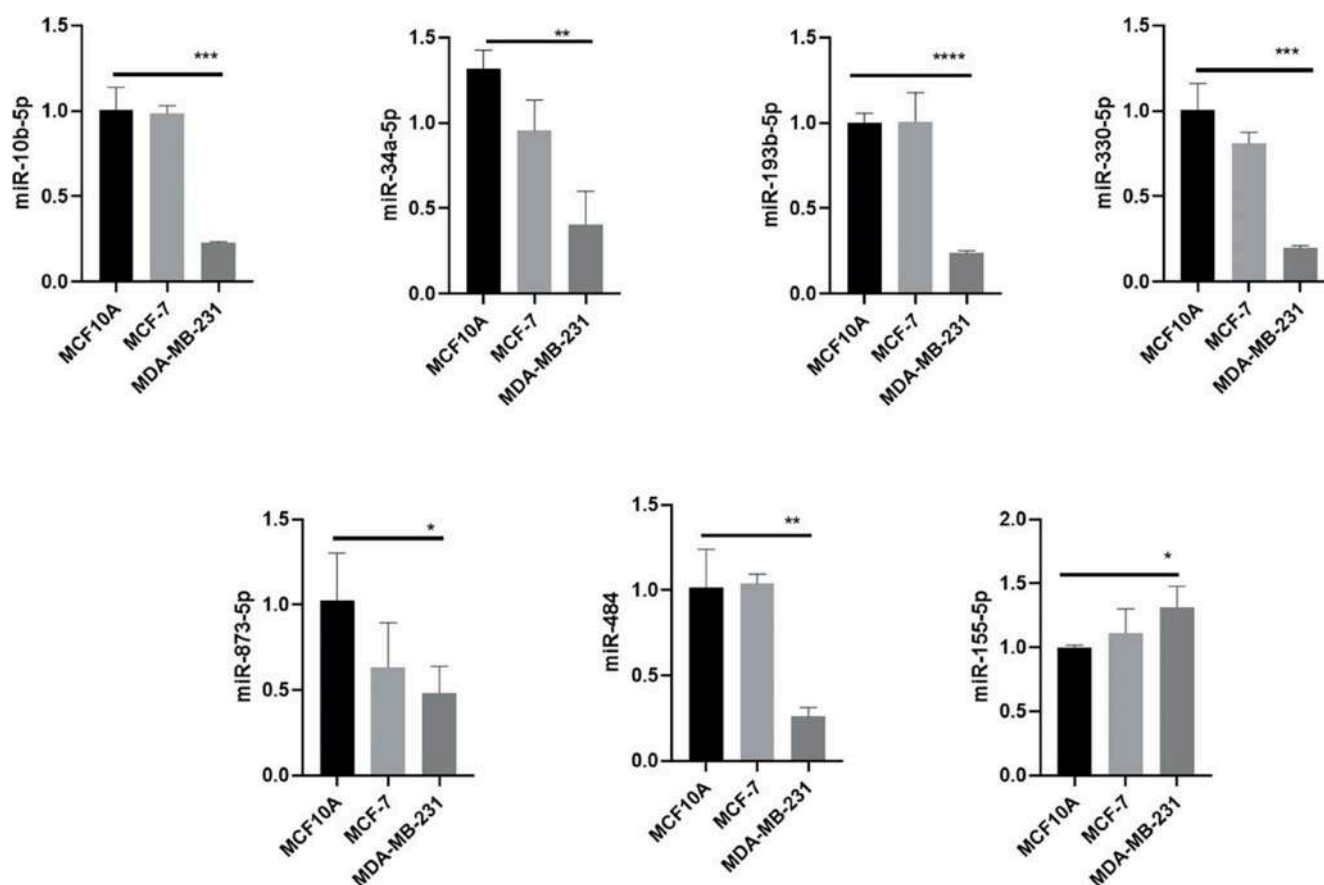


Figure 2. Endogenous expression levels of the selected miRNAs in breast cancer and normal cells. The expression levels of miRNAs were analyzed by RT-qPCR in normal breast epithelial cells (MCF10A), ER– MDA-MB-231 cells, and ER+ MCF-7 cells.

P<0.05; *P<0.01; **P<0.001; ***P<0.0001.

Collectively, these findings demonstrate that the prognostic value of several FOXM1-associated miRNAs differs according to breast cancer subtype, suggesting subtype-specific biological functions.

Endogenous miRNA Expression Profiles Differ Between TNBC and ER-Positive Breast Cancer Cells

To investigate whether the selected prognostic miRNAs exhibit subtype-specific expression patterns, their endogenous expression levels were analyzed by RT-qPCR in TNBC (MDA-MB-231), ER+ (MCF-7), and nontumorigenic breast epithelial (MCF10A) cells.

Compared with MCF-7 and MCF10A cells, MDA-MB-231 cells exhibited significantly lower expression of miR-10b-5p, miR-34a-5p, miR-193b-5p, miR-330-5p, miR-873-5p, and miR-484. In contrast, miR-155-5p expression was markedly elevated in MDA-MB-231 cells. No significant differences were observed between MCF-7 and MCF10A cells for the remaining miRNAs (Fig. 2).

These findings indicate that TNBC cells exhibit a distinct miRNA expression profile characterized by the suppression of multiple tumor-suppressive miRNAs and increased expression of the oncogenic miR-155-5p.

Expression Patterns of miRNAs in Breast Cancer Tissues Compared With Normal Tissues

To validate the clinical relevance of our experimental observations, the expression profiles of the selected miRNAs were further examined using the dbDEMOC database. The analysis revealed that miR-34a-5p, miR-484, and miR-10b-5p were significantly downregulated in breast cancer tissues compared with normal breast tissues (logFC=–2.52, –1.83, and –2.10, respectively). Conversely, miR-330-5p (logFC=0.98) and miR-155-5p (logFC=0.76) were upregulated in tumor tissues. Comparison of high-grade and low-grade breast tumors demonstrated increased expression of miR-873-5p (logFC=0.49) and miR-10b-5p (logFC=0.24) in high-grade tumors. In addition, miR-193b-5p expression was relatively

Table 1. Expression profiles of miR-34a-5p, miR-873-5p, miR-193b, miR-330-5p, miR-10b-5p, miR-484, and miR-155-5p in breast cancer tissues compared with normal tissues based on the dbDEMC database

miRNA ID	Cancer type	Cell line	Design	logFC	Expression status
hsa-miR-34a	Breast cancer	N/A	Cancer vs. normal	-2.52	Downregulated
hsa-miR-873-5p	Breast cancer	N/A	High grade vs. low grade	0.49	Upregulated
hsa-miR-193b	Breast cancer	N/A	ER-positive breast tumor vs. ER-negative breast tumor	0.28	Upregulated
hsa-miR-330-5p	Breast cancer	N/A	Cancer vs. normal	0.98	Upregulated
hsa-miR-10b-5p	Breast cancer	N/A	Cancer vs. normal	-2.10	Downregulated
hsa-miR-10b-5p	Breast cancer	N/A	Breast invasive carcinoma stage 3 vs. breast invasive carcinoma stage 2	0.24	Upregulated
hsa-miR-484	Breast cancer	N/A	Cancer vs. normal	-1.83	Downregulated
miR-155-5p	Breast cancer	N/A	Cancer vs. normal	0.76	Upregulated

logFC: Logarithm of fold change; ER: Estrogen receptor; N/A: Not applicable.

higher in ER-positive breast tumors than in ER-negative tumors (logFC=0.28) (Table 1).

Overall, these database findings support the subtype-specific dysregulation of FOXM1-associated miRNAs observed in our experimental analyses.

FOXM1 Knockdown Is Associated With Altered miRNA Expression Profiles in Breast Cancer Cells

To determine whether FOXM1 regulates the expression of clinically relevant miRNAs, MDA-MB-231 and MCF-7 cells were transfected with two independent FOXM1-targeting siRNAs, followed by RT-qPCR analysis. FOXM1 knockdown efficiency was confirmed only at the mRNA level in the present study. Protein-level validation was not repeated because the same FOXM1 siRNAs had previously been validated by our group to efficiently suppress FOXM1 protein expression by Western blot analysis.^{11–15}

Consistent with their endogenous expression profiles, MDA-MB-231 cells exhibited substantially lower basal levels of miR-10b-5p, miR-34a-5p, miR-193b-5p, miR-330-5p, miR-873-5p, and miR-484 than MCF-7 cells (Fig. 3a). FOXM1 knockdown significantly increased the expression of all six tumor-suppressive miRNAs in MDA-MB-231 cells, whereas miR-155-5p expression remained unchanged following FOXM1 inhibition (Fig. 3b, c).

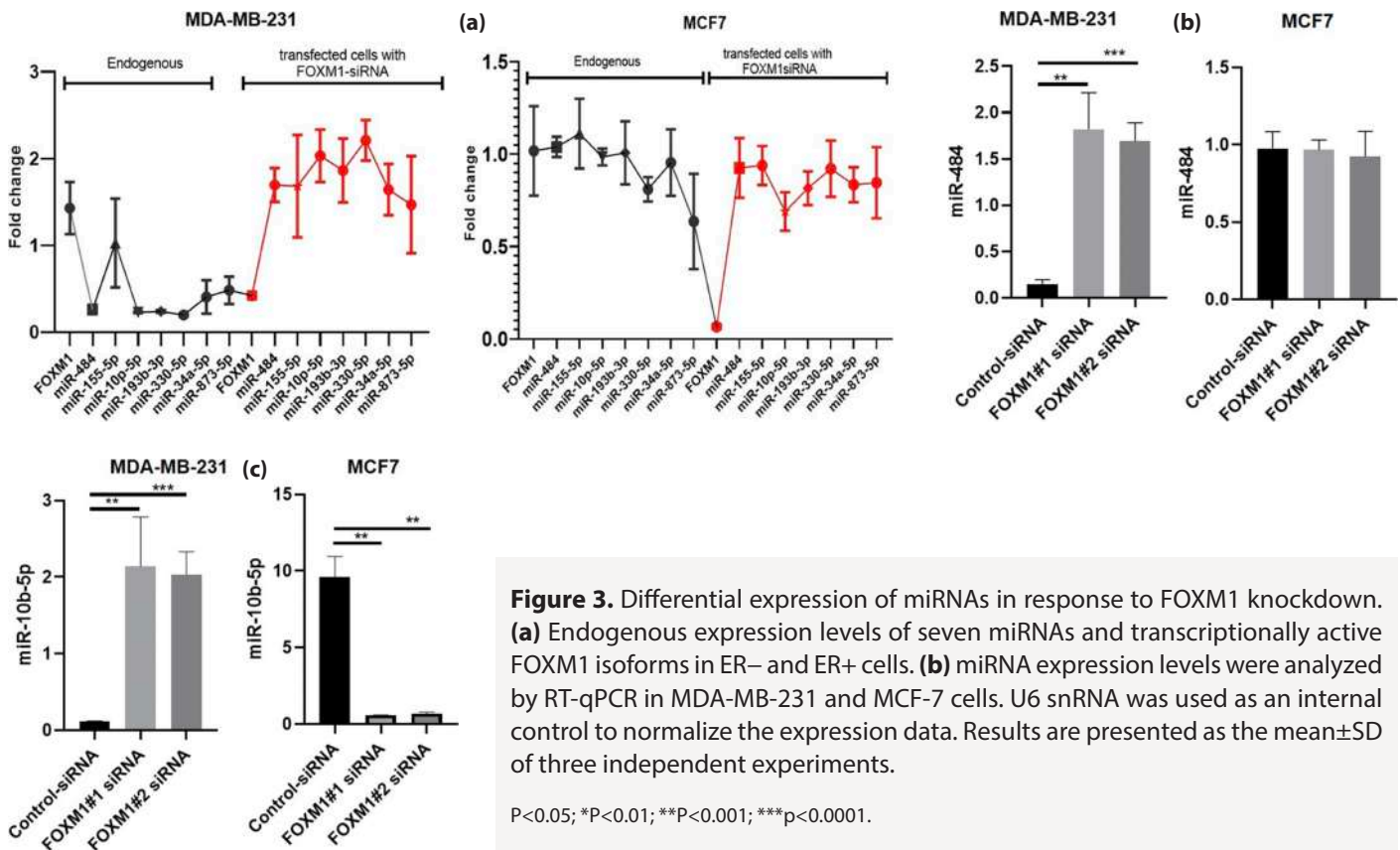
In contrast, FOXM1 silencing had minimal effects on miRNA expression in MCF-7 cells. Among the analyzed miRNAs, only miR-10b-5p showed a significant decrease following FOXM1 knockdown, whereas the expression levels of the remaining miRNAs were not significantly altered.

These findings indicate that FOXM1 inhibition is associated with subtype-specific alterations in the expression of clinically relevant miRNAs. The observed changes were considerably more pronounced in TNBC cells than in ER-positive breast cancer cells, suggesting that FOXM1 may contribute to the regulation of tumor-suppressive miRNAs in a subtype-dependent manner.

DISCUSSION

Breast cancer is a biologically heterogeneous disease in which distinct molecular subtypes exhibit substantial differences in clinical behavior, therapeutic response, and patient prognosis. Among these subtypes, triple-negative breast cancer (TNBC) remains the most aggressive because of its high metastatic potential, frequent chemoresistance, and lack of effective molecularly targeted therapies.^{12,13,16} Although aberrant miRNA expression has long been recognized as a hallmark of breast cancer progression, the upstream regulatory mechanisms responsible for subtype-specific miRNA dysregulation remain incompletely understood. In the present study, FOXM1 inhibition was associated with significant alterations in the expression of several clinically relevant miRNAs in TNBC cells, whereas only limited changes were observed in ER-positive breast cancer cells. These findings suggest that FOXM1 may contribute to subtype-specific miRNA regulation and support an association between FOXM1 signaling and the expression of tumor-suppressive miRNAs involved in TNBC biology.

FOXM1 is one of the most frequently overexpressed transcription factors in TNBC and has been implicated in cell-cycle progression, proliferation, epithelial–mesenchymal transition, metastasis, stemness, and therapeutic



resistance.^{5–8,11–15} The high prevalence of TP53 mutations in TNBC contributes to persistent FOXM1 activation through disruption of p53-mediated transcriptional repression, whereas ER-positive tumors generally exhibit lower FOXM1 expression because TP53 mutations occur less frequently. Although FOXM1 has previously been shown to regulate numerous oncogenic signaling pathways, its role in coordinating subtype-specific miRNA expression has remained largely unexplored. Our findings suggest that FOXM1 may contribute to the reduced expression of several prognostically significant tumor-suppressive miRNAs in TNBC cells. Although the present study demonstrates a clear association between FOXM1 inhibition and altered miRNA expression, the precise molecular mechanisms underlying this relationship remain to be determined. Future studies investigating direct transcriptional regulation, including promoter binding and chromatin occupancy analyses, will be required to establish whether FOXM1 regulates these miRNAs directly or indirectly.

An important observation of the present study is that the prognostic significance of several miRNAs differed markedly between ER-negative and ER-positive breast cancers. High expression of miR-10b-5p consistently predicted improved survival regardless of ER status, whereas miR-34a-5p, miR-330-

5p, and miR-873-5p exhibited opposite prognostic associations between patients with ER-negative and ER-positive breast cancer.^{17–26} These findings emphasize that the biological functions of individual miRNAs are highly dependent on the molecular context of the tumor rather than representing universal biomarkers. Such subtype-dependent behavior has been reported previously and is likely attributable to differences in transcriptional programs, receptor signaling, chromatin organization, and target gene availability across breast cancer subtypes.

Consistent with the clinical analyses, endogenous expression profiling demonstrated that multiple tumor-suppressive miRNAs were markedly downregulated in MDA-MB-231 cells compared with ER-positive MCF-7 and nontumorigenic MCF10A cells, whereas the oncogenic miR-155-5p was significantly upregulated. These findings support previous reports linking miR-155 to epithelial–mesenchymal transition, inflammatory signaling, immune modulation, and resistance to anticancer therapy.^{21–23} Interestingly, FOXM1 inhibition did not significantly alter miR-155-5p expression, suggesting that miR-155 may be regulated predominantly through alternative signaling pathways independent of FOXM1. This observation further highlights the selective nature of FOXM1-mediated miRNA regulation.

Collectively, these observations support a subtype-dependent association between FOXM1 signaling and clinically relevant miRNA expression. The differential response observed between TNBC and ER-positive cells further suggests that the influence of FOXM1 on miRNA expression may depend on the molecular context of individual breast cancer subtypes.

However, this study has several limitations. First, our findings are based on two representative breast cancer cell lines and therefore require validation in additional breast cancer models. Second, FOXM1 knockdown efficiency was confirmed only at the mRNA level, and protein-level validation was not performed in the present study. Third, miRNA expression was normalized using U6 snRNA as the endogenous reference gene. Although U6 is widely used for miRNA normalization, the inclusion of an additional validated reference gene would further strengthen the robustness of the analysis.¹¹ Fourth, because the seven selected miRNAs were analyzed using a hypothesis-driven targeted approach, no correction for multiple comparisons using the false discovery rate (FDR) was applied, which should be considered when interpreting the statistical findings. Finally, although FOXM1 knockdown was associated with significant alterations in miRNA expression, direct transcriptional regulation was not investigated using promoter-binding or chromatin occupancy assays, and the biological functions of the identified miRNAs were not evaluated in rescue experiments. These limitations should be addressed in future mechanistic and in vivo studies.

CONCLUSION

In conclusion, our findings demonstrate that FOXM1 inhibition is associated with subtype-specific alterations in the expression of clinically relevant miRNAs, particularly in TNBC cells. These observations suggest that FOXM1 may contribute to the dysregulation of tumor-suppressive miRNAs associated with the aggressive phenotype of TNBC. Although the present study establishes a strong regulatory association between FOXM1 signaling and miRNA expression, additional mechanistic studies are required to determine whether this regulation occurs through direct or indirect molecular mechanisms. Nevertheless, our findings provide further evidence supporting the FOXM1/miRNA axis as a potential therapeutic target for aggressive breast cancer.

Ethics Committee Approval: This study was conducted using established cell lines; therefore, ethical approval was not required as it did not involve human participants or animal subjects.

Informed Consent: Written informed consent was not required for this study.

Conflict of Interest: The authors have no conflicts of interest to declare.

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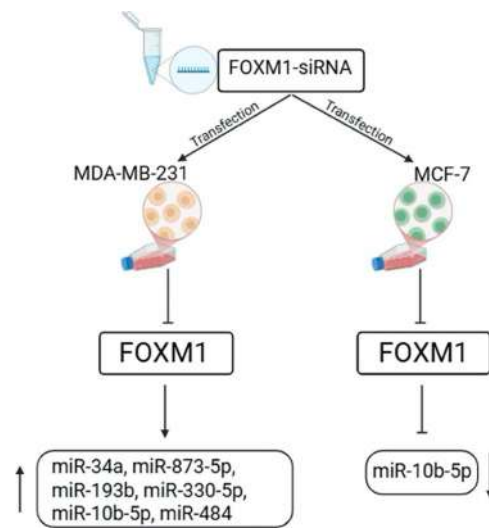
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Graphical abstract. Proposed association between FOXM1 signaling and the expression of clinically relevant miRNAs in breast cancer subtypes. Drawn using Biologic rendering software.

Machine Learning-Based Fatigue Detection from EEG Signals: A Methodological Pilot Framework with Potential for Nursing Applications

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ABSTRACT

Objective: Fatigue is a significant occupational health issue that adversely affects attention and decision-making. This study aimed to objectively predict fatigue levels using EEG signals and machine learning models and to develop a physiologically based assessment framework with potential future applications in nursing.

Materials and Methods: Time-domain, frequency-domain, entropy, Hjorth, and wavelet features were extracted from EEG data collected from 12 participants aged 21–40 years, with fatigue levels labeled using various scales.

Results: Classification performance varied across validation strategies. Under 5-fold cross-validation, Random Forest achieved the highest performance (accuracy=1.00, macro-F1=1.00), while KNN and NB also demonstrated strong performance. However, under LOOCV, sensitivity, precision, and macro-F1 values decreased despite relatively high accuracy. LOCO validation yielded more balanced results, with Random Forest maintaining the best performance (accuracy=0.97, macro-F1=0.91).

Conclusion: EEG-derived features provide valuable information for fatigue assessment. Although high performance was achieved under certain validation strategies, subject-independent evaluation revealed more realistic performance levels. The proposed framework offers a practical approach to fatigue monitoring. Future studies should focus on larger, domain-specific datasets and multimodal integration to enhance generalizability.

Keywords: EEG, fatigue prediction, machine learning, nursing.



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INTRODUCTION

Fatigue is a complex phenomenon involving both central and peripheral components. Central fatigue is linked to dysfunction within the central nervous system, resulting in reduced motivation, attention, and cognitive capacity.^{1–3} Neuroimaging studies reveal that structural and functional changes, along with neurotransmitter imbalances in the prefrontal cortex and basal ganglia, play a critical role in this process.⁴ Peripheral fatigue is associated with physiological changes at the



muscle level; force production decreases as a result of reduced muscle contractility, irregularities in calcium release, and disruptions in energy metabolism.⁵

The subjective and self-reported nature of fatigue presents significant challenges for its measurement. The existence of more than 250 assessment scales reflects its complexity and marked interindividual variability.^{6,7} Current evidence indicates that fatigue is a multidimensional phenomenon involving interacting central and psychological processes, leading to declines in both physical and mental performance.⁸

Prolonged shift durations and insufficient rest periods are major contributors to nurse fatigue, particularly in critical care settings. Fatigue negatively affects nurses' physical and mental health, increasing the risk of errors and compromising patient safety.⁹ Nurse fatigue has been linked to higher rates of medication errors, patient falls, and other safety incidents.^{10–13} Notably, Scott et al.¹² reported a 28% increase in patient safety incidents as shift duration increased. Consequently, machine learning–based approaches integrating subjective and objective fatigue measures offer promising strategies for understanding and managing fatigue in nursing and other professional groups.

In recent years, physiologically based objective measures have been increasingly used to address the limitations of subjective fatigue scales. Wearable technologies enabling automatic monitoring of indicators such as heart rate variability and sleep quality have advanced fatigue detection models.¹⁴ Electroencephalography (EEG) has emerged as a key indicator in fatigue detection because it directly reflects changes in mental state.¹⁵ Xu et al.¹⁶ showed that deep learning models can effectively capture complex EEG patterns, achieving 94.51% accuracy with a convolutional neural network (CNN) applied to multichannel EEG data. Yang et al.¹⁷ developed a hybrid fatigue detection model using publicly available EEG data from athletes. The model achieved 97.1% accuracy in classifying fatigue-related EEG patterns, demonstrating strong performance in early mental fatigue detection and performance optimization. Several studies have demonstrated the effectiveness of EEG-based AI models for fatigue detection, particularly in driving applications; reported accuracy rates using both traditional machine learning and deep learning approaches generally exceed 90%.^{18–22} These findings highlight the high accuracy and reliability of EEG-based AI models in driver fatigue detection and their potential to enhance driving safety. Chen et al.²³ assessed mental fatigue in healthcare personnel using multiscale entropy features extracted from single-channel EEG signals. Fatigue classification using Random Forest, XGBoost, and backpropagation neural network models achieved area under the curve (AUC) values of up to 95%, supporting EEG as a robust biomarker for mental fatigue detection.

KEY MESSAGES

- EEG-based machine learning models can effectively estimate fatigue using multidomain signal features.
- A data-driven hybrid labeling approach enables a more reliable representation of fatigue than single-scale methods.
- The proposed framework provides a practical and scalable approach for physiologically based fatigue monitoring in clinical settings.

While Chen et al.²³ presented a high-performance classification approach, the present study offers a more comprehensive and novel methodological pilot framework by integrating multiple fatigue scales and multidimensional EEG features. Given the critical importance of fatigue for patient safety, the limited evidence on objective fatigue assessment approaches specific to nursing represents a notable gap in the literature. In this context, rather than proposing a nursing-specific model, the present study provides a physiologically based, general methodological pilot framework with strong potential for future application in nursing by considering factors such as cognitive load and shift work conditions. This approach aims to serve as a foundation for future research focused on supporting employee well-being, enhancing patient safety, and improving the quality of care.

Aims and Objectives

This study introduces a novel framework for EEG-based fatigue detection by integrating multiple self-reported fatigue scales into a single composite label and combining this labeling strategy with a multidomain feature extraction and evaluation pipeline. In this respect, rather than proposing a model specifically tailored to nursing, the study presents a promising, physiologically based general methodological pilot framework with strong potential for future application in the nursing field. Unlike traditional approaches that rely on a single subjective scale, the proposed method uses a data-driven weighting scheme to combine different scale scores and create a composite fatigue index. To the best of our knowledge, such a hybrid label-generation strategy has not previously been reported in the literature.

In addition, the study employs a comprehensive feature representation that captures complementary information from the time, frequency, and time–frequency domains. Furthermore, the classification framework is evaluated using multiple validation strategies, such as 5-fold cross-validation, leave-one-out cross-validation (LOOCV), and leave-one-subject-out (LOSO) validation, enabling both sample-level and subject-independent performance assessments.

The results demonstrate that the proposed framework is more effective at capturing broad fatigue-related differences while also highlighting the challenges of subject-independent generalization. Overall, this study contributes to the literature by proposing a data-driven labeling strategy, integrating multidomain EEG features, and providing a robust evaluation framework for fatigue detection.

MATERIALS AND METHODS

Study Design

This study was designed as an investigation integrating both descriptive and methodological components. The descriptive aspect aims to reveal the relationships among variables in the existing dataset, while the methodological aspect seeks to develop a preliminary methodological framework for physiologically based models applicable to nurses using EEG-based machine learning approaches. This framework includes the development of a fatigue assessment model based on objective biophysiological indicators and a systematic evaluation of its potential applicability in nursing practice.

Participants and Dataset

The dataset, collected in 2021 as part of a study conducted by Kalanadhabhatta and colleagues, included a total of 12 participants (mean age=30.75±5.78 years), comprising 9 males and 3 females, aged 21–40 years. One participant had mild asthma, another had a history of seasonal asthma, and none of the other participants had any current or previous health issues. All participants provided informed consent before the study.²⁴

Experimental Procedure

The original study included three sessions conducted on separate days, with a maximum interval of 19 days, all held at the same time of day to control for circadian effects. Demographic data were collected before the first session. Personality traits (BFI-10) and chronotype (MCTQ) were assessed at baseline. Daily fatigue (FSS) and physical fitness (IFIS) were also evaluated. Before each session, sleepiness (SSS) and energy and mood (GVAS) were measured. Participants were equipped with four wearable devices to monitor physiological signals during each session. EEG data were recorded using the Muse device from an open-access dataset. Participants completed three sessions involving baseline EEG recording, physical exercise, cognitive tasks, and repeated fatigue assessments while physiological signals were continuously recorded.^{25–29} Detailed information regarding the scales used in this study is provided in Supplementary File 1.

EEG Recording and Preprocessing

The flowchart for this study is shown in Figure 1. The process consists of five main stages: labeling, data preprocessing,

feature extraction, classification, and performance evaluation. This flowchart provides a comprehensive overview of the process from EEG data to fatigue estimation.

Questionnaire-Based Labeling

In this study, participants' fatigue was assessed using scores from four self-report instruments: the SSS, GVAS, FSS, and IFIS. According to the dataset providers, some participants had missing FSS values. These missing values were estimated using SSS and GVAS data through a logistic regression model (fitglm), thereby minimizing information loss due to missing data.

Because each scale measures fatigue using different metrics, the scale scores were normalized and combined using the weighted-average method described in Equation 1. The weights were determined based on the variance values of the scales.

$$M_w = \frac{a.SSS + b.FSS + GVAS + d.IFIS}{a + b + c + d} \quad (1)$$

where a, b, c, and d denote the weighting coefficients corresponding to the SSS, FSS, GVAS, and IFIS, respectively, and M_w is the weighted mean score.

The variance was computed across all samples as defined in Equation 2. The corresponding weights (a, b, c, and d) were derived from these variance values, allowing each scale to contribute proportionally to its variability within the dataset.

$$w_i = \frac{var(x_i)}{\sum var(x_i)} \quad (2)$$

where var(x_i) represents the variance of the ith scale, and w_i represents its corresponding weight. This normalization ensures that the sum of all weights equals one.

In this context, scales with higher variance contribute more to the overall fatigue score because they provide greater discriminatory power for distinguishing fatigue levels among participants.

To the best of our knowledge, previous studies have not explicitly reported the integration of multiple fatigue-related scales into a single composite label using a data-driven weighting strategy.

The overall fatigue scores were divided into two categories using a data-driven threshold. Specifically, samples with fatigue scores less than or equal to the median value were labeled as low fatigue (Class 0), whereas those above the median were labeled as high fatigue (Class 1).

Feature Extraction and Selection

EEG signals were recorded from participants using the Muse device at a sampling frequency of 256 Hz from the TP9, AF7,

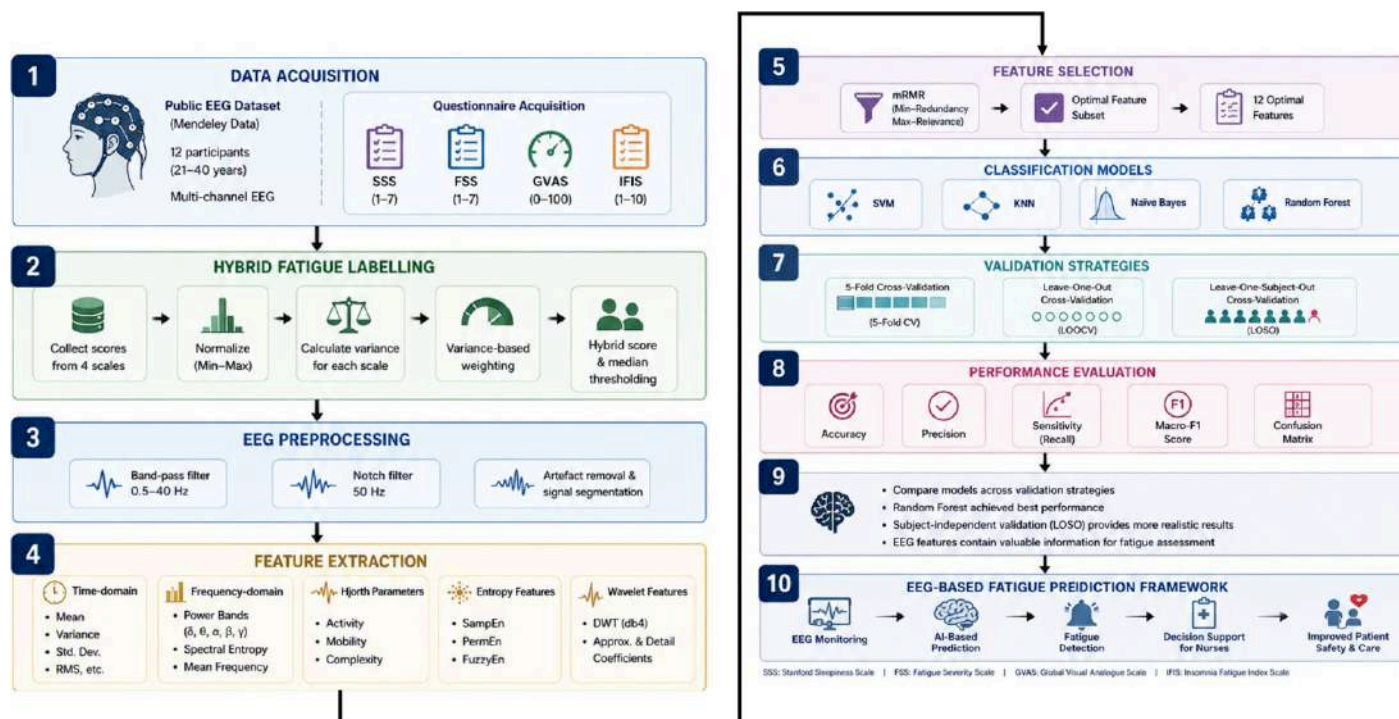


Figure 1. Flowchart of the EEG-based fatigue detection system.

AF8, and TP10 channels. The signals were then filtered using a 0.5–40 Hz Butterworth band-pass filter and a 50 Hz notch filter to remove noise.

The following feature sets were extracted for each participant and each recording:

- Frequency-based features: band powers in the delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), and beta (13–30 Hz) frequency bands, along with theta/alpha and theta/beta power ratios.
- Hjorth parameters: activity, mobility, and complexity, computed separately for each frequency band.
- Entropy features: spectral entropy computed for each frequency band to capture frequency-specific signal complexity.
- Time-domain features: mean, standard deviation, skewness, kurtosis, and root mean square (RMS) of the signal.
- Wavelet features: normalized subband energies derived from a 4-level discrete wavelet transform using the Daubechies 4 (db4) wavelet, including approximation (A4) and detail coefficients (D1–D4).

All features were computed on a per-channel basis and subsequently averaged across channels to obtain a participant-level representation.

To reduce feature dimensionality and improve generalization performance, feature selection was conducted using the minimum redundancy maximum relevance (mRMR) method. The mRMR algorithm ranks features by maximizing their relevance to the target variable while minimizing redundancy among features.³⁰ Using this procedure, the optimal feature subset was determined to comprise 12 features.

To prevent information leakage, feature selection was conducted within each cross-validation fold using only the training data. The selected features were then applied to the corresponding test data.

Classification Models and Evaluation Metrics

The extracted features were classified using support vector machine (SVM), Naive Bayes (NB), k-nearest neighbors (KNN), and Random Forest (RF) classification models based on the generated label information. Three validation strategies were employed to evaluate model performance under different validation conditions: 5-fold cross-validation, LOOCV, and LOSO validation.

In 5-fold cross-validation, the dataset was randomly divided into five subsets. In each iteration, four subsets were used for training, and one subset was used for testing. LOOCV was implemented to maximize the use of the limited data, with each sample serving once as the test sample. Additionally, LOSO validation

Table 1. Fatigue scales and weighting coefficients

Scales	SSS (a)	FSS (b)	GVAS (c)	IFIS (d)
Weight	0.19	0.42	0.25	0.15

SSS: Stanford Sleepiness Scale; FSS: Fatigue Severity Scale; GVAS: Global Energy and Affect Scale; IFIS: International Fitness Scale.

was performed to evaluate subject-independent performance, with all samples from one participant used as the test set and samples from the remaining participants used for training.

This multilevel validation framework facilitates the evaluation of both sample-level performance and subject-level generalization.

Ethical Approval

This study utilized publicly available anonymized data. Therefore, ethics committee approval was not required. The results were classified in (MATLAB R2024b, The MathWorks, Inc., Natick, MA, USA) using machine learning techniques.

RESULTS

Results of Labeling

In this study, participants’ overall fatigue was assessed using four different survey scales: the SSS, GVAS, FSS, and IFIS. Because each scale measures fatigue using different metrics, the scores were normalized and combined using a weighted-average method. The weights were determined based on the variance values of the scales, as shown in Table 1. The overall fatigue score was then calculated according to Equation 1.

Because no standardized clinical threshold for fatigue categorization was available in the literature, a data-driven

approach was adopted. The overall fatigue scores were divided into two classes using the median value as the threshold. Samples with scores less than or equal to the median were assigned to the low-fatigue class (Class 0), whereas those with scores above the median were assigned to the high-fatigue class (Class 1).

Statistical Structure of EEG Features

A feature matrix comprising 35 samples and 128 features was constructed. Features were extracted separately from each of the four EEG channels (TP9, AF7, AF8, and TP10), preserving channel-specific information rather than averaging across channels. The samples corresponded to 12 participants with 3 trials each, excluding one missing recording, while the feature set represented EEG characteristics extracted from multiple domains.

Frequency-domain features included relative band powers in the delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), and beta (13–30 Hz) bands, along with theta/alpha and theta/beta power ratios. In addition, Hjorth parameters (activity, mobility, and complexity) and spectral entropy were computed separately for each frequency band to capture band-specific signal dynamics.

Time-domain features included the mean, standard deviation, skewness, kurtosis, and root mean square (RMS) values of the EEG signal.

For time–frequency analysis, the EEG signal was decomposed into four levels using the Daubechies 4 (db4) wavelet, resulting in one approximation subband (A4) and four detail subbands (D1–D4).

Table 2. Classification results

Validation	Model	Accuracy	Sensitivity	Precision	Macro-F1
5-Fold	SVM	0.77	0.78	0.76	0.73
5-Fold	KNN	0.83	0.83	0.86	0.82
5-Fold	NB	0.83	0.84	0.85	0.83
5-Fold	RF	1.00	1.00	1.00	1.00
LOOCV	SVM	0.77	0.39	0.39	0.39
LOOCV	KNN	0.89	0.44	0.44	0.44
LOOCV	NB	0.86	0.43	0.43	0.43
LOOCV	RF	0.97	0.49	0.49	0.49
LOSO	SVM	0.65	0.61	0.49	0.51
LOSO	KNN	0.85	0.78	0.76	0.75
LOSO	NB	0.83	0.76	0.76	0.74
LOSO	RF	0.97	0.90	0.92	0.91

SVM: Support vector machine; KNN: K-nearest neighbors; NB: Naive Bayes; RF: Random Forest; LOOCV: Leave-one-out cross-validation; LOSO: Leave-one-subject-out.

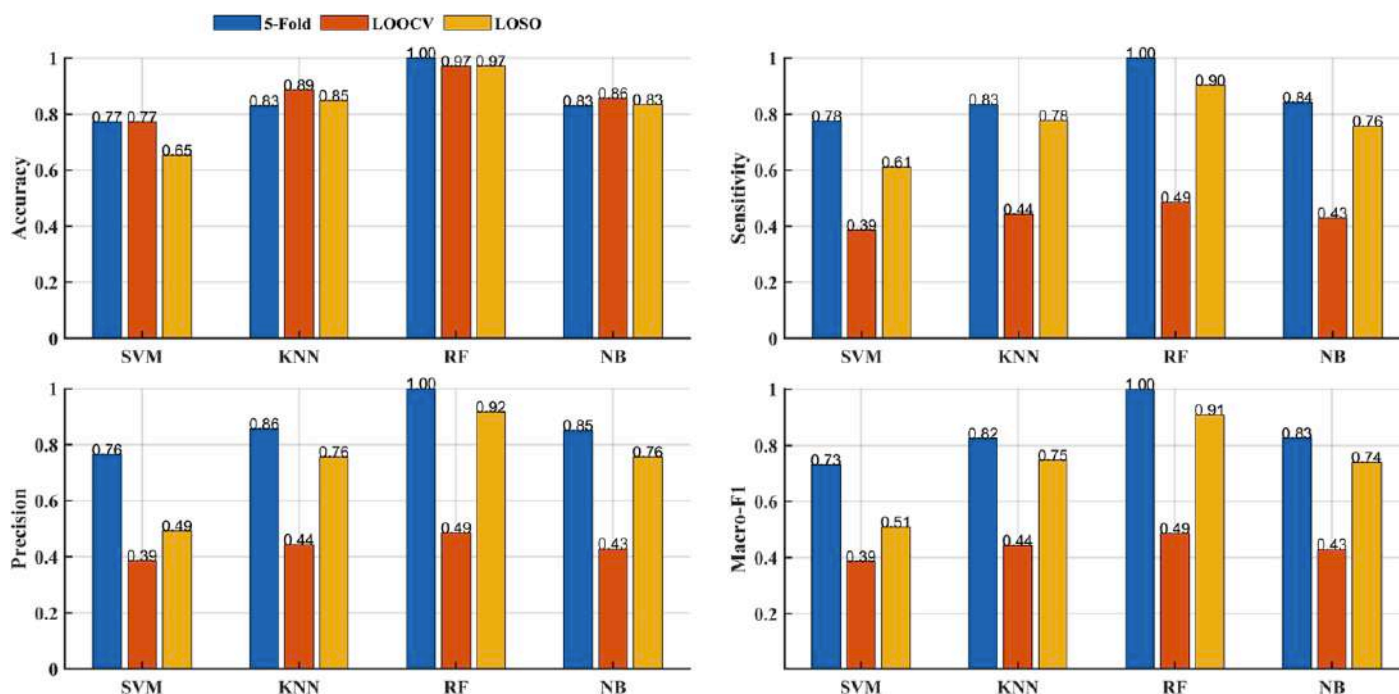


Figure 2. Performance metrics of classifiers across different validation strategies.

Results of Classification

The performance of four different classifiers (SVM, KNN, NB, and RF) was evaluated using multiple validation strategies, including 5-fold cross-validation, LOOCV, and LOSO validation.

For the SVM model, a radial basis function (RBF) kernel was employed; the KNN model used a neighbor count of $k=3$; the NB classifier was implemented assuming a Gaussian distribution; and the RF model was constructed using an ensemble of 100 decision trees.

The classification results are presented in Table 2, including accuracy, sensitivity, precision, and class-specific F1 scores. Additionally, the comparative performance of the classifiers across the different validation strategies is illustrated in Figure 2.

The classification performance of the four classifiers (SVM, KNN, NB, and RF) across the three validation strategies (5-fold CV, LOOCV, and LOSO) is presented in Table 2 and Figure 2. Under the 5-fold cross-validation scheme, the Random Forest (RF) classifier achieved perfect performance across all metrics (accuracy=1.00, macro-F1=1.00), while KNN and NB also demonstrated strong performance, with comparable macro-F1 scores of 0.82 and 0.83, respectively. In contrast, SVM exhibited relatively lower performance in this setting.

In the LOOCV scenario, although RF maintained high accuracy (0.97), sensitivity, precision, and the macro-F1 score decreased

substantially to approximately 0.49, indicating potential instability in generalization when evaluated using single-sample test folds. A similar trend was observed for SVM, KNN, and NB, for which accuracy remained relatively high, but class-specific performance metrics declined significantly.

LOSO validation, which provides a more realistic subject-independent evaluation, revealed a different performance pattern. RF again achieved the best overall performance (accuracy=0.97, macro-F1=0.91), followed by KNN and NB, which showed consistent and balanced results. In contrast, SVM demonstrated notably lower performance (macro-F1=0.51), indicating limited generalization across participants. Overall, RF emerged as the most robust classifier across all validation strategies, while KNN and NB provided competitive and stable alternatives, particularly under subject-independent evaluation conditions.

DISCUSSION

This study demonstrates the applicability of EEG-based machine learning models for fatigue detection. A notable discrepancy was observed among the validation strategies. While the Random Forest model achieved perfect performance under 5-fold cross-validation (accuracy=1.00), its performance decreased substantially under LOOCV and LOSO evaluations. This suggests that the 5-fold cross-validation results may have been influenced by data partitioning and may provide overly

Table 3. Comparison of related studies

Study	Year	Data	Classifier	Best accuracy (%)
Ren et al. ¹⁹	2021	EEG	RBF	92.71
Xu et al. ¹⁶	2023	EEG	CNN	94.5
Chen et al. ²³	2024	EEG	RF	87.0
Shen et al. ¹⁵	2025	Multimodal	Hybrid DL	96.1
Yang et al. ¹⁷	2024	EEG	LSTM	97.2
Geng et al. ²⁰	2025	EEG	SVM	94.32
Hafiz et al. ²²	2025	HRV	SVM	81.48
This study	2026	EEG	RF	100

ANN: Artificial neural network; CNN: Convolutional neural network; DL: Deep learning; HRV: Heart rate variability; LSTM: Long short-term memory network; RBF: Radial basis function; RF: Random forest; SVM: Support vector machine.

optimistic performance estimates, particularly given the limited sample size. In contrast, LOSO offers a more realistic subject-independent evaluation and revealed more moderate but reliable performance levels. Therefore, these results highlight the importance of employing subject-independent validation strategies when assessing EEG-based fatigue detection models. In addition to these findings, a notable decrease in performance was observed under LOOCV compared with 5-fold cross-validation. Although accuracy remained relatively high, sensitivity, precision, and macro-F1 scores declined substantially. This discrepancy may be attributed to the nature of LOOCV, in which each model is trained on a slightly reduced dataset and evaluated on a single sample, making performance more sensitive to data variability and noise.

In addition, the limited sample size and inherent variability of EEG signals may further amplify this effect, leading to unstable performance estimates. Therefore, LOOCV results should be interpreted with caution, as they may not fully reflect the generalization capability of the models. In contrast, LOSO validation provides a more realistic subject-independent evaluation and yielded more balanced performance results, suggesting that it may be a more appropriate validation strategy for EEG-based fatigue prediction tasks.

This study demonstrates a strong association between fatigue classes derived from self-report scales (SSS, FSS, GVAS, and IFIS) and EEG features, suggesting that EEG-derived features are associated with both the cognitive and physical aspects of fatigue.

The EEG-based classification results of this study provide a promising, physiologically based general methodological pilot framework with strong potential for future application in nursing. Given the need for real-time monitoring in high-risk settings (e.g., ICUs, emergency departments, operating rooms, and dialysis centers), these findings may inform the design of

future workload regulation systems. Consistent with the EEG-based fatigue framework of Liu et al.,³¹ the proposed modeling steps contribute methodologically to the development of algorithms for healthcare professionals working under high levels of stress. Moreover, in line with Alshammari et al.,³² the focus on fatigue-related factors highlights the role of such frameworks in improving worker well-being and reducing errors.

The applicability of this model in real-world clinical nursing settings presents several practical considerations. The comfort of wearable EEG devices, their suitability for prolonged use and user mobility, and their seamless integration into clinical workflows are critical issues. To this end, future systems should incorporate robust artifact removal techniques, real-time signal-processing algorithms, and user-friendly wearable designs.

The results obtained in this study reveal notable differences compared with the existing literature summarized in Table 3. Previous studies indicate that complex deep learning models (e.g., CNNs, LSTMs, and hybrid networks) generally achieve high accuracy in fatigue classification.

For instance, Xu et al.¹⁶ reported 94.5% accuracy using a CNN. Yang et al.¹⁷ achieved 97.2% accuracy using an LSTM.¹⁷ Xu et al.¹⁶ achieved high accuracy using CNN-based deep learning on multichannel EEG data, while Yang et al.¹⁷ demonstrated strong performance in fatigue classification using EEG data from athletes. In contrast, the present study differs by employing interpretable multidomain EEG feature extraction, introducing a composite fatigue-labeling strategy based on multiple self-report scales, and emphasizing subject-independent validation within a clinically oriented framework. Shen et al.¹⁵ reported 96.1% accuracy using a multimodal hybrid approach. However, more traditional machine learning models, such as those employed by Ren et al.¹⁹ and Geng et al.,²⁰ also demonstrated classification performance above 90%. Chen et al.²³ used a comprehensive feature-

engineering process involving multiscale entropy extraction from EEG signals and reported 87% accuracy with a Random Forest classifier. Nevertheless, this method is complex and computationally intensive because of the feature extraction process. Hafiz et al.²² focused solely on fatigue detection in nurses using HRV and achieved 81.48% accuracy with HRV features and an SVM classifier. Although HRV signals are relatively inexpensive to obtain, they only partially represent neurophysiological changes compared with EEG. Because HRV does not directly represent neural activity, it may not fully capture cortical-level processes such as cognitive load, reduced attention, or mental fatigue. In contrast to these studies, the present work emphasizes a balance between model simplicity and performance, offering a methodological framework that prioritizes interpretability and potential real-time applicability over maximum accuracy.

Among the evaluated classifiers, Random Forest generally demonstrated the highest performance across validation strategies, particularly under LOSO conditions, indicating its robustness to intersubject variability. KNN and NB also showed stable and competitive performance, whereas SVM exhibited comparatively lower generalization capability. These findings suggest that ensemble-based methods may be more suitable for EEG-based fatigue classification tasks.

Limitations

This study has several limitations. The small sample size ($n=12$), the use of an open-access dataset, and the fact that the EEG data were obtained from healthy individuals performing tasks in a laboratory setting rather than from nurses limit the generalizability of the findings and render them preliminary. The presence of two participants with a history of asthma should also be considered a potential limitation. The use of only four EEG channels may not capture all fatigue-related cortical activity, while self-report-based labeling using a percentile-based approach may introduce subjective bias because of the lack of clinically validated cutoff values. Accordingly, this study serves as a starting point for future nursing-focused research conducted in clinical settings with larger samples and multichannel EEG recordings.

CONCLUSION

This study demonstrates that fatigue can be objectively estimated by combining multidimensional EEG-derived features with machine learning models and presents a promising, physiologically based general methodological pilot framework with strong potential for future application in nursing. The pilot model achieved meaningful accuracy when integrated with self-report scales, indicating that EEG-derived features provide valuable information related to fatigue.

These findings highlight the potential of physiologically based fatigue-monitoring systems to enhance employee safety, patient safety, and continuity of care while providing a scientific basis for larger nursing-specific studies. Future research should include nurse samples, employ multichannel or high-density EEG, and incorporate performance and biophysiological measures to further improve model robustness and generalizability.

Ethics Committee Approval: Since this study utilized publicly available anonymized data, no ethics committee approval was required.

Informed Consent: Written informed consent was not required, as the study utilized publicly available anonymized data.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Author Contributions: Concept – SC, ED; Design – SC, ED; Supervision – SC, ED; Resource – SC, ED; Materials – ED; Data Collection and/or Processing – SC; Analysis and/or Interpretation – SC, ED; Literature Review – SC, ED; Writing – SC, ED; Critical Review – SC, ED.

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Clinical Relevance of Multiplex PCR for Respiratory Pathogens in Hospitalized Adults with Influenza-Like Illness

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ABSTRACT

Objective: Influenza-like illness (ILI) is a major cause of hospitalization during respiratory virus seasons. This study aimed to investigate the pathogens detected in patients presenting with ILI symptoms and to analyze the results in conjunction with clinical, laboratory, and radiological findings.

Materials and Methods: This retrospective, single-center study included 446 hospitalized adults with ILI between epidemiological week 40 of 2024 and week 20 of 2025. Respiratory pathogens were identified using a multiplex real-time polymerase chain reaction (PCR) assay detecting 18 viral and 5 bacterial pathogens. Demographic, clinical, laboratory, and radiological data were retrieved from electronic medical records. Disease severity was assessed using the CURB-65 score. Patients with *Streptococcus pneumoniae* detected by PCR were further evaluated according to radiological evidence of pneumonia.

Results: At least one respiratory pathogen was detected in 184/446 patients (41.2%), whereas no pathogen was identified in 262 patients (58.8%). The most frequently detected pathogens were *S. pneumoniae*, influenza viruses, and seasonal coronaviruses. The most common coinfection was influenza A plus *S. pneumoniae*. Among patients with pneumococcal PCR positivity, those with radiologically confirmed pneumonia were significantly older, had higher CURB-65 scores, and more frequently had concomitant influenza infection than patients without radiological evidence of pneumonia.

Conclusion: Multiplex molecular assays provide valuable information for respiratory pathogen detection, but molecular results, especially for bacterial pathogens such as *S. pneumoniae*, should be interpreted together with clinical and radiological findings.

Keywords: Adult, co-infection, influenza-like illness, molecular test, *Streptococcus pneumoniae*.



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INTRODUCTION

Influenza-like illness (ILI) is a general term used for respiratory infections caused by influenza and other respiratory viruses. These agents cannot be distinguished based solely on clinical findings.

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ILI imposes a significant economic burden by increasing healthcare costs and hospitalization rates.^{1,2}

According to the European Centre for Disease Prevention and Control (ECDC), ILI is defined by the acute onset of symptoms, with at least one systemic symptom (fever, headache, myalgia, or fatigue) and at least one respiratory symptom (cough, shortness of breath, or sore throat).³ However, these symptoms are not specific to any single pathogen and can occur in both viral and bacterial infections. Consequently, the frequent administration of empirical antibiotic therapy contributes to increasing antibiotic resistance and healthcare expenditures. This issue is particularly critical in countries where per capita daily antibiotic use is high.^{4,5}

The viruses most commonly responsible for acute respiratory infections include respiratory syncytial virus (RSV), influenza A and B viruses (IV), parainfluenza virus types 1, 2, and 3 (PIV), adenovirus (AV), coronavirus (CoV), rhinovirus (RV), enterovirus (EV), bocavirus (BoV), and metapneumovirus (MPV).⁶ Due to the numerous subtypes of these viruses and the lack of long-lasting immunity, diagnostic methods that directly detect viral nucleic acids are preferred.⁷ Although viral pathogens are the predominant cause of respiratory tract infections, several bacterial agents may present with clinical manifestations that overlap with those of ILI. *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *Legionella pneumophila*, and *Bordetella pertussis* are therefore included in multiplex respiratory panels because of their clinical relevance and the value of early etiological identification in guiding patient management.⁸ Recently developed rapid molecular diagnostic methods allow pathogen detection within hours of specimen collection. These tests facilitate the timely initiation of appropriate antibacterial or antiviral therapy and enable optimal cohorting of hospitalized patients. Compared with conventional diagnostic methods, rapid molecular tests can identify pathogens 2–3 days earlier, thereby reducing inappropriate antibiotic use.⁹

The aim of this study was to retrospectively investigate the pathogens detected in patients presenting with ILI during the 2024–2025 winter season and to analyze the results in conjunction with clinical, laboratory, and radiological findings.

MATERIALS AND METHODS

Study Design and Setting

This retrospective, single-center observational study was conducted at Balıkesir Atatürk City Hospital between October 2024 and May 2025, corresponding to epidemiological week 40 of 2024 through week 20 of 2025. The study population consisted of hospitalized patients admitted to hospital wards or intensive care units with symptoms consistent with ILI.

KEY MESSAGES

- Multiplex PCR provides rapid and comprehensive pathogen detection and may support timely clinical decision-making. However, its diagnostic interpretation differs between viral and bacterial targets. In particular, *S. pneumoniae* detection from nasopharyngeal specimens may reflect colonization or, potentially, nonspecific detection, limiting its ability to establish pneumococcal pneumonia on its own.
- Multiplex PCR results should be interpreted together with clinical severity, radiological findings, and, when available, conventional microbiological data.
- This integrated approach may help distinguish probable pneumococcal disease from colonization and support more appropriate antimicrobial treatment decisions.

Ethical Approval

Ethical approval was obtained from Balıkesir Atatürk City Hospital Scientific Research Ethics Committee (Approval Number: 2025/07/77, Date: 24.07.2025). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patients and Data Collection

A total of 446 patients with available respiratory polymerase chain reaction (PCR) panel results were included in the study. Patient demographic and clinical data, including age, sex, admission date, symptoms, comorbidities, laboratory findings, radiological findings, and clinical follow-up data, were retrieved from the hospital information management system and electronic medical records.

Computed tomography (CT) findings obtained concurrently with respiratory testing were evaluated through the Picture Archiving and Communication System (PACS). In addition, discharge summary reports were reviewed when available. The CURB-65 score was calculated for each patient (confusion, urea, respiratory rate, blood pressure, and age ≥ 65 years).

Patients were stratified into three age groups: 18–45 years, 46–64 years, and ≥ 65 years.

Diagnostic Criteria

ILI was defined as the presence of fever ($\geq 38^\circ\text{C}$) accompanied by respiratory symptoms such as cough and/or sore throat.

Definitions

Viral pathogens belonging to the same family were grouped for analysis (e.g., coronaviruses, including 229E, NL63, HKU1, and OC43).

Inclusion Criteria

Patients meeting the following criteria were included:

- Age ≥ 18 years
- Hospitalization due to ILI symptoms
- Availability of respiratory PCR panel results
- Complete clinical and laboratory data

Exclusion Criteria

Patients were excluded if they met any of the following criteria:

- Age < 18 years
- Fever lasting longer than 7 days within the previous 2 weeks
- Prior antibiotic use before hospital admission
- Outpatient status
- Incomplete or missing data

Clinical, Laboratory, and Radiological Investigations

Clinical findings, laboratory parameters, and radiological imaging results were evaluated retrospectively. Routine laboratory tests included hematological and biochemical parameters. Radiological evaluation primarily consisted of thoracic CT imaging when clinically indicated. Disease severity was assessed using the CURB-65 score. The score is based on five factors from which its name is derived:

- Confusion
- Urea
- Respiratory rate
- Blood pressure
- Age ≥ 65 years

Scoring was performed as follows: confusion (1 point), urea level > 7 mmol/L (1 point), respiratory rate ≥ 30 breaths/min (1 point), blood pressure (systolic < 90 mmHg or diastolic ≤ 60 mmHg) (1 point), and age ≥ 65 years (1 point). A total score of 0–1 indicates a low mortality risk, 2 points indicates a moderate mortality risk, and 3–5 points indicates a high mortality risk.

Sample Collection and Respiratory Panel Procedure

Respiratory PCR results obtained using the Bosphore Respiratory Pathogens Panel Kit v10 (Anatolia Geneworks) were analyzed.

Nasal, oropharyngeal, and nasopharyngeal swab samples were collected according to the manufacturer's recommendations. For nasopharyngeal swab collection, the patient's head was hyperextended, and a flocked swab was inserted through the

nostril into the posterior nasopharynx, rotated 2–3 times, and then withdrawn. The swab was immediately placed into a tube containing viral transport medium (VTM).

Samples were transported promptly to the laboratory. If storage was necessary, samples were maintained at room temperature (18–30°C) for up to 4 hours, at 2–8°C for up to 3 days, or at temperatures below –15°C for up to 30 days.

Real-time PCR analysis was performed in accordance with the manufacturer's instructions. Fluorescence detection was conducted using the FAM, HEX, Texas Red, and Cy5 channels. An endogenous internal control (Cy5) was used to evaluate sampling adequacy and procedural accuracy, while an exogenous internal control (Texas Red) was used to monitor nucleic acid extraction and PCR inhibition.

Threshold cycle (Ct) values were considered valid if ≤ 32 for internal controls and ≤ 28 for positive controls. Samples exceeding the threshold values in the FAM, HEX, Texas Red, or Cy5 channels were interpreted as positive. As results were reported qualitatively as positive or negative, analyses were based on qualitative data. Raw amplification data, Ct values, and amplification curves were not routinely archived and were therefore unavailable retrospectively.

The panel detected the following pathogens: parainfluenza virus (PIV) 1–4, enterovirus (EV), human metapneumovirus (hMPV), respiratory syncytial virus (RSV A/B), bocavirus (BoV), rhinovirus (RV), coronaviruses (229E, NL63, HKU1, OC43), adenovirus (AV), influenza A (H1N1, H3, H1), influenza B, parechovirus, *Haemophilus influenzae* type B, *Mycoplasma pneumoniae*, *Streptococcus pneumoniae*, *Legionella pneumophila*, and *Bordetella pertussis*.

Statistical Analysis

Statistical analyses were performed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). The normality of continuous variables (e.g., age and laboratory parameters) was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests. As the variables were not normally distributed, continuous variables were expressed as median (interquartile range) and compared between groups (e.g., pathogen-positive vs. pathogen-negative patients) using the Mann–Whitney U test.

Categorical variables, including pathogen detection status (positive/negative), age groups, sex, and the presence of single- or dual-pathogen infections, were presented as frequencies and percentages. Comparisons between categorical variables were performed using the chi-square test (e.g., pathogen detection by age group and sex and dual-pathogen infection by sex).

A p value of < 0.05 was considered statistically significant.

Table 1. General characteristics of the patient population

Patient characteristics	Pathogen (–) (n=262) (%)	Pathogen (+) (n=184) (%)	p
Sex			0.900
Female	113 (43.1)	80 (43.5)	
Male	149 (56.9)	104 (56.5)	
Age group (years)			0.400
18–45	20 (7.6)	13 (7.1)	
46–64	59 (22.5)	33 (17.9)	
≥65	183 (69.8)	138 (75.0)	
Clinical setting			0.300
Ward	194 (74.0)	144 (78.3)	
Intensive care unit (ICU)	68 (26.0)	40 (21.7)	

Table 2. Distribution of mono- and co-infections by sex and age group

	Mono-infection (n=148) (%)	Co-infection (n=36) (%)	p
Sex			0.030
Female	70 (47.3)	10 (27.8)	
Male	78 (52.7)	26 (72.2)	
Age group (years)			0.090
18–45	12 (8.1)	1 (2.8)	
46–64	30 (20.3)	3 (8.3)	
≥65	106 (71.6)	32 (88.9)	

RESULTS

A total of 446 hospitalized adult patients with ILI were included in the study. Respiratory PCR testing identified at least one pathogen in 184 patients (41.2%), while no pathogen was detected in 262 patients (58.8%). The demographic characteristics of the study population are summarized in Table 1.

Pathogen detection was most frequent among patients aged ≥65 years, followed by those aged 46–64 years. The distribution of detected pathogens according to age group and sex is presented in Table 2. Both single- and dual-pathogen infections were most commonly observed in patients aged ≥65 years.

Among patients with dual-pathogen infections, males constituted the majority (72.2%), and this difference was statistically significant (p=0.030) (Table 2).

The distribution of pathogens detected by respiratory PCR testing showed that *S. pneumoniae*, influenza viruses, and

Table 3. Distribution of pathogens detected by PCR testing

Respiratory pathogen	Subtype	Number
<i>S. pneumoniae</i> (n=64)		64
Influenza (n=59)	Influenza A	26
	Influenza H3	26
	Influenza H1N1	3
	Influenza B	3
	Influenza H1	1
Coronavirus (n=32)	Coronavirus HKU1	19
	Coronavirus NL63	7
	Coronavirus OC43	5
	Coronavirus 229E	1
Rhinovirus/Enterovirus (n=28)		28
Respiratory syncytial virus (n=17)		17
Metapneumovirus (n=11)		11
Adenovirus (n=3)		3
Parainfluenza virus 2 (n=3)		3
Bocavirus (n=2)		2
Parechovirus (n=1)		1
Total		220

PCR : Polymerase chain reaction.

coronaviruses were the most frequently identified pathogens (Table 3). Among cases with co-detected pathogens, the distribution of respiratory pathogens is summarized in Figure 1, with the most common combinations being influenza A + *S. pneumoniae*, RV/EV + *S. pneumoniae*, and RSV + *S. pneumoniae*.

Patients with respiratory PCR positivity for *S. pneumoniae* were categorized based on radiological findings. Patients with imaging features consistent with typical pneumonia—such as lobar involvement, consolidation, or air bronchograms shortly after symptom onset—were classified as Group 2 (radiologically confirmed pneumonia). Patients without these radiological findings were classified as Group 1 (no radiological evidence of pneumonia).

Demographic data, laboratory findings such as CRP and WBC, CURB-65 scores, and clinical findings were analyzed for Groups 1 and 2 (Table 4). In the pneumonia group, median age, CURB-65 score, and the frequency of concomitant influenza infection were significantly higher.

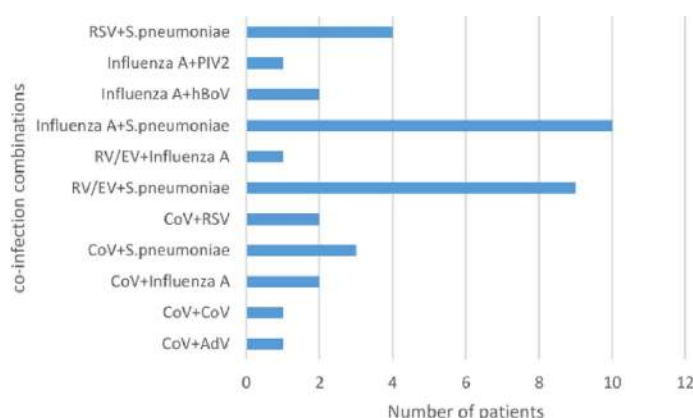
DISCUSSION

This study aimed to elucidate the demographic, epidemiological, clinical, and radiological characteristics of adults hospitalized with ILI during the 2024–2025 season.

Table 4. Evaluation of patients with pneumococcal PCR positivity according to radiological findings (n=64)

Variable	Group 1 (n=47)	Group 2 (n=17)	p
Age, median (IQR)	67 (59–75)	80 (69–90)	0.001
Sex (M/F)	30/17	11/6	0.900
CRP, median (IQR)	7.5 (2.02–13)	10.3 (5.94–18.85)	0.060
WBC, median (IQR)	11,870 (7,897–15,370)	10,880 (7,980–13,540)	0.800
CURB-65, median (IQR)	2 (0–2)	3 (2–3)	<0.0001
Co-infection with influenza	3 (6.4%)	7 (41.2%)	0.002
ICU admission	6 (12.8%)	6 (35.3%)	0.060

Group 1: Radiologically unconfirmed pneumonia (negative); Group 2: Radiologically confirmed pneumonia (positive). CRP: C-reactive protein; CURB-65: Confusion, urea, respiratory rate, blood pressure, and age ≥ 65 years; ICU: Intensive care unit; IQR: Interquartile range; M/F: Male/female; WBC: White blood cell.

**Figure 1.** Distribution of co-infection combinations.

During this season, 446 nasopharyngeal swab (NPS) samples were analyzed in our laboratory, with a positivity rate of 41.2%. In 2018, using a different version of the same device and kit, 3,074 NPS samples from both pediatric and adult patients across 13 hospitals in Türkiye were analyzed, yielding a positivity rate of 48%.¹⁰ In a study conducted in Taiwan using respiratory tract samples from hospitalized adult patients, similar to the patient population in our study, the positivity rate was 22%.¹¹ Mengelle et al.¹² reported a very high viral pathogen positivity rate of 88.7% in a pediatric population. Although PCR tests for respiratory infections are highly sensitive, positivity rates may vary depending on patient demographics, the duration and severity of symptoms, the analytical performance of the test, sample type, and seasonal factors.^{13,14} Consequently, positivity rates differ among studies.

It is noteworthy that in our study, the most frequently detected agent in nasopharyngeal swab samples obtained from patients presenting with ILI findings was *S. pneumoniae*. In adults, nasopharyngeal carriage is theoretically important

for the development of invasive pneumococcal disease (IPD), although it is not considered a strong independent risk factor. A high nasopharyngeal microbial load may facilitate bacterial invasion and microaspiration into the lungs, leading to pneumonia. Evidence suggests that influenza virus can modify host cells to enhance pneumococcal adhesion, invasion, and disease potential.¹⁵ Among the 64 patients with pneumococcal detection, 17 (27%) had radiological findings compatible with pneumonia, while 47 (73%) did not, suggesting colonization. Influenza and *S. pneumoniae* coinfection was observed in 10 patients; 7 (41.2%) were in the radiologically confirmed pneumonia group, while 3 (6.4%) were in the colonization group. This difference was statistically significant ($p=0.002$). Similarly, Karabulut et al.¹⁶ reported a substantial rate of viral–bacterial co-detection (35.53%), highlighting the frequent etiological overlap encountered in routine clinical practice. Consistent with these findings, a meta-analysis of 27 studies showed that *S. pneumoniae* was the most frequently detected bacterial coinfecting pathogen (35%).¹⁷ Therefore, in patients with ILI, secondary pneumococcal infections, in addition to viral agents, should be considered, emphasizing the importance of pneumococcal vaccination during influenza seasons.

Multiplex PCR assays and syndrome-specific panels allow the simultaneous detection of multiple pathogens in a single sample. However, interpreting *S. pneumoniae* results on respiratory panels is challenging because of frequent nonpathogenic colonization of the upper respiratory tract mucosa or potential cross-reactivity with closely related streptococci such as *S. mitis* and *S. oralis*.¹⁸ In this study, the *lytA* gene was used for pneumococcal detection. Although useful, the *lytA* gene is not entirely specific, as homologous sequences exist in *S. mitis* and *S. oralis*, potentially causing false-positive results.^{19,20} Furthermore, the presence of pneumococcal target genes in nonpneumococcal streptococci isolated from oral specimens has raised concerns regarding the specificity of

qPCR-based approaches. Miellet et al.²¹ emphasized that the reliability of molecular pneumococcal detection depends on appropriate specimen collection, the selection of suitable target genes, and the interpretation of results within the clinical context. Despite the high sensitivity and rapid turnaround time of multiplex molecular assays, their role in routine clinical practice—particularly in distinguishing colonization from true infection and in comparison with conventional diagnostic methods—remains under debate. Current guidelines support the use of molecular pneumonia panels because of their superior sensitivity and faster pathogen detection; however, as highlighted by Walker et al.,²² their integration into clinical practice requires methodological standardization and careful interpretation of results in conjunction with clinical findings. Supporting these limitations, Simões et al.²³ reported that real-time PCR targeting *lytA* misidentified three isolates. Thus, PCR-based pneumococcal detection from colonized sites may yield inaccurate results.

In our study, the CURB-65 score was significantly higher in the radiologically confirmed pneumonia group, indicating that pneumococcal PCR positivity alone has limited diagnostic value but may support the likelihood of true pneumonia when combined with clinical scoring. The Turkish Thoracic Society recommends molecular diagnostics in hospitalized patients or those with severe pneumonia, emphasizing interpretation alongside the clinical presentation.²⁴

In their study of pediatric patients, Pernica et al.²⁵ showed that quantitative evaluation of *S. pneumoniae* in the nasopharynx would not provide practical benefit to clinicians attempting to distinguish viral from bacterial respiratory tract infections. Another pediatric case-control study suggested that a colonization density threshold of $>6.9 \log_{10}$ copies/mL yielded 64% sensitivity and 92% specificity for differentiating clinical pneumonia.²⁶ An important limitation of our study is that molecular test results were reported only qualitatively (positive/negative). Therefore, the relationship between Ct values and pathogen burden could not be assessed.

During the 2024–2025 season, according to the WHO global influenza report, influenza A(H1N1)pdm09 predominated in the Americas, Western Africa, Northern Europe, and Oceania, while influenza A(H3N2) predominated in East and South Africa and in West, East, and South Asia. Influenza A(H1N1)pdm09 and influenza A(H3N2) were identified as co-dominant in Southeast Asia.²⁷ In Türkiye, according to Sentinel ILI surveillance from September 30, 2024, to April 20, 2025, influenza A and its subtype A(H3N2) were the most frequently detected.²⁸ Unlike the surveillance data, our study identified influenza A using the MP2 gene region without subtyping, and influenza A and A(H3) were detected at similar frequencies.

In Türkiye, Sentinel Surveillance from September 30, 2024, to April 20, 2025, showed that the most frequent non-influenza respiratory viruses were RV and CoV. In our study, following influenza, the most frequently detected pathogens were CoV, RV, RSV, and hMPV. Kaaijk et al.²⁹ similarly reported seasonal coronaviruses and rhinoviruses as the most common pathogens after influenza in adults aged >60 years. Although hMPV incidence decreased during the COVID-19 pandemic, positivity rates returned to pre-pandemic levels over three consecutive seasons.³⁰

In our study, 47 patients (73%) were PCR positive for *S. pneumoniae*, yet radiological and culture findings did not provide corresponding evidence. While molecular tests are now the reference standard for viral detection,⁷ PCR interpretation for typical bacterial pathogens such as pneumococcus is challenging because of nasopharyngeal colonization. Strålin et al.³¹ showed that sputum samples were superior to nasopharyngeal aspirates for determining pneumococcal etiology in community-acquired pneumonia by PCR. Consistent with this observation, Pickens emphasized the limited value of multiplex PCR testing performed on upper respiratory tract specimens for the etiological diagnosis of bacterial pneumonia, as bacterial pneumonia primarily affects the lower respiratory tract.³² Nevertheless, when interpreted in the appropriate clinical context, rapid and sensitive multiplex molecular panels may still support the diagnosis and management of patients with community-acquired pneumonia.

One limitation of our study is that the rapid respiratory panel was validated only for nasopharyngeal specimens. Although lower respiratory tract specimens may be more appropriate for determining the bacterial etiology of pneumonia, their processing requires additional extraction steps, which may prolong turnaround time. Consequently, nasopharyngeal specimens are frequently preferred in routine clinical practice because they are easier to obtain and allow more rapid reporting. Furthermore, PCR positivity does not necessarily indicate active infection, and qualitative molecular results alone cannot reliably distinguish infection from colonization. In a study by Søgaaard et al.,³³ respiratory panels demonstrated high sensitivity and specificity but a low positive predictive value for the detection of bacterial pathogens, suggesting that molecular detection of bacterial targets may have limited ability to distinguish true infection from colonization. Quantitative or semiquantitative molecular assays may provide additional diagnostic value by estimating bacterial burden. Endicott-Yazdani et al.³⁴ demonstrated, using real-world data, that semiquantitative respiratory panel results may be useful for assessing the clinical significance of detected microorganisms. Nevertheless, molecular bacterial burden should not be

interpreted in isolation but rather in conjunction with clinical findings, radiological features, and culture results. In the present study, Ct values were not systematically recorded; therefore, the association between molecular signal intensity and the clinical relevance of detected bacterial pathogens could not be evaluated. Future prospective studies reporting Ct values together with clinical, radiological, and conventional microbiological findings may provide additional diagnostic value and contribute to distinguishing active infection from colonization. However, Ct values should be interpreted in the context of specimen type, assay platform, and the overall clinical presentation.

CONCLUSION

In conclusion, multiplex PCR assays are valuable for rapid respiratory pathogen detection; however, *S. pneumoniae* positivity in nasopharyngeal samples should be interpreted cautiously, as it may represent colonization rather than active infection. Clinical and radiological correlation remains essential for accurate interpretation.

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Investigation of Reasons for Early Withdrawal of Participants from Clinical Trials: The Example of TURKOVAC Vaccine Clinical Trials

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ABSTRACT

Objective: This study aimed to describe the timing and recorded reasons for early withdrawal among participants in four TURKOVAC vaccine clinical trials and to explore differences according to sex, age group, study design, and follow-up duration.

Materials and Methods: This retrospective, descriptive secondary analysis used aggregate withdrawal data from TURKOVAC Primary Phase III (n=1,290), Hybrid COV-Booster TR Phase II (n=222), Inactivated Booster Phase III (n=4,340), and TURKOVAC after BNT162b2 Phase IIb (n=65). Reasons for withdrawal were classified into standardized categories. Descriptive statistics and exploratory chi-square or Fisher's exact tests were used.

Results: Of the 5,917 enrolled participants, 656 (11.1%) withdrew early. Withdrawal rates were 9.5%, 14.4%, 11.5%, and 1.5% across the four studies, respectively (p=0.005). The most frequent reasons were withdrawal of consent (296/656, 45.1%) and loss to follow-up (281/656, 42.8%). Sex-specific withdrawal rates were generally similar; however, the Inactivated Booster Phase III study showed a higher withdrawal rate among male participants (12.3% vs. 9.8%, p=0.015). Younger participants had higher withdrawal rates in the TURKOVAC Primary Phase III study and in the pooled analysis. Across studies, the proportion of withdrawals occurring within 6 months ranged from 47.5% to 100.0%.

Conclusion: Early withdrawal was mainly associated with withdrawal of consent, loss to follow-up, and pandemic-related external vaccination. One adverse-event-coded withdrawal (1/656, 0.2%) was recorded; the aggregate dataset did not identify this event as vaccine-related, and causality could not be assessed in this secondary analysis. Overall, attrition did not appear to be predominantly safety-driven. Literature-informed implications for participant retention include close attention to early follow-up, clear communication, feasible visit procedures, and contingency planning for external vaccination.

Keywords: Clinical trials as topic, COVID-19 vaccines, participant adherence, participant dropout, TURKOVAC, vaccination.



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INTRODUCTION

Vaccination is one of the most effective public health interventions for preventing infectious diseases and reducing morbidity and mortality at both the individual and population levels.¹ The COVID-19 pandemic, declared by the World Health Organization on March 11, 2020, accelerated vaccine development worldwide and created an unprecedented need for rapid yet methodologically rigorous vaccine trials.²

In Türkiye, the Health Institutes of Türkiye (TÜSEB) supported several vaccine development projects during the pandemic. TURKOVAC, an inactivated SARS-CoV-2 vaccine developed in collaboration with Erciyes University, completed phase studies and was subsequently made available in Türkiye following national regulatory evaluation. The efficacy, immunogenicity, and safety of the primary TURKOVAC schedule and booster regimens have been reported previously.^{3,4}

The success of clinical trials depends not only on recruitment but also on participant retention throughout scheduled follow-up. Premature discontinuation reduces the effective sample size, may compromise statistical power, threatens data completeness, and creates operational and ethical burdens.^{5–7} Retention is particularly challenging in vaccine trials involving healthy volunteers because participants may perceive limited direct benefit after vaccination while still facing repeated visits, reminders, and protocol restrictions. Compared with the broader clinical trial retention literature, empirical reports focusing specifically on early withdrawal patterns in vaccine trials remain limited. Available evidence from vaccine trials suggests that retention may be influenced by trial burden, participant motivation, socioeconomic and logistical barriers, staff-participant communication, and the perceived value of continued follow-up.^{8–10}

The COVID-19 pandemic also created distinctive retention challenges. Public vaccination campaigns, evolving booster recommendations, fear of infection, and changing perceptions of risk could alter participants' motivation to remain in a trial after enrollment. In COVID-19 vaccine development, large-scale vaccine deployment and external access to authorized vaccines have been described as factors that may affect ongoing phase 2/3 trials and long-term follow-up.⁸ A Latin American COVID-19 vaccine trial-readiness report similarly noted that adherence may decline when the perceived benefit of continued participation wanes and national immunization policies change during follow-up.⁹ Therefore, examining why participants withdrew from vaccine trials conducted during this period may provide practical insights for future trial design, particularly when healthy volunteers are followed for several months.

KEY MESSAGES

- Early withdrawal occurred in 11.1% of 5,917 participants across four TURKOVAC vaccine clinical trials.
- Withdrawal of consent and loss to follow-up accounted for most early discontinuations. One adverse-event-coded withdrawal was recorded, but the aggregate dataset did not identify the event as vaccine-related; causality could not be assessed in this secondary analysis.
- Literature-informed implications for participant retention include close attention to early follow-up, clear communication, feasible visit procedures, and contingency planning for external vaccination; these strategies were not evaluated as interventions in this study.

This study investigated the recorded reasons and timing of early withdrawal among participants enrolled in four TURKOVAC vaccine clinical trials. Given the descriptive nature of the dataset, the analysis focused on standardized withdrawal categories, timing patterns, and exploratory comparisons according to sex, age group, study type, and follow-up duration. By summarizing withdrawals across several TURKOVAC protocols, this study adds vaccine-trial-specific evidence to a literature in which participant retention is often discussed broadly, whereas reasons for early withdrawal are less frequently described in detail in vaccine studies.

MATERIALS AND METHODS

Study Design and Reporting Framework

This study was designed as a retrospective, descriptive secondary analysis of participant withdrawal data recorded in four completed TURKOVAC clinical trials. The present analysis did not evaluate vaccine efficacy or immunogenicity; rather, it focused on participant retention and the recorded reasons for early withdrawal. The reporting of this secondary analysis was organized in accordance with STROBE principles for observational studies, where applicable.¹¹

Source Studies

For consistency, the four source studies are referred to throughout as follows: TURKOVAC Primary Phase III (Study 1; NCT04942405), Hybrid COV-Booster TR Phase II (Study 2; NCT04979949), Inactivated Booster Phase III (Study 3; NCT05077176), and TURKOVAC after BNT162b2 Phase IIb (Study 4; NCT05210179). Supplementary Figure S1 presents enrollment demographics across the source studies, and Supplementary Figures S2–S5 present the source-trial

participant disposition diagrams for Studies 1-4, respectively. Published reports are available for Studies 1 and 2.^{3,4} Trial registrations provide protocol identifiers and sponsor information for Studies 3 and 4.^{12,13}

Participants and Data Source

Participants were enrolled in the original clinical trials according to protocol-specific inclusion and exclusion criteria. These protocol-specific criteria are provided in the electronic appendices and were not redefined for the present secondary analysis. Withdrawal data were obtained from sponsor-accessible electronic case report forms and study records after completion of follow-up and data-query processes. The analysis used aggregate counts by study, sex, age group, withdrawal reason, and withdrawal timing.

Definitions and Classification of Early Withdrawal

Early withdrawal was defined as discontinuation of study participation before completion of the scheduled protocol follow-up. Loss to follow-up was defined as the inability to contact a participant despite repeated attempts. Withdrawal of consent was defined as a participant's voluntary decision to discontinue study participation. Protocol deviation referred to significant noncompliance with protocol requirements or study procedures. External fourth-dose vaccination referred to receipt of an additional COVID-19 vaccine outside the study protocol during the follow-up period. Investigator decision/noncompliance included investigator-initiated removal because of behavioral or procedural noncompliance. Inclusion/exclusion criteria not met referred to cases in which protocol eligibility criteria were subsequently found not to have been fulfilled.

Ethical Considerations

The original clinical trials were conducted under study-specific ethical and regulatory authorizations distinct from the present secondary analysis. Study 1 (NCT04942405) was approved by the Hacettepe University Clinical Research Ethics Board (No. KA-21070; June 21, 2021).³ Study 2 (NCT04979949) was approved by the Ankara City Hospital Ethics Committee (No. E2-21-640; June 22, 2021).⁴ Study 3 (NCT05077176) was approved by the Ankara City Hospital Clinical Research Ethics Committee (No. E2-21-889; October 5, 2021).¹² Study 4 is registered as NCT05210179, with the Health Institutes of Türkiye (TÜSEB) as the sponsor; ClinicalTrials.gov reports an actual study start date of January 24, 2022.¹³ The public registry does not report a study-specific ethics committee approval number for Study 4; accordingly, no unverified approval identifier is stated here. All source trials were conducted in accordance with the Declaration of Helsinki.¹⁴ They were also conducted under the applicable Ministry of Health/Turkish Medicines and Medical

Devices Agency (TİTCK) clinical trial authorizations, and all source-trial participants provided written informed consent. The present study used de-identified aggregate withdrawal data from completed trials, involved no new participant contact or intervention, and was conducted under sponsor/data-holder authorization from TÜSEB; no separate reference number was assigned to this authorization. Under Article 2(2) of the Regulation on Clinical Trials of Medicinal Products for Human Use, retrospective studies are outside the scope of that clinical trial regulation.¹⁵ Therefore, no new TİTCK clinical trial authorization was required for this secondary analysis.

Statistical Analysis

Categorical variables were summarized as numbers and percentages. Exploratory categorical comparisons of Study 1 baseline characteristics used Pearson chi-square tests when expected cell counts were adequate and Fisher's exact tests for sparse 2×2 tables; continuous-variable tests were not reconstructed from aggregate source data. Overall withdrawal rates were calculated using enrolled participants as the denominator. Sex-specific and age-specific withdrawal rates were calculated within each corresponding stratum. Exploratory comparisons of withdrawal rates across sex, age group, and study were performed using chi-square tests; Fisher's exact test was used for 2×2 tables with sparse cells. Distributions of withdrawal reasons by sex and age among withdrawn participants were compared using Pearson chi-square tests after sparse categories were collapsed into an "other" group where necessary. Because some cells remained sparse, these reason-distribution tests were considered exploratory. These subgroup analyses were not adjusted for multiple comparisons. Multivariable logistic regression was not performed because the secondary dataset available for this analysis was aggregate rather than individual-level and did not include center-level variables, socioeconomic factors, visit-level exposure data, or detailed participant-reported reasons for withdrawal of consent or loss to follow-up. Statistical significance was set at $p < 0.05$. Analyses were performed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Study Characteristics and Overall Early Withdrawal

Across the four studies, 5,917 participants were enrolled, and 5,261 (88.9%) completed the scheduled follow-up. A total of 656 participants (11.1%) withdrew early. Withdrawal rates differed across studies ($p = 0.005$), ranging from 1.5% in TURKOVAC after BNT162b2 Phase IIb (Study 4) to 14.4% in Hybrid COV-Booster TR Phase II (Study 2) (Table 1). TURKOVAC Primary Phase III (Study 1) had a 14-month follow-up period and 122 withdrawals among 1,290 participants. Hybrid COV-

Table 1. General characteristics and early withdrawal across TURKOVAC studies

Study	Standardized short name	Design and follow-up	Enrolled n	Completed n (%)	Early withdrawal n (%)
Study 1	TURKOVAC Primary Phase III	Randomized, observer-blinded, active-controlled, two-dose phase III trial; 14-month safety follow-up	1,290	1,168 (90.5)	122 (9.5)
Study 2	Hybrid COV-Booster TR Phase II	Double-blind, randomized, controlled, multicenter booster trial; 6-month safety follow-up	222	190 (85.6)	32 (14.4)
Study 3	Inactivated Booster Phase III	Open-label, multicenter phase III booster trial; 12-month safety follow-up	4,340	3,839 (88.5)	501 (11.5)
Study 4	TURKOVAC after BNT162b2 Phase IIb	Two-arm, open-label, multicenter phase IIb booster trial; 6-month safety follow-up	65	64 (98.5)	1 (1.5)
Total	–	–	5,917	5,261 (88.9)	656 (11.1)

Booster TR Phase II (Study 2) had 32 withdrawals among 222 participants. Inactivated Booster Phase III (Study 3), the largest study, had 501 withdrawals among 4,340 participants. TURKOVAC after BNT162b2 Phase IIb (Study 4) had one withdrawal among 65 participants.

Baseline Characteristics in TURKOVAC Primary Phase III (Study 1) by Vaccine Arm

In TURKOVAC Primary Phase III (Study 1), 1,290 participants were randomized (648 to CoronaVac and 642 to TURKOVAC). Table 2 presents the modified intention-to-treat (mITT) baseline analysis population (n=915: 459 CoronaVac and 456 TURKOVAC), rather than the full randomized population. As reported in the source publication, 375 participants with positive baseline anti-SARS-CoV-2 antibodies were excluded from the mITT population.³ Baseline labels and counts were verified against the published Study 1 table. No statistically significant between-arm differences were observed in the exploratory categorical comparisons shown in Table 2.

Sex- and Age-Specific Withdrawal Rates

Most participants who withdrew were male in absolute numbers because enrollment was male-predominant. When stratum-specific denominators were considered, sex-specific withdrawal rates were similar in TURKOVAC Primary Phase III (Study 1; female, 8.7% vs. male, 9.7%; $p=0.572$) and Hybrid COV-Booster TR Phase II (Study 2; 18.8% vs. 12.4%; $p=0.207$). Inactivated Booster Phase III (Study 3) showed a modestly higher withdrawal rate among male participants (12.3% vs. 9.8%; $p=0.015$), and the pooled comparison was also statistically significant (11.6% vs. 9.8%; $p=0.041$). Age-specific analyses showed higher withdrawal rates among

younger participants in TURKOVAC Primary Phase III (Study 1; $p<0.001$) and in the pooled dataset ($p<0.001$), whereas age-related differences were not statistically significant in Hybrid COV-Booster TR Phase II (Study 2) or Inactivated Booster Phase III (Study 3) (Table 3). The pooled sex- and age-group comparisons were unadjusted exploratory aggregate analyses combining studies with different designs, follow-up durations, and participant structures. Because Study 3 contributed 4,340 of the 5,917 participants (73.4%), the pooled estimates were heavily influenced by Study 3 and should not be interpreted as adjusted individual-level effects.

Reasons for Early Withdrawal

The most common reasons for early withdrawal were withdrawal of consent (296/656, 45.1%) and loss to follow-up (281/656, 42.8%). Together, these two categories accounted for 577 of 656 withdrawals (88.0%). Protocol deviation was concentrated in TURKOVAC Primary Phase III (Study 1; 33/122, 27.0%), whereas external fourth-dose vaccination was observed in Hybrid COV-Booster TR Phase II (Study 2; 6/32, 18.8%) and Inactivated Booster Phase III (Study 3; 17/501, 3.4%), consistent with pandemic-era external vaccination pressures. The distribution of grouped withdrawal reasons differed across studies ($p<0.001$), indicating that withdrawal patterns varied according to study design and context (Table 4). Detailed study-specific distributions of withdrawal reasons by sex and age group are provided in Supplementary Tables S1, S2, and S3. One withdrawal in Study 1 was coded as an adverse event (1/656, 0.2% of all withdrawals). The aggregate secondary dataset did not document this event as vaccine-related; therefore, vaccine causality cannot be inferred from the present analysis.

Table 2. TURKOVAC primary phase III (Study 1) baseline characteristics by vaccine arm with exploratory comparisons

Characteristic	CoronaVac arm (n=459)	TURKOVAC arm (n=456)	p
Age, years, median (IQR)	39 (32–44)	38 (34–44)	Not calculated*
Age group, n (%)			0.407
18–29 years	78 (17.0)	69 (15.1)	
30–39 years	170 (37.0)	188 (41.2)	
40–55 years	211 (46.0)	199 (43.6)	
Sex, n (%)			0.202
Male	325 (70.8)	340 (74.6)	
Female	134 (29.2)	116 (25.4)	
BMI, kg/m ² , median (IQR)	25.96 (23.53–29.04)	26.26 (23.56–28.60)	Not calculated*
BMI category, n (%)			0.301
<25 kg/m ²	176 (38.3)	172 (37.7)	
25–30 kg/m ²	198 (43.1)	215 (47.1)	
≥30 kg/m ²	85 (18.5)	69 (15.1)	
Hypertension, n (%)	6 (1.3)	5 (1.1)	0.770
Diabetes mellitus, n (%)	2 (0.4)	4 (0.9)	0.450
Allergic conditions, n (%)	5 (1.1)	12 (2.6)	0.084
Neurological/psychiatric conditions, n (%)	6 (1.3)	6 (1.3)	0.991
Respiratory diseases, n (%)	5 (1.1)	1 (0.2)	0.217
Thyroid diseases, n (%)	7 (1.5)	1 (0.2)	0.069
Any medical history, n (%)	69 (15.0)	74 (16.2)	0.619

Table 2 presents the modified intention-to-treat (mITT) baseline analysis population (n=915), rather than all 1,290 randomized participants. Of the 1,290 randomized participants (648 CoronaVac and 642 TURKOVAC), 375 participants with positive baseline anti-SARS-CoV-2 antibodies were excluded from the mITT population, leaving 459 CoronaVac and 456 TURKOVAC participants, respectively. * Continuous-variable tests were not reconstructed from aggregate source data. Categorical p values were recalculated from published counts using Pearson chi-square tests, with Fisher's exact tests used for sparse 2x2 tables. Medical history labels and counts were verified against the published Study 1 baseline table.

Reasons for Withdrawal According to Sex and Age

Exploratory analyses of withdrawal-reason distributions among participants who withdrew showed no clear sex-related differences in Studies 1–3. Age-related differences were not evident in Study 1 but were statistically significant in Study 3, in which loss to follow-up was relatively more frequent among withdrawn participants aged 18–29 years (Table 5). Table 5 presents the grouped contingency counts used for each test and defines the components collapsed into “other”; full uncollapsed counts are provided in Supplementary Tables S1, S2, and S3. For the Study 3 age-group analysis, the single withdrawn participant aged 60 years or older was combined with the 40–59-year stratum to form an age category of ≥40 years. These comparisons were unadjusted and exploratory.

Timing of Early Withdrawal

Most withdrawals occurred during the earlier follow-up period. In TURKOVAC Primary Phase III (Study 1), 58/122

withdrawals (47.5%) occurred within 6 months, and 89/122 (73.0%) occurred within 12 months. In Hybrid COV-Booster TR Phase II (Study 2), all withdrawals occurred within 6 months. In Inactivated Booster Phase III (Study 3), 229/501 withdrawals (45.7%) occurred within 3 months, and 380/501 (75.8%) occurred within 6 months. In TURKOVAC after BNT162b2 Phase IIb (Study 4), the single withdrawal occurred at approximately 3 months (Table 6). Figure 1 summarizes enrollment, follow-up duration, early withdrawal, and grouped withdrawal reasons across the four studies.

DISCUSSION

This secondary analysis of four TURKOVAC vaccine clinical trials showed that approximately one in nine participants withdrew before completing the scheduled follow-up. The predominant recorded reasons were withdrawal of consent and loss to follow-up. One adverse-event-coded withdrawal occurred in Study 1; however, the aggregate dataset did not establish that the event was vaccine-related. Accordingly,

Table 3. Sex- and age-specific early withdrawal rates by study

Study	Comparison	Stratum 1	Stratum 2	Stratum 3	p
Study 1	Sex-specific withdrawal rate	Female: 30/345 (8.7)	Male: 92/945 (9.7)	–	0.572
Study 1	Age-specific withdrawal rate	18–29 years: 40/241 (16.6)	30–39 years: 46/493 (9.3)	≥40 years: 36/556 (6.5)	<0.001
Study 2	Sex-specific withdrawal rate	Female: 13/69 (18.8)	Male: 19/153 (12.4)	–	0.207
Study 2	Age-specific withdrawal rate	18–29 years: 5/33 (15.2)	30–39 years: 10/83 (12.0)	≥40 years: 17/106 (16.0)	0.734
Study 3	Sex-specific withdrawal rate	Female: 131/1,339 (9.8)	Male: 370/3,001 (12.3)	–	0.015
Study 3	Age-specific withdrawal rate	18–29 years: 61/412 (14.8)	30–39 years: 128/1,088 (11.8)	≥40 years: 312/2,840 (11.0)	0.074
Study 4	Sex-specific withdrawal rate	Female: 1/30 (3.3)	Male: 0/35 (0.0)	–	0.462 [†]
Study 4	Age-specific withdrawal rate	18–29 years: 0/12 (0.0)	30–39 years: 0/16 (0.0)	≥40 years: 1/37 (2.7)	Descriptive only
Total	Sex-specific withdrawal rate	Female: 175/1,783 (9.8)	Male: 481/4,134 (11.6)	–	0.041
Total	Age-specific withdrawal rate	18–29 years: 106/698 (15.2)	30–39 years: 184/1,680 (11.0)	≥40 years: 366/3,539 (10.3)	<0.001

†: Fisher's exact test.

Table 4. Standardized reasons for early withdrawal across studies

Reason for early withdrawal	Study 1, n (%)	Study 2, n (%)	Study 3, n (%)	Study 4, n (%)	Total, n (%)
Withdrawal of consent	40 (32.8)	13 (40.6)	243 (48.5)	0 (0.0)	296 (45.1)
Loss to follow-up	44 (36.1)	13 (40.6)	224 (44.7)	0 (0.0)	281 (42.8)
Protocol deviation	33 (27.0)	0 (0.0)	0 (0.0)	0 (0.0)	33 (5.0)
External fourth-dose vaccination	0 (0.0)	6 (18.8)	17 (3.4)	0 (0.0)	23 (3.5)
Investigator decision/noncompliance	3 (2.5)	0 (0.0)	5 (1.0)	1 (100.0)	9 (1.4)
Adverse event	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)
Death	1 (0.8)	0 (0.0)	3 (0.6)	0 (0.0)	4 (0.6)
Inclusion/exclusion criteria not met	0 (0.0)	0 (0.0)	9 (1.8)	0 (0.0)	9 (1.4)
Total	122 (100.0)	32 (100.0)	501 (100.0)	1 (100.0)	656 (100.0)

The single adverse-event-coded withdrawal occurred in Study 1. The aggregate secondary dataset did not identify this event as vaccine-related; therefore, causality cannot be inferred from the available aggregate data.

the observed attrition pattern appears to have been predominantly operational, motivational, and contextual rather than safety-driven, although this secondary analysis cannot establish causality or exclude unrecorded or indirectly safety-related contributions.

The findings are consistent with the broader clinical trial literature indicating that retention may be influenced by participant-centered planning, communication, visit feasibility, reminder systems, and the perceived burden-benefit balance of continued participation.^{5–7,16–19} These specific retention

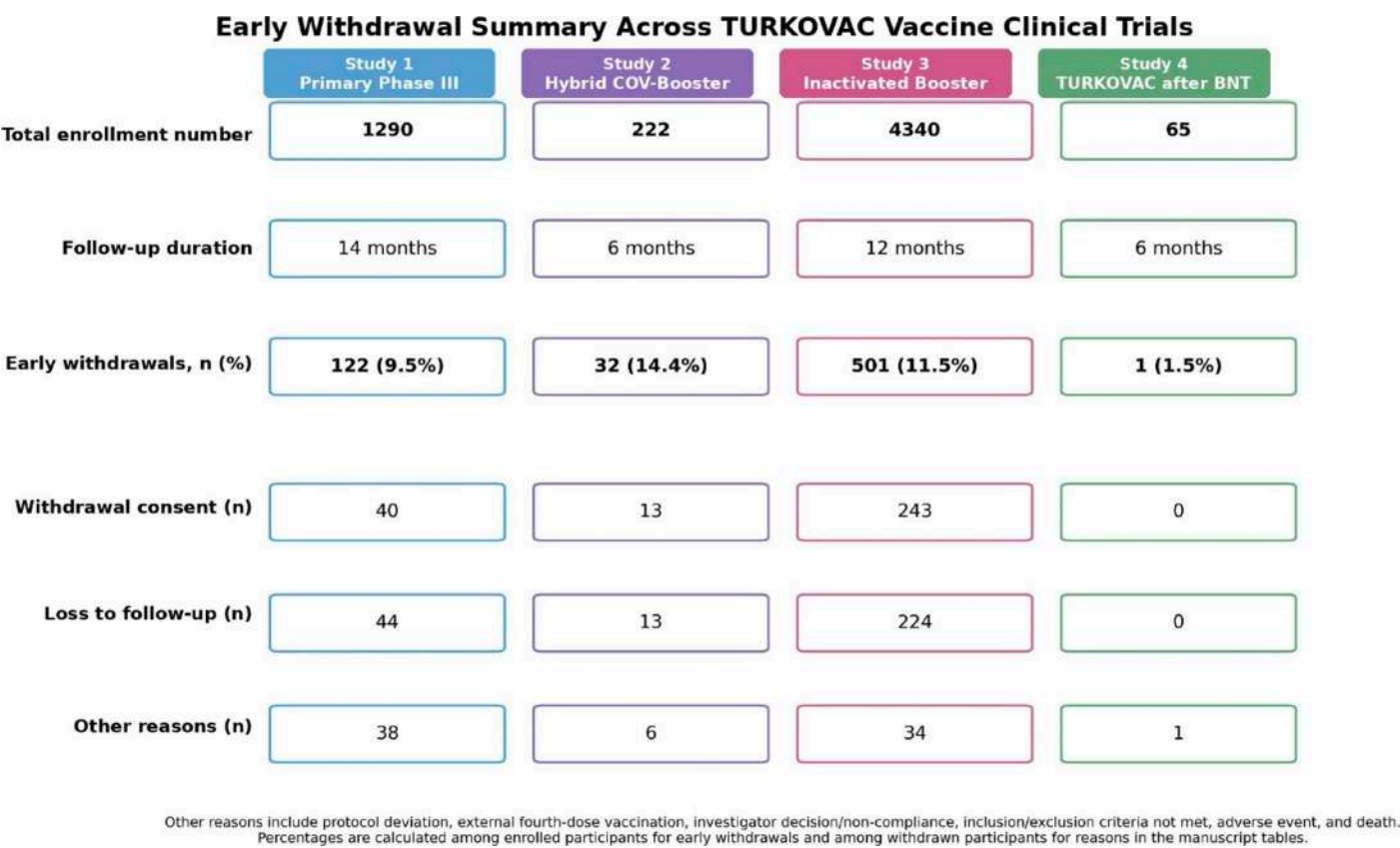


Figure 1. Consolidated summary of enrollment, follow-up duration, early withdrawal, and grouped reasons for early withdrawal across the four TURKOVAC vaccine clinical trials. “Other reasons” include protocol deviation, external fourth-dose vaccination, investigator decision/noncompliance, inclusion/exclusion criteria not met, adverse event, and death.

measures were not evaluated as interventions in the present dataset. They should therefore be interpreted as literature-informed implications for future protocol development rather than as strategies demonstrated to be effective in this study. The present analysis contributes vaccine-trial-specific descriptive evidence regarding when and why early withdrawal was recorded across four TURKOVAC studies.

The pandemic context is important when interpreting these results. In the booster studies, some participants withdrew because they received an additional vaccine dose outside the study protocol. This factor is not a routine source of attrition in many clinical trials but was highly plausible during a period of rapidly evolving national booster recommendations. Similar concerns have been raised in COVID-19 vaccine trials, in which external vaccine deployment, placebo crossover, and policy changes may complicate continued follow-up and the interpretation of long-term trial data.^{8,9,20} Future vaccine trials conducted during public health emergencies should anticipate this conflict by clearly explaining protocol restrictions, documenting external vaccination, and defining

how such events will be addressed in retention plans and statistical analyses.

The sex-specific analysis helped clarify the apparent male predominance among participants who withdrew. Although most withdrawn participants were male in absolute numbers, this largely reflected the male-predominant enrollment structure. Sex-specific withdrawal rates were similar in TURKOVAC Primary Phase III (Study 1) and Hybrid COV-Booster TR Phase II (Study 2), whereas Inactivated Booster Phase III (Study 3) showed a modest but statistically significantly higher withdrawal rate among male participants. Accordingly, absolute male withdrawal counts should not be interpreted as evidence of male-specific attrition unless the corresponding denominators are considered.

The age-related findings were more informative. In TURKOVAC Primary Phase III (Study 1) and in the pooled dataset, younger participants had higher withdrawal rates. In Inactivated Booster Phase III (Study 3), the distribution of withdrawal reasons differed by age, with loss to follow-up being relatively

Table 5. Exploratory comparisons of withdrawal-reason distributions among withdrawn participants

Study	Reasons compared	Grouped contingency counts used in test	Comparison by sex	Comparison by age group	Interpretation
Study 1	Withdrawal of consent; loss to follow-up; protocol deviation; other	Sex: Male, 30/33/26/3; Female, 10/11/7/2. Age: 18-29 years, 15/16/8/1; 30-39 years, 12/19/13/2; 40-55 years, 13/9/12/2. Other = investigator decision/noncompliance + adverse event + death.	p=0.837	p=0.610	No clear sex- or age-related difference.
Study 2	Withdrawal of consent; loss to follow-up; other/ external fourth-dose vaccination	Sex: Male, 7/7/5; Female, 6/6/1. Age: 18-29 years, 3/2/0; 30-39 years, 7/2/1; 40-60 years, 3/9/5. Other = external fourth-dose vaccination.	p=0.415	p=0.065	No statistically significant age-related difference; exploratory only.
Study 3	Withdrawal of consent; loss to follow-up; other	Sex: Male, 179/164/27; Female, 64/60/7. Age: 18-29 years, 17/41/3; 30-39 years, 59/63/6; ≥40 years, 167/120/25. Other = external fourth-dose vaccination + inclusion/exclusion criteria not met + investigator decision + death.	p=0.742	p=0.001	The distribution of withdrawal reasons differed by age; loss to follow-up was relatively more frequent among withdrawn participants aged 18-29 years.
Study 4	Not tested	Only one early withdrawal; no contingency test was performed.	Not tested	Not tested	Descriptive only.

All comparisons were unadjusted exploratory analyses among withdrawn participants. Full uncollapsed counts are provided in Supplementary Tables S1-S3. Sparse categories were collapsed only for the contingency tests, as specified above. For Study 3, the single withdrawn participant aged 60 years or older was combined with the 40–59-year stratum to form a ≥40-year category for the age-group contingency test.

Table 6. Timing of early withdrawal across TURKOVAC studies

Study	Within 1 month	Within 3 months	Within 6 months	Completion pattern
Study 1	16 (13.1%)	34 (27.9%)	58 (47.5%)	89 (73.0%) by 12 months; the remaining withdrawals occurred later during the 14-month follow-up
Study 2	0 (0.0%)	7 (21.9%)	32 (100.0%)	All withdrawals occurred within the 6-month follow-up
Study 3	56 (11.2%)	229 (45.7%)	380 (75.8%)	492 (98.2%) by 12 months
Study 4	0 (0.0%)	1 (100.0%)	1 (100.0%)	Single withdrawal at approximately 3 months

more frequent in the 18–29-year age group. This may reflect greater mobility, work or school constraints, changing perceptions of risk, or lower tolerance for the burden of follow-up among younger adults. Because the dataset did not include individual-level socioeconomic or behavioral variables, this interpretation should be made cautiously.

The study also illustrates why retention analyses should distinguish among protocol deviation, eligibility problems, investigator decisions, and loss to follow-up. These categories may indicate different operational responses, although the

effectiveness of such responses was not evaluated in this study. Based on the broader retention literature, protocol deviations may warrant greater attention to scheduling, eligibility checks, and procedural monitoring; loss to follow-up may justify stronger contact-verification or reminder procedures; withdrawal of consent may motivate clearer expectation-setting and participant engagement; and external vaccination may require better alignment between trial protocols and public health implementation. These are literature-informed implications rather than causal findings of the present analysis.

Limitations

This study has several limitations. First, it is a retrospective secondary analysis of four vaccine trials conducted in a single country during the COVID-19 pandemic; therefore, the findings may not be generalizable to non-pandemic trials, therapeutic trials, or settings with different health system structures. Second, the source population consisted primarily of healthy volunteers, whose motivations and barriers to retention may differ from those of patients participating in treatment trials. Third, the analysis relied on reasons recorded in electronic case report forms; detailed qualitative reasons for withdrawal of consent and loss to follow-up were unavailable. Fourth, the included studies were heterogeneous in design, sample size, vaccination schedule, and follow-up duration. Fifth, some subgroups and withdrawal-reason categories were sparse, particularly in TURKOVAC after BNT162b2 Phase IIb (Study 4); therefore, grouped reason-distribution tests should be interpreted as exploratory. Finally, individual-level covariates and center-level characteristics were unavailable, precluding multivariable modeling. The pooled sex- and age-group analyses were unadjusted aggregate comparisons across heterogeneous studies; because Study 3 accounted for 73.4% of the pooled enrollment, the pooled findings were strongly weighted toward that study.

CONCLUSION

Across four TURKOVAC vaccine clinical trials, early withdrawal was primarily attributable to withdrawal of consent and loss to follow-up, with additional pandemic-specific attrition resulting from external vaccination outside the study protocol. One adverse-event-coded withdrawal was recorded, but the aggregate dataset did not identify it as vaccine-related; therefore, the available data support only the cautious conclusion that attrition was not predominantly safety-driven. Literature-informed implications for future vaccine trials include close attention to the first 3–6 months of follow-up, clear communication, feasible follow-up procedures, and contingency planning for external public health interventions during emergency settings.

Ethics Committee Approval: No separate ethics committee approval was obtained for the present retrospective secondary analysis of de-identified aggregate data. The original source trials were conducted under study-specific ethics approvals as detailed in the Ethical Considerations section, and the present secondary analysis was conducted under sponsor/data-holder authorization from TÜSEB.

Informed Consent: No new informed consent was obtained for the present secondary analysis. All participants had provided written informed consent for participation in the original source trials.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Use of AI for Writing Assistance: AI was used to assist with language editing and manuscript revision. The authors reviewed and verified all content and take full responsibility for the final manuscript.

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Supplementary Table S1. Withdrawal Reasons by Sex and Age Group in TURKOVAC Primary Phase III (Study 1)

Reason for withdrawal	Male, n (%)	Female, n (%)	18-29 years, n (%)	30-39 years, n (%)	40-55 years, n (%)	Total, n (%)
Loss to follow-up	33 (35.9)	11 (36.7)	16 (40.0)	19 (41.3)	9 (25.0)	44 (36.1)
Withdrawal of consent	30 (32.6)	10 (33.3)	15 (37.5)	12 (26.1)	13 (36.1)	40 (32.8)
Protocol deviation	26 (28.3)	7 (23.3)	8 (20.0)	13 (28.3)	12 (33.3)	33 (27.0)
Investigator decision	1 (1.1)	1 (3.3)	1 (2.5)	0 (0.0)	1 (2.8)	2 (1.6)
Adverse event	0 (0.0)	1 (3.3)	0 (0.0)	1 (2.2)	0 (0.0)	1 (0.8)
Participant noncompliance	1 (1.1)	0 (0.0)	0 (0.0)	1 (2.2)	0 (0.0)	1 (0.8)
Death	1 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.8)	1 (0.8)
Total	92 (100.0)	30 (100.0)	40 (100.0)	46 (100.0)	36 (100.0)	122 (100.0)

This supplementary table provides detailed counts supporting the revised subgroup and withdrawal-reason analyses.

Supplementary Table S2. Withdrawal Reasons by Sex and Age Group in Hybrid COV-Booster TR Phase II (Study 2)

Reason for withdrawal	Male, n (%)	Female, n (%)	18-29 years, n (%)	30-39 years, n (%)	40-60 years, n (%)	Total, n (%)
Withdrawal of consent	7 (36.8)	6 (46.2)	3 (60.0)	7 (70.0)	3 (17.6)	13 (40.6)
Loss to follow-up	7 (36.8)	6 (46.2)	2 (40.0)	2 (20.0)	9 (52.9)	13 (40.6)
External fourth-dose vaccination	5 (26.3)	1 (7.7)	0 (0.0)	1 (10.0)	5 (29.4)	6 (18.8)
Total	19 (100.0)	13 (100.0)	5 (100.0)	10 (100.0)	17 (100.0)	32 (100.0)

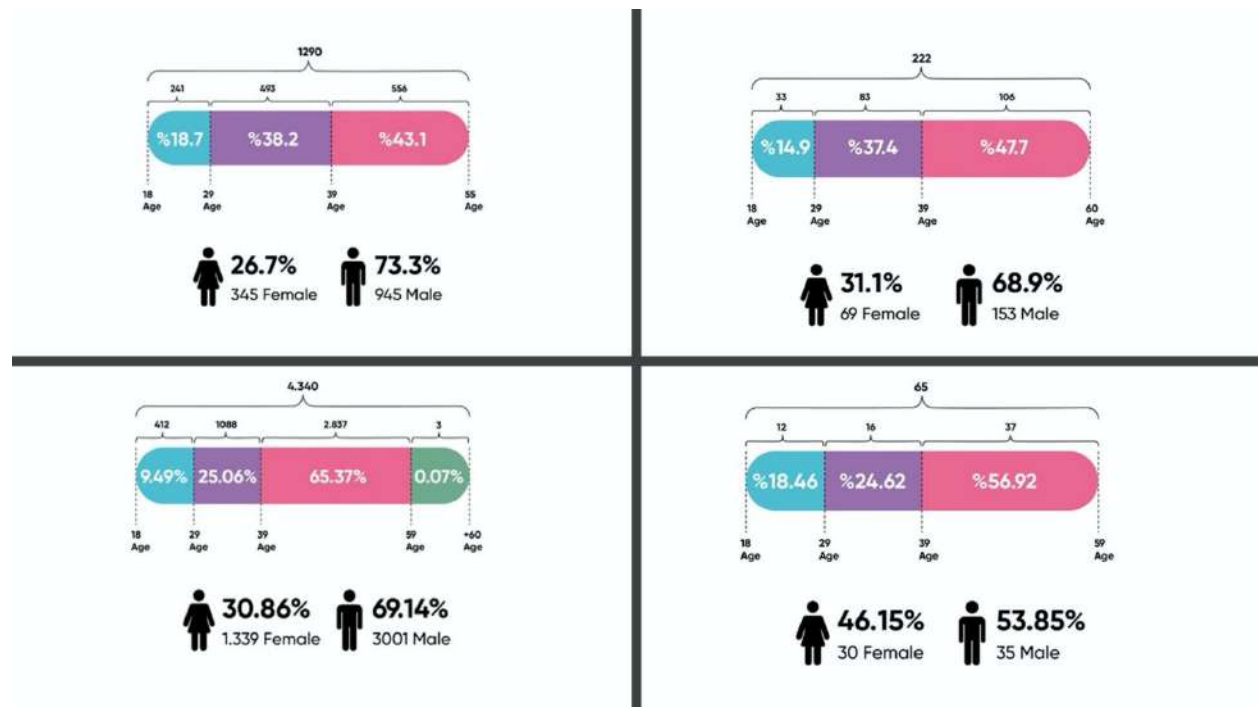
This supplementary table provides detailed counts supporting the revised subgroup and withdrawal-reason analyses.

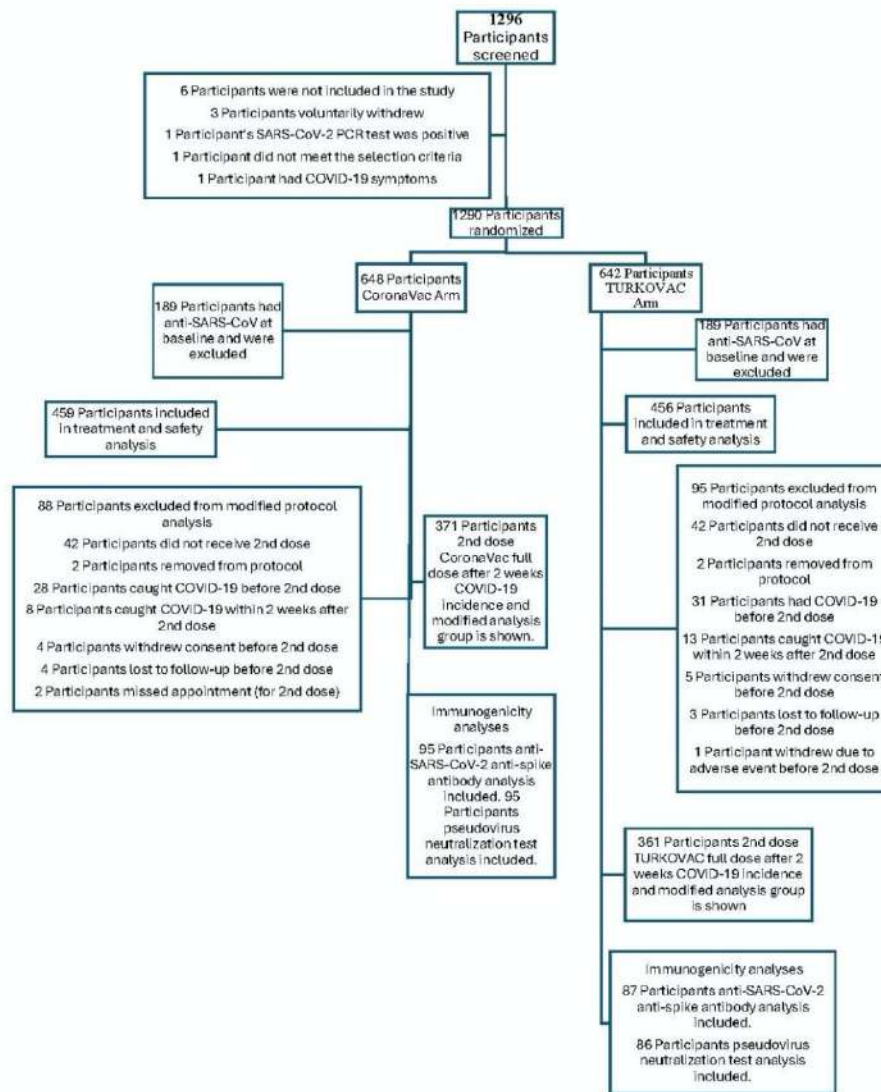
Supplementary Table S3. Withdrawal Reasons by Sex and Age Group in Inactivated Booster Phase III (Study 3)

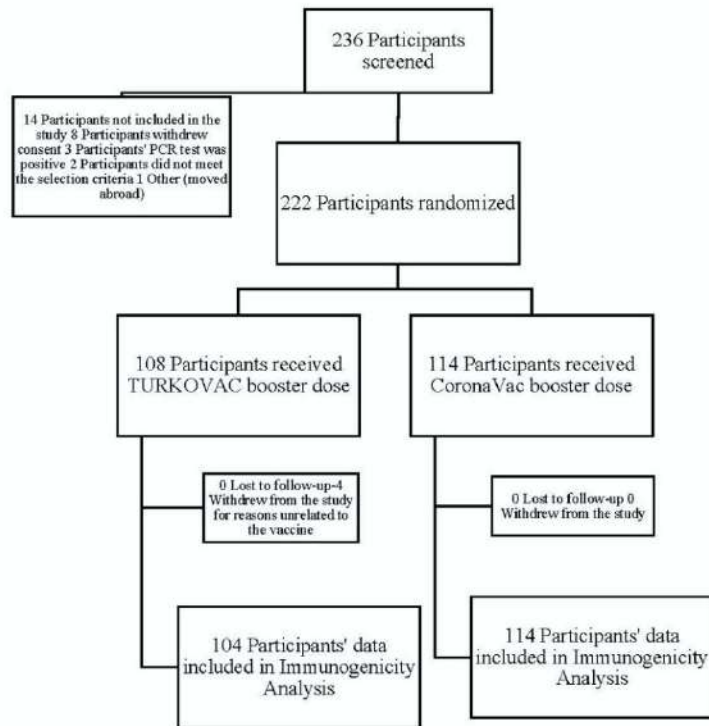
Reason for withdrawal	Male, n (%)	Female, n (%)	18-29 years, n (%)	30-39 years, n (%)	40-59 years, n (%)	≥60 years, n (%)	Total, n (%)
Withdrawal of consent	179 (48.4)	64 (48.8)	17 (27.9)	59 (46.1)	167 (53.7)	0 (0.0)	243 (48.5)
Loss to follow-up	164 (44.3)	60 (45.8)	41 (67.2)	63 (49.2)	120 (38.6)	0 (0.0)	224 (44.7)
External fourth-dose vaccination	12 (3.2)	5 (3.8)	1 (1.7)	4 (3.1)	12 (3.9)	0 (0.0)	17 (3.4)
Inclusion/exclusion criteria not met	8 (2.2)	1 (0.8)	0 (0.0)	1 (0.8)	7 (2.2)	1 (100.0)	9 (1.8)
Investigator decision	4 (1.1)	1 (0.8)	1 (1.6)	1 (0.8)	3 (1.0)	0 (0.0)	5 (1.0)
Death	3 (0.8)	0 (0.0)	1 (1.6)	0 (0.0)	2 (0.6)	0 (0.0)	3 (0.6)
Total	370 (100.0)	131 (100.0)	61 (100.0)	128 (100.0)	311 (100.0)	1 (100.0)	501 (100.0)

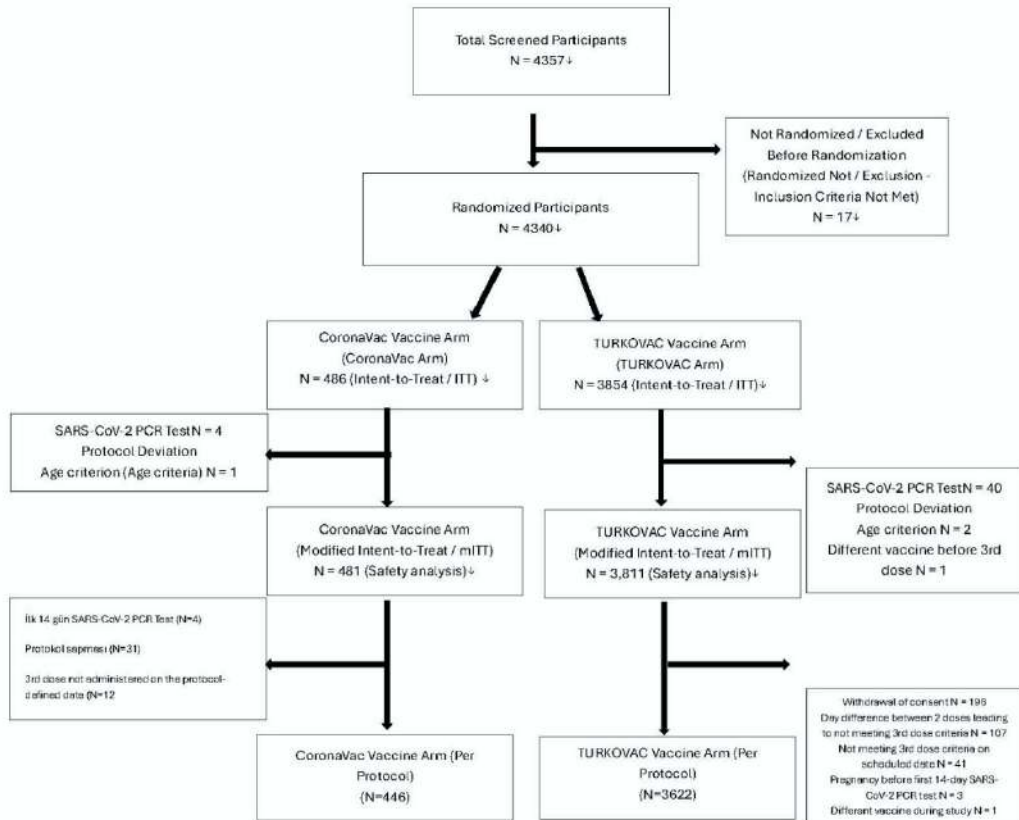
This supplementary table provides detailed counts supporting the revised subgroup and withdrawal-reason analyses.

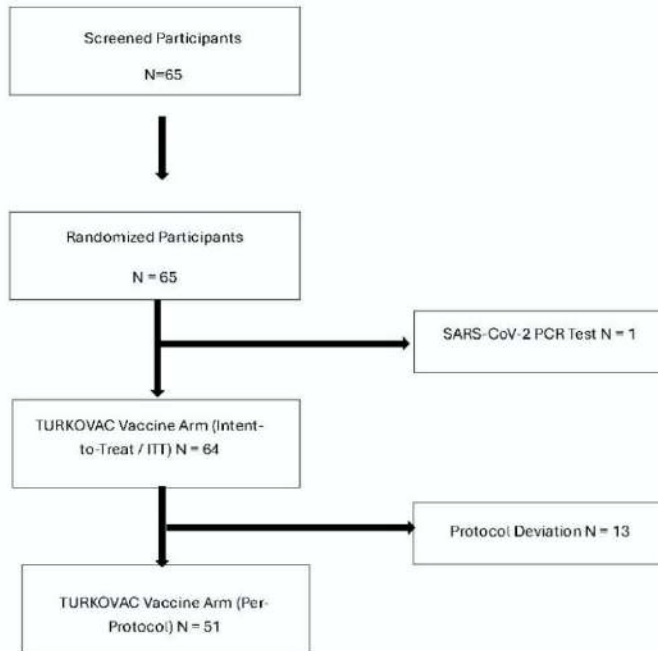
Supplementary Figures S1-S5. Supplementary Figure S1 presents enrollment demographics across the four source studies. Supplementary Figures S2-S5 present the source-trial participant disposition diagrams for Studies 1-4, respectively. These figures should be interpreted as protocol/source-trial disposition figures and not as substitutes for the secondary withdrawal analysis tables.











Investigation of the Frequency of Condylar Canal Variations and Their Relationship with Clinical Symptoms

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ABSTRACT

Objective: The condylar canal (CC) is an important anatomical structure located in the posterior cranial fossa and frequently exhibits variations that may influence surgical approaches and clinical interpretation. This study aimed to investigate the frequency of CC variations and evaluate their possible relationship with clinical findings.

Materials and Methods: A total of 113 cranial CT scans (34 females and 79 males) were retrospectively analyzed, and detailed morphometric measurements of the foramen magnum and CC were performed. In addition, anamnesis data regarding clinical symptoms, including headache, nausea, dizziness, neck pain, blurred vision, and epistaxis, were assessed.

Results: The results demonstrated that the CC was absent in some individuals, unilateral in others, and bilateral in a considerable proportion. However, no statistically significant associations were found between CC variations and patient demographics or clinical symptoms ($p>0.05$). Similarly, morphometric analysis of the foramen magnum revealed no significant differences among the groups ($p>0.05$).

Conclusion: These findings suggest that although the CC demonstrates considerable anatomical variability, its presence or absence does not appear to correlate with clinical symptoms. Our study provides valuable radiological and anatomical data that may help neurosurgeons, radiologists, and anatomists better understand CC variations. To our knowledge, this is among the first studies to examine CC variations together with clinical symptomatology, thereby providing new insights into the literature.

Keywords: Anatomical variation, clinical correlation, computed tomography, condylar canal, foramen magnum.



INTRODUCTION

The functional significance of the condylar canal (CC) lies in its role as the primary osseous conduit for the posterior condylar emissary vein (PCEV), which constitutes a critical collateral connection between the intracranial sigmoid sinus and the extracranial vertebral venous plexus.¹ Under normal physiological conditions, cerebral venous outflow occurs predominantly through the internal jugular veins. However, during positional changes, jugular vein compression, or states of increased intracranial pressure, emissary veins act as essential safety valves that help maintain hemodynamic equilibrium within the posterior cranial fossa. From a mechanistic perspective, structural variations of the CC, such as critical narrowing (hypoplasia) or complete absence (agenesis), can significantly restrict this alternative venous drainage pathway. This restriction may potentially trigger localized venous congestion and transient retro-obstruent hypertension within the dural sinuses. Such altered venous dynamics are well-known triggers for the activation of perivascular nociceptive fibers within the dura mater, clinically manifesting as chronic or tension-type suboccipital headaches. Furthermore, venous congestion extending toward the petrosal and internal auditory veins may directly disrupt the microfluidic environment of the inner ear, providing a plausible biological rationale for neuro-otological symptoms such as pulsatile tinnitus, vertigo, and dizziness. Therefore, assessment of the morphometric configuration of the CC is not merely an anatomical exercise but may also be important for understanding unexplained posterior fossa symptomatology.^{2–5}

The CC is situated in the fossa condylaris, posterior to the hypoglossal canal and within the lower part of the jugular foramen.^{6–8} The condylar canal and the accompanying emissary vein represent important anatomical landmarks in skull base surgical procedures. Functionally, the condylar canal provides a conduit that facilitates communication between the intracranial and extracranial compartments.⁸ Condylar emissary veins, meningeal arteries, and nerves supplying the dura mater pass through this canal in the posterior cranial fossa.⁹ The condylar canal has also been referred to as the condyloid canal, condylar foramen, and posterior condylar canal.^{10,11} The hypoglossal canal, also known as the anterior condylar canal, is distinct from the posterior condylar canal.⁸

In clinical conditions associated with impaired cranial venous drainage, such as intracranial hypertension, vascular malformations, and internal jugular vein obstruction, emissary veins may provide alternative pathways to facilitate venous drainage.¹⁰ The posterior condylar vein is a major emissary vein of the cranial venous system. It establishes a venous connection between the vertebral venous plexus and the sigmoid-jugular

KEY MESSAGES

- Multiplanar CT reconstructions revealed considerable normal variation in condylar canal anatomy.
- Bilateral, unilateral, and absent condylar canal variants showed a balanced distribution. No statistically significant correlation was found between these variants and neuro-otological symptoms.
- Morphometric dimensions of the foramen magnum did not differ significantly among the groups. Documentation of these osseous landmarks is essential to avoid iatrogenic skull base injuries.

venous complex. This vein is observed more commonly on the right side than on the left. The higher prevalence of the condylar canal on the right has been attributed to a more direct venous pathway to the heart, which may increase the likelihood of emissary foramen formation on that side.⁶

The condylar foramen represents one of the largest emissary foramina of the skull. The posterior condylar vein passing through this foramen provides a significant alternative pathway for venous outflow, particularly when drainage into the sigmoid sinus-jugular venous complex is compromised.¹² Venous imaging studies in patients with achondroplasia and complex craniosynostosis have demonstrated venous outflow obstruction at the skull base, with the jugular foramina being particularly affected.¹³ Anatomically, the condylar canal represents a variable osseous pathway in the posterior cranial base that allows venous communication between the intracranial and extracranial compartments.¹⁴

Accurate skull base morphometry is crucial for reducing morbidity and mortality in craniovertebral surgery.⁸ In addition to examination in dry bones, CT and MRI are used to visualize the condylar canal.⁸ Although the morphological variability of the condylar canal and its emissary veins is well documented in the classical osteological literature, there remains a substantial paucity of objective clinical data investigating whether these structural variations directly affect patient symptomatology. In this study, we primarily hypothesized that individuals with complete bilateral absence (agenesis) or severe hypoplasia of the condylar canal would exhibit a significantly higher prevalence of congestion-related neuro-otological symptoms, such as persistent suboccipital headache, dizziness, or tinnitus, due to structural restriction of collateral venous outflow pathways from the posterior cranial fossa. Therefore, the objective of this retrospective study was to determine the frequency and dimensions of CC variations using multiplanar computed tomography (CT) reconstructions

and to systematically analyze their potential correlations with documented clinical symptoms, thereby bridging the gap between gross anatomy and neurosurgical practice.

MATERIALS AND METHODS

This retrospective study included 113 patients, both adults and children (34 females and 79 males; age range, 11–98 years), who underwent routine cranial computed tomography (CT) examinations at Malatya Turgut Özal University. The primary indications for CT imaging included evaluation of chronic nonspecific headache, exclusion of acute neurological events, and neuro-otological assessment.

Patients with poor image quality; motion or metal artifacts; previous skull base or neurosurgical procedures; craniovertebral junction anomalies; bone tumors involving the occipital region; traumatic cranial injuries; or lesions affecting the condylar canal were excluded from the study.

All CT datasets were obtained using a standardized protocol with a 3-mm slice thickness and were analyzed using RadiAnt DICOM Viewer software. The study protocol was approved by the Malatya Turgut Özal University Non-Interventional Clinical Research Ethics Committee (Approval Number: 2022/136, Date: 05.09.2022).

The presence, absence, and morphometric characteristics of the condylar canal were independently evaluated by one neurosurgeon and three senior anatomists. Interobserver reliability was assessed using the intraclass correlation coefficient (ICC). The ICC was 0.91 (95% CI, 0.87–0.94), indicating excellent agreement among observers.

Groups and Measurement Parameters

Group 1: This group included crania with bilateral CCs (Fig. 1).

CCD: Distance between the two condyles.

CCTD: Transverse diameter of the CCs (right and left) (Fig. 2).

CCSD: Anteroposterior diameter of the CCs (right and left) (Fig. 3a).

CCBC-A: Angle between the CC and the cranial base.

CCBC-D: Distance between the CC and the skull base on axial sections.

CCFM-A: On the sagittal axis extending anteroposteriorly through the foramen magnum, the angle between the line extending from the right CC and the transverse axis extending between the CCs.

FMS: Anteroposterior diameter of the foramen magnum (Fig. 3b).

FMT: Transverse diameter of the foramen magnum (Fig. 4a).

Group 2: This group included individuals without a CC (Fig. 4b). Only the transverse and sagittal diameters of the foramen magnum were examined in this group.

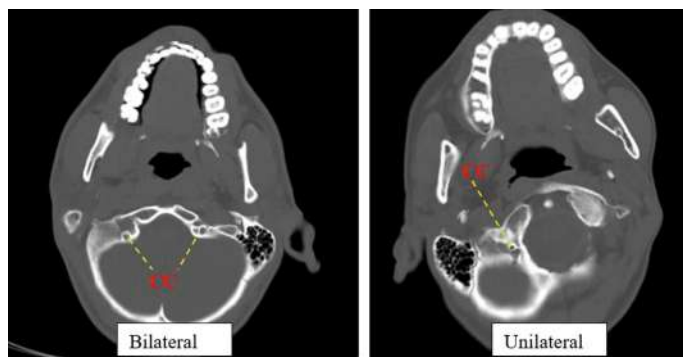


Figure 1. Bilateral and unilateral condylar canals (CCs). Computed tomography (CT) images demonstrating CC morphology. Examples of bilateral and unilateral CCs are shown.

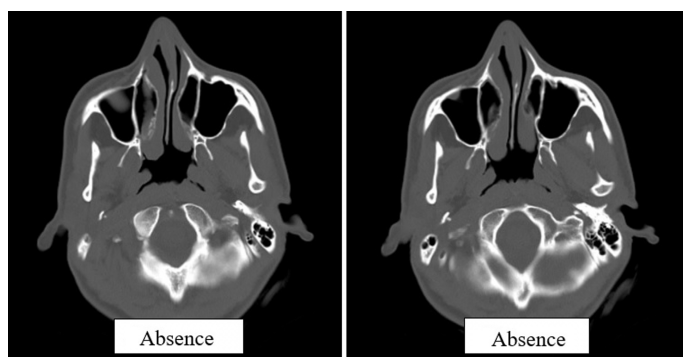


Figure 2. Transverse diameter of the condylar canal (CCTD). Measurement of the transverse diameter of the CC (CCTD) on axial CT images. The maximum mediolateral diameter of the canal was measured.

FMS: Anteroposterior diameter of the foramen magnum.

FMT: Transverse diameter of the foramen magnum.

FMD: Diameter of the foramen magnum.

Group 3: This group included crania with a unilateral CC.

CCTD: Transverse diameter of the CC (right or left).

CCSD: Anteroposterior diameter of the CC (right or left).

CCBC-A: Angle between the CC and the cranial base.

FMS: Anteroposterior diameter of the foramen magnum.

FMT: Transverse diameter of the foramen magnum.

Sample Size Determination

The adequacy of the sample size was determined using an a priori power analysis performed with G*Power software (Version 3.1; Heinrich Heine University, Düsseldorf,

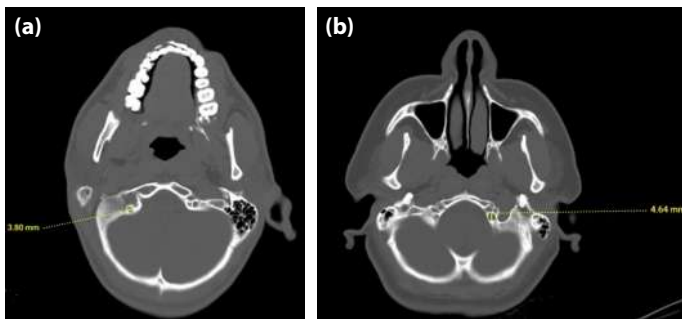


Figure 3. (a) Anteroposterior diameter of the condylar canal (CCSD). Measurement of the anteroposterior diameter of the CC (CCSD) on axial CT images. The maximum anteroposterior dimension was recorded. **(b)** Anteroposterior diameter of the foramen magnum (FMS). Measurement of the anteroposterior diameter of the foramen magnum (FMS) on axial CT images, defined as the distance between the basion and opisthion.

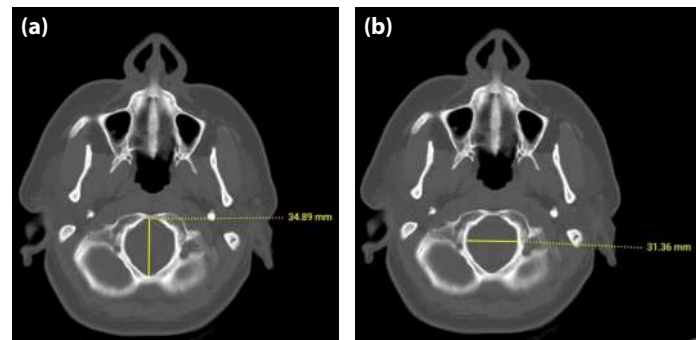


Figure 4. (a) Transverse diameter of the foramen magnum (FMT). Measurement of the transverse diameter of the foramen magnum (FMT) on axial CT images, defined as the maximum mediolateral distance. **(b)** Absence of the condylar canal (CC) in different patients. CT images illustrating the absence of the CC in different patients. Unilateral and bilateral absence of the canal is demonstrated.

Germany). Because this study is among the first to evaluate the relationship between condylar canal variations and clinical symptoms, no previous literature was available from which to derive a specific reference effect size. Therefore, a moderate effect size of 0.30 was assumed in accordance with Cohen's criteria. Based on this moderate effect size of 0.30, a significance level (α) of 0.05, a statistical power of 0.95, and a 95% confidence level, the minimum required sample size was calculated as 111 participants. Consequently, the final study sample of 113 patients was considered sufficient to achieve adequate statistical power for detecting meaningful clinical effects.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA). The normality of continuous variables was assessed using the Shapiro–Wilk test. Distributional characteristics of the data were used to determine whether parametric or nonparametric statistical methods were appropriate. Continuous variables were presented as mean±standard deviation (SD) for normally distributed data and as median with minimum and maximum values for nonnormally distributed data. Categorical variables were expressed as frequencies and percentages. Homogeneity of variances was evaluated using Levene's test. Comparisons among three or more independent groups were performed using the Kruskal–Wallis test for nonnormally distributed data. When significant differences were identified, post hoc pairwise comparisons were performed using the Mann–Whitney U test with Bonferroni correction to control for multiple comparisons. Categorical variables were analyzed using contingency tables

and the chi-square (χ^2) test. A two-tailed p-value of <0.05 was considered statistically significant.¹⁵ The ICC was used to assess interrater reliability.

RESULTS

The anatomical and morphometric dimensions of the condylar canal (CC) demonstrated wide normal variations across the study population. Among the 113 patients analyzed, a bilateral CC configuration was observed in 35 patients, a unilateral CC was identified in 38 patients, and the canal was completely absent (agenesis) in 40 patients. Demographic analysis revealed no statistically significant differences among these morphological groups in terms of sex distribution ($\chi^2=3.636$, $p=0.162$). Similarly, no statistically significant differences were detected among the groups in terms of age distribution ($H=0.753$, $p=0.686$), with a mean age of 46.13 ± 23.29 years in the absent group, 48.63 ± 23.88 years in the unilateral group, and 51.46 ± 26.96 years in the bilateral group. Furthermore, clinical history findings revealed no statistically significant associations between specific CC variations and patient symptoms ($p>0.05$) (Table 1, 2).

The anatomical and morphometric dimensions of the condylar canal demonstrated wide normal variations between the unilateral and bilateral groups, as detailed below. In our study population, no statistically significant differences were observed among the morphological groups according to age or sex ($p>0.05$). The condylar canal was observed to be unilateral, bilateral, or completely absent across the cohorts. According to the clinical history findings, no statistically significant differences were detected among the

Table 1. Comparison of demographic characteristics (age and sex) according to condylar canal status

Variables	Group/value	Absent	Unilateral	Bilateral	Total	χ^2 /Kruskal–Wallis	p
Sex	Female, n	8	15	11	34	3.636	0.162
	Female, %	20.0	39.5	31.4	30.1		
	Male, n	32	23	24	79		
	Male, %	80.0	60.5	68.6	69.9		
Age (years)	Mean $\pm$ SD	46.13 $\pm$ 23.29	48.63 $\pm$ 23.88	51.46 $\pm$ 26.96	—	0.753	0.686
	Median (min–max)	40.5 (11–85)	44.5 (12–98)	48 (16–97)	—		

SD: Standard deviation; Min: Minimum; Max: Maximum. Statistical significance was set at $p < 0.05$.**Table 2.** Comparison of clinical symptoms according to condylar canal status

Variables	Absent	Unilateral	Bilateral	Total	χ^2	p
Symptoms					17.435	0.134
Asymptomatic, n	5	4	1	10		
Asymptomatic, %	12.5	10.5	2.9	8.8		
Headache, n	17	18	21	56		
Headache, %	42.5	47.4	60.0	49.6		
Nausea, n	8	6	4	18		
Nausea, %	20.0	15.8	11.4	15.9		
Dizziness/imbalance, n	5	2	2	9		
Dizziness/imbalance, %	12.5	5.3	5.7	8.0		
Neck pain, n	2	5	2	9		
Neck pain, %	5.0	13.2	5.7	8.0		
Blurred vision, n	3	0	3	6		
Blurred vision, %	7.5	0.0	8.6	5.3		
Epistaxis, n	0	3	2	5		
Epistaxis, %	0.0	7.9	5.7	4.4		

Statistical significance was set at $p < 0.05$.**Table 3.** Comparison of transverse and sagittal measurements of the foramen magnum among the groups

Variable	Group	Mean $\pm$ SD	Median (min–max)	Kruskal–Wallis test	p
FMTD (mm)	Absent	22.0 $\pm$ 5.6	23.3 (12.5–35.1)	1,584	0,453
	Unilateral	23.0 $\pm$ 4.6	21.9 (13.3–30.6)		
	Bilateral	24.0 $\pm$ 4.6	24.5 (17.1–33.6)		
FMSD (mm)	Absent	18.3 $\pm$ 6.0	17.5 (10.1–30.7)	0,229	0,892
	Unilateral	19.6 $\pm$ 7.4	16.0 (10.4–33.3)		
	Bilateral	20.0 $\pm$ 8.5	16.7 (10.8–40.2)		

SD: Standard deviation; Min: Minimum; Max: Maximum; FMTD: Transverse diameter of the foramen magnum; FMSD: Sagittal diameter of the foramen magnum. Statistical significance was set at $p < 0.05$.

groups regarding patient symptoms ($p > 0.05$). Furthermore, morphometric analysis of the foramen magnum dimensions across all study cohorts demonstrated no statistically

significant differences ($p > 0.05$) (Table 3). Measurements showed that the CC could be unilateral, bilateral, or completely absent (Table 4–6) (Fig. 1, 2). According to the

Table 4. Descriptive morphometric measurements of the foramen magnum in the absent condylar canal group

Variables	Mean±SD	Median (min–max)
FMT (mm)	22.0±5.6	23.3 (12.5–35.1)
FMS (mm)	18.3±6.0	17.5 (10.1–30.7)

SD: Standard deviation; Min: Minimum; Max: Maximum; FMT: Transverse diameter of the foramen magnum; FMS: Sagittal diameter of the foramen magnum.

Table 5. Morphometric measurements in the unilateral condylar canal group (mm)

Variables	Mean±SD	Median (min–max)
CCTD (right) (mm)	3.07±0.84	3.13 (1.59–4.64)
CCSD (left) (mm)	2.26±0.86	2.05 (0.95–3.96)
FMTD (mm)	23.0±4.6	21.9 (13.3–30.6)
FMSD (mm)	19.6±7.4	16.0 (10.4–33.3)
FMD (mm)	26.42±6.33	24.7 (15.07–38.7)

SD: Standard deviation; Min: Minimum; Max: Maximum; CC: Condylar canal; CCTD: Transverse diameter of the condylar canal; CCSD: Sagittal diameter of the condylar canal; FMTD: Transverse diameter of the foramen magnum; FMSD: Sagittal diameter of the foramen magnum; FMD: Diameter of the foramen magnum.

clinical history findings, there was no statistically significant difference among the CC groups ($p>0.05$) (Table 2). In all groups, there were no statistically significant differences in the transverse and sagittal measurements of the foramen magnum ($p>0.05$) (Table 3).

DISCUSSION

Precise and comprehensive mapping of skull base anatomy is of paramount importance for neurosurgeons and neuroradiologists to optimize surgical trajectories and avoid iatrogenic neurovascular injuries. The current literature includes a substantial body of morphometric data concerning the condylar canal (CC) and its anatomical variations, predominantly derived from dry osteological specimens.^{1,16–21} However, misinterpretation of these normal variants as osteolytic lesions, pathological fractures, or dural arteriovenous fistulas remains a critical diagnostic pitfall in clinical practice. To the best of our knowledge, the present study represents the first multiplanar computed tomography (CT) investigation specifically designed to correlate these cross-sectional structural variations with a defined set of clinical neuro-otological symptoms. By combining the expertise of anatomists, radiologists, and neurosurgeons, this multidisciplinary study aimed to address an important gap in the literature by determining whether these osseous polymorphisms have subtle subclinical manifestations or actively contribute to the pathogenesis of specific patient complaints.

Table 6. Morphometric measurements in the bilateral condylar canal group

Variables	Mean±SD	Median (min–max)
CCTD (right) (mm)	3.57±0.71	3.52 (2.06–5.09)
CCSD (right) (mm)	2.67±0.97	2.49 (0.91–5.01)
CCTD (left) (mm)	3.31±0.77	3.28 (1.81–5.10)
CCSD (left) (mm)	2.47±1.05	2.11 (0.98–4.95)
CCD (mm)	41.9±11.5	39.9 (24.3–76.0)
FMTD (mm)	24.0±4.6	24.5 (17.1–33.6)
FMSD (mm)	20.0±8.5	16.7 (10.8–40.2)
CCBC-D (right) (mm)	5.35±1.65	5.67 (2.68–8.51)
CCBC-D (left) (mm)	4.95±1.59	5.29 (1.55–7.60)

SD: Standard deviation; Min: Minimum; Max: Maximum; CC: Condylar canal; CCTD: Transverse diameter of the condylar canal; CCSD: Sagittal diameter of the condylar canal; CCD: Distance between the two condylar canals; FMTD: Transverse diameter of the foramen magnum; FMSD: Sagittal diameter of the foramen magnum; CCBC-D: Distance between the condylar canal and the skull base on axial sections.

No statistically significant difference has been reported between sexes in the prevalence of the intermediate condylar canal.^{14,22,23} In our study, there was no statistically significant difference among the groups according to age or sex ($p>0.05$). The similar findings in our study may indicate that sex does not affect the presence of the canal. Although the CC may be present unilaterally or bilaterally, it may also be completely absent in some individuals.²⁴ According to Boyd et al.,²⁴ the posterior condylar canal was detected bilaterally in 46.6% of cases, was absent in 23.81%, was present only on the right side in 16.5%, and was present only on the left side in 13.88%. Ginsberg et al.¹ reported that the canal was present bilaterally in 55.9% of cases and unilaterally in 17.9%. Tatar et al.¹⁸ assessed the bilateral presence of the CC and reported that it was observed on the left side in 22 of 32 skulls (68.75%) and on the right side in 19 (61.2%). Kumar et al.²⁵ observed that the CC was present bilaterally in 48.33% of cases, unilaterally on the left in 18.33%, unilaterally on the right in 15%, and was completely absent in 16.66%. Muthukumar et al.⁶ found the CC on the right side in 4 crania and on the left side in 16 crania. Dakshayani and Shivanal¹⁹ observed that the posterior condylar canal was present bilaterally in 36% of cases, unilaterally on the left in 24%, unilaterally on the right in 20%, and was completely absent in 20%. Among the participants in our study, three groups were identified: those with unilateral CC (39.5% female, 60.5% male), those with bilateral CC (31.4% female, 68.6% male), and those without CC (20% female, 80% male). The distribution of CC variations in our study was consistent with the literature. In addition, clinical data related to CC variations were evaluated, and no statistically significant

differences were detected among the groups according to the clinical history findings. Therefore, CC variation did not appear to be associated with a distinct clinical presentation. Intermediate CC was not observed in our study; similarly, Smith et al.¹⁴ reported a prevalence of 3% for intermediate CC and found no statistically significant difference between sexes. The reported anatomical dimensions indicate an approximate sagittal diameter of 35 mm and a transverse diameter of 30 mm.²⁶ Muthukumar et al.⁶ reported a mean foramen magnum length of 36.75 ± 2.50 mm, an anteroposterior length of 33.3 mm, and a width of 27.9 mm.

The mean morphometric measurements of the foramen magnum, including both transverse and sagittal diameters, were expressed in millimeters (mm) in accordance with international anatomical reporting standards. Morphometric analysis of the foramen magnum dimensions across all study cohorts demonstrated no statistically significant differences ($p > 0.05$). Specifically, the mean transverse diameter of the foramen magnum was 22.0 ± 5.6 mm in individuals without a CC, 23.0 ± 4.6 mm in the unilateral CC group, and 24.0 ± 4.6 mm in the bilateral CC group. Similarly, the mean sagittal diameter of the foramen magnum was 18.3 ± 6.0 mm in individuals with an absent CC, 19.6 ± 7.4 mm in those with a unilateral CC, and 20.0 ± 8.5 mm in patients with a bilateral CC. No statistically significant differences were detected among the groups in these transverse and sagittal anatomical measurements ($p > 0.05$).¹²

In our study, both anteroposterior and transverse diameters were measured. In cases with bilateral CCs, the mean transverse diameter was 3.57 ± 0.71 mm, the mean anteroposterior diameter was 2.67 ± 0.97 mm on the right and 2.47 ± 1.05 mm on the left, and the mean distance between the two canals was 41.9 ± 11.5 mm. In cases with a unilateral CC, the mean transverse diameter was 3.07 ± 0.84 mm, whereas the mean anteroposterior diameter was 2.26 ± 0.86 mm. The CC measurements obtained in our study were comparable to those reported in the literature. Our study provides additional morphometric data on the anteroposterior and transverse dimensions of the CC.

Limitations

This study has several limitations. First, its retrospective design may have limited the accuracy and completeness of the clinical data obtained from patient records. Second, although the overall sample size was mathematically adequate, the low frequency of specific clinical symptoms within the subgroups may have reduced the statistical power to detect subtle associations, thereby increasing the risk of type II errors. Additionally, imaging was performed using 3-mm CT slices, which may have limited the detailed evaluation of small anatomical structures. The absence of advanced

imaging modalities, such as MR venography, also limited the assessment of functional venous drainage. Finally, this was a single-center study, which may limit the generalizability of the findings to broader populations. Future multicenter studies with larger cohorts and multimodal imaging techniques are warranted to validate and expand upon our findings.

CONCLUSION

In conclusion, our findings demonstrate that the condylar canal (CC) exhibits considerable normal anatomical variability, presenting as bilateral, unilateral, or completely absent configurations across the study population. Within the methodological limitations of this retrospective, single-center study, no statistically significant correlations were detected between specific CC variants and clinical or demographic parameters in this particular sample. Although the overall sample size provided adequate statistical power, the low frequency of specific clinical symptoms across the cohorts increased the risk of type II errors, whereby subtle clinical associations may have remained undetected. Therefore, these descriptive findings should be interpreted as indicating a lack of detectable statistical correlation within the current sample rather than definitive evidence of clinical irrelevance. Recognition of these morphometric variations remains essential in neurosurgical planning and radiological evaluation to prevent inadvertent vascular and hemorrhagic complications during skull base interventions. Future prospective, multicenter studies using advanced multimodal imaging techniques and larger patient cohorts are warranted to further evaluate and validate the potential clinical relevance of these anatomical variations.

Ethics Committee Approval: Ethics committee approval was obtained from Malatya Turgut Özal University Non-Interventional Clinical Research Ethics Committee (Approval Number: 2022/136, Date: 05.09.2022).

Informed Consent: Written informed consent was not required due to the retrospective nature of this study.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Author Contributions: Concept – GBU; Design – MAKçiçek; Supervision – GBU; Resource – AET, ZÖ; Data Collection and/or Processing – MAYdın, AK; Analysis and/or Interpretation – Fİ, AET, MAKçiçek; Literature Review – MFA, HP; Writing – GBU; Critical Review – GBU, Fİ.

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Evaluation of Pompe Disease from a Broad and Systemic Perspective

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ABSTRACT

Pompe disease is a lysosomal storage disorder caused by α -1,4-glucosidase deficiency. The broad spectrum of clinical manifestations results from glycogen accumulation in the muscles and central nervous system (CNS). In this study, we aimed to evaluate the clinical course of Pompe disease. This brief report included six patients. The collected data included demographic characteristics, clinical findings, echocardiography, electroencephalography, electromyography, and brain magnetic resonance imaging. High-dose enzyme replacement therapy reduced the severity of hypertrophic cardiomyopathy in patients with infantile-onset Pompe disease. Three patients exhibited speech difficulties, one patient was diagnosed with epilepsy, and MRI revealed white matter abnormalities in three patients. The neurological findings observed in patients with CNS involvement suggest that those with infantile-onset Pompe disease should be closely monitored for neurocognitive development. Novel treatments capable of crossing the blood-brain barrier may benefit these patients.

Keywords: α -1,4-glucosidase deficiency, cardiomyopathy, Pompe disease.



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INTRODUCTION

Pompe disease (α -1,4-glucosidase deficiency, OMIM #232300) is a lysosomal storage disorder caused by autosomal recessive mutations in the GAA gene. These mutations result in loss of α -1,4-glucosidase function, leading to excessive glycogen accumulation in tissues, particularly the myocardium and skeletal muscles. Pompe disease is classified into two subgroups based on age at onset, organ involvement, and the rate of disease progression: infantile-onset Pompe disease (IOPD) and late-onset Pompe disease (LOPD).^{1,2}

Infantile-onset Pompe disease typically manifests with hypertrophic cardiomyopathy, generalized muscle weakness, and hypotonia within the first few months of life. In most untreated cases, death occurs within the first year of life due to cardiorespiratory failure.²



Alglucosidase alfa, an enzyme replacement therapy (ERT) approved by the US Food and Drug Administration (FDA) in 2006, is used to treat Pompe disease.³ Before ERT was introduced into clinical practice, patients with IOPD and hypertrophic cardiomyopathy typically died before reaching 1 year of age because effective treatment was unavailable.⁴ However, an increased frequency of central nervous system (CNS) findings associated with prolonged survival has been reported in patients with IOPD because ERT is unable to cross the blood-brain barrier.⁵

In this study, we aimed to evaluate the echocardiographic, clinical, and CNS findings of six patients with Pompe disease who received ERT at our clinic.

METHODS

This retrospective, single-center cohort study included six patients who were diagnosed with Pompe disease and followed up in the Nutrition and Metabolism Department of Erciyes University between June 2008 and May 2023.

All procedures conducted in studies involving human participants adhered to the ethical standards of the institutional and/or national research committee, the 1964 Declaration of Helsinki and its subsequent amendments, or comparable ethical standards. The study was approved by Erciyes University Clinical Research Ethics Committee (Approval Number: 2023/610, Date: 25.09.2023).

Data on demographic characteristics, clinical findings, muscle enzyme measurements, genetic analyses, and the results of echocardiography (ECHO), electroencephalography (EEG), electromyography (EMG), and brain magnetic resonance imaging (MRI) were recorded.

Statistical analyses were performed using R software (version 4.3.2; R Foundation for Statistical Computing, Vienna, Austria; available online at <http://www.r-project.org/>).

RESULTS

Among the patients, one was diagnosed with LOPD and five with IOPD. The median age of the participants was 90 (62.3–130.5) months, and 33.3% (n=2) were female. The parents of 50% (n=3) of the patients were consanguineous. The median age at diagnosis was 3.5 (1–25.5) months, the median birth weight was 3400 (2800–3795) g, and the median gestational age at birth was 39.5 (38–40) weeks. For patients with IOPD, treatment was initiated at a median age of 3 (1–6) months, whereas treatment for the patient with LOPD was initiated at 84 months. The patients received ERT for a median duration of 87.5 (57–108.8) months. Table 1 provides details on the patients' ages, treatment durations, and enzyme and molecular analysis results.

Patients with IOPD presented with hypotonia, respiratory distress, and cyanosis, whereas the patient with LOPD presented with gait disturbance and fatigue. Speech difficulties were observed in 50% (n=3) of the patients, and one patient had difficulty rising from a seated position. Details of the physical examination findings are presented in Table 1.

Patients #2 and #3 experienced seizures, and EEG revealed epilepsy in patient #2, prompting the initiation of antiepileptic treatment. Table 1 summarizes the EEG, EMG, and brain MRI findings.

Patient #1, who was subsequently diagnosed with LOPD, was referred to our clinic after an elevated CK level was detected. Alpha-glucosidase activity was measured at 1.13 $\mu\text{mol/L}$ (reference range: 1.5–10) by leukocyte enzyme analysis, and a single variant allele, c.32-13T>G (2737del), was identified in the GAA gene. Alpha-glucosidase activity was remeasured to confirm the diagnosis (0.9 $\mu\text{mol/L}$). Genetic analysis of the parents identified a single c.32-13T>G (2737del) variant allele in the GAA gene in the mother. Alpha-glucosidase activity was normal in both parents. Myogenic motor unit potentials (MUPs) were detected on the patient's EMG. Because the clinical and laboratory findings were consistent with LOPD and no alternative causes could explain the symptoms or laboratory findings, the patient was diagnosed with LOPD. ERT was initiated at a dose of 40 mg/kg every 2 weeks.

At diagnosis, the patient's pulmonary function tests showed a forced expiratory volume in 1 second (FEV1) of 111.7% and a forced vital capacity (FVC) of 94.8%. After treatment, pulmonary function tests showed an FEV1 of 124% and an FVC of 125.2%. On the 6-minute walk test, the patient walked 440 m at diagnosis, and this distance remained unchanged at the end of the second year of treatment.

DISCUSSION

This study showed that the CNS manifestations of IOPD become more apparent over time. As ERT prolongs survival in patients with IOPD, the management of IOPD may require more extensive CNS evaluation. This finding also suggests a need for novel therapies to address the progression of neurological symptoms.

A comprehensive investigation by the Infantile-Onset Pompe Disease Natural History Study Group found that the mean age at symptom onset in patients with IOPD was 2 months and that the mean age at diagnosis was 4.7 months.⁶ Similarly, our study showed that the mean age at diagnosis of Pompe disease was 3.5 months.

Khan et al.⁷ reported that high-dose ERT reduced the need for mechanical ventilation in patients with Pompe disease

Table 1. Clinical, laboratory, and imaging findings of the patients

Patient	P1	P2	P3	P4	P5	P6
Age (months)	204	68	106	90	45	90
Age at diagnosis (months)	84	6	1	1	3	4
ERT duration (months)	120	62	105	89	42	86
Enzyme activity (μmol/L/s) (reference range: 1.5–10)	1.13	0.2	N/A	0.17	0.76	0.73
GAA gene	c.32-13T>G, heterozygous	c.896T>G, heterozygous; c.2662G>A, heterozygous	c.2646+2T>A, homozygous	c.266G>A, homozygous; c.2303C>G, homozygous	c.2456G>C, homozygous	c.2104C>T, homozygous
AST (0–32 U/L)	145	273	86	122	189	159
CPK (26–192 U/L)	1409	867	534	450	600	596
Presenting symptom	Gait disturbance, fatigue	Hypotonia	Respiratory distress	Hypotonia	Respiratory distress	Cyanosis after birth
Speech difficulties	–	+	+	–	–	+
Mechanical ventilation requirement	–	–	–	–	–	–
Difficulty standing up	–	–	–	–	–	+
Independent walking	–	–	–	–	–	–
Need for wheelchair	–	–	–	–	–	–
Supraventricular tachycardia	–	+	–	–	+	+
Seizure	–	+	+	–	–	–
EEG	N/A	High-amplitude delta- frequency, sharply contoured wave activity in the posterior hemisphere	Normal	N/A	N/A	N/A
EMG	Myogenic MUPs	N/A	N/A	Myogenic MUPs	N/A	N/A
Age at brain MRI (years)	13	2	7	4	N/A	4

Table 1 (cont). Clinical, laboratory, and imaging findings of the patients

Patient	P1	P2	P3	P4	P5	P6
Brain MRI	Normal	Normal	Widespread patchy and confluent T2/FLAIR signal increases in the bilateral cerebral hemispheric white matter, extending from the subcortical regions to the periventricular areas and internal capsules. The corpus callosum is thinned.	Symmetrical T2/FLAIR hyperintense signal abnormalities in the bilateral centrum semiovale and periventricular deep white matter. Symmetrical lesions are present in the bilateral deep cerebral white matter.	N/A	Symmetrical T2/FLAIR signal increases at the bilateral supraventricular level. The corpus callosum is present in the midline and appears diffusely thinned.
ECHO (at diagnosis)	Normal	Hypertrophic cardiomyopathy	Hypertrophic cardiomyopathy	Normal	Hypertrophic cardiomyopathy	Hypertrophic cardiomyopathy
ECHO (last examination)	Normal	Mild left ventricular hypertrophy	Mild left ventricular hypertrophy	Normal	Hypertrophic cardiomyopathy	Mild hypertrophic cardiomyopathy

ERT: Enzyme replacement therapy; EEG: Electroencephalography; EMG: Electromyography; MRI: magnetic resonance imaging; ECHO: Echocardiography.

by improving respiratory function.⁷ No patients in our study required a wheelchair or respiratory support. After ERT, the patient with LOPD demonstrated increases in FEV1 and FVC, indicating improved pulmonary function. This finding was consistent with previous studies reporting a positive association between ERT duration and respiratory function and muscle strength in patients with LOPD.^{7,8}

Speech and swallowing difficulties are observed in patients with Pompe disease because of impaired respiratory and orofacial muscle function. In addition, impaired myelination and neuronal cell loss in Pompe disease may exacerbate neurological symptoms. In a study by Su et al.,⁹ which evaluated 14 patients with Pompe disease, speech impairments were detected in two-thirds of the patients. In our study, speech difficulties were observed in half of the patients.

Compared with LOPD, IOPD is more severe, and ERT has been shown to improve survival in patients with IOPD. Previous research indicates that some cognitive abilities and neurological functions may deteriorate as survival is prolonged in patients with IOPD.⁵ Indeed, postmortem examinations of patients with IOPD have revealed glycogen deposits in brain tissue, particularly in glial cells of the brainstem, thalamus, basal ganglia, hippocampus, and cerebellum.¹⁰ Because current ERT cannot cross the blood-brain barrier, it is ineffective in preventing glycogen accumulation in the brain. In this study, MRI scans revealed periventricular deep white matter abnormalities, indicating progressive brain damage, in three patients with IOPD. This finding suggests that the management of IOPD may require more extensive CNS evaluation as patients age and that novel ERT capable of crossing the blood-brain barrier is needed. In contrast, Van den Dorpel et al.¹¹ reported no abnormalities on brain MRI in patients with LOPD. Similarly, the patient with LOPD in our study had a normal brain MRI scan. This finding may result from greater residual enzyme activity in patients with LOPD, which may protect against brain damage.¹¹

EEG was performed in two patients after the onset of seizures. Of these two patients, one was diagnosed with epilepsy. Kenney-Jung et al.¹² reported six patients with IOPD and CNS symptoms, noting that epilepsy was identified in five of them. The authors also reported no comorbidities in patients with epilepsy. Similarly, the patient with IOPD and epilepsy in our study had no comorbidities or family history of epilepsy. The lower rate of epilepsy in our study compared with that reported by Kenney-Jung et al.¹² (20% vs. 83.3%) may be attributable to the shorter follow-up period in our study. Our finding, which is consistent with the literature, highlights the importance of awareness of the potential development of epilepsy in patients with IOPD.

This study has several limitations. Owing to the rarity of Pompe disease, only six patients could be enrolled despite a 15-year recruitment period. Longer follow-up periods are needed to more clearly delineate the progression of CNS involvement.

Our study demonstrated that CNS involvement worsened over time. With the improvement in life expectancy achieved by ERT, the management of CNS manifestations is likely to become increasingly important in the long-term care of patients with IOPD. This finding also highlights the unmet need for next-generation ERT formulations capable of crossing the blood-brain barrier to prevent progressive neurological damage. These findings further emphasize the need for enhanced supportive care strategies in the long-term management of IOPD.

Ethics Committee Approval: Ethics committee approval was obtained from Erciyes University Clinical Research Ethics Committee (Approval Number: 2023/610, Date: 25.09.2023).

Informed Consent: Written informed consent was not required due to the retrospective nature of this study.

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Primary Intratesticular Leiomyosarcoma: A Case Report and Literature Review

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ABSTRACT

Background: Primary intratesticular leiomyosarcomas are rare neoplasms. Diagnosis is established after radical inguinal orchiectomy through histological and immunohistochemical analysis. According to the literature, testicular leiomyosarcoma may exhibit indolent behavior and can potentially be cured with early detection and surgical intervention.

Case Report: A 63-year-old man presented with painless enlargement of the right testis. High inguinal orchiectomy was performed. Histopathological evaluation of the excised specimen demonstrated low-grade leiomyosarcoma.

Conclusion: The clinical, radiological, and histopathological characteristics of this tumor are described, along with an overview of current therapeutic approaches.

Keywords: Leiomyosarcoma, orchiectomy, testicular mass, testis.



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INTRODUCTION

Leiomyosarcoma of the testis is a rare tumor, with the first case reported by Yachia and Auslaender in 1989. Since then, approximately 39 cases have been documented.¹ In younger patients, this neoplasm has been associated with prior radiotherapy and anabolic steroid use. The clinical management is similar to that of other testicular masses, with painless scrotal swelling being the most common clinical presentation.² For diagnostic evaluation, ultrasonography effectively identifies a hypoechoic mass with or without calcification, whereas cross-sectional imaging is necessary to assess for metastatic disease. Definitive diagnosis is achieved after radical inguinal orchiectomy through histological and immunohistochemical analysis. The literature indicates that testicular leiomyosarcoma may demonstrate indolent behavior and can potentially be cured with early diagnosis and surgical intervention.

CASE REPORT

A 63-year-old man was referred to the urology clinic for evaluation of lower urinary tract symptoms. His medical history included hypertension, managed with enalapril, and previous surgeries, including right inguinal hernia repair in 2022 and right testicular sperm extraction (TESE) in 1987.

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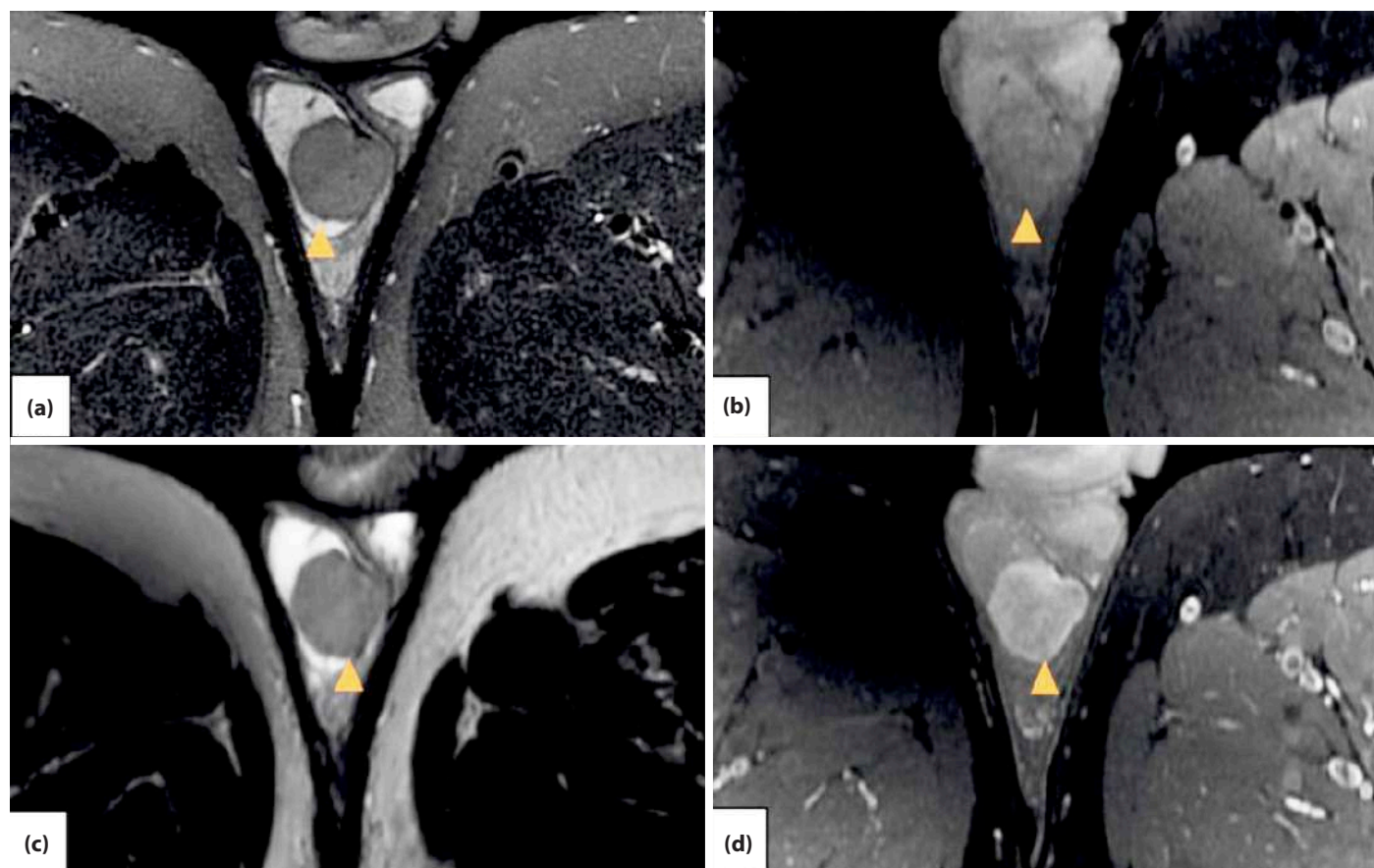


Figure 1. Pelvic MRI findings of the tumor. **(a)** Axial T2-weighted image. **(b)** Axial T1-weighted image. **(c)** Axial T2-weighted fat-suppressed image. **(d)** Axial T1-weighted contrast-enhanced image.

Physical examination revealed that the right testis was solid, irregular, and atrophic, whereas the left testis was also atrophic but regular and soft. Testicular tumor markers, including alpha-fetoprotein, lactate dehydrogenase, and beta-human chorionic gonadotropin, were within normal limits. Scrotal Doppler ultrasonography (USG) was performed as the initial radiological assessment and identified a well-circumscribed, heterogeneous lesion measuring 26×24 mm in the right testis. Doppler imaging demonstrated an isohypoechoic lesion with central vascularity relative to the testicular parenchyma. Millimetric echogenic foci consistent with microlithiasis were observed in the parenchyma of both testes, more prominently on the left. The left testis was reduced in size, but no intratesticular solid or cystic mass was identified.

One month after USG, contrast-enhanced pelvic magnetic resonance imaging (MRI) identified a hypointense mass in the right testis on axial T2-weighted images (Fig. 1a), characterized by regular borders, necrosis, and cystic degeneration. The mass appeared isointense on axial T1-weighted images (Fig. 1b). Axial

T2-weighted fat-suppressed images (Fig. 1c) confirmed the absence of a fat component within the tumor. Significant contrast enhancement was observed on axial T1-weighted contrast-enhanced fat-suppressed images (Fig. 1d). Axial diffusion-weighted imaging ($b=800 \text{ sec/mm}^2$) and apparent diffusion coefficient analysis revealed no definite restricted diffusion within the tumor. Thoracoabdominal computed tomography (CT) scans did not detect any additional primary or metastatic lesions. Based on the imaging findings, a clinical diagnosis of testicular malignancy was established. The patient subsequently underwent high inguinal orchiectomy under spinal anesthesia. No complications were observed during the postoperative period.

Macroscopic examination of the resected specimen demonstrated a 3×2.2 cm gray-white solid tumor confined to the testis (Fig. 2). Microscopic examination revealed atrophic testicular tissue surrounding the mesenchymal tumor (Fig. 3a). The tumor was composed of spindle cells arranged in intersecting bundles, with increased cellularity, low mitotic activity, and mild to moderate pleomorphism

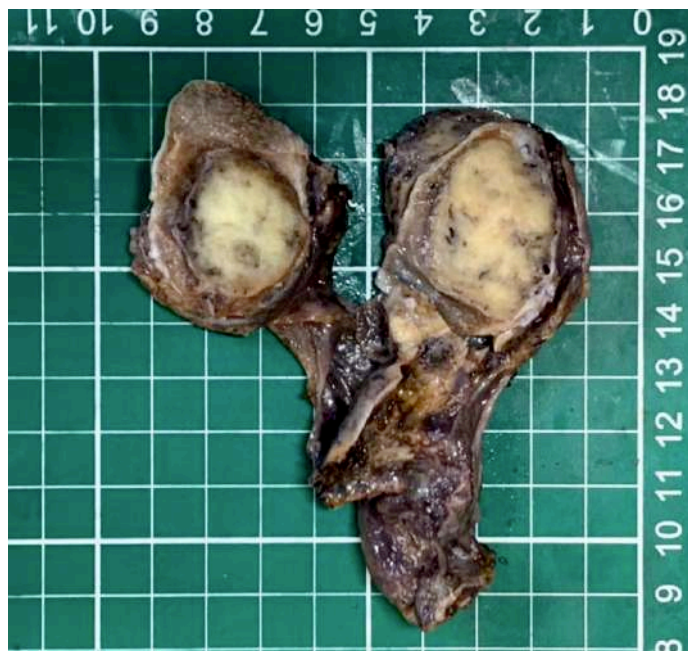


Figure 2. Macroscopic image of the intratesticular tumor mass.

(Fig. 3b). Necrosis and lymphovascular invasion were absent. According to the FNCLCC grading system, the tumor had a total score of 3 (differentiation: 2, mitotic count: 1, necrosis: 0), corresponding to grade 1.

Immunohistochemical analysis demonstrated diffuse positivity of the tumor cells for vimentin, desmin, smooth muscle actin (SMA), and caldesmon. The tumor cells were negative for cytokeratin AE1/AE3, myogenin, S100, SOX10, inhibin, HMB45, Melan-A (MART1), PLAP, CD34, and CD117 (Fig. 3c, d). The Ki-67 proliferation index was low (3–5%).

In the differential diagnosis of dedifferentiated liposarcoma with smooth muscle differentiation, microscopic examination did not reveal any fatty component. Immunohistochemical analysis was negative for CDK4 and MDM2. In addition, fluorescence in situ hybridization (FISH) analysis did not demonstrate CDK4 or MDM2 amplification. Other differential considerations included testicular teratoma with smooth muscle differentiation; however, no teratomatous component was identified. Based on the combined histopathological and immunohistochemical findings, the tumor was diagnosed as primary low-grade intratesticular leiomyosarcoma. The tumor infiltrated the tunica albuginea, with no involvement of the tunica vaginalis, epididymis, or spermatic cord. All surgical margins were tumor-free. The patient did not receive postoperative chemotherapy or radiotherapy. No metastasis or recurrence was observed during the 2-year follow-up period.

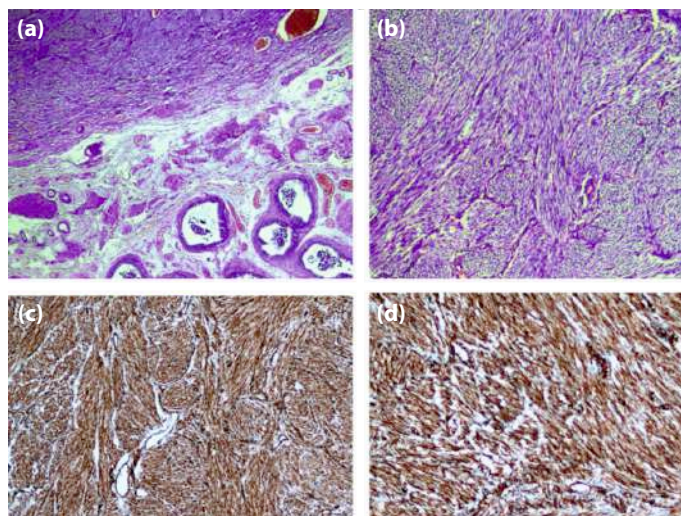


Figure 3. (a) Atrophic testicular tissue and epididymis surrounding the tumor (H&E, $\times 40$). (b) Tumor composed of spindle cells forming intersecting smooth muscle bundles (H&E, $\times 200$). (c) Diffuse positive staining for SMA in the tumor ($\times 200$). (d) Diffuse positive staining for desmin in the tumor ($\times 400$).

DISCUSSION

Leiomyosarcomas are malignant soft-tissue tumors that arise from tissues containing smooth muscle. Scrotal leiomyosarcomas are categorized as either paratesticular or intratesticular.³ Paratesticular leiomyosarcomas are more common, with approximately 100 documented cases.⁴ In contrast, intratesticular leiomyosarcoma, which develops from smooth muscle cells of the tunica propria, tunica albuginea, seminiferous tubules, or blood vessel walls, is rare and has been reported in only a few cases in the literature.¹

Primary intratesticular leiomyosarcomas most commonly present between the fourth and seventh decades of life, with a mean age at diagnosis of 50 years. The precise etiology of testicular leiomyosarcoma is unknown; however, potential contributing factors include hormonal stimulation, such as prolonged anabolic steroid use, radiotherapy, and chronic inflammation.^{5,6}

Scrotal ultrasonography is the primary radiological diagnostic tool. When ultrasonographic findings are inconclusive, MRI can effectively differentiate benign from malignant paratesticular tumors because of its superior soft-tissue contrast and high sensitivity to contrast enhancement.⁷ Evaluation for distant metastases, including pulmonary and lymph node metastases, is performed using thoracoabdominopelvic CT.

Definitive diagnosis of intratesticular leiomyosarcoma depends on histopathological examination. Demonstrating the absence of germ cell elements within the tumor is essential for establishing the diagnosis of primary leiomyosarcoma.⁸ Histological criteria for intratesticular leiomyosarcoma are limited in the literature. To date, 39 cases of intratesticular leiomyosarcoma have been reported.¹ Appendix 1 summarizes the clinical features, pathological findings, demographic characteristics, therapeutic approaches, and clinical outcomes of previously reported cases, as well as those of the current case. Diagnostic criteria for uterine spindle cell leiomyosarcoma, including nuclear atypia, mitotic count, and coagulative necrosis with nuclear debris, may also be applied to intratesticular leiomyosarcoma. Immunohistochemical studies are recommended to support the diagnosis of a primary intratesticular smooth muscle tumor.

High inguinal radical orchiectomy is the standard treatment for intratesticular leiomyosarcoma. Current literature suggests that adjuvant therapy, including chemotherapy and radiotherapy, is generally not recommended because of the low metastatic potential of these tumors.⁹ Distant metastases have been reported in only 4 cases, all involving high-grade tumors. Although the role of adjuvant therapy in advanced disease remains poorly defined, chemotherapy is typically indicated in such cases.¹⁰ The standard regimen for invasive or metastatic leiomyosarcoma includes doxorubicin or a combination of doxorubicin and ifosfamide.² In the present case, the tumor was low-grade, stage I, and confined to the testis, with no evidence of metastasis; therefore, adjuvant chemotherapy or radiotherapy was not administered after radical orchiectomy.

The prognosis of testicular leiomyosarcoma is generally more favorable than that of leiomyosarcomas arising in other organs. Follow-up every 6 months, including urogenital examination and CT imaging, is recommended.⁷ Close monitoring is essential because late metastasis can occur even in low-grade tumors, and mortality due to pulmonary involvement has been reported up to 24 months after orchiectomy.⁹

CONCLUSION

Because of the rarity of this tumor, data on its clinical course, diagnostic criteria, prognostic factors, survival rates, and treatment options remain limited. Radical orchiectomy is considered the standard initial treatment. Nevertheless, optimal management strategies have not been well established because of insufficient research and the absence of formal guidelines. Existing studies indicate that early diagnosis and treatment are associated with a high cure rate.

Ethics Committee Approval: This is a single case report, and therefore ethics committee approval was not required in accordance with institutional policies.

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Appendix 1. Summary of the 38 published case reports

Case no.	Authors	Age	Side	Clinical stage	Treatment	Follow-up (months)	Outcome
1	Yachia ¹	55	R	I	orchiectomy	24	survived
2	Pellice ²	37	L	I	orchiectomy	24	survived
3	Washecka ³	47	R	I	orchiectomy	49	survived
4	Washecka ³	40	R	I	orchiectomy	42	survived
5	Froehner ⁴	32	R	I	orchiectomy+LND	79	survived
6	Hachi ⁵	70	L	I	orchiectomy	14	death (lung metastasis)
7	Ali ⁶	65	R	I	orchiectomy	12	survived
8	Takizawa ⁷	76	L	I	orchiectomy	12	survived
9	Canales ⁸	30	R	I	orchiectomy	6	survived
10	Yoshimine ⁹	73	L	III	orchiectomy+CTx	9	survived
11	Sattary ¹⁰	27	L	I	orchiectomy	30	survived
12	Singh ¹¹	26	L	I	orchiectomy	not mentioned	survived
13	Wakhlū ¹²	0,8	L	I	orchiectomy	12	survived
14	Borges ¹³	19	L	I	orchiectomy+CTx+RT	16	survived (retroperitoneal recurrence)
15	Fadl-Elmula ¹⁴	20	L	II	orchiectomy+CTx+RT	11	survived (cerebral metastasis+ local recurrence+paraortic LN metastasis)
16	Joshi ¹⁵	65	R	I	orchiectomy	6	survived
17	Raspolini ¹⁶	77	L	I	orchiectomy	12	survived
18	Tobe ¹⁷	71	R	I	orchiectomy	7	survived
19	Labanaris ¹⁸	73	R	I	orchiectomy	28	survived
20	Giridhar ¹⁹	55	L	I	orchiectomy+CTx	11	death (soft tissue and bone metastasis)
21	Komeya ²⁰	70	L	I	orchiectomy	34	survived (retroperitoneal metastasis)
22	Moona ²¹	45	R	II	orchiectomy+CTx	not mentioned	survived (paraortic LN metastasis)
23	Bakhshi ²²	60	R	I	orchiectomy+RT	12	survived
24	Bostanci ²³	68	L	I	orchiectomy	12	survived
25	Damle ²⁴	68	R	I	orchiectomy	6	survived
26	Hmida ²⁵	78	R	II	orchiectomy	24	survived
27	Rana ¹⁴	50	L	I	orchiectomy	4	survived
28	Suvarchala ²⁶	19	L	I	orchiectomy	15	survived
29	Abdullazade ²⁷	49	L	I	orchiectomy	24	survived
30	González ²⁸	80	R	III	orchiectomy+CTx	3	death (liver, lung metastasis)
31	Oranusi ²⁹	61	R	I	orchiectomy	32	survived
32	Kammeyer ³⁰	3,5	R	I	orchiectomy	not mentioned	survived
33	Rajagopal ³¹	70	R	not mentioned	orchiectomy+RT	not mentioned	survived (local recurrence)
34	Siraj ³²	27	L	I	orchiectomy	24	survived
35	Berg ³³	54	R	IV	orchiectomy+CTx	not mentioned	survived (pulmonary and subcutaneous metastasis)
36	Nazar ³⁴	53	L	I	orchiectomy	12	survived
37	Abdallah ¹⁴	42	R	I	orchiectomy	12	survived
38	Joshi ¹⁵	70	L	I	orchiectomy	3	survived
39	Current case	63	R	I	orchiectomy	24	survived

CTx: Chemotherapy; RT: Radiotherapy; LND: Lymph node dissection; LN: Lymph node.

Appendix 1 (cont). References

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Recurrent Sarcoidosis Presenting With Bilateral Leg Edema: A Case Report

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ABSTRACT

Background: Bilateral lower extremity edema may serve as an early clinical manifestation of pulmonary sarcoidosis. Identifying sarcoidosis as the underlying cause can be challenging in patients with or without a prior history of the disease, as lower extremity edema is not typically considered part of its clinical spectrum.

Case Report: A 63-year-old man presented with a complex medical history, including non-Hodgkin lymphoma, bilateral renal cell carcinoma, colon cancer, and pleomorphic sarcoma of the upper extremity. He also had a history of recurrent pulmonary sarcoidosis. He reported a 6-month history of bilateral pitting lower extremity edema that developed several months before the onset of dyspnea.

Conclusion: In patients with multiple comorbidities, recognizing sarcoidosis as a potential cause of bilateral lower extremity edema requires careful exclusion of systemic and malignant etiologies. Sarcoidosis should be considered when more common causes have been excluded.

Keywords: Differential diagnosis, edema, lymphedema, pulmonary sarcoidosis, sarcoidosis.



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INTRODUCTION

Bilateral lower extremity lymphedema is an uncommon manifestation of sarcoidosis and may even be the initial presenting symptom, sometimes occurring in the absence of pulmonary involvement. These features pose diagnostic challenges for clinicians when determining the underlying cause of lymphedema. A thorough history and physical examination are essential to exclude more common causes and identify any related symptoms or physical findings. In patients with a known history of sarcoidosis, lymphedema should be recognized as a potential manifestation and presenting feature of the disease.

CASE REPORT

A 63-year-old White man presented with a 6-month history of progressive dyspnea, initially exertional and later occurring at rest, and bilateral lower extremity edema that had begun several

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months earlier. His past medical history included stage IIIB non-Hodgkin lymphoma, partial resections for bilateral papillary renal cell carcinoma, and resection of stage I colon cancer, all in remission. He had previously experienced three biopsy-confirmed episodes of pulmonary sarcoidosis in his late twenties, early thirties, and mid-fifties.

One year before the present evaluation, he underwent radiation followed by surgical excision of stage I undifferentiated pleomorphic sarcoma in the subdeltoid region of the left shoulder, with continued surveillance. A chest CT in August 2025 showed several new nodular opacities, including an 8-mm right lower lobe nodule and clustered nodules in the left upper lobe. Abdominal MRI showed no lymphadenopathy.

On physical examination, breath sounds and jugular venous pressure were normal, with no evidence of pulmonary venous congestion. His lower legs showed bilateral pitting edema that was nontender to palpation. Laboratory studies showed a normal complete blood cell count, total protein level, and albumin level. The creatinine level was 2.78 mg/dL, unchanged from his baseline. The estimated GFR was 42 mL/min/1.73 m². Because of the normal jugular venous pressure, absence of pulmonary venous congestion on physical examination, and normal echocardiogram, BNP was not measured. Pulmonary function tests in November 2025 demonstrated a mixed obstructive–restrictive pattern with mildly to moderately reduced gas transfer (DLCO, 11.05 mL/min/mm Hg). Duplex ultrasonography ruled out deep venous thrombosis in both legs, and echocardiography showed normal biventricular function with no evidence of elevated pulmonary pressures.

A repeat chest CT in December 2025 showed a new 10-mm solid, noncalcified nodule in the left upper lobe, additional 4-mm indeterminate nodules, and a 3-mm right upper lobe nodule. Several previously documented 5-mm nodules in the left upper lobe had regressed. No mediastinal or hilar lymphadenopathy was present. Whole-body PET imaging demonstrated linear interstitial FDG uptake and a low-grade FDG-avid 7-mm right apical nodule that had slightly increased in size since August 2025. These findings, particularly the fluctuating distribution and partial spontaneous regression, favored inflammatory granulomatous disease rather than metastatic malignancy. MRI of the left arm revealed no evidence of sarcoma recurrence.

Given the fluctuating parenchymal abnormalities, the patient’s nonsmoking status, and his history of biopsy-confirmed sarcoidosis, a shared decision was made to initiate empirical corticosteroid therapy with prednisone 20 mg daily for 1 month. The decision considered the biopsy-related challenges associated with the nodule’s location, the PET scan results, the

Table 1. Reported cases of sarcoidosis presenting with bilateral or distal lower-extremity edema								
Study	Age	Sex	Race	Initial symptom*	Cutaneous involvement	Inguinal lymphadenopathy	Retroperitoneal lymphadenopathy	Pulmonary involvement
Silver et al. ¹ (1966)**	30	Female	Black	Yes	No	Yes	Yes	No
Nathan et al. ² (1974)	32	Female	Black	Yes	Yes	No	No	Yes
Sweeney et al. ³ (1980)	30	Male	Black	No	No	No	Yes	Yes
Cantini et al. ⁴ (1997) patient 1	32	Male	Not reported	Yes	No	Not reported	Not reported	Yes
Cantini et al. ⁴ (1997) patient 2	48	Female	Not reported	Yes	Yes	Not reported	Not reported	Yes
Cantini et al. ⁴ (1997) patient 3	34	Female	Not reported	Yes	No	Not reported	Not reported	Yes
Cantini et al. ⁴ (1997) patient 4	31	Male	Not reported	Yes	No	Not reported	Not reported	Yes
Cantini et al. ⁴ (1997) patient 5	28	Female	Not reported	Yes	Yes	Not reported	Not reported	Yes
Tomoda et al. ⁵ (1999)	55	Male	Not reported	Yes	Yes	Yes	Yes	No
Huang et al. ⁶ (2016)	48	Female	Asian	Yes	Yes	No	Not reported	Yes
* "Initial symptom" refers to bilateral or distal lower extremity edema being the first recognized manifestation of sarcoidosis. **Silver HM, Tsangaris NT, Eaton OM. Lymphedema and lymphography in sarcoidosis. Arch Intern Med. 1966;117:712-714.								

patient's history of recurrent sarcoidosis, and the high pretest probability of the disease. He experienced marked improvement in dyspnea within 3 days and complete resolution of bilateral edema within 1 week. Follow-up chest CT after 4 weeks showed radiographic resolution of all nodular infiltrates. At the 3-month follow-up, there was no recurrence of the patient's symptoms.

DISCUSSION

Bilateral peripheral edema is a rare but recognized manifestation of sarcoidosis and may even be the presenting feature.^{1–6} Published cases show that sarcoidosis-associated edema typically reflects lymphatic involvement, such as inguinal or retroperitoneal lymphadenopathy, or direct soft-tissue infiltration causing lymphatic obstruction.^{1–4,6} In some patients, tenosynovitis can lead to a pitting distal edema pattern referred to as remitting extremity swelling syndrome.⁴

Among the 10 well-documented cases in the literature (Table 1), most occurred in adults aged 28–55 years, and several were associated with cutaneous sarcoid lesions, including subcutaneous nodules, plaques, erythema nodosum, or ulcerated nodules. Lymphadenopathy or pulmonary disease often accompanied these presentations. Our patient differs from prior reports in that he presented with bilateral lower extremity edema before the onset of respiratory symptoms and without radiologically detectable lymphadenopathy or cutaneous involvement.

The patient's extensive oncologic history created a diagnostic challenge, particularly in distinguishing recurrent sarcoidosis from metastatic disease. In this patient, the waxing and waning pattern of the pulmonary nodules, with some appearing while others regressed, was more compatible with an inflammatory granulomatous process than with metastatic disease. The absence of progressive lymphadenopathy and the presence of only mildly increased FDG uptake also supported sarcoidosis. Deep venous thrombosis was excluded by duplex ultrasonography, and no findings suggested anemia, hypoalbuminemia, or volume overload. In contrast, metastatic nodules arising from lymphoma or renal cell carcinoma would be expected to enlarge steadily and follow a more persistent radiologic course. The patient's rapid and complete resolution of both edema and dyspnea after the initiation of corticosteroids further supports sarcoidosis as the underlying diagnosis.

In patients with bilateral lower extremity edema in the absence of obstructive lymphadenopathy, cutaneous lesions, or tenosynovitis, alternative mechanisms must be considered to explain the symptoms. Elevated proinflammatory cytokines, such as tumor necrosis factor- α (TNF- α), interferon- γ (IFN- γ), IL-6, and IL-18, are common in patients with sarcoidosis.⁷ These cytokines increase vascular permeability, allowing protein

and fluid to move from blood vessels into the interstitium. Genetic polymorphisms in these proteins may alter gene expression and contribute to lower extremity peripheral edema.

CONCLUSION

Lower extremity edema in sarcoidosis may be subtle, easily misattributed to comorbidities, or overlooked entirely, particularly in older patients and those with a history of malignancy. It is likely underreported or unrecognized as a clinical entity in sarcoidosis. Additional well-characterized reports are needed to refine the understanding of its clinical spectrum, underlying mechanisms, and diagnostic implications.

Ethics Committee Approval: This is a single case report, and therefore ethics committee approval was not required in accordance with institutional policies.

Informed Consent: Written informed consent was obtained from the patient.

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The Past, Present, and Future of Cutaneous Larva Migrans in Türkiye: From Selim Kurtoğlu's 1981 Case to Current Clinical and Epidemiological Reality

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ABSTRACT

Objective: To place Selim Kurtoğlu's 1981 Turkish report of cutaneous larva migrans (CLM) within the context of pre-digital medical history and the updated Türkiye-related literature.

Materials and Methods: We conducted a historical documentary review of the 1981 article and a scoping search of PubMed/MEDLINE, Google Scholar, TR Dizin, DergiPark, journal websites, bibliographic catalogs, and accessible archives. The search was updated on July 27, 2026.

Results: Robert James Lee's 1874 report served as the international historical anchor. Kurtoğlu's 1981 article described a 2.5-month-old boy from Nevşehir with widespread pruritic, tunnel-like lesions and cited a 1947 Turkish parasitology item by Şekban under the term "köstebek hastalığı"; however, neither the cited item nor Şekban's biographical details could be independently verified. The updated search identified 11 subsequent digitally accessible Türkiye-related CLM publications and one 2026 Turkish report of a CLM mimic. The CLM literature included travel-associated disease, probable local acquisition, pediatric and atypical presentations, a 22-patient regional series, and discussions related to climate.

Conclusion: Kurtoğlu's 1981 report demonstrates that the history of CLM in Türkiye is older and more complex than digital databases alone suggest. The 1947 Şekban citation remains a historical trace awaiting archival confirmation, and older Turkish medical periodicals and local archives are essential for reconstructing the national chronology.

Keywords: Archives, epidemiology, history of medicine, larva migrans, parasitic, skin diseases, Türkiye.



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INTRODUCTION

The classical global starting point for the medical description of creeping eruption is the 1874 report by Robert James Lee, MD, FRCP, an English physician whose appointments included assistant physician and later physician at the Hospital for Sick Children, Great Ormond Street,

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London.^{1,2} The report appeared in Transactions of the Clinical Society of London. It provided a recognizable description of a migrating cutaneous eruption but predated contemporary parasitological classification.

In Türkiye, Prof. Dr. Selim Kurtoğlu published “Kutanöz Larva Migrans: Bir Olayın Raporu” in *Sağlık Dergisi* (Health Journal) in 1981. The report described a 2.5-month-old infant from the Nevşehir region and cited an earlier Turkish parasitology item attributed to Şekban in 1947 under the folk term “köstebek hastalığı.”³ Because the original 1947 source has not been retrieved, it is treated here as an archival lead rather than as a verified Turkish CLM case.

Historical and Contemporary Terminology

Historical texts sometimes used CLM, creeping eruption, larva currens, epidermitis linearis migrans, and other terms for migratory dermatoses with overlapping meanings. Contemporary usage should distinguish the clinical sign “creeping eruption” from hookworm-related CLM and differentiate both from Strongyloides-related larva currens, which typically advances more rapidly and may indicate persistent systemic infection.^{4–6} This terminology is consolidated here and applied consistently throughout the manuscript.

Hookworm-related CLM is usually characterized by a pruritic, slowly migrating, serpiginous eruption following contact with soil or sand contaminated by animal hookworm larvae.⁴ Türkiye-related evidence is dispersed across pre-digital local journals, travel-associated reports, probable locally acquired cases, and small regional series. The present study therefore analyzes Kurtoğlu’s 1981 report as a historical source and compares it with the updated literature, focusing on chronology, clinical and epidemiological patterns, diagnostic terminology, treatment, archive accessibility, and future surveillance.

METHODS

Study Design

This was a historical documentary study combined with a scoping literature analysis. It was not designed as a systematic review or meta-analysis, and no quantitative synthesis was planned. The principal question was whether the currently accessible digital literature adequately represents the chronology and clinical significance of CLM in Türkiye.

Archival and Historical Sources

The principal archival source was Kurtoğlu’s 1981 article in *Sağlık Dergisi*, volume 55, issue 1–3, pages 33–37.³ A scanned/facsimile copy was reviewed for bibliographic information, patient age and region, clinical description, terminology,

treatment, and public health comments. The cited 1947 item was searched by author surname, title phrase, year, folk terminology, and spelling variants. The expanded search did not establish Şekban’s full name, specialty, institutional affiliation, journal title, volume, issue, or page numbers; therefore, no conjectural citation was added to the reference list.

Literature Search, Eligibility, and Data Extraction

The search was last updated on July 27, 2026. PubMed/MEDLINE, Google Scholar, TR Dizin, DergiPark, journal websites, bibliographic catalogs, and accessible full-text archives were searched using combinations of “cutaneous larva migrans,” “creeping eruption,” “hookworm-related cutaneous larva migrans,” “larva migrans Turkey,” “kutanöz larva migrans,” “kütanöz larva migrans,” “köstebek hastalığı,” “larva currens,” and “Türkiye.” The reference lists of eligible articles were also screened.

Eligible publications reported CLM in Türkiye, imported cases diagnosed in Türkiye, or Turkish data relevant to local epidemiology, climate, travel, or public health. A 2026 Turkish report of cutaneous pili migrans was included separately because it directly addressed a clinically important CLM mimic; it was not counted as a CLM case. For each publication, we extracted the study type, number and age of patients, geographic and acquisition context, travel history, lesion site, treatment, outcome, and specific contribution.

Source Access, Permissions, and Ethics

No rights-cleared institutional copy of Kurtoğlu’s 1981 article with a stable public URL was identified. Copyright status and republication permission for a page facsimile or patient photograph could not be confirmed; consequently, neither is reproduced. Deposit in an institutional repository or publication as supplementary material would be appropriate after written permission is obtained from the rights holder and the journal. Ethics committee approval was not required because the study used published and publicly accessible sources and involved no new patient contact or unpublished patient-level data.

RESULTS

Historical Anchor Points

Lee’s 1874 report provides the classical international historical anchor.¹ In the chronology of CLM in Türkiye, Kurtoğlu’s 1981 article is the earliest retrievable clinical report identified in this study.³ Kurtoğlu’s citation of Şekban’s 1947 “köstebek hastalığı” item is historically important, but the source remains unverified and therefore cannot establish the first national clinical case. Figure 1 distinguishes the 1947 archival trace from verified reports.

Historical visibility of cutaneous larva migrans in Türkiye

Verified evidence, archival traces, and changes in clinical interpretation

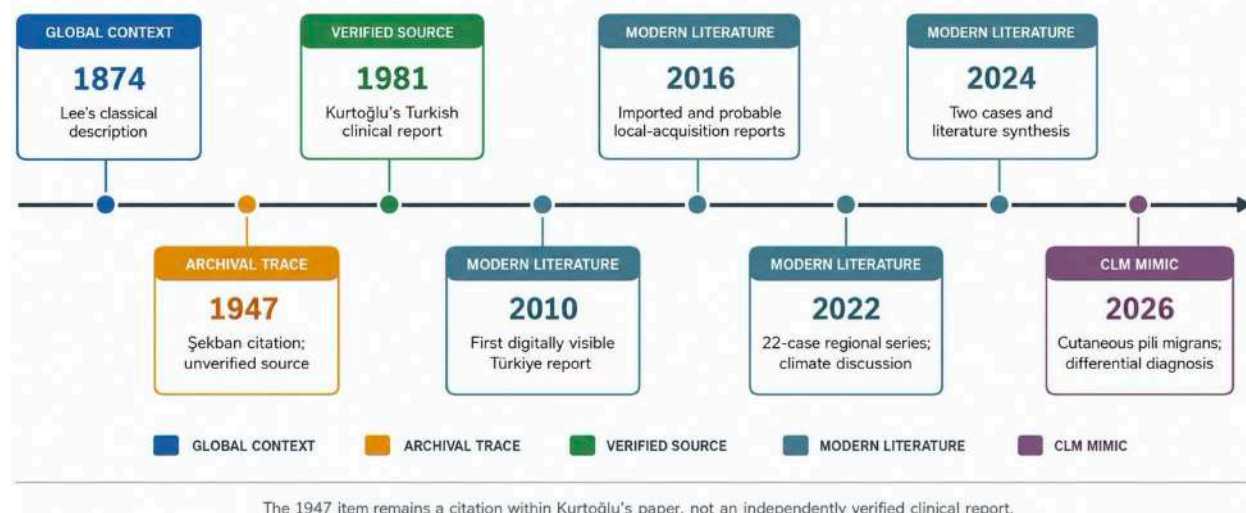


Figure 1. Chronological anchors in the visibility and interpretation of cutaneous larva migrans in Türkiye. The 1947 Şekban item is presented as a citation within Kurtoğlu's paper and as an unverified historical trace, rather than as a confirmed clinical case. The 2026 cutaneous pili migrans report is presented as a CLM mimic relevant to the differential diagnosis, rather than as an additional CLM case.

Kurtoğlu's 1981 Report

The report originated from the Department of Pediatrics, Gevher Nesibe Faculty of Medicine, Kayseri University, and concerned a 2.5-month-old male infant from Yuva village in Nevşehir.³ The infant had systemic symptoms and an approximately 1-month history of raised, intensely pruritic, tunnel-like tracks involving the chest, abdomen, anterior and posterior trunk, arms, and ears. The distribution was unusually widespread compared with the more common distal-extremity pattern. Because the original clinical records, microscopy findings, and pathology material are unavailable, the present study interprets the publication from a historical perspective and does not attempt to re-diagnose the patient. The extracted features are summarized in Table 1.

Kurtoğlu's discussion extended beyond the individual case to animal reservoirs, contaminated soil, the Niğde–Nevşehir region, hygiene, parasitological investigation, and public education.³ These observations gave the report epidemiological as well as clinical value. Kurtoğlu's discussion anticipated concerns now framed within the human–animal–environment interface and the One Health concept.

Later Türkiye-Related Literature

The updated search identified 11 digitally accessible Türkiye-related CLM publications published after 1981 and one Turkish report of a CLM mimic. The 2010 and early 2016

publications included a travel-associated case diagnosed in Türkiye, a three-patient cluster of probable local acquisition, and another imported case.^{7–9} Further reports published from 2016 to 2018 described zosteriform disease associated with local exposure, an imported presentation, and a prolonged bullous case following domestic environmental exposure.^{10–12} Publications from 2020 to 2021 emphasized pediatric disease, atypical localization, diagnostic delay, and probable local acquisition.^{13–15} A 22-patient Eastern Black Sea series and a two-case travel-associated report broadened the regional and epidemiological evidence base.^{16,17} The 2026 publication addressed cutaneous pili migrans as a CLM mimic rather than as an additional CLM case.¹⁸ Detailed clinical and epidemiological characteristics are provided in Table 2 rather than repeated in the text.

The 2020 pediatric report described a 2-year-and-7-month-old boy from rural Samsun with no history of international travel, exposure to sand or soil contaminated by dogs and cats, atypical truncal lesions, an incomplete response to 5 days of albendazole, and regression after oral ivermectin.¹³ The 2026 report described cutaneous pili migrans in a 17-month-old girl: a black linear hair shaft on the sole was identified dermoscopically and removed, with no recurrence at 3 months.¹⁸ This latter publication expands the differential diagnosis but does not add a CLM patient to the national case count.

Table 1. Key clinical, historical, and archival elements extracted from Kurtoğlu’s 1981 report in *Sağlık Dergisi*

Domain	Information extracted from the 1981 report	Contemporary interpretation
Bibliographic source	Kurtoğlu S. “Kutanöz Larva Migrans: Bir Olayın Raporu.” <i>Sağlık Dergisi</i> . 1981;55(1–3):33–37.	Verified Turkish-language published source that is difficult to retrieve through routine digital searches.
Patient and place	2.5-month-old boy from Yuva village, Nevşehir.	Very early pediatric report from central Türkiye.
Clinical pattern	Raised, intensely pruritic, tunnel-like tracks on the chest, abdomen, anterior and posterior trunk, arms, and ears.	Widespread and atypical distribution; the historical source is analyzed without retrospective re-diagnosis.
Terminology	CLM, larva currens, creeping eruption, epidermitis linearis migrans, and migratory parasitic dermatoses discussed in proximity.	Reflects the terminology of the period; current usage distinguishes hookworm-related CLM from Strongyloides-related larva currens.
Diagnosis and treatment	Needle extraction/biopsy and treatment options available at the time, including local measures and oral mebendazole, were discussed.	Typical CLM is now usually diagnosed clinically; albendazole and ivermectin are standard systemic treatment options.
Public health observations	Animal reservoirs, soil contamination, the Niğde–Nevşehir region, hygiene, parasitological investigation, and public education were discussed.	Anticipated concerns now framed within the human–animal–environment interface and the One Health concept.
Archival status	The article was reviewed from a scan/facsimile; the cited 1947 Şekban item was not retrieved.	A rights-cleared institutional deposit and a stable URL are desirable; the 1947 citation remains an archival lead.

Diagnosis, Treatment, and Evidence Gaps

Across time periods, diagnosis depended primarily on a compatible exposure history and the presence of a pruritic migrating track. Kurtoğlu discussed extraction and biopsy alongside the treatment options available at the time, including mebendazole.³ Contemporary guidance generally supports a clinical diagnosis in typical cases and places albendazole or ivermectin at the center of therapy.^{19,20} Modern reports also demonstrate why morphology matters: a black, relatively straight filament and a dermoscopic hair-shaft appearance support cutaneous pili migrans, whereas hookworm-related CLM is usually erythematous, pruritic, and tortuous.¹⁸

The principal evidence gaps include incomplete digitization of older Turkish journals, reliance on case reports and small series, inconsistent documentation of travel and local exposure, and the absence of linked human, animal, and environmental data. These limitations preclude estimation of the national incidence or confident attribution of temporal trends to climate. Proposed priorities are summarized in Table 3.

DISCUSSION

The central contribution of this study is historical: Kurtoğlu’s 1981 pediatric report restores a pre-digital Turkish source to the CLM chronology while maintaining a clear evidentiary boundary around the unverified 1947 Şekban citation. The updated search also demonstrates that the modern

literature cannot be reduced to a narrative of imported disease; it includes travel-associated cases, probable domestic acquisition, pediatric and atypical presentations, and a regional case series.

Digital Visibility Does Not Equal National Disease Onset

If only indexed databases are searched, Türkiye-related CLM appears to begin in 2010. Kurtoğlu’s report demonstrates the distortion created by this approach. Conversely, the 1947 citation should not be elevated from an archival clue to a confirmed case without the underlying document and adequate bibliographic identification. This two-level chronology—verified source versus archival trace—is more defensible than either ignoring pre-digital literature or overinterpreting an inaccessible citation.

The clinical literature supports a similarly balanced interpretation. Cases linked to hazelnut-grove work, barefoot stream exposure, contaminated rural soil, gardening, and animal waste make exclusive importation unlikely.^{8,10,12,13,15} However, the evidence is too sparse and selectively reported to establish endemicity, frequency, or a climate-driven increase at the national level. The 22-patient Eastern Black Sea series, which included tea harvesting and soil contact, is important for hypothesis generation, but exposure surveillance and denominator data are needed before causal claims can be made.¹⁶

Table 2. Clinical and epidemiological features of digitally accessible Türkiye-related publications (2010–2026)

Citation/ year	Patients	Region and acquisition context	Travel/ local status	Lesion site/ pattern	Treatment and outcome	Specific contribution
Yavuzer et al., 2010 ⁷	1; 27-year-old Australian woman	Evaluated in Nevşehir after travel to the Brazilian Amazon; wore sandals	Imported; diagnosed in Türkiye	Dorsum of the right foot; serpiginous track	Albendazole 400 mg/day; marked regression	First modern, digitally accessible Türkiye-diagnosed report; not evidence of local acquisition
Çalışkan et al., 2016 ⁸	3 men; 23–54 years	Düzce, Western Black Sea region; hazelnut-grove mowing with a motorized trimmer; no foreign travel	Probable local acquisition	Abdomen, trunk, and neck; one case with secondary bacterial infection	Albendazole 400 mg/day for 3 days; all lesions regressed; one patient also received antibacterial therapy	Three-case occupational/environmental cluster supporting domestic acquisition and atypical lesion sites
Oral et al., 2016 ⁹	1; 54-year-old Turkish man	Evaluated in Istanbul after travel to the Brazilian Amazon	Imported	Right arm; 4–5-cm pruritic serpiginous lesion	Albendazole 400 mg/day; improvement within days	Additional travel-associated report involving a Turkish patient identified in the updated search
Aktaş et al., 2016 ¹⁰	1; 43-year-old woman	Karabük; recent contact with sheep and cow feces during cultivation	Probable local acquisition	Left submammary area; unilateral zosteriform pattern	Albendazole 400 mg/day for 3 days plus topical metronidazole; treatment course repeated; complete clearance	Unusual morphology and site associated with domestic animal-waste exposure
Sönmezer et al., 2017 ¹¹	1; 36-year-old man	Diagnosed in Ankara after travel to the Brazilian Amazon and barefoot exposure	Imported	Bilateral feet; secondary infection	Albendazole plus antibacterial treatment; rapid improvement	Imported CLM with a typical history of travel and barefoot exposure
Rastgar et al., 2018 ¹²	1; 44-year-old man	Sakarya; walked barefoot in a stream; no tropical travel	Probable local acquisition	Medial malleolar/ankle region; serpiginous and bullous lesions; 6-month course	Albendazole 400 mg/day for 3 days plus topical metronidazole; regression without recurrence	Prolonged, bullous, locally acquired case following moist-soil exposure
Kökçü Karadağ et al., 2020 ¹³	1; 2-year-7-month-old boy	Rural Samsun; played in sand/soil contaminated with dog and cat feces; no travel	Probable local acquisition	Back and trunk; atypical pediatric distribution	Albendazole for 5 days produced an incomplete response; oral ivermectin resulted in regression	Pediatric probable local-acquisition report highlighting atypical truncal distribution and successful ivermectin treatment after an incomplete response to albendazole

Table 2 (cont). Clinical and epidemiological features of digitally accessible Türkiye-related publications (2010–2026)

Citation/ year	Patients	Region and acquisition context	Travel/ local status	Lesion site/ pattern	Treatment and outcome	Specific contribution
Subaşı Gökşin et al., 2020 ¹⁴	1; 26-year-old man	Denizli; travel to Thailand and hostel stay 2 months earlier	Imported	Right periumbilical abdomen; buttock papules; initially treated as scabies	Cryotherapy plus albendazole 400 mg/ day for 5 days; healed with post-inflammatory pigmentation	Atypical lesion site and diagnostic delay following travel
Önder et al., 2021 ¹⁵	1; 45-year-old man	Ordu; weekend gardening; no recent foreign travel	Probable local acquisition	Thigh to hip, trunk, and back; approximately 10 cm	Albendazole 800 mg/day for 3 days; complete resolution	Local garden exposure with eosinophilia and histopathological evaluation
Can and Yürekli, 2022 ¹⁶	22 patients; retrospective series	Eastern Black Sea region; tea harvesting/soil contact	Local regional series	Feet, arms, and trunk reported	Not specified in the accessible report summary	Largest Türkiye series; generated a hypothesis linking increased case numbers with temperature and environmental change
Aydın et al., 2024 ¹⁷	2 men; 43 and 47 years	Diagnosed in Türkiye after frequent travel to Somalia and barefoot sand sports	Imported	Plantar, medial, and dorsal foot lesions	Albendazole 400 mg/ day for 3 days; one patient required a repeated course; both improved	Two cases plus an updated synthesis of Türkiye-related literature
Güven et al., 2026 ¹⁸	1; 17-month- old girl	Aydın; embedded hair shaft rather than infectious acquisition	CLM mimic: cutaneous pili migrans	Left sole; black, thin, relatively linear/curvilinear filament	Curettage and forceps extraction; no recurrence at 3 months	Dermoscopy- based differential diagnosis that may prevent unnecessary antiparasitic treatment; not counted as CLM

Modern diagnostic practice also benefits from a broader differential diagnosis. Typical hookworm-related CLM is usually diagnosed clinically, whereas biopsy rarely demonstrates the larva.¹⁹ Larva currens should prompt consideration of strongyloidiasis, whereas cutaneous pili migrans can be recognized by a visible black filament, a relatively linear trajectory, a dermoscopic hair-shaft structure, and resolution after extraction.^{6,18} Distinguishing these entities helps avoid unnecessary anthelmintic therapy and prevents potentially important Strongyloides infection from being mislabeled as ordinary CLM.

Treatment terminology has evolved from local methods and older anthelmintics to systemic albendazole and ivermectin. Contemporary guidance and reviews support these systemic options.^{19,20} Comparative and prospective

clinical evidence also supports both agents.^{21–23} Türkiye-related reports nevertheless show variation in dose, duration, adjunctive therapy, and response. A standardized case registry should therefore capture exposure, lesion progression, anatomic site, laboratory findings when obtained, treatment regimen, adherence, response time, relapse, and adverse events.

Prevention requires attention to animal reservoirs and contaminated environments. Dog and cat hookworms, occupational or recreational soil contact, and suitable microclimatic conditions create a human–animal–environment problem.^{24–26} Priorities for Türkiye include digitizing older medical periodicals, verifying the Şekban lead, improving clinician recognition of both local and imported disease, mapping human reports alongside

Table 3. Proposed historical, research, and public health priorities for cutaneous larva migrans in Türkiye

Priority	Rationale	Minimum data or action	Expected output
Digitize older medical periodicals	Pre-digital Turkish reports may be missed in routine searches.	Rights-cleared scans, bibliographic metadata, optical character recognition, and stable institutional URLs.	A searchable national archive and durable access to sources such as the 1981 report.
Verify the 1947 Şekban citation	The citation may alter the national chronology but cannot be accepted without the original source.	Search bound journal volumes, library catalogs, institutional archives, author indexes, and contemporary bibliographies.	A verified full citation and biographical note, or a documented negative archival result.
Standardized human case registry	Case reports cannot estimate frequency or regional clustering.	Age, sex, province, date, travel history, local soil/sand exposure, animal contact, lesion site, diagnostic basis, treatment, and response.	A comparable national dataset distinguishing imported cases from probable local acquisition.
Human–veterinary linkage	Transmission depends on dog and cat hookworm reservoirs.	Map human cases alongside local animal infection and deworming data.	Risk maps and targeted prevention strategies for beaches, parks, gardens, and rural settings.
Environmental sampling	Soil and sand contamination is central to transmission.	Standardized sampling at beaches, playgrounds, gardens, agricultural sites, and rural areas.	Evidence-based identification of environmental hotspots.
Climate and seasonality	Case counts alone cannot establish climate-related causation.	Link cases to temperature, rainfall, humidity, occupation, and exposure opportunities using appropriate denominators.	Regional models that test, rather than assume, climate-related changes.
Clinical education and differential diagnosis	Atypical sites, local acquisition, larva currens, and CLM mimics may be overlooked.	A practical diagnostic checklist including travel history, migration rate, pruritus, morphology, black filament, dermoscopy, and immune status.	Earlier diagnosis, appropriate therapy, and fewer unnecessary anthelmintic treatments.

veterinary and environmental findings, and monitoring climate and seasonality without inferring causation from case counts alone. Travel medicine remains relevant, whereas public health strategies for strongyloidiasis should remain distinct.^{27,28}

Strengths and Limitations

This study combines direct review of a poorly accessible Turkish source with an updated, structured scoping search and a publication-level comparison of acquisition context, lesion site, treatment, and contribution. It also explicitly distinguishes verified history, an unverified archival lead, true CLM reports, and a clinically relevant CLM mimic.

The study was not a registered systematic review, and older Turkish publications may still be missing. The 1947 source and Şekban's biographical details could not be verified. The 1981 article is interpreted as a historical document without

access to the original records or diagnostic material. Most modern evidence consists of case reports, and publication bias precludes estimates of incidence, geographic burden, or temporal trends. Emerging reports of treatment-refractory CLM further support careful follow-up but cannot be generalized to Türkiye without local data.²⁹

CONCLUSION

Kurtoğlu's 1981 report demonstrates that the history of CLM in Türkiye is older and more layered than digital databases alone suggest. The 1947 Şekban citation remains an important historical trace awaiting archival confirmation. The history of CLM in Türkiye cannot be written without considering older Turkish medical periodicals and local archives. Modern publications provide evidence of both travel-associated and probable locally acquired disease, but stronger surveillance is required before the national burden or climate-related changes can be quantified.

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