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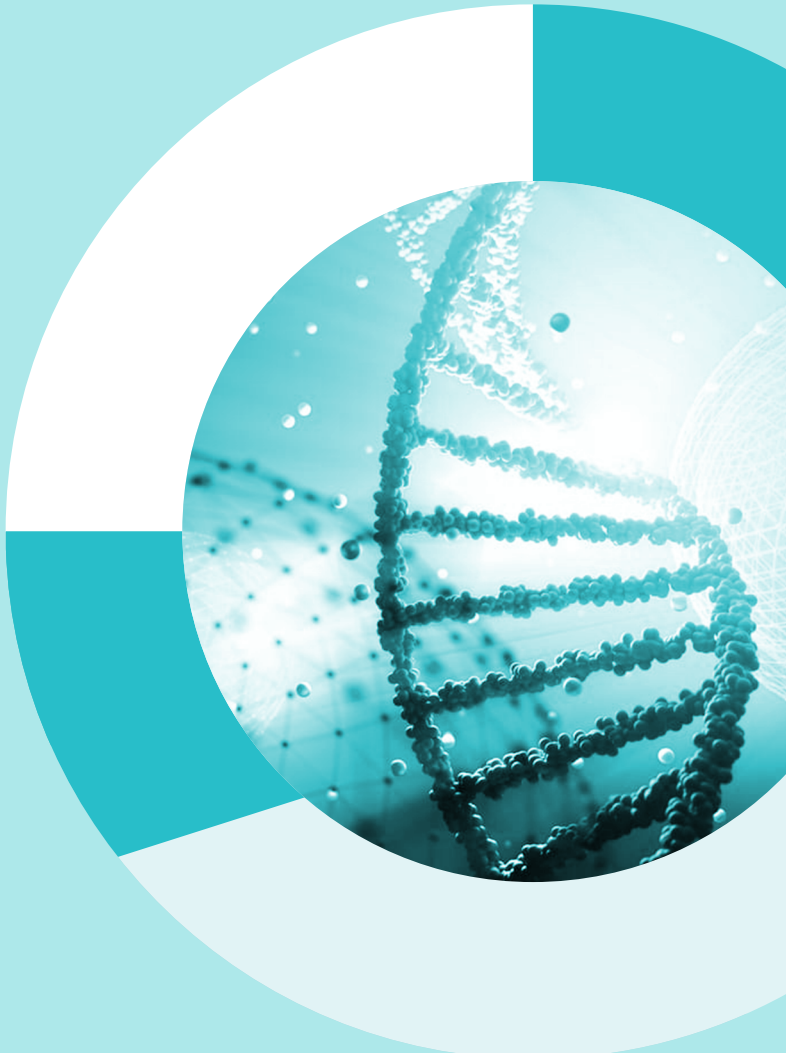

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REVIEW

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Different Maxillary Molar Intrusion Mechanics Supported By Mini-Screws: A Finite Element Method

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Abstract

To evaluate and compare the biomechanical effects of three different maxillary molar intrusion mechanics supported by mini-screws using finite element analysis. A three-dimensional maxillary model, including cortical bone, trabecular bone, teeth, and periodontal ligaments, was constructed from high-resolution cone-beam computed tomography data (0.1 mm slice thickness). Three intrusion mechanics were simulated: (1) a cast appliance with a palatal bar, (2) a prefabricated "Mousetrap" appliance with a transpalatal arch, and (3) an appliance with both buccal and palatal mini-screw anchorage. In each scenario, an intrusive force of 100 g was applied, and displacement patterns were analyzed in the transverse, sagittal, and vertical directions. Material properties, mesh characteristics, and boundary conditions were standardized across all simulations. Buccally placed mini-screws effectively minimized transverse displacement of posterior teeth. Palatal anchorage alone resulted in greater transverse tipping, especially in the second molars. Combined buccal-palatal mini-screw anchorage produced controlled tooth movement in all three spatial dimensions and reduced tipping compared to other scenarios. Occlusal wing extensions transmitted forces but did not adequately prevent transverse tipping of the second molar. Dual buccal-palatal anchorage offers superior three-dimensional control during maxillary molar intrusion, whereas palatal-only anchorage compromises transverse stability.

Keywords: Finite element analysis, orthodontic anchorage procedures, tooth movement techniques

INTRODUCTION

Tooth movement apically along its long axis within the alveolar socket is defined as intrusion.[1] Intrusion mechanics are used in cases of Class I, II, or III open bites and in situations where a molar tooth elongates toward an extraction space.[1-3] Traditional approaches include active vertical correctors[1], springs and magnets in bite blocks[2,4], occipital headgear[3,5], and maxillary traction devices.[5,6] Despite good patient compliance, achieving absolute anchorage control with these conventional methods is often challenging.[7]

Skeletal anchorage involves enhancing the anchorage of the reactive unit by temporarily placing devices into the bone, thereby reducing or eliminating the need for dental or soft tissue support.[8] These devices, including mini-implants, miniplates, and microscrews, can be placed transosteally, subperiosteally, or endosteally, and can be attached mechanically or through osseointegration.[8,9] Skeletal anchorage has been widely applied to maxillary molar intrusion.[6-29]

Finite element analysis (FEA) allows for the creation of models incorporating the physical properties of structures to calculate stresses, strains, and displacements under applied forces.[30,31] This method, widely used in engineering, has become an important tool in dental research for simulating craniofacial structures and visualizing tooth displacement in response to applied forces.[32]

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Although previous studies have evaluated molar intrusion with skeletal anchorage using FEA[22], no published work has comprehensively compared the specific modern methods assessed in this study.

Understanding the biomechanics of molar intrusion has direct clinical implications for the management of vertical discrepancies. Patients with anterior open bite or excessive lower facial height often present with functional problems, such as incomplete incisor contact, impaired mastication, and an increased risk of temporomandibular joint dysfunction. Aesthetically, these patients may also be dissatisfied because of increased gingival display or elongated facial proportions. Successful molar intrusion can address these concerns by inducing counterclockwise mandibular autorotation, thereby improving both occlusion and facial balance.

However, the effectiveness of intrusion is largely dependent on the stability of anchorage. Uncontrolled tipping or asymmetric displacements may not only compromise treatment efficiency but also increase the risk of root resorption, periodontal damage, or relapse. Identifying which anchorage configuration-palatal, buccal, or combined-provides the most favorable biomechanical environment is therefore of critical importance. By clarifying these differences under standardized conditions through finite element modeling, this study offers valuable guidance for clinicians seeking to optimize treatment mechanics, minimize complications, and improve long-term stability.

Therefore, the aim of this study was to compare the biomechanical effects of three distinct maxillary molar intrusion protocols supported by skeletal anchorage. Displacements in transverse, sagittal, and vertical dimensions were assessed using FEA to determine which method provides superior three-dimensional control and minimizes unwanted side effects.

MATERIALS AND METHODS

A digitally reconstructed cone-beam computed tomography (CBCT) dataset, not associated with a real patient, was used to construct the 3D finite element model; therefore, neither ethics committee approval nor informed consent was required. Figure 1 illustrates the stepwise process, from CBCT data acquisition and 3D model reconstruction through finite element meshing, intrusion scenarios, loading conditions, and output analysis. The dataset had a voxel size of 0.1 mm and was processed using 3D Slicer (v4.11) and Mimics (v21.0, Materialise, Leuven, Belgium). The maxilla, teeth, and periodontal ligament (PDL) were segmented and reconstructed according to Hounsfield unit (HU) thresholds (teeth >1200 HU, cortical bone >450 HU, and trabecular bone 150-450 HU). The PDL was modeled as a 0.2 mm uniform layer surrounding the tooth roots. The models were imported into ANSYS Workbench (v19.2, ANSYS Inc., USA) for mesh generation and FEA.

The maxilla, dentition, PDL, and mini-screws were meshed with 10-node tetrahedral elements (SOLID187). The average element size was 0.3-0.5 mm, resulting in approximately 450,000 nodes and 1,600,000 elements. Mesh convergence was verified by ensuring that changes in displacement remained below 5%. Linear elastic, isotropic material properties were assigned based on previous studies. The elastic modulus and Poisson's ratio for cortical bone, trabecular bone, teeth, PDL, stainless steel, titanium, and acrylic are summarized in Table 1.

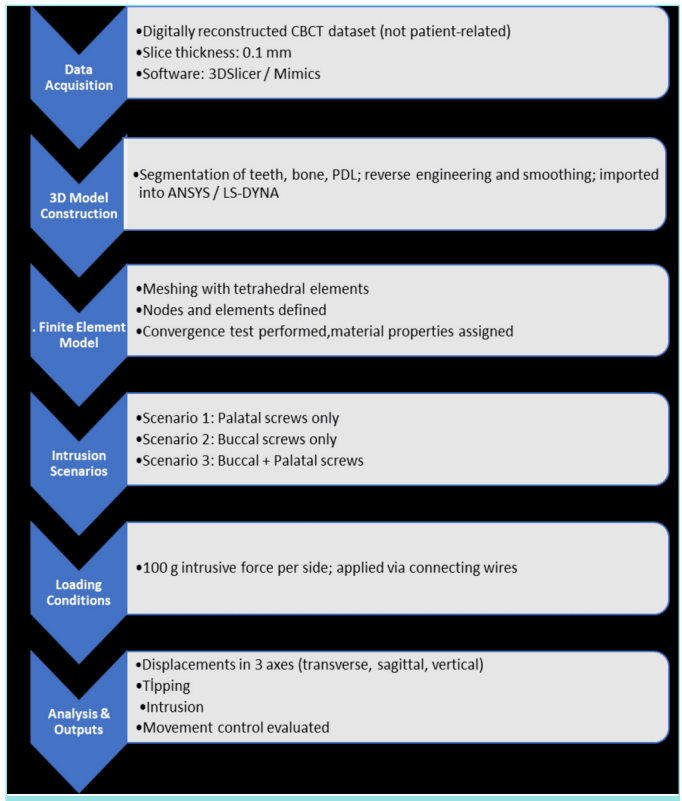

Figure 1. Flowchart summarizing the study design. The process included CBCT dataset reconstruction, 3D segmentation, finite-element meshing, simulation of three intrusion scenarios (palatal screws, buccal screws, and combined anchorage), application of a 100-g intrusive force per side, and evaluation of displacement, tipping, and intrusion patterns.

CBCT: Cone-beam computed tomography, PDL: Periodontal ligament.

Table 1. Linear elastic, isotropic material properties were used for all components

Material	Elastic modulus (MPa)	Poisson's ratio (ν)
Cortical bone	13,700	0.26
Trabecular bone	1,370	0.30
Tooth	19,613.3	0.15
Periodontal ligament	69	0.49
Stainless steel	200,000	0.30
Titanium	110,000	0.33
Acrylic	1,800	0.35

Three different intrusion scenarios were simulated :

- Scenario 1: palatal screws only (Figure 2)
- Scenario 2: buccal screws only (Figure 3)
- Scenario 3: buccal and palatal screws combined (Figure 4)

In all scenarios, a total intrusive force of 100 g per side was applied. In Scenarios 1 and 3, the force was delivered via elastic chains, while in Scenario 2 it was generated through lever arm activation. Displacements

were measured along the transverse, sagittal, and vertical planes, and tooth movements were evaluated accordingly. Boundary conditions were defined by constraining all degrees of freedom at nodes in the inferior and posterior bone regions, with symmetry about the Y-Z plane. Bonded contacts were assigned between all contacting surfaces, assuming no relative motion during loading.

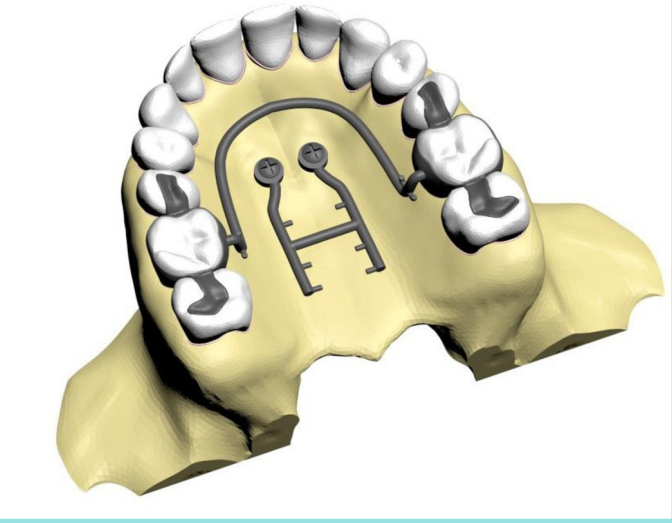

Figure 2. Cast appliance with palatal bar design used in Scenario 1.

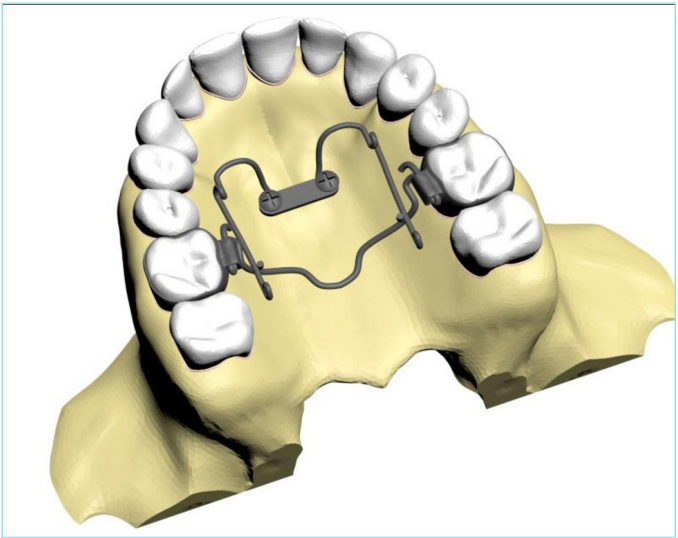

Figure 3. Prefabricated "Mousetrap" appliance used in Scenario 2. The appliance includes a transpalatal arch with hooks positioned near the molars' center of resistance and connected to lever arms to apply force.

RESULTS

In the transverse direction, all scenarios showed palatal tipping of the crowns and buccal tipping of the roots, though the degree varied among teeth. In the first scenario, the second molar exhibited the greatest tipping, the first premolar the least, and the second premolar and the first molar tipped at similar levels. In the second scenario, the greatest tipping was observed in the first molar, whereas the first premolar and the second molar exhibited less pronounced tipping. In the third scenario, all crowns tipped palatally, but the movements were more parallel overall, with the first premolar exhibiting the least tipping and the first molar exhibiting slightly more tipping than the second premolar (Table 2).

In the sagittal direction, crowns tended to tip distally while roots tipped mesially. The second premolar was generally the most affected, showing the greatest distal tipping in the first and second scenarios, while the second molar showed the least distal tipping. In the third scenario, the first premolar demonstrated the most distal tipping, whereas the second molar was the only tooth showing mesial tipping, resulting in the least overall distal tipping among the three scenarios (Table 3).

Vertically, the greatest intrusion occurred in the second molar (first scenario), the first molar (second scenario), and the second premolar (third scenario). Palatal aspects exhibited greater intrusion than buccal aspects. Across all scenarios, the smallest overall intrusion was observed in the third scenario, while values in the first and second scenarios were similar, with slightly greater intrusion in the second (Table 4).

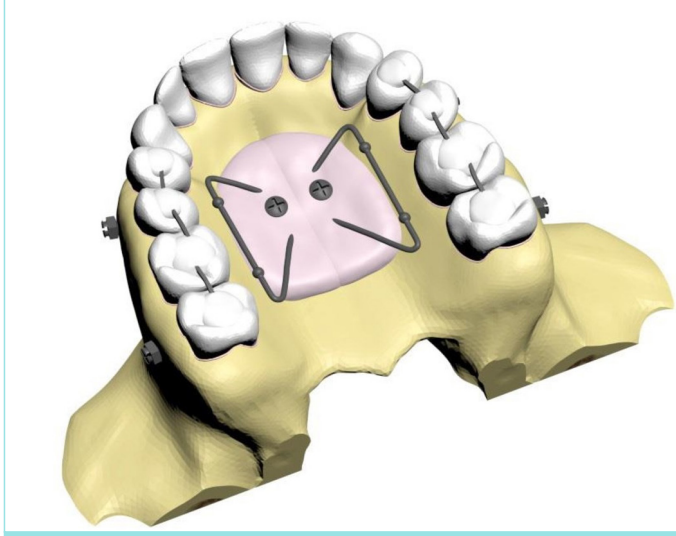

Figure 4. Acrylic palatal plate with buccal and palatal mini-screw anchorage used in Scenario 3. The occlusal wire unites posterior teeth to distribute forces evenly.

Table 2. Values of transversal displacement of crown and root (mm)

Transversal (X) axis	1. Premolar crown	1. Premolar apex	2. Premolar crown	2. Premolar apex	1. Molar crown	1. Molar apex	2. Molar crown	2. Molar apex
Scenario 1	-0.0003852	0.00005408	-0.0006454	0.0002388	-0.0007383	0.0001318	-0.0000729	0.0001559
Scenario 2	-0.0001833	0.00002587	-0.0003448	0.0001283	-0.0004060	0.0001039	-0.0003579	0.00009886
Scenario 3	-0.00003386	0.00001535	-0.0003581	-0.0000864	-0.0003841	-0.00002589	-0.0004106	-0.00005892

Table 3. Values of sagittal displacement of crown and root (mm)

Sagittal (Y) axis	1. Premolar crown	1. Premolar apex	2. Premolar crown	2. Premolar apex	1. Molar crown	1. Molar apex	2. Molar crown	2. Molar apex
Scenario 1	0.0004302	-0.00006298	0.0001064	-0.00008342	0.00008934	-0.00004438	0.00008108	0.0001124
Scenario 2	0.0006166	-0.00004377	0.0001012	-0.00006024	0.00006620	-0.00005821	0.00005440	0.0001073
Scenario 3	0.00009224	-0.00004807	0.00003437	0.00002330	0.00008432	-0.00002454	-0.00001158	0.00001198

Table 4. Values of vertical displacement of crown and root (mm)

Vertical (Z) axis	1. Premolar crown	1. Premolar apex	2. Premolar crown	2. Premolar apex	1. Molar crown	1. Molar apex	2. Molar crown	2. Molar apex
Scenario 1	0.0001144	0.0001716	0.0001289	0.0002071	0.0002078	0.0003980	0.0002402	0.0004097
Scenario 2	0.0001067	0.0001313	0.0001528	0.0001874	0.0002091	0.0003313	0.0002035	0.0002862
Scenario 3	0.0003373	0.0002145	0.0003495	0.0002391	0.0002586	0.0002230	0.0001782	0.0001664

DISCUSSION

Intrusion of posterior teeth has historically been challenging because traditional methods provide limited anchorage.[1-5] The advent of temporary anchorage devices has allowed clinicians to apply forces from multiple directions, improving control.[6-9] However, the mechanical differences between various appliances require further investigation.[33,34] This highlights the importance of FEM-based comparative analyses, which provide valuable theoretical guidance when clinical experimentation is limited.

In Scenario 1, all posterior crowns tipped palatally and roots tipped buccally due to the palatal-occlusal position of the application of force relative to the center of resistance (Figure 2). The first premolar tipped the least because it was excluded from the appliance design. The palatal bar effectively minimized the palatal tipping of the first molars, consistent with the findings of Wilmes et al.[33] Occlusal wing extensions transmitted intrusive forces to the second molars and the second premolars, but did not sufficiently prevent palatal movement of the second molars.[33] Sagittally, second premolars tipped more than molars, partly due to having a single root and smaller mesiodistal root surface area, reducing resistance to tipping forces.[33,35] Occlusal wing extensions had minimal effect on distal tipping control of second premolars.[35] Greater vertical intrusion of the second molars was observed, likely due to a higher trabecular bone content in that region combined with force transmission via the occlusal wing.[35] These findings suggest that palatal anchorage and occlusal extensions can partially improve control but remain insufficient for comprehensive 3D stability.

In Scenario 2, tipping patterns were similar to those in Scenario 1, but greater tipping occurred in the first molars due to the reduced rigidity of the 0.9 mm transpalatal arch (TPA) compared with the cast palatal bar in Scenario 1 (Figure 3). This finding aligns with previous studies showing that a TPA alone provides insufficient transverse anchorage.[35] In the sagittal plane, second premolars tipped more than molars, consistent with their single-rooted morphology and reduced resistance to tipping.[33,35] Occlusal wing extensions again showed minimal effect in controlling the distal tipping of the second premolars.[34] Vertically, intrusion was greatest in the first molars, consistent with Kawamura et al.[30], who reported that palatal-only forces favor intrusion of the first molars.[35] There fore, palatal anchorage alone may achieve localized intrusion but lacks the multidimensional control required for clinical predictability.

In Scenario 3, palatal tipping was least pronounced overall, likely due to force application from both buccal and palatal mini-screws (Figure 4). This dual anchorage reduced transverse movement compared with Scenarios 1 and 2, consistent with other studies in which labio-palatal or mesio-distal mini-implant placement helped prevent molar tipping.[34,35] In the sagittal plane, mesial tipping of the second molar and an overall reduction in distal tipping may be attributed to the unifying occlusal wire and bidirectional force application.[33,34] Similar mesial tipping of second molars when forces are applied between the first and second molars has been reported by Wilmes et al.[33] Second premolars intruded most in the vertical dimension, possibly because single-rooted teeth are more susceptible to intrusion when equal forces are applied.[31] Across all scenarios, Scenario 3 achieved the least intrusion while providing the best three-dimensional movement control.[34,35] A force of 100 g per side is consistent with literature recommendations of 100-250 g for upper posterior intrusion.[35] This demonstrates that dual anchorage offers the most balanced and clinically relevant biomechanics for posterior intrusion.

CONCLUSION

Although this study provides valuable insights into the biomechanics of maxillary molar intrusion, several methodological limitations should be considered. The model was based on a digitally reconstructed CBCT dataset not associated with a real patient, allowing standardization but limiting clinical representativeness. The PDL was modeled as a uniform, linearly elastic layer, although its true behavior is non-linear and viscoelastic. Bonded contacts were assumed between all interfaces, which may underestimate micromovements; the analysis was performed under static, linear conditions and did not account for time-dependent biological changes such as bone remodeling. Moreover, the study lacked experimental or clinical validation and was limited to a single anatomical model, thereby reducing generalizability. Despite these simplifications, the displacement patterns were consistent with previous FEM and clinical studies, supporting the method's usefulness as a theoretical framework and highlighting the need for future in vivo validation.[12,30,35]

This FEA provided valuable insights into the biomechanics of maxillary molar intrusion with different skeletal anchorage systems. Although methodological limitations-such as using a digitally reconstructed CBCT dataset not linked to a real patient, simplified PDL modeling, bonded contact assumptions, and absence of biological time-dependent factors-restrict direct clinical translation, the overall displacement patterns

were consistent with previous FEM and clinical studies, supporting the model's predictive validity.

In conclusion, our data support the conclusion that buccal miniscrews reduce the transverse displacement of the posterior teeth, while palatal-only anchorage provides less transverse control. Dual buccal-palatal anchorage yielded the most favorable three-dimensional control, minimizing tipping and enhancing stability. Occlusal wing extensions contributed to the transmission of intrusive forces but were insufficient to fully prevent transverse tipping of second molars. Taken together, these findings indicate that dual anchorage may be an effective biomechanical strategy for posterior intrusion and that it provides a sound basis for future experimental and clinical investigations.

MAIN POINTS

- Mini-screws placed buccally minimized transverse displacement of the posterior teeth.
- Palatal anchorage alone reduced control of transverse movements.
- Combined buccal-palatal mini-screws provided superior three-dimensional control and reduced tipping.
- Occlusal wing extensions alone did not adequately prevent transverse tipping of second molars.

Footnotes

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Authorship Contributions

Concept: T.T., Design: T.T., Data Collection and/or Processing: D.K., Analysis and/or Interpretation: D.K., Literature Search: D.K., Writing: D.K.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The analyses were carried out by a specialized engineering firm.

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RESEARCH ARTICLE

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Therapeutic Ineffectiveness of Calcitonin Gene-Related Peptide Antagonists: A Real-World Pharmacovigilance Analysis

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Abstract

BACKGROUND/AIMS: Migraine is treated with a variety of drugs with different pharmacological mechanisms of action. Among these drugs, calcitonin gene-related peptide (CGRP) antagonists represent a novel approach to the treatment of migraine. Aim of this study was to evaluate "drug ineffective" reports of CGRP antagonists obtained from an adverse event reporting system by disproportionality analysis.

MATERIALS AND METHODS: To ascertain the signal strength of the "drug ineffective" event associated with CGRP antagonists, reports available in Food and Drug Administration Adverse Event Reporting System (FAERS) until 31 December 2024 were included in the study. The OpenVigil 2.1-MedDRA-v24 software package was utilised for data mining and the analysis of disproportionality of these reports.

RESULTS: After data mining of reports in FAERS, it was found that there were 3,610 reports of small molecule CGRP receptor antagonists and 4,956 reports of monoclonal antibodies against CGRP related to drug ineffectiveness. Disproportionality analysis revealed that rimegepant [reporting odds ratio (ROR) 10.879; proportional reporting ratio (PRR) 6,708], ubrogepant (ROR 5,865, PRR 4,487), eptinezumab (ROR 5.7, PRR 4,397), and zavegepant (ROR 3,559; PRR 3,064) exhibited a positive signalling strength for drug ineffectiveness.

CONCLUSION: A retrospective review of reports of CGRP antagonist drugs that provide effective treatment for migraine showed that half of these drugs had a positive signal indicating drug ineffectiveness. However, this information, obtained using the pharmacovigilance database, needs to be supported by other studies conducted in the clinic.

Keywords: CGRP antagonist, drug ineffective, adverse event reporting system

INTRODUCTION

Calcitonin gene-related peptide (CGRP) has been identified as an important mediator of vasodilation and pain in migraine patients. The administration of CGRP antagonists has been demonstrated to result in the termination of migraine attacks and the prevention of migraine occurrence.[1] There are currently two groups of drugs that act on CGRP in the treatment of migraine: small molecule CGRP receptor antagonists and monoclonal antibodies against CGRP.[2] All monoclonal antibodies against CGRP, including erenumab, fremanezumab, galcanezumab, and eptinezumab, as well as the small molecule CGRP receptor antagonist atogepant, are used in the prophylaxis of episodic and chronic migraine. Other small molecule CGRP receptor antagonists, rimegepant, are indicated for the prophylaxis of episodic migraine and the acute treatment of migraine. Ubrogepant and zavegepant are indicated only for the acute treatment of migraine.[3] The American Headache Society position statement lists CGRP-targeted migraine therapies as a first-line option for migraine prevention.[4]

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In recent years, there have been groundbreaking developments in migraine treatment.[5] Along with these advances in treatment, reports of therapeutic ineffectiveness have also been shown to increase over the years.[6] Furthermore, therapeutic ineffectiveness has been reported to be one of the main reasons for discontinuation of used migraine drugs.[7] Nevertheless, there are various drug groups with different mechanisms of action in the treatment of migraine, ineffectiveness varies even within these groups. For instance, clinical evidence demonstrates the efficacy of metoprolol and propranolol in the treatment of migraine, while acebutolol, which utilizes a similar mechanism, has been shown to be ineffective.[8] A network meta-analysis showed that monoclonal antibodies against CGRP were most effective and had fewer adverse effects compared to placebo, followed by small molecule CGRP receptor antagonists.[9] Nevertheless, ineffectiveness or serious adverse effects have been shown to affect the discontinuation of CGRP treatment.[10]

Adverse events (AEs), including 'drug ineffective' are defined as medically undesirable events resulting from the use of a pharmaceutical product. The most comprehensive database of AEs is the United States Food and Drug Administration's Adverse Event Reporting System (FAERS).[11] FAERS is a publicly accessible database containing real-world data on AEs. This database contains basic information on patients' demographic details, drug usage details, reporting sources, drug indications, and patient outcomes. The data are primarily submitted voluntarily by healthcare professionals and patients.[12] There are differences between patient and healthcare professional reports. Some studies have shown that patient reports may be similar to, or contain more detail than, healthcare professionals' reports.[13] It is important that changes in the effect of drugs are recorded in FAERS. Disproportionality analysis of the data obtained from large databases of spontaneous AE reporting systems such as FAERS has been an important method for drug safety monitoring.[14,15] Regarding pharmacovigilance, the proportional reporting ratio (PRR)[16] and the reporting odds ratio (ROR)[17] are the most popular methodologies utilized for the detection of AE signals. FAERS contains duplicate or incomplete reports. OpenVigil 2.1 is a software tool specifically designed for the extraction, cleaning, mining, and analysis of AE data from the FAERS database that calculates ROR and PRR values.[18]

The effectiveness of migraine treatment in clinical practice is assessed by looking at factors such as the monthly migraine day count, the percentage change in migraine severity relative to baseline, the need for acute attack drugs, the degree of adverse effects of the drug and patient-reported outcome measures.[19,20] The objective of the present study was to evaluate the "drug ineffective" signaling strength of these drugs through a disproportionality analysis of reports covering all CGRP antagonists used in the treatment of migraine, employing data from an AE reporting system.

MATERIALS AND METHODS

Data Source and Collection

In this observational, retrospective pharmacovigilance study, the adverse drug reactions reported in the public version of the FAERS database were used. The medical dictionary for regulatory activities (MedDRA) includes "drug ineffective" as a preferred term (PT).[21] The present study used OpenVigil 2.1-MedDRA-v24 (OpenVigil, Kiel, Germany) to query the FAERS database. Drug ineffective AE reports for the generic name "atogepant", "eptinezumab", "eptinezumab-jjmr", "erenumab", "erenumab-aooe", "fremanezumab", "galcanezumab", "galcanezumab-gnlm", "rimegepant", "rimegepant sulfate", "ubrogepant", "zavegepant" and "zavegepant hydrochloride" have been selected for the period from the last quarter of 2003 to the end of 2024. Adverse reaction reports in which the reporter identified these drugs as "primary suspect" were included in this study.

AE reports of ineffective drugs in the FAERS database were queried for each drug individually using the OpenVigil analysis tool. The association between drug use and AEs was analyzed by selecting "frequency" as the analysis method and "entire cases" as the case type in the query tab of this tool.

Statistical Analysis

A disproportionality analysis was performed using OpenVigil version 2.1. PRR and ROR were employed to detect safety signals. All algorithms are based on 2×2 contingency tables. In this study, PTs with a reported frequency of ≥3 were selected for the initial screening procedure. ROR and PRR values with a signal strength of 2 or higher were considered as positive signals.[22]

The human-related datasets utilized in this study are available to the public for research purposes. Patient consent was not necessary in this study due to the nature of the data source. The research did not require ethics committee approval because the authors did not participate in data collection or know the participants in the present study.

RESULTS

Adverse Event Reports Information

To determine the signal strength of the "drug ineffective" event associated with CGRP antagonists, reports available in the FAERS through December 31, 2024, were included in the study. Of these reports, 11,982 were related to drug inefficacy, with 65.1% (n=7,388) of these reports being related to monoclonal antibodies against CGRP. A greater proportion of the reports was from female patients (59.5%, n=7,135). Among the subsets of reports for which age data were available (6,570 patients without age data), the most common age group was 18-64 years (n=4,381, 36.6%). Most reporters were consumers (n=9,477, 79.1%), with only 20.9% (n=2,500) being healthcare professionals. Details of demographic data related to drug usage are presented in Table 1.

Disproportionality Analysis Information

After data mining of reports in FAERS, 3,610 reports of small molecule CGRP receptor antagonists and 4,956 reports of monoclonal antibodies against CGRP associated with drug ineffective were found were associated with drug ineffectiveness. In this study, ROR and PRR, were used to analyse AE signals, and 4 potential positive signals were identified from analyzing reports until the end of 2024.

Small Molecule Calcitonin Gene-Related Peptide Receptor Antagonists

In the current study, rimegepant was found to be the CGRP antagonist with the highest number of reports and signal magnitude (n=3,005, ROR 10.879, PRR 6,708). Other drugs with a positive signal are ubrogepant (ROR 5,865, PRR 4,487) and zavegepant (ROR 3,559; PRR 3,064). Atogepant was found not to generate a positive signal in terms of signal strength (ROR 1,072, PRR 1,067). The results of the disproportionality analysis of small molecule drugs, which block the CGRP receptor, are shown in Table 2.

Monoclonal Antibodies Against Calcitonin Gene-Related Peptide

Among the monoclonal antibodies against CGRP, only eptinezumab was found to generate a positive signal (ROR 5.7, PRR 4,397). The other drugs, fremanezumab (ROR 1,153, PRR 1,142), galcanezumab (ROR 0,978, PRR 0.98) and erenumab (ROR 0,725, PRR 0,738), had no positive signal strength, although there were reports of drug ineffectiveness events. The results of the disproportionality analysis of monoclonal antibodies against CGRP were shown in Table 3.

DISCUSSION

To our knowledge, this is the first study on the ineffectiveness of all CGRP antagonist drugs using a large database. Therefore, this study has the potential to be used as a basic resource for future research on the therapeutic ineffectiveness of drugs used in the treatment of migraine.

The results of a recent study demonstrated that consumers were more inclined to report a lack of therapeutic effectiveness than doctors.[6] Similarly, in the present study, a large proportion of those who reported (around four-fifths) were identified as consumers. There is a consensus in the European Headache Federation (EHF) that the evaluation of response to drugs used in migraine treatment should be patient-centred.[23] Considering this information, the report by most consumers that the migraine treatment was ineffective may be considered to increase the value of this study. The study revealed that rimegepant, ubrogepant, eptinezumab, and zavegepant exhibited a positive signal strength for drug ineffectiveness.

One of the most common reasons for discontinuation of small molecule CGRP antagonists is that the drug is ineffective (38%).[24] Ineffectiveness of the drug has also been reported as one of the reasons for switching between drugs in this group.[25] In this study, 36.3% of reports of small molecule CGRP antagonists were found to be ineffective. Compared to other small molecule CGRP receptor antagonists, atogepant had a low rate of ineffectiveness (6.74%) and exhibited no measurable signalling effect.

There are few direct head-to-head studies comparing rimegepant with other migraine treatments[26] and the current study is one of the studies comparing CGRP antagonists with each other. In this evaluation, rimegepant was found to have the highest number of primary suspect cases and the strongest positive signalling strength. In one study, it was determined that most of the patients (69.9%) switched to rimegepant treatment due to the ineffectiveness of other migraine drugs. It was stated that approximately half of the patients in this study (44.9%) were relieved of migraine pain with rimegepant treatment.[27] In a study analysing the data using FAERS data of rimegepant, until the end of the first quarter of 2023, it can be observed that the ROR (14.09) and PRR (10.88) values show a decreasing trend in over 2 years.[28] In this study, it is remarkable that 42.2% of the primary suspect AEs due to rimegepant use were classified as drug ineffective. Although there is a patient population in which rimegepant is effective in studies, it is important to keep in mind that there are patients in whom rimegepant is ineffective; to evaluate this issue during the treatment process and to personalise the treatment.

Table 1. Basic information on drug ineffectiveness adverse event reports related to CGRP antagonists*

		Small molecule CGRP receptor antagonist				Monoclonal antibodies against CGRP			
Category	**Number of cases (n)**	**Rimegepant (n=3,344)**	**Ubrogepant (n=626)**	**Atogepant (n=537)**	**Zavegepant (n=87)**	**Erenumab (n=3,545)**	**Eptinezumab (n=1,619)**	**Galcanezumab (n=1,570)**	**Fremanezumab (n=654)**
Gender	Female	1,975	383	400	68	2,050	831	965	463
	Male	305	91	75	6	347	186	212	89
	Unknown	1,064	152	62	13	1,148	602	393	102
Age (years)	<18	7	-	-	-	6	3	1	3
	18-64	1,254	84	103	35	1,493	887	312	213
	65-84	249	19	20	3	258	196	82	105
	≥85	10	2	1	49	8	3	4	2
	Not specified	1,824	521	413	0	1,780	530	1,171	331
Reporter type	Healthcare professional	426	121	49	42	1,188	297	256	121
	Consumer	2,917	505	488	45	2,355	1,322	1,313	532
	Not specified	1	-	-	-	2	-	1	1
Report year	2018	-	-	-	-	643	-	7	16
	2019	-	-	-	-	866	-	331	157
	2020	250	72	-	-	696	24	442	73
	2021	739	139	2	-	271	192	320	100
	2022	1,352	235	261	-	235	365	248	106
	2023	578	146	224	37	253	456	115	92
	2024	425	34	50	50	581	582	107	110

*The Food and Drug Administration Adverse Event Reporting System public dashboard was used when preparing this table. Active substances are ranked according to the total number of reports.

CGRP: Calcitonin gene-related peptide.

Table 2. Disproportionality analysis information of small molecule CGRP receptor antagonist reported to cause drug ineffective*

Drugs	Total reports (n)	Reports (n)	ROR	95% CI	PRR	χ^2	DE/D (%)
Rimegepant	7,117	3,005	10.879	10.378; 11.403	6,708	15515.929	42.22
Ubrogepant	1,709	484	5,865	5,279; 6,516	4,487	1395.609	28.32
Zavegepant	367	71	3,559	2,747; 4,612	3,064	103.178	19.35
Atogepant	742	50	1,072	0,805; 1,429	1,067	0.16	6.74

*The information in this table was analysed using the OpenVigil tool. It is widely accepted that a chi-square value exceeding 4 is statistically significant.
CI: Confidence interval, χ^2: Chi-squared with Yates', D: Drug, DE: Both the drug was used, and the event occurred, PRR: Proportional reporting ratio, ROR: Reporting odds ratio, CGRP: Calcitonin gene-related peptide.

Table 3. Disproportionality analysis information of monoclonal antibodies against CGRP reported to cause drug ineffective*

Drugs	Total reports (n)	Reports (n)	ROR	95% CI	PRR	χ^2	DE/D (%)
Eptinezumab	5,407	1,499	5.7	5.37; 6.05	4,397	4187.718	27.72
Fremanezumab	7,007	505	1,153	1,053; 1,262	1,142	9,301	7.21
Galcanezumab	21,728	1,344	0,978	0,926; 1,034	0.98	0,586	6.19
Erenumab	34,500	1,608	0,725	0,689; 0,762	0,738	159.536	4.66

*The information in this table was analysed using the OpenVigil tool. It is widely accepted that a chi-square value exceeding 4 is statistically significant.
CI: Confidence interval, χ^2: Chi-squared with Yates', D: Drug, DE: Both the drug was used, and the event occurred, PRR: Proportional reporting ratio, ROR: Reporting odds ratio, CGRP: Calcitonin gene-related peptide.

In this study, 7.2% of the reports of the monoclonal antibodies against CGRP drugs were reported as primary suspect due to drug ineffectiveness. According to a few studies conducted in recent years, the ineffectiveness rate in patients receiving monoclonal antibodies against CGRP therapies (except eptinezumab) was 20.3% (n=472); 21.3% (n=169); and 23.2% (n=281).[10,29,30] In the present study, the total number of cases with "drug ineffective" AEs are higher than these studies. However, the total number of AEs reported is also quite high. It might be considered that the presence of eptinezumab in this study and the fact that the main data source is a large database might result in proportional differences. Despite these differences, the main indication of these studies is that monoclonal antibodies against CGRP may be effective in at least three quarters of patients.

A meta-analysis of four randomized controlled trials found that eptinezumab showed excellent efficacy and low side effects in the treatment of migraine.[31] Real-world data on the use of eptinezumab are limited. However, approximately one-third of patients who switched to eptinezumab due to treatment failure after treatment with monoclonal antibodies against CGRP, (erenumab, galcanezumab, fremanezumab) experienced ≥30% reduction in monthly migraine days, while no patient achieved ≥75%.[32] According to the present study, 27.72% of the adverse effects of eptinezumab were due to drug ineffectiveness, and it was found that it ranked 2nd among CGRP monoclonal antibodies in terms of the number of reports. In contrast to eptinezumab, other CGRP monoclonal antibodies had effectiveness that was inversely proportional to the number of cases, and they did not generate positive signal strength. This study shows that eptinezumab is the only drug that has ineffective signalling power among monoclonal antibodies against CGRP. It is recommended to pay attention to this feature of eptinezumab, when switching between these drugs within the same class. In one study, it was shown that the effect of eptinezumab was less in patients who had CGRP antagonist in previous treatment, when patients who used and discontinued monoclonal antibodies against CGRP due to ineffectiveness and similar reasons were compared with patients who did not use the antagonist.[33] Several studies support the recommendation to switch to a second CGRP antagonist monoclonal antibody in the event of an ineffective response to the first CGRP antagonist monoclonal antibody.[34-36] However, there is currently insufficient evidence on the potential benefits of switching from CGRP drugs to monoclonal antibodies.

In the current study, erenumab was found to have the lowest signalling strength, despite having the highest number of ineffectiveness reports, after rimegepant. In many studies, patients who switched to erenumab due to drug ineffectiveness were reported to benefit from erenumab; these findings support the results of our study.[37,38]

The ineffectiveness of drugs used in migraine should be carefully evaluated. Poor efficacy of migraine drugs, especially in the acute treatment of migraine, may lead to progression of migraine.[39,40] The EHF has published a consensus on recommended doses and durations to be used in the assessment of drug ineffectiveness.[41] The concepts of effective and ineffective for the use of triptans as migraine-specific acute treatment are defined by the EHF.[23] Migraine drugs, which are newer than triptans, may also need clearer definitions of ineffectiveness.

Study Limitations

This study has several limitations. The study was limited to reports in which CGRP antagonist drugs were the primary suspect. The study is limited to reports where "drug ineffective" PT was selected. No restrictions were imposed on the therapeutic indication of the drugs during data analysis; such as for episodic migraine or chronic migraine. There is no information about the duration of drug use in this study. This is an important limitation, especially because of late response to monoclonal antibodies.[42] A causal relationship between the ineffectiveness of these drugs and their intended outcomes could not be established. Although MedDRA is used to code AEs in FAERS, no single

naming scheme is used for drugs. While it is stated that this process is performed automatically in OpenVigil version 2.0.2, it is also noted that some raw FAERS data may be discarded when no drug name can be reccognized.[43] Additionally, this study cannot provide information on the number of reports excluded from evaluation during data mining with OpenVigil, i.e., duplicate or incomplete reports. Despite these limitations inherent in pharmacovigilance studies, spontaneous reporting systems are valuable, as they allow the use of large amounts of data for the safety assessment of potential AEs.

CONCLUSION

The availability of CGRP antagonists is important to ensure effective treatment for people affected by migraine. Nevertheless, it should be remembered that there have been some reports suggesting that these drugs, which began to be prescribed after erenumab was licensed in 2018, are ineffective. The evaluation of the ineffectiveness of CGRP antagonists provided by this study needs to be supported by other studies that are conducted in the clinic. Given the ineffectiveness of monoclonal antibodies against CGRP in certain patients and the associated financial burden, it would be advantageous for healthcare professionals and patients to be aware of the findings presented in this study.

MAIN POINTS

- Reports of "drug ineffective" among post-marketing reports play a crucial role in ensuring pharmacovigilance.
- Recognition of drug ineffectiveness in migraine treatment is one of the main conditions that shape treatment.
- There are many "drug ineffective" reports for the eight calcitonin gene-related peptide antagonist drugs used in migraine treatment.
- This study has shown that half of this group of drugs positive signalled "drug ineffective".

ETHICS

Ethics Committee Approval: The research did not require ethics committee approval because the authors did not participate in data collection or know the participants in the present study.

Informed Consent: Due to the nature of the data source, patient consent was not obtained in this study.

DISCLOSURES

Financial Disclosure: The authors declared that this study received no financial support.

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RESEARCH ARTICLE

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Necrotizing Enterocolitis (NEC) in Premature Infants: A 4-Year Review of Our Cases

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Abstract

BACKGROUND/AIMS: This study aims to evaluate the outcomes of necrotizing enterocolitis (NEC) in premature infants, focusing on identifying potential biomarkers and risk factors that may guide early diagnosis and management strategies, while also addressing the limitations of current diagnostic criteria.

MATERIALS AND METHODS: A retrospective observational cohort study was conducted on 56 premature infants initially diagnosed with NEC at a single tertiary neonatal intensive care unit (NICU). Of these, 32 infants fulfilled the Modified Bell Criteria and were included in the analysis. Statistical tests were used to determine significant differences between infants with complications and those without.

RESULTS: The incidence of NEC in our NICU was 1.58%, lower than the 2-7% range typically reported in the literature. The mean gestational age was 29.25±2.99 weeks. Variability was observed in platelet and white blood cell counts, with thrombocytopenia suggested as a potential marker for more severe cases. Electrolyte disturbances, particularly changes in chloride (Cl) and sodium (Na) levels, were significantly associated with more severe NEC. Mortality was observed in 12.5% of cases, all of which were classified as stage IIIb NEC. Eight cases were excluded by the Modified Bell Criteria despite clinical diagnosis, highlighting the limitations of the current framework.

CONCLUSION: This study highlights the importance of early diagnosis and prompt intervention in NEC management. The findings suggest that electrolyte disturbances, particularly fluctuations in Na and Cl levels, may serve as predictive biomarkers of NEC severity. Furthermore, the results emphasise the need for updated diagnostic criteria to improve the accuracy and comprehensiveness of NEC detection in neonates.

Keywords: Necrotizing enterocolitis, diagnosis, electrolyte disturbances, platelet count, mortality rate

INTRODUCTION

Necrotizing enterocolitis (NEC) is a severe neonatal disease characterized by inflammation and tissue damage in the intestines.[1] The primary pathophysiological feature of the disease involves disruption of the intestinal flora and reduced resistance of the intestinal system to pathogenic microorganisms.[2] Consequently, NEC occurs more frequently in premature infants, although it can also develop-albeit less frequently in infants born at or beyond 32 weeks of gestation.[1]

Accurate diagnosis and staging of NEC are among the most critical steps in the treatment process. The most widely used classification system for this purpose is the Bell staging criteria criterion, developed by Bell et al.[3] in 1978. However, the Bell Criteria do not incorporate more

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sensitive biochemical markers or advanced imaging modalities.[3] This shortcoming limits the early and accurate identification of NEC, leading to subsequent modifications of the Bell system over time.

In recent years, numerous studies have sought to address these diagnostic gaps by investigating potential biomarkers. In particular, inflammatory protein markers have been proposed as tools for determining both the diagnosis and severity of NEC. For example, some studies have reported that interleukin-8, interleukin-24, and C-C motif chemokine ligand 20 can differentiate between stage II and stage III NEC and may also assist in distinguishing NEC from sepsis.[4] However, the routine clinical use of these biomarkers remains limited, and they are currently considered supportive but insufficient diagnostic tools on their own.

Imaging methods also hold potential in aiding NEC diagnosis. A meta-analysis that reviewed key studies reported that abdominal ultrasonography could provide sensitive information for the early detection of NEC.[5] Nevertheless, heterogeneity in methods, poor reporting quality, and the presence of uncertain bias have limited its practical use in clinical decision-making.[5]

In addition, artificial intelligence (AI) and machine learning-based models represent new and promising approaches, as they analyse multivariable clinical, and laboratory data to predict NEC risk.[6] Recent studies[6-8] have suggested that such models may facilitate both earlier and more accurate NEC diagnosis, while also reducing subjectivity in interpretation. However, these technologies still face limitations regarding data variability, model transparency, and clinical validity.[6-8]

In this context, the adequacy of the Bell Criteria-still regarded as the "gold standard" for NEC diagnosis remains controversial when considered against current technological advancements and clinical requirements.[7-10] The aim of the present study is therefore to evaluate NEC through the framework of the Bell Criteria and to question its current validity, as repeatedly emphasised in the literature.

MATERIALS AND METHODS

Study Location and Duration

This study was conducted at a single tertiary-care neonatal intensive care unit (NICU) in Türkiye. The study period spanned from January 1, 2020, to October 24, 2023, covering a total duration of nearly four years. During this period, cases of neonates diagnosed with NEC were retrospectively analyzed.

The study was conducted in strict accordance with the ethical principles outlined in the Declaration of Helsinki. This study was approved by the Karabük University Non-Interventional Clinical Research Ethics Committee (approval number: 2023/1495, date: 08.11.2023). Informed consent forms were obtained from all participants in the study.

Inclusion and Exclusion Criteria

Inclusion criteria:

- **Diagnosis of NEC:** All patients included in the study must have been diagnosed with NEC according to the international classification of diseases (ICD) code P77.
- **Gestational age:** Only premature infants born between 24 and 32 weeks of gestation were included in the study. Infants born at 32 weeks + 0 days (32w + 0d), were included, while those born at 32w + 1d or later were excluded from the study.
- **Clinical stage according to the Modified Bell Criteria:** Only infants diagnosed with NEC according to the Modified Bell Criteria were included. These criteria assess the severity of the disease from stage I (suspected) to stage III (severe), including conditions such as intestinal perforation.
- **Laboratory and radiological findings:** Infants showing laboratory signs such as elevated leukocyte count, increased C-reactive protein (CRP), and radiological findings consistent with NEC (e.g., bowel dilation, free air in the abdominal cavity) were included in the study.

Exclusion criteria:

- **Failure to meet NEC criteria:** Infants who did not meet the Modified Bell Criteria for NEC were excluded. This includes cases with severe gastrointestinal disorders but without a diagnosis of NEC.
- **Severe genetic or congenital anomalies:** Infants with severe genetic syndromes or significant congenital anomalies, such as major cardiac defects, were excluded from the study.
- Infants born at or after 32 weeks and 1 day of gestation who were diagnosed with NEC were excluded.
- **Missing data:** Infants with incomplete clinical or laboratory data necessary for staging NEC and assessing its progression were excluded from the study. These data are essential for accurately staging the disease and monitoring its course.
- **Other gastrointestinal disorders:** Infants with other gastrointestinal conditions that may mimic NEC (e.g., Hirschsprung disease or intestinal atresia) were excluded from the study.

Identification of Study Participants

Initially, 56 patient records were selected based on the neonatal NEC diagnosis, identified through the hospital's P77 automation system, according to the ICD classification through the hospital's P77 automation system, according to the ICD classification. Each patient record was then reviewed and re-assessed based on the Modified Bell Criteria. Of the 56 records, only 32 patients met the criteria for NEC diagnosis, while the remaining 24 patients did not. However, it is important to note that these 24 patients were all clinically diagnosed with NEC by the attending physicians. Among them, 14 patients were excluded because their gestational age was over 32 weeks of gestation as the study was restricted to patients born at or before 32 weeks. Furthermore, 2 patients with known genetic conditions were excluded accordingly, according to the study protocol's exclusion criteria for genetic disorders. Additionally, three patient records were excluded due to insufficient data, which led to their removal from the study. As a result, the remaining five patients, despite not meeting the Modified Bell Criteria, had been diagnosed with NEC by the attending physicians and were recorded as such in the hospital's P77 automation system.

Biomarkers and Analytical Parameters

In this study, various laboratory parameters and biomarkers were assessed to evaluate the clinical progression and severity of NEC. These included common laboratory values such as white blood cell (WBC) count, platelet (PLT) count, CRP, sodium (Na), potassium (K), chloride (Cl), urea, creatinine (Cr), and liver enzymes [alanine

aminotransferase (ALT) and aspartate aminotransferase (AST)]. These parameters were collected and analyzed to monitor the patient's condition and to help identify any deviations indicative of NEC progression or complications.

Additionally, clinical data was recorded, including:

1. **Average gestational age:** The mean gestational age at birth for the study participants.
2. **Average length of hospitalization:** The average duration of hospital stay for patients diagnosed with NEC.
3. **Mortality rate**: The percentage of patients who experienced mortality during the study period.
4. **Recovery rate:** The percentage of patients who showed complete recovery from NEC.
5. **NEC incidence:** The frequency of NEC diagnoses within the study population, helping to evaluate the incidence rate during the specified study period.

These biomarkers and clinical parameters were used to assess the disease's clinical course, predict potential outcomes, and guide therapeutic decisions.

Data Collection and Validation

Data for this study were retrieved from the hospital's electronic health record system, which provided past patient records. Neonates diagnosed with NEC, according to the ICD classification, specifically those with the diagnosis of "P77 - fetal and neonatal NEC," were identified. Neonates with the diagnosis of "P77 - fetal and neonatal NEC," according to the ICD classification, specifically those diagnosed with NEC, were identified.

Statistical Analysis

The data were analyzed using Intellectus Statistics (online software). The normality of the distribution was assessed using the Shapiro-Wilk test, which indicated that the data were not normally distributed. Descriptive statistics for continuous variables are reported as mean, minimum, and maximum values, while categorical variables are presented as frequencies and percentages. For blood parameters, mean and standard deviation values are reported, with p-values <0.05 considered statistically significant.

RESULTS

During the study period, a total of 2015 patients were admitted to our NICU. Among these, 32 cases fulfilled the Modified Bell Criteria for NEC diagnosis, yielding an NEC incidence of 1.58% among NICU admissions. Notably, this incidence does not represent the incidence among all births but rather within the admitted NICU population. Additionally, due to the retrospective nature of the study, some clinically diagnosed NEC cases may not have been recorded, which could lead to underestimation. This limitation is inherent to retrospective studies and should be considered when interpreting the incidence data.

The mean gestational age was 29.25±2.99 weeks. The minimum gestational age was 23 weeks, and the maximum gestational age was 32 weeks. The median gestational age was 30.5 weeks, indicating that 50% of the infants were born before 30.5 weeks and 50% after. Additionally, the median gestational age of 30.5 weeks suggests that the majority of infants were born between 28 and 32 weeks (Table 1).

Table 1. Descriptive characteristics of the neonates

Characteristics	Mean ± SD	Min.-Max.
Gestational age	29.25±2.99	(23-32)
Hospitalization duration (days)	35.62±17.05	(6-65)
Mortality	**n**	**%**
- No mortality	28	87.50
- Mortality present	4	12.50
Recovery		
- Recovery present	28	87.50
- No recovery	4	12.50
Complication status		
- No complications	26	81.25
- Complications present	6	18.75
Risk factors		
- Prematurity, sepsis, antibiotic usage	10	31.25
- Prematurity, antibiotic usage	22	68.75
Complications		
- Perforation	6	18.75
- No perforation	26	81.25

SD: Standard deviation, Min.: Minimum, Max.: Maximum.

The average length of hospital stay for the patients was 35.62 days (±17.05 days), with a range between 6 and 65 days. Out of the 32 patients, 4 died, resulting in a mortality rate of 12.5% and a recovery rate of 87.5%.

When examining the complication status, it was found that 81.25% of the infants did not experience any complications, while 18.75% developed complications. Among the complications, perforation was the most frequently encountered condition, identified in 18.75% of the cases. In terms of risk factors, 31.25% of the infants had a combination of prematurity, sepsis, and antibiotic use, while 68.75% had both prematurity and antibiotic use. These findings indicate that prematurity and antibiotic use are prevalent risk factors in this patient group.

Clinical Staging and Outcomes

According to the Modified Bell Criteria, the patients were distributed as follows:

- **Stage I (suspicious NEC):** 14 patients (43.8%)
- **Stage II (localized NEC):** 10 patients (31.2%)
- **Stage III (advanced NEC):** 4 patients (12.5%)
- **Stage IIIb (IV) (perforation):** 4 patients (12.5%)

Laboratory Findings

Several laboratory parameters were evaluated to assess the progression and severity of NEC. Table 2 compares the laboratory findings of infants with and without complications.

The average gestational age of infants with complications was 26.67±4.23 weeks, while the average gestational age of those without complications was 29.85±2.36 weeks. This difference was not statistically significant (p=0.107). However, the length of hospital stay for infants with complications (11.67±8.02 days,) was significantly longer compared to those without complications (41.15±13.34 days, p=0.000).

No significant difference was found between infants with and without complications in terms of WBC count (p=0.480). Similarly, PLT count did not show a significant difference between the two groups (p=0.225).

Although this difference is borderline statistically significant (p=0.069), Na levels tend to be lower in infants with complications (135±2.68) compared to those without complications (138.23±4.16), although this difference is borderline statistically significant (p=0.069). Cl levels were significantly higher in infants with complications (112.33±1.03) than in those without complications (106.38±6.28) (p=0.014). This finding indicates that electrolyte imbalances occur more often in infants with complications. As for K levels, no significant difference was found between the two groups (p=0.308).

Urea levels tend to be higher in infants with complications (73.67±80.85) compared to those without complications (32.46±57.45), although this difference is only borderline statistically significant (p=0.069). No significant difference was found between the two groups in terms of Cr levels (p=0.189). AST levels did not show a significant difference between infants with and without complications (p=0.480). However, ALT levels were significantly lower in infants with complications (5.00±0.89) compared to those without complications (28.38±35.2) (p=0.000) (Table 2).

Given that the dataset did not follow a normal distribution and the sample size was relatively small, non-parametric statistical tests were used in this analysis. These tests were selected to provide reliable comparisons between groups and to appropriately account for potential skewness in the data.

DISCUSSION

In our study, the NEC incidence among NICU admissions was 1.58%, which is lower than the 2-7% range commonly reported in the literature.[9] This may reflect the effectiveness of treatment protocols or changes in NEC diagnostic criteria. McElroy and Lueschow.[8] highlighted adjustments in diagnostic approaches for premature infants that may explain this decrease. Although machine learning tools are not used in our unit, the low incidence likely reflects advances in neonatal care-such as improved feeding strategies, immune support, and antibiotic use-which facilitate the early NEC management and contribute to reduced mortality, supporting the success of our protocols.

According to our findings, the mean gestational age of premature infants was 29.25±2.99 weeks, with a median gestational age of 30.5 weeks. Our findings align with the literature, which indicates that NEC is more commonly observed in infants born between 28 and 32 weeks of gestation.[10-13] The clinical implication of these findings highlights the necessity for more vigilant monitoring for NEC in preterm infants and emphases the need for improved management strategies for this high-risk group.

The analysis revealed that the average length of hospital stay for patients was 35.62±17.05 days. This duration is comparable to the reported hospitalisation times for medical NEC cases in the study by Velazco et al.[14] However, in Velazco et al.[14] research, the average hospital stay for NEC patients who underwent surgical treatment was reported as 63±36.94 days (medical group: 34±22.61 days). This finding aligns with the general trend in the literature, which associates longer hospital stays with NEC cases requiring surgical intervention.[14,15] In our study, however, all patients were evaluated together, without separately analyzing the surgical and medical treatment groups, leading to an overall calculation of hospital stay. In this context, one might have expected longer hospitalisation times by focusing solely on medical NEC cases; nevertheless, similar results were obtained as those observed in Velazco et al.[14] study, with shorter hospitalisation durations. Several factors could play a role in these discrepancies in length of stay. However, the most likely explanation may stem from the

Table 2. Impact of complication status on laboratory findings

Features	Complication				Test used*	p-value
	Perforation		Absent			
	Mean ± SD	Median (Min.- Max.)	Mean ± SD	Median (Min.- Max.)		
Gestasyonal age	26.67±4.23	25 (23-32)	29.85±2.36	31 (25-32)	1.692	0.107
Hospitalization duration (days)	11.67±8.02	7 (6-22)	41.15±13.34	38 (22-65)	3.680	**0.000**
WBC	128.33±106.79	6.6 (5.3-26.6)	209.92±257.33	8.9 (3.7-22.9)	0.870	0.480
PLT	200.33±183.74	124 (44-433)	263±123.39	235 (47-541)	1.258	0.225
Na	135±2.68	135 (132-138)	138.23±4.16	137 (134-151)	1.857	0.069
K	53±8.530	5.8 (4.2-5.9)	48.46±7.71	4.8 (3.2-6.1)	-1.066	0.308
Cl	112.33±1.03	113 (111-113)	106.38±6.28	104 (96-120)	-2.428	**0.014**
Urea	73.67±80.85	24 (19-178)	32.46±57.45	9 (3- 220)	-1.840	0.069
Creatinine	9.67±8.78	0.4 (0.4-2.1)	18±46.2	0.3 (0.07-1.7)	-1.368	0.189
AST	44±41.91	20 (14-98)	40.46±21.59	37 (18-76)	0.870	0.480
ALT	5.00±0.89	5 (4-6)	28.38±35.2	14 (4-33)	3.295	**0.000**

*Shapiro-Wilk test.
WBC: White blood cell, PLT: Platelet, Na: Sodium, K: Potassium, Cl: Chloride, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, SD: Standard deviation, Min.: Minimum, Max.: Maximum.

diversity in hospital treatment protocols, patient care processes, and clinical management strategies.

In our study, the mortality rate was determined to be 12.5% (4/32), and all mortality cases occurred in patients in stage IIIb (intestinal perforation). This finding is consistent with the high mortality rates observed in surgical NEC cases in the study by Flahive et al.[16] Flahive et al.[16] reported a mortality rate of 23.5% in stage II and higher NEC cases, with the rate rising to 50.9% in infants with low birth weight (<1000 g). Other studies in the literature have also indicated that mortality rates in surgical NEC cases can range from 30% to 50%.[16-18] The lower mortality rate observed in our study may be attributed to the fact that, in the majority of cases, early intervention led to recovery without the need for surgical intervention. Nevertheless, it was observed in our study that mortality rates in surgical NEC cases remain significantly higher, which is consistent with the current literature.

Our data highlight the importance of monitoring electrolyte disturbances, such as increased Cl and decreased Na levels, in the early identification of severe forms of NEC. These findings are in line with previous studies that have explored the biochemical alterations in NEC and their correlation with disease severity.[19,20] The significant relationship of Cl and Na fluctuations with the risk of complications suggests that these biomarkers could potentially offer predictive insight into the clinical course of NEC, particularly if monitored alongside other emerging markers such as inflammatory cytokines and gut-specific proteins. Understanding electrolyte imbalances in relation to other biomarkers could provide predictive insights and guide interventions. Another critical finding is the increase in urea levels, which monitors kidney function. Leading studies in the literature indicate that such increases are critical in advanced-stage NEC cases, particularly those requiring surgical intervention, and could elevate the mortality risk of patients.[21]

In our study, the mean PLT count in the overall population showed a wide variabilityvaried widely. This finding suggests that some infants may have had thrombocytopenia. However, there was no significant difference in PLT counts between infants with and without complications. Although this variability was not statistically significant, the existing literature suggests that thrombocytopenia may reflect disease progression.[20,22]

Furthermore, although K and urea levels were not statistically significant, Na and Cl levels were found to be significant. In particular, the detection of pronounced hyperchloremia and hyponatremia in infants with complications indicates that these electrolyte disturbances may be associated with severe forms of NEC. This finding suggests that Cl and Na levels may serve not only as markers for disease monitoring but also as biomarkers for early diagnosis. The relationship between electrolyte imbalances and NEC severity is consistent with previous literature.[19] These parameters may provide clinicians with important clues in predicting NEC severity.

The relationship between risk factors, including prematurity and antibiotic usage, also emerged as a significant finding in this study. The combination of prematurity and antibiotic use accounted for the majority of medical cases, with 68.75% of infants exposed to antibiotics in conjunction with prematurity. This is consistent with findings in other studies suggesting that antibiotic therapy, particularly the use of broad-spectrum antibiotics in premature infants, may disrupt the normal gut flora and contribute to the pathogenesis of NEC.[23] This highlights the need to carefully manage antibiotic exposure in this vulnerable population and suggests that antimicrobial stewardship strategies should be a key component of NEC prevention.

In this study, 32 cases of NEC were confirmed using the Bell Criteria; however, the NEC diagnosis for the remaining 5 patients, although being clinically established by clinicians, could not be validated. As a result, while a total of 37 NEC diagnoses should have been made, only 32 patients received this diagnosis, leading to a diagnostic loss of approximately 13.5%. This situation caused an incomplete diagnosis in these patients. The inadequacy of the Bell Criteria in diagnosing NEC is not specific to this study. Therefore, the growing need for more sensitive and specific diagnostic methods is frequently emphasized in the literature.[7,8] This finding demonstrates that the Bell Criteria are insufficient in certain cases and emphasizes the necessity for new diagnostic methods to ensure accurate and timely diagnosis of NEC, particularly in its early stages.

Although our study included both medically and surgically managed NEC cases, the small number of surgical cases (n=4) precluded subgroup analysis. This represents a limitation, as stratification by treatment modality could provide further insights into outcome differences. Future multicentre studies with larger cohorts are needed to address this gap.

Study Limitations

This study had a retrospective design, which may have caused some clinically diagnosed but unrecorded NEC cases to be missed. The NEC incidence was calculated only among patients admitted to the NICU and therefore does not reflect the general birth population. The presence of outliers in some parameters, such as electrolyte values, may have limited the reliability of the statistical analyses. In addition, the relatively small sample size and the study being conducted in a center may restrict the generalisability of our findings.

CONCLUSION

Our findings highlight the need for multidimensional diagnostic approaches. In particular, the significant association of electrolyte disturbances such as hyponatremia and hyperchloremia with NEC suggests that these parameters may serve as potential biomarkers in diagnosis and prognosis. The integration of such laboratory data with AI algorithms may enable the development of more sensitive and earlier diagnostic models for NEC.

MAIN POINTS

- Changes in chloride and sodium levels were significantly associated with necrotizing enterocolitis (NEC) severity.
- All mortality cases occurred in infants with stage IIIb NEC, indicating high-risk at advanced stages.
- The Bell Criteria failed to capture some clinically diagnosed cases, highlighting diagnostic limitations.

ETHICS

Ethics Committee Approval: The study was conducted in strict accordance with the ethical principles outlined in the Declaration of Helsinki. This study was approved by the Karabük University Non-Interventional Clinical Research Ethics Committee (approval number: 2023/1495, date: 08.11.2023).

Informed Consent: Informed consent forms were obtained from all participants in the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Y.D., S.E., Concept: Y.D., B.D., Design: Y.D., Data Collection and/or Processing: B.D., G.Ş.B., Analysis and/or Interpretation: B.D., G.Ş.B., Literature Search: Y.D., S.E., Writing: Y.D.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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RESEARCH ARTICLE

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Multicenter and Multivariate Analysis of Complications Associated with Biliary and Vascular Anomalies in Patients Who Underwent Laparoscopic Cholecystectomy

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Abstract

BACKGROUND/AIMS: In this retrospective study, the aim was to analyze complications associated with biliary and vascular anomalies in patients who underwent cholecystectomy.

MATERIALS AND METHODS: A total of 925 patients who underwent cholecystectomy between 1993 and 2018 were included in the study. The patients' gender, age, diagnosis, comorbidities, antibiotic use, drainage use and duration, operation, hospital stay, and complications were analyzed.

RESULTS: Mean age was significantly higher in the no-anomaly group ($p<0.05$). Drainage and duration of hospitalization were significantly higher in the biliary anomaly group ($p<0.05$). Differences in gender, diagnosis, endoscopic retrograde cholangiopancreatography, hypertension, diabetes mellitus, chronic artery disease, chronic obstructive pulmonary disease, antibiotic usage, drainage, and operation duration between anomaly groups were not significant ($p>0.05$). Having an anomaly was significantly correlated with hospitalization duration ($r=0.088$; $p<0.01$). Biliary anomaly was also significantly correlated with hospitalization duration ($r=0.105$; $p<0.05$). Vascular anomaly was not significantly correlated with the research parameters ($p>0.05$). Complications were significantly correlated with gender ($r=0.097$; $p<0.01$), diagnosis ($r=-0.072$; $p<0.05$), operation duration ($r=0.129$; $p<0.01$), hospitalization duration ($r=0.257$; $p<0.01$), biliary anomaly ($r=0.127$; $p<0.01$), and no anomaly ($r=-0.122$, $p<0.01$). The effect of operation duration on complications was significant at the multivariate level ($B=0.033$; $p<0.01$). Receiver operating characteristic analysis showed that the area under the curve for operation time was 0.701, indicating that operation time has significant predictive value at the 70.1% level for complications in cholecystectomy patients. When operation time exceeded 29 minutes, sensitivity for complications was 82.8% and specificity was 37.7%. When operation time exceeded 31 minutes, sensitivity was 72.4% and specificity was 59.2% for complications.

CONCLUSION: Prolonged operation times in cholecystectomy patients with biliary anomalies significantly increase the risk of complications compared with the non-anomaly and vascular anomaly groups. Depending on the duration of the operation, complications may be predicted and precautions taken.

Keywords: Cholecystectomy, anomaly, biliary, vascular anomaly, complication

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INTRODUCTION

Cholecystectomy, performed when the gallbladder is non-functional and requires removal, is considered the gold standard for most gallbladder pathologies, including acute cholecystitis.[1] With the development of laparoscopic methods, interest in laparoscopic cholecystectomy (LC) has increased, and it has become a minimally invasive alternative to open cholecystectomy.[2] After LC, patients can be discharged after a stay of less than two days, and return to work within two weeks, and have a mortality rate of less than 0.2%.[3] Patients can usually resume their daily diet within one to two days after the operation and report very low levels of pain.[4] On the other hand, complications can still develop even when the minimally invasive approach is used.

Biliary atresia (BA) is an anomaly affecting the pancreas and biliary system.[5] BA is a pathological condition that occurs after bile duct obstruction, which begins before or shortly after birth.[6] Anomalies that are more common in patients with congenital aberrations of the gallbladder are rare but important in surgical operations.[7] BA occurs in 0.5-0.8 per 10,000 births in Western countries, whereas rates are higher in Eastern countries.[8] Although the incidence is low, studies have reported severe inflammation and complications in patients with BA.[9] A vascular anomaly, another biliary anomaly, has been associated with choledochal cysts.[10] Vascular anomalies in infants and children may present with ascites or abdominal pain and may also be detected on imaging.[11] BA, which is more common in girls than in boys, may cause complications during surgical procedures.[12] Because the gallbladder and biliary tree are connected to the portal venous system, venous drainage may predispose them to lesser-known vascular events, such as gallbladder bed perfusion abnormalities[13], which are associated with postoperative complications.[14]

Studies have reported bile leakage, iatrogenic injuries, and other complications following cholecystectomy in patients with biliary and vascular anomalies.[15-17] However, there were insufficient studies examining the complications of these anomalies. Therefore, this study aimed to analyze complications in patients with biliary and vascular anomalies who underwent LC.

MATERIALS AND METHODS

Research Model

The study used a retrospective, mixed-model design that included descriptive and relational screening models. In the study, patients who underwent LC over a 25-year period were analyzed, and the relationships between complications and patients' clinical and demographic parameters were examined.

Patients

The study included 925 patients who applied for and underwent surgery for LC at different health centers between 1993 and 2018. Patient selection was based on patients individuals who had been treated by the researchers' teams throughout their careers at the centers where they had worked. Although there were differences between institutions differed, the research team was the same across institutions, and a similar procedure was performed on all patients. Using the time-based sampling method, all patients with 25 years' experience who met the inclusion criteria were included in the study.

The inclusion criteria were as follows:

- Being 18 years of age or older,
- Having had LC,
- Not having any comorbidities that would prevent the study,
- Patient data must be complete in the file,

The exclusion criteria for the study were as follows:

- Being under 18 years of age,
- Having missing data in the file,
- Having a health condition that would affect follow-up and research,

USG results may be subject to deviations in the identification of identifying biliary and vascular anomalies. Therefore, during the operation, the surgeon performed an intraoperative examination and made a decision. In cases where observational exploration was inconclusive, the decision was supported by postoperative radiological examination.

Data Set

Data on gender, age, diagnosis, endoscopic retrograde cholangiopancreatography (ERCP), comorbid diseases, antibiotic use, drainage, length of stay, and complications were obtained from patient files. Patients were also divided into anomaly groups.

Ethical Approval

Ethical approval was obtained from the Üsküdar University Non-Interventional Research Ethics Committee (approval number: 04, date: 26.05.2025). Because the research involved a retrospective review of medical records, patient consent forms were not obtained. The study was conducted in accordance with the Declaration of Helsinki. Due to the retrospective nature of the study, Üsküdar University Non-Interventional Research Ethics Committee waived the need to obtain informed consent.

Statistical Analysis

Frequency analysis was used to describe nominal and ordinal data, and Fisher's exact and chi-square tests were used to assess differences in these data. Measurement data were summarized using the mean, standard deviation, median, and range. The Kolmogorov-Smirnov test was used to assess the normality of the measurement data. The Kruskal-Wallis test was used to analyze differences in the measurement data. Binary logistic regression analysis was performed because Spearman's rho correlation and linearization deviations[18,19] were observed during relational screening analysis. Receiver operating characteristic (ROC) analysis was performed to evaluate the diagnostic value of operation time for predicting complications. All analyses were performed using SPSS 25.0 for Windows at the 95% confidence level.

RESULTS

Age mean was significantly higher in no-anomaly group ($p<0.05$). Drainage and hospitalization duration were significantly higher in Biliary anomaly group ($p<0.05$). Differences between anomaly groups in gender, diagnosis, ERCP, hypertension, diabetes mellitus, chronic

artery disease, chronic obstructive pulmonary disease, antibiotic usage, drainage, and operation duration were not significant (p>0.05) (Table 1).

Having an anomaly was significantly correlated with duration of hospitalization (r=-0.088, p<0.01). Biliary anomaly was also significantly correlated with hospitalization duration (r=0.105; p<0.05). Vascular anomaly was not significantly correlated with research parameters (p>0.05) (Table 2).

Spearman's rho correlation analysis showed that complication was significantly correlated with gender (r=0.097, p<0.01), diagnosis (r=-0.072, p<0.05), operation duration (r=0.129, p<0.01), hospitalization duration (r=0.257, p<0.01), biliary anomaly (r=0.127, p<0.01), and noanomaly (r=-0.122, p<0.01) (Table 3).

Binary logistic regression analysis showed that only the effect of operation duration on complications was significant at the multivariate level (B=0.033; p<0.01). The effects of gender, diagnosis, hospitalization duration, biliary anomaly (presence versus absence) were not statistically significant (p>0.05) (Table 4).

In the no-anomaly group, complications were significantly correlated with gender (r=-0.106, p<0.01), operation duration (r=-0.127, p<0.01), drainage (r=-0.084, p<0.05), and hospitalization duration (r=-0.233, p<0.01). In the biliary anomaly group, complications were significantly correlated with hospitalization duration (r=0.752; p<0.01) (Table 5).

The complication rate was highest in the biliary anomaly group (26.1%), followed by the vascular anomaly group (12.5%) and the no-anomaly group (Figure 1).

Among patients who developed complications, hospitalization duration was highest in the biliary anomaly group, followed by the no-anomaly and vascular anomaly groups. Among patients without complications, the hospitalization duration was highest in the biliary anomaly group, followed by the no-anomaly group (Figure 2).

ROC analysis showed that the area under the curve for operation time was 0.701, indicating that operation time has a significant predictive value of 70.1% for complications in cholecystectomy patients (Figure 3). For operation time over 29 minutes, sensitivity was 82.8% and specificity was 37.7% for complication. For operation time over 31 minutes, sensitivity was 72.4% and specificity was 59.2% for complication.

Table 1. Baseline characteristics and difference analysis results between patient groups

	Anomaly			p-value
	No-anomaly (n=894; 96.6%)	Biliary (n=23; 2.5%)	Vascular (n=8; 0.9%)	
Gender, n (%)				
Female	614 (68.7)	16 (69.6)	6 (75.0)	0.923[a]
Male	280 (31.3)	7 (30.4)	2 (25.0)	
Age	52.18±14.71 52.00 (18.00-97.00)	40.35±15.86 36.00 (18.00-68.00)	41.50±11.63 40.50 (26.00-59.00)	0.001[b]
Diagnosis, n (%)				
Acute cholecystit	133 (14.9)	5 (21.7)	-	0.501[a]
Cholelitiasis	721 (80.6)	17 (73.9)	7 (87.5)	
Polyp	28 (3.1)	1 (4.3)	1 (12.5)	
Other	12 (1.3)	-		
ERCP, n (%)				
Pre operation	36 (83.7)	2 (100.0)	-	0.710[c]
Post operation	7 (16.3)	-	-	
HT, n (%)	152 (17.0)	2 (8.7)	1 (12.5)	0.494[a]
DM, n (%)	30 (3.4)	1 (4.3)	-	0.737[a]
CAD, n (%)	78 (8.7)	3 (13.0)	-	0.380[a]
COPD, n (%)	12 (1.3)	-	-	0.662[a]
Antibiotic usage, n (%)				
Single	529 (92.5)	14 (100.0)	4 (100.0)	0.597[a]
Dual	40 (7.0)	-	-	
Mixed	3 (0.5)	-	-	
Operation duration	36.20±19.92 30.00 (10.00-180.00)	39.21±14.07 35.00 (20.00-80.00)	32.86±26.12 30.00 (15.00-90.00)	0.118[b]
Drainage, n (%)	611 (74.2)	22 (96.7)	7 (87.5)	0.016[a]
Hospitalization duration	3.09±3.10 2.00 (0.00-36.00)	5.23±4.73 3.00 (2.00-18.00)	2.75±2.06 2.50 (1.00-5.00)	0.042[b]
Complication, n (%)	50 (6.8)	6 (26.1)	1 (12.5)	0.018[a]

[a]: Chi-square test, [b]: Kruskal-Wallis test, [c]: Fisher's exact test.
HT: Hypertension, DM: Diabetes mellitus, CAD: Chronic artery disease, COPD: Chronic obstructive pulmonary disease, ERCP: Endoscopic retrograde cholangiopancreatography.

Table 2. Spearman's rho correlation between having anomaly and research parameters according to anomaly groups

	No-anomaly		Biliary		Vascular	
	r	p	r	p	r	p
Gender	0.009	0.787	-0.003	0.933	-0.013	0.702
Diagnosis	-0.002	0.956	-0.027	0.411	0.049	0.137
Operation duration	-0.030	0.392	0.060	0.086	-0.041	0.240
ERCP	0.093	0.545	-0.093	0.545		
Hospitalization duration	**-0.088***	0.034	**0.105***	0.012	-0.007	0.865

*p<0.05.
ERCP: Endoscopic retrograde cholangiopancreatography.

Table 3. Spearman's rho correlation between complication and research parameters

Complication	r	p
Gender	**0.097****	0.003
Age	0.045	0.175
Diagnosis	**-0.072***	0.029
Operation duration	**0.129****	0.000
Hospitalization duration	**0.257****	0.000
Biliary	**0.127****	0.000
Vascular	0.023	0.488
No-anomaly	**-0.122****	0.000

*p<0.05, **p<0.01.

Table 4. Binary logistic regression analysis on effect of significant correlated parameters on complication

	B	S.E.	Wald	df	p	OR	95% CI for OR	
							Lower	Upper
Gender	-0.883	0.525	2.828	1	0.093	0.413	0.148	1.158
Diagnosis			2.863	3	0.413			
Acute cholecystit	-1.233	1.226	1.012	1	0.314	0.291	0.026	3.221
Cholelitiasis	-1.501	1.180	1.620	1	0.203	0.223	0.022	2.250
Polyp	-0.082	1.542	0.003	1	0.957	0.921	0.045	18.902
Operation duration	0.033	0.007	19.323	1	0.000	1.033	1.018	1.048
Hospitalization duration	0.108	0.061	3.099	1	0.078	1.114	0.988	1.257
Biliary	-20.784	16525.532	0.000	1	0.999	0.000	0.000	.
No-anomaly	-19.297	16525.532	0.000	1	0.999	24.194	0.000	.
Constant	-1.963	1.627	1.456	1	0.228	0.140		

CI: Confidence interval, OR: Odds ratio, S.E.: Standard error.

DISCUSSION

In this study, the factors affecting complications in BA and Variations in anatomy (VA) cases complications after cholecystectomy in BA cases were analyzed. The results showed that, in BA cases, longer operation time was significantly associated with a higher risk of complications.

LC cholecystectomy is a procedure associated with lower patient mortality and morbidity and with faster recovery compared with open cholecystectomy.[20] However, in cases of anomalies such as BA and VA, patients may experience undesirable surgical outcomes or complications.[21-25] Lee et al.[21] reported that reoperation is required in cases of major biliary injury in laparoscopic approach (LA) operations, and described the relationship between biliary injury and complications. Radunovic et al.[22] reported that major BA and VA complications after LA are clinically significant and may be more likely to result in mortality than other complications. Alexander et al.[23] reported that LC soneal complications vary and that the BA parameter is important among them. Deziel et al.[24] reported in their studies that although cholecystectomy performed with LA is associated with a low complication rate, anomalies may increase this rate. Murphy et al.[25] examined the main causes of complications after LA and reported that patient-related factors were the most important cause of major complications. Kim et al.[26] evaluated hepato-biliary-pancreatic cancercancers and reported that BA diagnosis is more common after cholecystectomy. Perry et al.[27] reported that BA may be a operation delay reason for cholecystectomy timing related decisions.a reason

to delay an operation in decisions related to cholecystectomy timing. Varshney and Kapoor.[28] reported that BA surgical repair is related withrelated to cholecystectomy operationoperative parameters. Yue and Hu.[29] reported that acute BA and complications are related withassociated with cholecystectomy parameters. The conclusion from these studies is that anomalies, such as BA and VA, and patient characteristics have an important effect on complications after LA.

Although there have been studies on LA complications in the literature[21-25], there are insufficient studies correlating them with BA and VA. In this limited study, complications after LA are associated with patient characteristics and anomalies.[25] In our study, BA was significantly correlated with hospitalization duration (r=0.105). Complications were significantly correlated with gender (r=0.097), diagnosis (r=-0.072), operation duration (r=0.129), hospitalization duration (r=0.257), biliary anomaly (r=0.127), and no-anomaly (r=-0.122). The effect of operation duration on complications was significant at the multivariate level (B=0.033). According to the multivariate analysis results, the significant effect of operation duration on complications in BA cases may inform the management of the treatment process based on this variable.

Correlations between complications and gender, operation time, hospitalization duration, and the presence or absence of biliary anomaly were highly significant, whereas only a weak correlation was observed between diagnosis and complications. A weak correlation was also observed between anomaly status and hospitalization duration in the no-anomaly and biliary-anomaly groups.

Study Limitations

The most important limitation of the study is that, owing to the rarity of BA cases, there is insufficient published literature on BA complications; therefore, there are not enough studies to compare the results obtained in the study. Although complications after LA have been analyzed relatively extensively, those occurring in BA cases have been examined less frequently. Although this is a limitation of the study, it also contributes to the study's status as a pioneer in the field.

Another limitation of the study is that it is prospective; therefore, many patients are lost to follow-up. The study examines cases over a 25-year period. Because collecting and compiling patient data was less feasible in the past than it is today, significant data gaps exist, especially in patient files before 2000. This is another important limitation of the study.

Contribution of the Research to Literature and Surgical Practice

The study's most important contribution to the literature is that it examines the complications in BA and VA patients using a relational screening model and reveals the relationship between operation time and complications. In this respect, the study is designed using a relational screening model, unlike a limited number of studies with a 25-year or longer duration. This situation may provide a basis for further studies aimed at reducing the occurrence of complications in patients with BA after LC.

The contribution of the research to surgical practices is that it numerically demonstrates the relationship between operation duration and complications, and shows that when operations are prolonged for any reason, the surgeon contributes to the management of postoperative complications and recommends that these patients receive increased attention. In this respect, the research makes a positive contribution to surgical practices by addressing them in a pragmatic manner.

Table 5. Spearman's rho correlation between complication and research parameters according to anomaly groups

	No-anomaly		Biliary		Vascular	
	r	p	r	p	r	p
Gender	**-0.106****	0.001	0.037	0.865	-0.218	0.604
Age	-0.059	0.076	0.045	0.839	0.247	0.555
Diagnosis	0.063	0.058	-0.195	0.373	-0.143	0.736
Operation duration	**-0.127****	0.000	0.096	0.696		
HT	0.025	0.449	0.168	0.443	-0.143	0.736
DM	-0.032	0.337	-0.127	0.565		
CAD	-0.023	0.490	0.064	0.772		
COPD	-0.012	0.723				
Drainage	**-0.084***	0.016	-0.359	0.093	0.143	0.736
Drainage duration	-0.108	0.193				
Antibiotic	-0.070	0.095				
Hospitalization duration	**-0.233****	0.000	**0.752****	0.003		

*p<0.05, **p<0.01.
HT: Hypertension, DM: Diabetes mellitus, CAD: Chronic artery disease, COPD: Chronic obstructive pulmonary disease.

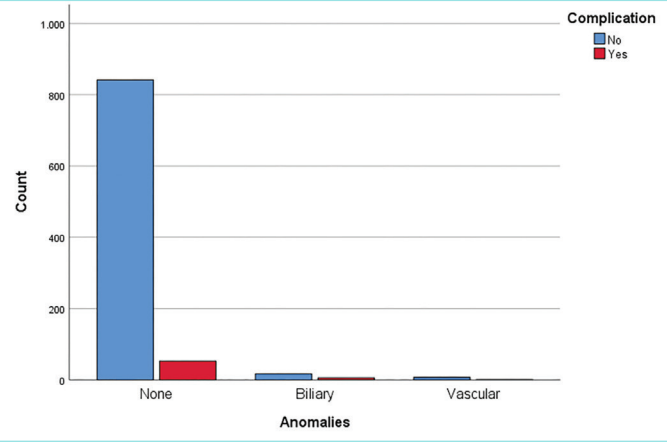

Figure 1. Complications according to anomaly groups.

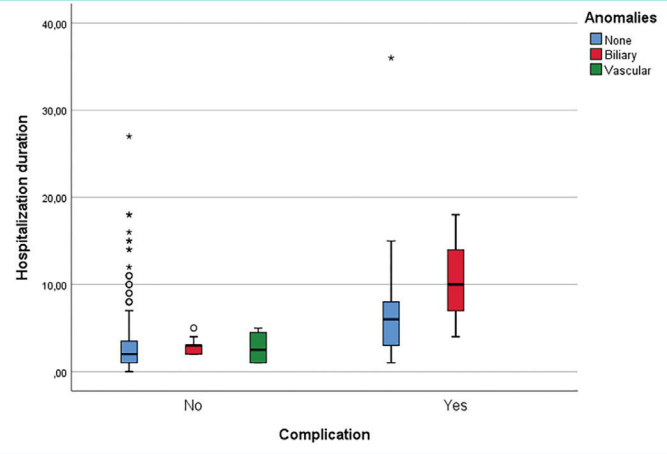

Figure 2. Hospitalization durations of anomaly groups according to complications.

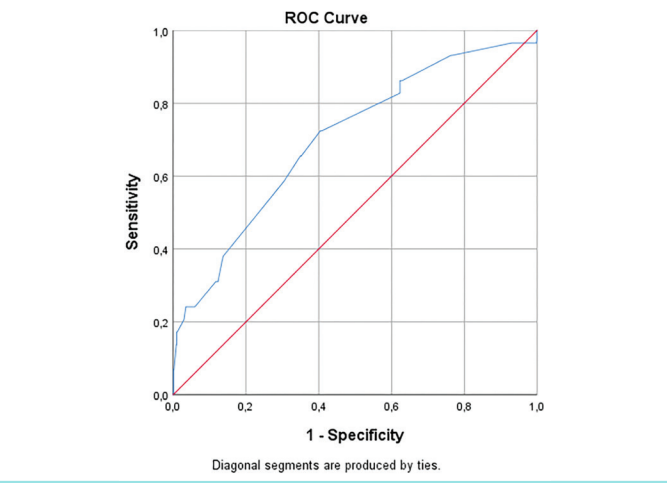

Figure 3. ROC analysis for predictive value of operation time on complication.

ROC: Receiver operating characteristic.

CONCLUSION

Prolonged operation time in cholecystectomy patients with BA is associated with a significantly higher complication rate than in the non-anomaly and VA groups. Depending on the duration of the operation, it may be possible to predict complications and take precautions. At this point, since the surgeon already tends to perform the operation as quickly and with the least-invasive procedures possible, a short operation time does not necessarily prevent complications. Complications are more commonly observed in patients undergoing prolonged operations for various reasons.

Although BA and VA are rare anomalies, they have important implications for both the health system and patients' quality of life because of postoperative complications. However, because they are rare, few studies have been conducted on them. Therefore, coordinated, multicenter studies that recruit larger patient cohorts are needed.

MAIN POINTS

- Operation time has significant predictive value for complication in cholecystectomy patients.
- Prolonged operation time in cholecystectomy patients with biliary anomalies significantly increases complications.
- Complications and take precautions in biliary anomalies may be predicted by operation time.

ETHICS

Ethics Committee Approval: Ethical approval was obtained from the Üsküdar University Non-Interventional Research Ethics Committee (approval number: 04, date: 26.05.2025).

Informed Consent: The research involved a retrospective review of medical records, patient consent forms were not obtained. Due to the retrospective nature of the study, Üsküdar University Non-Interventional Research Ethics Committee waived the need to obtain informed consent.

Footnotes

Authorship Contributions

Surgical and Medical Practices: V.M., K.E., Concept: V.M., K.E., K.Y., Design: K.E., Data Collection and/or Processing: V.M., K.E., K.Y., Analysis and/or Interpretation: K.Y., Literature Search: V.M., Writing: V.M., K.Y.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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RESEARCH ARTICLE

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Analysis of Germline Variants with Low Allele Frequencies in Turkish Healthy Cohort

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Abstract

BACKGROUND/AIMS: Within a specific population, rare variants with minor allele frequencies (MAFs) below 0.01% may underlie a particular genetic disorder when Mendelian diseases are investigated. Although MAFs can be determined by universal databases, the importance of a variant with a low MAF may vary across populations. Hence, documentation of rare variants in healthy individuals could be remarkably valuable. However, such approaches have not yet been applied in the Turkish population. Thus, we aimed to identify rare germline variants in a healthy Turkish population.

MATERIALS AND METHODS: We re-analyzed whole-exome sequencing data from 80 healthy Turkish individuals and filtered variants using a MAF threshold of <0.01%. We further assessed the pathogenicity of the filtered variants according to the American College of Medical Genetics criteria.

RESULTS: There were numerous rare variants, some of which were common to all participants. Importantly, those variants were classified as of unknown significance or as likely pathogenic; however, this classification should be revised because the variants were observed in healthy individuals.

CONCLUSION: We propose that these variants could be benign, as they were detected in healthy Turkish participants. Finally, the rare variants in the Turkish population should still be reported to guide clinicians during routine molecular diagnosis.

Keywords: Exome sequencing, Turkish population, rare variants, minor allele frequencies

INTRODUCTION

Molecular causes of genetic disorders have been identified using genetic variants. Although many variants have been reported for specific genetic disorders, additional variants remain to be discovered.[1] Thus, studies in human genetics focus on exploring variants associated with, or resulting in, a particular phenotype.[2] Here, using the single-gene single-phenotype approach, Mendelian diseases are generally diagnosed by detecting rare variants in a population.[3]

Rare variants are defined as deoxyribonucleic acid (DNA) alterations with minor allele frequencies (MAFs) of less than 0.01% in a specific population.[4] According to the American College of Medical Genetics (ACMG) criteria[5], a variant with low allelic frequency is classified with moderate piece of evidence for pathogenicity (PM2). Thus, low MAFs could be informative for evaluating the unreported variant. However, the MAF cutoff may vary between populations.[6] Moreover, unreported or not molecularly proven variants, even those with low MAFs, are classified as variants of unknown significance (VUS), which limits molecular diagnosis.[7]

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To determine the pathogenicity of a variant, population databases are required. Although global databases such as gnomAD[8], ExAc[9], and 1000 Genomes Project[10] are available, some countries have established population-specific databases to reduce biases arising from population-based differences.[11-13] Similarly, Türkiye has reported a whole-exome and whole-genome database[14] and launched a portal for variant search (https://tgd.tuseb.gov.tr/) based on data from healthy participants. Nonetheless, the portal allows only single-variant searches, restricting cumulative evaluation of variants with low MAFs. Moreover, some variants of interest were not listed in the database. Hence, a more comprehensive report on variants with low frequencies in healthy cohorts is still needed.

In the present study, we re-analyzed whole exome sequencing (WES) data from 80 healthy Turkish individuals with respect to rare variants. After filtering the variants according to MAF values (<0.01%) from universal databases, we used the Franklin by Genoox tool (https://franklin.genoox.com/)to clarify variant pathogenicity according to ACMG criteria and listed the non-benign variants. Our results indicated that numerous variants had low MAFs, including variants in critical genes that were classified as VUS or likely pathogenic (LP). However, the proportion of those variants in the healthy cohort led to the conclusion that those variants, especially those linked to early-onset genetic disorders, should be re-categorized as benign in the Turkish population. The present study is the first to underscore the importance of rare variants in healthy individuals in the Turkish population, and such studies are still required.

MATERIALS AND METHODS

Ethics and Participants

In the present study, the WES data previously obtained from 80 healthy individuals in the sport genetics studies[15,16] were re-analyzed. In previous studies, only polymorphisms of the participants were evaluated using an exome-wide association study. Sixty participants were elite Turkish athletes, and twenty were healthy, unrelated Turkish individuals. In the present study, all participants (27 females, 33.75%; 53 males, 66.25%; age >18 years) were regarded as healthy individuals and were not grouped, as none had any known (declared) genetic disorders. The study was approved by the Gazi University Non-Interventional Clinical Research Ethics Committee (approval number: 09, date: 05.04.2021). Under this ethical approval, all variants were already approved for analysis. Both written and verbal consents were obtained from the participants before the study. Moreover, the data were publicly available at https://doi.org/10.6084/m9.figshare.24496216.v1.

Variant Prioritization

In the study, data generated by WES, which was performed using the Twist Human Comprehensive Exome Panel (Twist Biosciences, USA) and the Illumina NextSeq500 (Illumina Inc., USA) on genomic DNAs from peripheral blood samples of the participants, were further examined. For the analysis of rare variants, the variants in variant call format for each participant were annotated using the ANNOVAR tool[17] with the hg19 human reference genome. Next, variants located in exons or exon-intron boundaries were prioritized using VarAFT software[18], where variants with read depth >10 were filtered by MAF <0.01%, considering only morbid genes.[19] To limit the number of variants and to avoid manipulating any possible *de novo* variants, only homozygous variants were evaluated, while hemizygous or homozygous variants on chromosome X were documented regardless of participant sex. Finally, the pathogenicity of the variants was determined using the Franklin by Genoox tool (https://franklin.genoox.com/) according to the ACMG criteria.[5] Moreover, we queried the Turkish Genome Project (TGP) database (TGP; https://tgd.tuseb.gov.tr/) for each variant, using a single-variant search when the variant was listed, and excluded variants reported in the database.

Statistical Analysis

No statistical analysis was performed in the study.

RESULTS

Each participant had nearly 700,000 variants before filtering. Applying a MAF <0.001 filter to homozygous variants in morbid genes yielded approximately 15 variants per patient. Variant assessment using Franklin by Genoox according to the ACMG criteria showed approximately three variants per patient classified as VUS or LP; on average, one such variant per patient was not documented in the TGP database. Those variants listed in Table 1 were detected in at least one participant. Variants that met ACMG criteria BS2 (observed in a homozygous state in population databases more than expected for disease), BS3 (well-established functional studies show no damaging effect on protein function or splicing), BP3 (in-frame deletions/insertions in a repetitive region with no known function), or BP6 (reported as benign but lacking evidence for independent laboratory evaluation), as well as variants classified as VUS by the Franklin tool were not reported in the results.

DISCUSSION

In the present study, we re-analyzed the WES data from 80 healthy Turkish individuals and documented homozygous rare variants that were recurrently observed in the participants and not reported in the TGP database. Regarding the early-onset status of diseases linked to genes harboring detected variants, we documented 62 variants in 60 genes associated with diverse genetic disorders.

Rare variants are regarded as absent from control (healthy) individuals, and their frequencies are extremely low in population-based genome databases.[20] Thus, they are annotated with moderate evidence of pathogenicity (PM2) according to the ACMG criteria (5). Even though marking a variant with the PM2 criterion is suggestive of pathogenicity, studies have pointed out benign or likely benign variants in the presence of the PM2 criterion.[21] Hence, reporting rare variants in healthy individuals may be useful to determine whether variants meeting the PM2 criterion are benign or likely benign. In the present cohort, 60 variants were annotated with the PM2 criterion, indicating that their frequencies were markedly low in public genome databases.

The frequency of a variant of interest can be determined using various genomic databases. Nonetheless, those databases are population-specific, and the exact frequencies vary across populations.[22] Recently, Türkiye initiated a genome project called TGP, containing genomic information from 557 so-called healthy individuals. The platform allows users to search for individual variants of interest and provides a valuable opportunity to determine variant frequencies in a manner specific to the Turkish population. However, we noted that the platform does not document variants on the sex chromosomes, particularly indel variants. Therefore, listing rare variants in healthy Turkish individuals is fundamental to the assessment of variant pathogenicity in medical genetics in Türkiye.

Table 1. List of the rare variants with VUS or LP classification and not reported in the TGP database in 80 healthy participants

Gene	Variant	Depth/allele frequency	Pathogenicity	ACMG Criteria	Disease, inheritance pattern (OMIM#)
ADAMTS17	NM_139057.4: c.353G>A: p.Arg118His	11/0.91	VUS	PM2, BP4	-Weill-Marchesani 4 syndrome, AR (613195)
ADGRG6	NM_198569.3: c.2906G>A: p. Arg969Gln	38/1.0	VUS	PM2	Lethal congenital contracture syndrome 9, AR (616503)
ASPM	NM_018136.5: c.8357C>T: p.Ala2786Val	53/1.0	VUS	PM2, BP4	-Microcephaly 5, primary, AR (608716)
ASPN	NM_017680.5: c.153_154insTGATGA: p.Asp52*	27/1.0	VUS	PM2	-{Lumbar disc degeneration}, ? (603932) -{Osteoarthritis susceptibility 3}, AD, (607850)
ATXN1	NM_001128164.2: c.678_679insGCA: p.His226_Leu227insAla	75/0.91	VUS	PM2, PM4	-Spinocerebellar ataxia 1, AD (164400)
ATXN3	NM_004993.6: c.916_917insCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAG: p.Gln305_Gly306insAlaAlaAlaAlaAlaAlaAlaAlaAlaAlaAlaAla	85/0.98	VUS	PM2, PM4	-Machado-Joseph disease, AD (109150) -{Parkinson disease, late-onset, susceptibility to}, AD (168600)
	NM_004993.6: c.917_918insC: p.Asp307Glyfs*11	79/0.96	VUS	PM2	
BPTF	NM_182641.4: c.8027_8028insCCTCCAGCCCCTCCAGCC: p.Ala2676_Pro2677insLeuGlnProLeuGlnPro	60/1.0	VUS	PM2, PM4	-{Kaposi sarcoma, susceptibility to}, AD (148000) -Neurodevelopmental disorder with dysmorphic facies and distal limb anomalies, AD (617755)
CNTNAP1	NM_003632.3: c.2992+10G>C	18/1.0	VUS	PM2, BP4	-Hypomyelinating neuropathy, congenital, 3, AR (618186) -Lethal congenital contracture syndrome 7, AR (616286)
DIP2B	NM_173602.3: c.1122G>T: p.Leu374Phe	28/1.0	VUS	PM2, PP2	-Intellectual developmental disorder, autosomal dominant, FRA12A type, AD (136630)
DKC1	NM_001363.5: c.1490_1491insAAG: p.Thr497_Lys498insSer	30/0.97	VUS	PM2, PM4	-?Cataracts, hearing impairment, nephrotic syndrome, and enterocolitis 1, XLD (301108) -Dyskeratosis congenita, X-linked, XLR (305000)
DLG5	NM_004747.4: c.4938C>G: p.Ile1646Met	81/1.0	VUS	PM2, BP4	-Yuksel-Vogel-Bauser syndrome, AR (620703)
DMD	NM_004006.3: c.5788C>T: p.Arg1930Cys	32/1.0	VUS	PM2	-Becker muscular dystrophy, XLR (300376) -Cardiomyopathy, dilated, 3B, XL (302045) -Duchenne muscular dystrophy, XLR (310200)
DMXL2	NM_001378457.1: c.88-2_88-1insTTTTTTTTTTTTT	24/1.0	VUS	PVS1, PM2	-?Deafness, autosomal dominant 71, AD (617605) -?Polyendocrine-polyneuropathy syndrome, AR (616113) -Developmental and epileptic encephalopathy 81, AR (618663)
DUOX2	NM_001363711.2: c.3175C>T: p.Arg1059Cys	60/1.0	VUS	PM2, PP3	-Thyroid dyshormonogenesis 6, AR (607200)
FAM20C	NM_020223.4: c.951_952insACAGGTGAGCCCTTCCTTCCTCCCTCCATCCGCG: p.Asp318Thrfs*118	44/0.98	VUS	PVS1, PM2, PM4	-Raine syndrome, AR (259775)
FBXL3	NM_012158.4: c.483C>G: p.Ile161Met	39/1.0	VUS	PM2	-Intellectual developmental disorder with short stature, facial anomalies, and speech defects, AR (606220)
FCSK	NM_145059.3: c.1873C>T: p.Arg625Trp	94/1.0	VUS	PM2	-Congenital disorder of glycosylation with defective fucosylation 2, AR (618324)
FLVCR1	NM_014053.4: c.1463A>G: p.Tyr488Cys	41/1.0	VUS	PM2	-Neurodevelopmental disorder with microcephaly, absent speech, and hypotonia, AR (621060) -Retinopathy-sensory neuropathy syndrome, AR (609033)
FRMPD4	NM_001368397.1: c.3862C>T: p.Arg1288Trp	55/1.0	VUS	PM2	-Intellectual developmental disorder, X-linked 104, XL (300983)

Table 1. Continued

Gene	Variant	Depth/allele frequency	Pathogenicity	ACMG Criteria	Disease, inheritance pattern (OMIM#)
GCM2	NM_004752.4: c.283G>C: p.Asp95His	53/1.0	VUS	PM2	-Hyperparathyroidism 4, AD (617343) -Hypoparathyroidism, familial isolated 2, AD/AR (618883)
GFI1	NM_005263.5: c.925-3_925-2insTCTCTC	12/0.92	VUS	PM2	-?Neutropenia, nonimmune chronic idiopathic, of adults, AD (607847) -Neutropenia, severe congenital 2, AD (613107)
HTT	NM_002111.8:c.51_52insAGCAGCAGCAGCAGC:p.Phe17_Gln18insSerSerSerSerSer	74/1.0	VUS	PM2, PM4	-Huntington disease, AD (143100) -Lopes-Maciel-Rodan syndrome, AR (617435)
INPP5E	NM_019892.6:c.1159+8_1159+9insTGGCTGGAGGGGTGGGCG	28/1.0	VUS	PM2	-Impaired intellectual development, truncal obesity, retinal dystrophy, and micropenis syndrome, AR (610156) -Joubert syndrome 1, AR (213300)
INTU	NM_015693.4: c.1091+6A>C	32/1.0	VUS	PM2, BP4	-?Orofaciodigital syndrome XVII, AR (617926) -?Short-rib thoracic dysplasia 20 with polydactyly, AR (617925)
JPH3	NM_001271604.4: c.430_431insTGCTGC: p.Leu143_Pro144insLeuLeu	104/0.98	VUS	PM2, PM4	-Huntington disease-like 2, AD (606438)
KCNN3	NM_002249.6: c.243_244insGCAGCAGCAGCAGCA: p.Pro81_Pro82insAlaAlaAlaAlaAla	93/1.0	VUS	PM2, PM4	-Zimmermann-Laband syndrome 3, AD (618658)
KDM5C	NM_004187.5:c.2517-6_2517-5insACT	22/1.0	VUS	PM2	-Intellectual developmental disorder, X-linked syndromic, Claes-Jensen type, XLR (300534)
KDM6B	NM_001348716.2:c.751_752insACCACC: p.Pro250_Leu251insTyrHis	59/0.95	VUS	PM2, BP3	-Stolerman neurodevelopmental syndrome, AD (618505)
KIF4A	NM_012310.5: c.1924A>G: p.Met642Val	22/1.0	VUS	PM2, PM4	-Intellectual developmental disorder, X-linked 100, XLR (300923) -Taurodontism, microdontia, and dens invaginatus, XLR (313490)
KLHL15	NM_030624.3: c.1805G>A: p.Arg602His	22/1.0	VUS	PM2, PP2	-Intellectual developmental disorder, X-linked 103, XLR (300982)
KRT10	NM_000421.5: c.1684_1685insAGCTCCGGCGGCGGATACGGCGGCGGCAGC: p.Ser562*	34/1.0	LP	PVS1, PM2	-?Ichthyosis histrix, Lambert type, AD (146600) -Epidermolytic hyperkeratosis 2A, AD (620150) -Epidermolytic hyperkeratosis 2B, AR (620707) -Ichthyosis with confetti, AD (609165) -Ichthyosis, annular epidermolytic 1, AD (607602)
LFNG	NM_001166355.2:c.137_138insGATG: p.Asp47Metfs*11	83/1.0	VUS	PM2	-Spondylocostal dysostosis 3, AR (609813)
LRSAM1	NM_001005373.4: c.593C>T: p.Ala198Val	67/1.0	VUS	PM2, BP4	-Charcot-Marie-Tooth disease, axonal, type 2P, AD/AR (614436)
MLC1	NM_015166.4: c.1053_1054insCGGGGAGGTGAGTGGCCTGTGGGGTGGGGGTGC: p.Ala351_Gly352insArgGlyGlyGluTrpProValGlyTrpGlyCys	41/1.0	VUS	PM2, PM4	-Megalencephalic leukoencephalopathy with subcortical cysts 1, AR (604004)
NEFH	NM_021076.4:c.1939_1940insGTCCCCT GAGAAGGCCAA:p.Ala646_Lys647insSer ProLeuArgArgPro	95/0.97	VUS	PM2, BP4	-{?Amyotrophic lateral sclerosis, susceptibility to}, AD, AR (105400) -Charcot-Marie-Tooth disease, axonal, type 2CC, AD (616924)
OGT	NM_181672.3: c.922A>G: p.Ser308Gly	33/0.97	VUS	PM2, PP2	-Intellectual developmental disorder, X-linked 106, XLR (300997)
OTC	NM_000531.6: c.76C>T: p.Arg26Trp	29/1.0	VUS	PM2, PM5, PP2	-Ornithine transcarbamylase deficiency, XL (311250)

Table 1. Continued

Gene	Variant	Depth/allele frequency	Pathogenicity	ACMG Criteria	Disease, inheritance pattern (OMIM#)
OTOG	NM_001292063.2: c.89G>A: p.Arg30His	57/1.0	VUS	PM2, BP4	-Deafness, autosomal recessive 18B, AR (614945)
PAPPA2	NM_020318.3: c.2162A>G: p.Tyr721Cys	32/1.0	VUS	PM2	-Short stature, Dauber-Argente type, AR (619489)
PAPSS2	NM_001015880.2: c.381+1_381+2insAAAAA	12/1.0	VUS	PVS1	Brachyolmia 4 with mild epiphyseal and metaphyseal changes, AR (612847)
PCNT	NM_006031.6: c.427_428insTGGGATGTTCACAGTCAGTGACCACCCACCAGAACA: p.Arg143delinsLeuGlyCysSerGlnSerValThrThrHisGlnAsnSerSer	24/1.0	VUS	PM2, PM4	-Microcephalic osteodysplastic primordial dwarfism, type II, AR (210720)
POGZ	NM_015100.4: c.3606C>G: p.Asp1202Glu	64/1.0	VUS	PM2, PP2	-White-Sutton syndrome, AD (616364)
POLR3A	NM_007055.4: c.3503A>C: p.His1168Pro	60/1.0	VUS	PM2, PP2	-Leukodystrophy, hypomyelinating, 7, with or without oligodontia and/or hypogonadotropic hypogonadism, AR (607694) -Wiedemann-Rautenstrauch syndrome, AR (264090)
POU3F4	NM_000307.5: c.517G>C: p.Gly173Arg	34/1.0	VUS	PM2	-Deafness, X-linked 2, XLR (304400)
PPFIBP1	NM_003622.4:c.2764A>G:p.Met922Val	72/1.0	VUS	PM2	-Neurodevelopmental disorder with seizures, microcephaly, and brain abnormalities, AR (620024)
RBM10	NM_005676.5: c.1166T>C: p.Met389Thr	34/0.97	VUS	PM2, PP2, BP4	-TARP syndrome, XLR (311900)
SEC63	NM_007214.5: c.1936-6_1936-5dup	18/.94	VUS	-	-Polycystic liver disease 2, AD (617004)
SH3BP2	NM_001122681.2: c.1507G>A: p.Glu503Lys	52/1.0	VUS	PM2	-Cherubism, AD (118400)
SLC12A2	NM_001046.3: c.284_285insGCGGCGGCGGCG: p.Ala95_Ala96insArgArgArgArg	14/0.93	VUS	PM2, PM4	-Deafness, autosomal dominant 78, AD (619081) -Delpire-McNeill syndrome, AD (619083) -Kilquist syndrome, AR (619080)
SLC4A10	NM_001178016.2: c.81+2T>C	49/1.0	VUS	PM2	-Neurodevelopmental disorder with hypotonia and characteristic brain abnormalities, AR (620746)
SLC44A4	NM_025257.3: c.845T>C: p.Val282Ala	73/1.0	VUS	PM2, BP4	-?Deafness, autosomal dominant 72, AD (617606)
SLC9A6	NM_001379110.1: c.1581+8T>A	32/1.0	VUS	PM2	-Intellectual developmental disorder, X-linked syndromic, Christianson type, XL (300243)
SMC1A	NM_006306.4: c.1911+3G>A	54/1.0	VUS	PM2, BP4	-Cornelia de Lange syndrome 2, XLD (300590) -Developmental and epileptic encephalopathy 85, with or without midline brain defects, XLD (301044)
SMS	NM_004595.5: c.536G>A: p.Arg179Gln	66/1.0	VUS	PM2, PP2	-Intellectual developmental disorder, X-linked syndromic, Snyder-Robinson type, XLR (309583)
SPNS2	NM_001124758.3: c.1607+5dup	43/1.0	VUS	PM2, PP3	-?Deafness, autosomal recessive 115, AR (618457)
SV2A	NM_014849.5: c.730G>T: p.Val244Phe	57/1.0	VUS	PM2	-Developmental and epileptic encephalopathy 113, AR (620772)
TTC8	NM_144596.4: c.909G>C: p.Glu303Asp	51/1.0	VUS	PM2, PP3	-?Retinitis pigmentosa 51, AR (613464) -Bardet-Biedl syndrome 8, AR (615985)

Table 1. Continued

Gene	Variant	Depth/allele frequency	Pathogenicity	ACMG Criteria	Disease, inheritance pattern (OMIM#)
USP9X	NM_001039591.3: c.3496C>T: p.His1166Tyr	21/1.0	VUS	PM2, PP2	-Intellectual developmental disorder, X-linked 99, XLR (300919) -Intellectual developmental disorder, X-linked 99, syndromic, female-restricted, XLD (300968)
WNK3	NM_020922.5: c.4870+8T>A	25/1.0	VUS	PM2	-Prieto syndrome, XLR (309610)
	NM_020922.5: c.3652-8_3652-7insTTGTT	13/1.0	VUS	PM2	
ZNF711	NM_001330574.2: c.547A>G: p.Thr183Ala	36/1.0	VUS	PM2, BP4	-Intellectual developmental disorder, X-linked 97, XL (300803)

VUS: Variants of unknown significance, LP: Likely pathogenic, AD: Autosomal dominant, AR: Autosomal recessive, XLR: X-linked recessive, XLD: X-linked dominant, ACMG: American College of Medical Genetics, OMIM: Online Mendelian Inheritance in Man, PVS1: Pathogenic very strong 1.

Among the rare variants detected in the healthy cohort, two variants (*FAM20C*: NM_020223.4:c.951_952insACAGGTGAGCCCTTCCTTCCTCCCTCCATCCGCG:p.Asp318Thrfs*118 and *KRT10*:NM_000421.5:c.1684_1685insAGCTCCGGCGGCGGATACGGCGGCGGCAGC:p.Ser562*) were classified as LP based on PM2 and the very strong pathogenic very strong 1 (PVS1) criteria for pathogenicity. The PVS1 criterion applies to variant types that result in loss of protein function, including frameshifts, altered splicing, and exonic deletions.[23] Those variants were detected in at least three healthy participants in the Turkish population. *FAM20C* (Golgi-associated secretory pathway kinase) encodes a protein that functions as a Golgi casein kinase, regulates phosphorylation of secreted proteins, and is linked to Raine Syndrome (Online Mendelian Inheritance in Man #259775), which is characterized by neonatal osteosclerotic bone dysplasia.[24] However, the LP variant in the *FAM20C* gene was detected in five independent healthy individuals in the Turkish population, and therefore is unlikely to be the cause of lethal Raine Syndrome. Interestingly, the frameshift variant localizes to the kinase domain of the protein.[25] Thus, further molecular studies are required to determine how protein function is conserved in the presence of this variant. The other variant in *KRT10* was observed in three participants. The *KRT10* gene encodes a type I keratin that is mainly expressed in epidermal cells. The mutations in this gene have been associated with rare skin anomalies. Importantly, the C-terminus of the protein was identified as a mutation hotspot.[26,27] According to the ENSEMBL database[28], *KRT10* gene (NM_000421.5) encodes a 584 amino acid-length protein. Hence, the variant detected in the healthy participants was localized to the end of the protein, which may not affect the protein's structure and function.[29]

During classification, unreported or unproven variants are classified as VUS.[30] In the present study, 60 variants were classified as VUS. The classification would change when the variant is detected in healthy individuals.[31] Therefore, we propose that the classification of those variants be re-evaluated in medical genetics in Türkiye.

In the present study, we report biallelic variants identified in healthy individuals. Among the 60 genes affected in the homozygous state, 14 have been linked exclusively to dominant genetic disorders. In specific cases, the homozygous variants in dominant diseases have been reported to be neutral[32], further proving the non-pathogenic nature of the listed variants.

Study Limitations

Although the study submitted variant lists for a limited number of healthy individuals, similar studies with larger group of participants or a wider sequencing approach as whole genome sequencing should be conducted to define more variants that would be reclassified in Turkish population.

CONCLUSION

The present study documented rare germline and homozygous variants in genes linked to early-onset genetic disorders in healthy Turkish individuals. Although the variants were classified as VUS or LP, their detection in the healthy cohort may indicate that those variants are benign. Moreover, the listed variants were not reported in the TGP database. Thus, reporting rare variants in a population-specific manner, as in the present study, is still valuable for guiding medical geneticists when prioritizing variants. Nevertheless, possible multigenic or multifactorial inheritance patterns should be considered when referring to those variants.

MAIN POINTS

- Rare variants are common in Turkish healthy cohort.
- The reported rare variants of unknown significance may be reclassified as benign in the Turkish population.
- Further population-based studies could facilitate variant prioritization.

ETHICS

Ethics Committee Approval: The study was approved by the Gazi University Non-Interventional Clinical Research Ethics Committee (approval number: 09, date: 05.04.2021).

Informed Consent: Informed consent was obtained from all subjects involved in the study.

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Footnotes

Authorship Contributions

Concept: S.G.E., C.B., H.H.K., Design: S.G.E., G.A., C.B., H.H.K., Data Collection and/or Processing: C.B., H.H.K., Analysis and/or Interpretation: S.G.E., F.Y.T., G.A., H.H.K., Writing: S.G.E., F.Y.T., G.A., C.B., H.H.K.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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RESEARCH ARTICLE

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Liver Echinococcosis - A Single-Center Retrospective Study

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Abstract

BACKGROUND/AIMS: Liver echinococcosis, endemic to Mediterranean and livestock-farming regions, is characterized by hydatid cyst formation, primarily affecting the liver. Surgical management remains the primary treatment for large or complex cysts. The study aimed to evaluate surgical outcomes, complications, and recurrence rates associated with the Papadimitriou procedure, hepatic resections, and splenectomy in patients with liver echinococcosis at a single center.

MATERIALS AND METHODS: A retrospective analysis was conducted on 144 patients who underwent surgical treatment for liver echinococcosis over 13 years. Patients were divided into two groups: those undergoing urgent surgeries and those undergoing elective surgeries. Data on cyst localization, surgical approach, postoperative complications, albendazole use, and outcomes were analyzed.

RESULTS: Among the 144 patients, 75% had right-lobe cysts and 25% had left-lobe involvement. The Papadimitriou procedure was performed in 88.9% of cases, and 33.6% of cases required biliostasis management. Cholecystectomy was performed in 17.4% of patients and segmental hepatic resection in 2.1%. Elective surgery accounted for 91% of cases. Postoperative complications (subphrenic abscess, hemorrhage, biliary leakage) each occurred in 0.7% of cases. Albendazole was administered postoperatively to 18.8% of patients. The mean hospital stay was 10.8 days.

CONCLUSION: The Papadimitriou procedure demonstrated excellent outcomes with low morbidity and recurrence rates, particularly when combined with postoperative albendazole therapy. Early surgical intervention and comprehensive postoperative care are essential for achieving optimal treatment outcomes.

Keywords: Liver echinococcosis, hydatid cyst, Papadimitriou, liver resection, single center

INTRODUCTION

Liver echinococcosis, also known as hydatid disease of the liver, is a zoonotic parasitic infection caused by Echinococcus granulosus. The disease remains endemic in regions with close contact between livestock and dogs, with the highest prevalence reported in the Mediterranean, the Middle East, South America, and other areas where extensive farming is practiced.[1] Human infection occurs through ingestion of parasite eggs, usually via food or water contaminated with dog feces. After ingestion, the larvae (oncospheres) hatch in the intestine, penetrate the intestinal wall, and spread through the portal circulation, most commonly reaching the liver. The liver is involved in approximately 75% of cases, followed by the lungs. Hydatid liver cysts often remain asymptomatic for long periods and are frequently discovered incidentally on imaging. When symptoms occur, they generally include vague abdominal discomfort or right upper quadrant pain and occasionally a palpable abdominal mass.[2] In one clinical series, abdominal pain was reported in 87% of patients, hepatomegaly or an abdominal mass in 60%, and approximately 11% were asymptomatic. Acute complications, such as rupture of a cyst into the peritoneal cavity or biliary tree, or secondary infection of the cyst, can present with severe abdominal pain or jaundice and carry significant risks, including anaphylaxis, and therefore require prompt intervention. Management of hepatic hydatid cysts depends on cyst size, location, symptoms, and the presence of complications.

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Small, asymptomatic cysts may be managed conservatively with antiparasitic therapy, such as albendazole or mebendazole, along with careful observation.[3] Percutaneous aspiration techniques, such as puncture, aspiration, injection, re-aspiration, have emerged as an option for selected uncomplicated cysts. However, for large cysts (>5-10 cm) or complicated cases, surgical intervention remains the definitive treatment, providing immediate parasite eradication and preventing potentially serious sequelae.[4] Surgical approaches for hepatic hydatid disease range from conservative, cyst-directed procedures to radical hepatic resections. The primary objectives are complete parasite eradication, preservation of maximal functional liver tissue, and minimization of operative morbidity. A widely employed conservative technique is the Papadimitriou procedure, which involves partial cystectomy (partial pericystectomy or cyst unroofing), removing the cyst contents and the superficial cyst wall, while leaving the deeper pericystic tissue, which is adherent to vital structures, intact.[5] Adjunctive measures, including the use of scolicidal agents, closure of biliary communications, and omentoplasty, are employed to sterilize the cyst and manage the residual cavity. This organ-preserving approach aims to minimize operative risk while effectively treating the disease. In contrast, radical hepatic resections, such as segmentectomy or lobectomy, involve the removal of the cyst along with the surrounding liver tissue. Although radical surgery may reduce recurrence by ensuring the complete elimination of parasitic elements, it is associated with higher morbidity and a significant loss of functional hepatic parenchyma. Such procedures are typically reserved for giant cysts, deeply located lesions, or cases in which extensive involvement of a hepatic lobe makes conservative management impractical or unsafe.[6] Hydatid cysts can also involve other organs, with the spleen third-most commonly affected site, accounting for only 0.5-4% of cases. In instances of multisystem involvement, splenectomy may be required to achieve complete disease control. Despite advances in pharmacologic therapy, surgery remains the mainstay of treatment for hepatic hydatid disease, especially in endemic regions. The choice between conservative cystectomy and radical resection continues to be debated and should be individualized according to cyst characteristics, patient factors, and available resources.[7,8]

This study aims to evaluate the outcomes, complications, and recurrence rates of the Papadimitriou partial cystectomy, compared with those of hepatic resections. Furthermore, it assesses the role of adjunctive albendazole therapy and seeks to provide additional insight into the efficacy, safety, and optimal surgical strategies for the management of hepatic echinococcosis, particularly in resource-limited settings.

MATERIALS AND METHODS

Study Design and Patients

A retrospective, descriptive study was conducted, that included all patients who underwent surgical treatment for hepatic hydatid cysts at our hospital between 2013 and 2025. A total of 144 patients with imaging-confirmed hepatic echinococcosis were included. Patients were retrospectively evaluated for age, gender, cyst location, imaging methods and laboratory tests used for cystic echinococcosis diagnosis, treatment administered, and presence of relapse or complications. The study was approved by the Ss. Cyril and Methodius University in Skopje Faculty of Medicine for Human Research Ethics Committee (approval number: 03-4312/2, date: 14.07.2025).

Imaging and Diagnosis

Abdominal ultrasonography, computed tomography (CT), or magnetic resonance imaging (MRI) were used depending on cyst location. Ultrasonography, capable of detecting lesions as small as 1 cm, served as the initial diagnostic modality, while contrast-enhanced CT, which had 100% sensitivity for hepatic hydatids, provided detailed anatomical information to guide surgical planning (Figure 1). Cysts were classified according to the Gharbi/ World Health Organization criteria, using imaging features to aid treatment selection. Hydatid serology, primarily using enzyme-linked immunosorbent assay[9], was performed when available and was positive in most cases; however, treatment proceeded in patients with imaging-consistent findings regardless of serological results.

Patient Groups

Patients were divided into two groups: elective surgeries (n=131, 91%) and urgent surgeries (n=13, 9%). Elective cases included patients with sizable cysts that were identified during routine workup or that caused chronic symptoms. Urgent cases required emergency intervention because of complications such as cyst rupture into the peritoneal cavity or biliary tree; secondary infection resulting in sepsis or peritonitis; or severe compression of the inferior vena cava or biliary obstruction leading to jaundice.

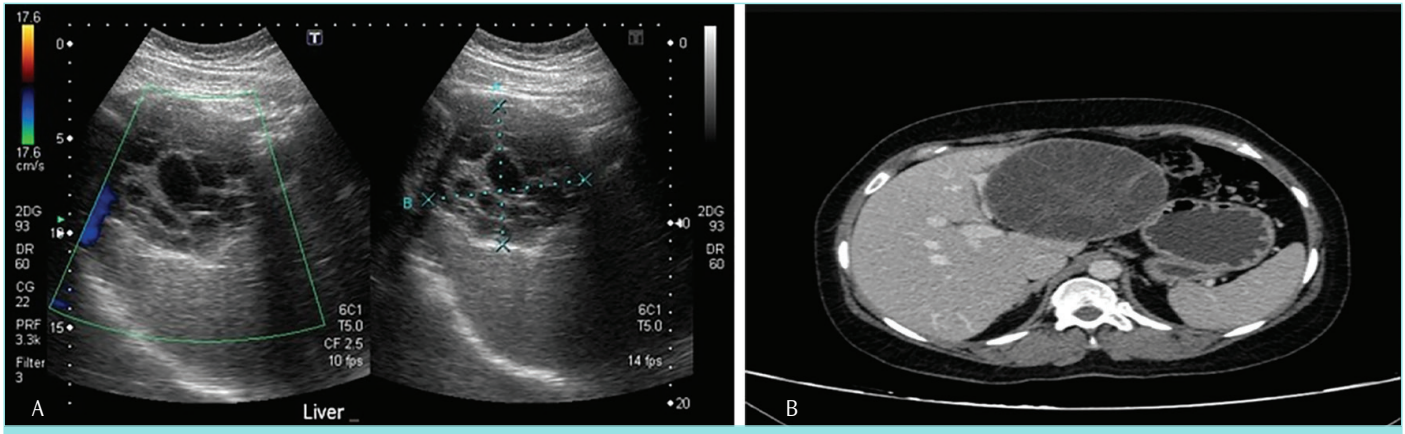

Figure 1. Imaging of hepatic hydatid cysts. (A) Abdominal ultrasonography demonstrates a cystic lesion with daughter cysts in the right hepatic lobe. (B) Contrast-enhanced computed tomography demonstrates the same lesion with a well-defined cyst wall and internal septations.

Preoperative Evaluation and Optimization

All patients underwent preoperative assessment with abdominal ultrasonography and contrast-enhanced CT. Preoperative optimization of comorbidities was performed as needed. Routine preoperative albendazole therapy was not administered; however, a short (1-2-week) course was initiated in selected urgent cases or situations with a high risk of intraoperative cyst dissemination to sterilize cyst contents and reduce the risk of spillage. Broad-spectrum antibiotic prophylaxis was administered perioperatively, particularly in patients with infected cysts, along with standard venous thromboembolism prophylaxis.

Surgical Techniques

All surgical procedures were performed via open laparotomy under general anesthesia. Strict intraoperative precautions were employed to prevent spillage of hydatid fluid and to minimize the risk of secondary cyst implantation or an anaphylactic reaction. The operative field was isolated with gauze packs soaked in scolicidal solutions (20% hypertonic saline or povidone-iodine) before cyst manipulation.

Selection of Surgical Approach

The choice between partial cystectomy and radical hepatic resection was based on cyst characteristics and operative considerations:

- Papadimitriou's partial cystectomy was the first-line technique for the majority of cases, particularly for cysts that were superficial, peripherally located, or that did not replace an entire hepatic segment. This approach was also preferred for single or multiple cysts that could be safely managed without significant loss of liver tissue.

Radical hepatic resection (wedge resection or segmentectomy) was reserved for cases with:

- Giant cysts replacing an entire segment or lobe.
- Complex, clustered cysts where multiple cystectomies would be unsafe or technically challenging.
- Suspicion of co-existing malignancy requiring en bloc resection.

Surgical steps:

- Cyst aspiration followed by injection of a scolicidal agent, allowing sufficient time for parasitic sterilization.
- Cystotomy with evacuation of cyst contents, including daughter cysts and hydatid sand, with careful removal of the germinal membrane.
- Partial pericystectomy, excising the superficial cyst wall while preserving fibrous pericyst adherent to major vascular or biliary structures to avoid hemorrhage or bile duct injury.
- Inspection and closure of biliary communications with fine absorbable sutures to ensure biliostasis.
- Management of the residual cavity by omentoplasty (placement of a greater omentum flap) or capitonnage in smaller, superficial cavities.

A closed-suction drain was placed in the residual cavity or subhepatic space in all cases. Cholecystectomy was performed concurrently in 25 patients (17.4%) when indicated by cyst proximity, coexisting cholelithiasis, or the need for biliary exploration. In cases of biliary involvement, the common bile duct was explored, debris was removed, and T-tube drainage was placed when necessary. No splenectomies were performed in this cohort, though institutional protocols dictated that any identified splenic hydatid cysts would be treated with splenectomy or partial splenectomy as indicated.

Postoperative Management and Follow-Up

Following surgery, all patients were monitored in the surgical ward, with intensive care admission for 24-48 hours in cases of emergency surgery or intraoperative complications. Abdominal drains were maintained until output was minimal and non-bilious. Serial liver function tests and complete blood counts were obtained to detect bile leakage, hemorrhage, or infection. Any suspected bile leak was further evaluated using imaging and, if necessary, endoscopic retrograde cholangiopancreatography with biliary stenting. Broad-spectrum intravenous antibiotics were administered perioperatively and continued postoperatively in cases involving ruptured or infected cysts. Postoperative albendazole therapy was administered to 27 patients (18.8%), typically initiated within the first postoperative week once oral intake was tolerated, at a dose of approximately 10 mg/kg/day for 1-3 months. Indications included intraoperative spillage, multiple cysts, and residual cysts in other organs. Structured follow-up included clinical examination and imaging at 1 month, 6 months, and annually thereafter for a minimum of 2 years, with a mean follow-up of 3.5 years (range 2-10 years). Abdominal ultrasonography was the primary imaging modality, with CT or MRI used for suspicious lesions or detailed anatomical assessment. Patient compliance was high, with less than 5% lost to follow-up; outcomes for these patients were supplemented with data from outpatient records and telephone interviews. Recurrence was defined as the appearance of a new cystic lesion at a previous surgical site or elsewhere in the liver, confirmed by imaging and clinical correlation.

Statistical Analysis

Descriptive statistics were used to summarize patient demographics, cyst characteristics, surgical procedures, complications, and outcomes. Categorical variables (e.g., postoperative complications, recurrence) were expressed as frequencies and percentages. Continuous variables (e.g., length of hospital stay) were presented as means. Comparative analyses of categorical outcomes between groups (e.g., complications with Papadimitriou vs. other techniques, recurrence in albendazole-treated vs. untreated) were performed using Fisher's exact test because of the small number of events. Continuous variables for which subgroup data were available were compared using Independent Samples t-tests. A p-value <0.05 was considered statistically significant. All statistical analyses were performed using SPSS for Windows, version 25.0 (IBM Corp., Armonk, NY, USA).

RESULTS

A total of 144 patients underwent surgical treatment for liver echinococcosis during the study period. The cohort included both sexes, predominantly from rural, livestock-raising areas, reflecting the endemic nature of hydatid disease in our region. Demographic and baseline characteristics of the patients are presented in Table 1.

Clinical Presentation

The most common symptoms were upper abdominal pain or discomfort (approximately 80% of patients) and palpable mass or hepatomegaly (20% of patients). A minority were diagnosed incidentally on imaging. Thirteen patients (9%) presented emergently with complications such as cyst rupture or acute cholangitis.

Postoperative Outcomes

- **Mortality:** 0% (no intraoperative or postoperative deaths)
- **Morbidity:** Overall complication rate was 2.1%, with three major complications:
  - Subphrenic abscess (0.7%)
  - Intra-abdominal bleeding (0.7%)
  - Biliary leakage (0.7%)
- Minor wound infections occurred in a few cases (approximately 2-3%)

Hospital stay: 5-20 days (mean 10.8 days)

All complications were successfully managed without permanent sequelae.

Albendazole Use and Recurrence

- Postoperative albendazole was administered to 27 patients (18.8%) and was typically initiated once oral intake resumed (10 mg/kg/day for 1-3 months).
- Recurrence was observed in 2 patients (1.4%); neither had received postoperative albendazole. No recurrences occurred in the albendazole-treated group (0%).

These results suggest that postoperative albendazole therapy may significantly reduce recurrence risk.

Overall, our center achieved high success rates, with low morbidity (2.1%), zero mortality, and very low recurrence (1.4%), which demonstrates the efficacy of organ-preserving surgery (Papadimitriou procedure) for the majority of hepatic hydatid cyst cases. The outcomes are summarized in Table 2.

Comparative analysis showed that all postoperative complications (n=3) occurred in patients undergoing the Papadimitriou procedure (2.3%), while no complications were observed in patients treated with other surgical techniques; this difference was not statistically significant (Fisher's exact test, p=1.0). Recurrence was observed only in the untreated group (2/117, 1.7%), whereas no recurrences occurred among patients who received postoperative albendazole (0/27, 0%). This difference was not statistically significant (Fisher's exact test, p=1.0).

Cyst Characteristics

- **Location:** Right lobe involvement in 75% (113 patients), left lobe in 25% (36 patients), with some patients having bilobar disease.
- **Size:** Cyst diameters ranged from 3-20 cm (mean, approximately 8-10 cm).

Table 1. Demographic and baseline characteristics of patients

Parameter	Value
Total patients	144
Age, mean ± SD (range)	42.3±15.7 years (18-78)
Gender, n (%)	Male: 82 (57%) Female: 62 (43%)
Residence, n (%)	Rural: 110 (76%) Urban: 34 (24%)
Symptom presentation, n (%)	Abdominal pain/discomfort: 115 (80%) Palpable mass/hepatomegaly: 29 (20%) Asymptomatic/incidental: 16 (11%)

SD: Standard deviation.

Table 2. Summarized key operative and outcome data

Parameter	Value
Total patients	144
Elective surgeries	131 (91%)
Urgent/emergency surgeries	13 (9%)
Papadimitriou partial cystectomy	128 (88.9%)
Hepatic resections (wedge/segmental)	3 (2.1%)
Cholecystectomy	25 (17.4%)
Biliostasis required (suturing bile ducts)	43 (33.6%)
Subphrenic abscess (complication)	1 (0.7%)
Intra-abdominal bleeding (complication)	1 (0.7%)
Biliary leakage (complication)	1 (0.7%)
Overall major complication rate	2.1%
Mortality	0%
Hospital stay range (mean)	5-20 days (mean 10.8 days)
Postoperative albendazole use	27 patients (18.8%)
Recurrence rate	2 patients (1.4%)
Recurrence in the albendazole-treated group	0%

- **Multiplicity:** Approximately 15% had multiple hepatic cysts. Surgical procedures Papadimitriou's partial cystectomy was performed in 128 patients (88.9%), with preservation of maximal liver parenchyma.
- Hepatic resections were necessary in only 3 patients (2.1%) and involved wedge or segmental resections.
- Cholecystectomy was performed in 25 patients (17.4%), primarily to facilitate exposure or address coexistent cholelithiasis.
- Biliostasis was achieved in 43 cases (33.6%) by suturing biliary communications identified within the cyst cavity.
- Among the operations, 131 (91%) were elective, while 13 (9%) were urgent due to complications such as intrabiliary rupture, peritonitis following free rupture, or cyst infection.

DISCUSSION

This 13-year single-center study provides valuable insights into the outcomes of different surgical strategies for hepatic echinococcosis. The most salient finding is the excellent efficacy of the Papadimitriou partial cystectomy technique, which was performed in approximately 89% of

patients. This organ-sparing procedure achieved cure (no recurrence) in the vast majority of patients, with minimal morbidity (approximately 2% complication rate) and no mortality.[10] By contrast, formal liver resection was required in only 2.1% of cases, suggesting that even large or multiple cysts were usually manageable without sacrificing major portions of the liver. Our findings support a conservative surgical approach. Careful prevention of cyst spillage and secure maintenance of biliostasis are essential for achieving excellent outcomes. Even giant cysts (>15-20 cm) were successfully managed through careful evacuation and partial cystectomy with omentoplasty, preserving liver parenchyma and minimizing the risks of major hepatectomy.[11]

Preservation of liver tissue is particularly important in regions where patients may have underlying liver disease or multiple cysts affecting both lobes. The Papadimitriou technique not only preserves functional hepatic units but also reduces the incidence of serious complications compared with radical surgery. In our cohort, postoperative bleeding and bile leakage occurred in only two patients following partial cystectomy, demonstrating that even these risks are minimal when meticulous surgical technique is applied.[12] Comparisons in the literature, such as the study by Shi et al.[13], suggest that while radical surgery may offer a slight reduction in recurrence, it comes with a higher complication rate (e.g., bile leakage and longer hospital stays). Our findings support a conservative surgical approach. With careful measures to prevent cyst spillage and to secure biliostasis, we achieved an exceptionally low recurrence rate, comparable to radical surgery, but with fewer risks. Biliostasis was critical to this success, as one-third of the cysts had biliary communications that required suturing, thereby keeping the bile leak rate below 1%. These results emphasize that conservative surgery is not a "minimal or simple procedure", it requires thorough inspection, evacuation, and careful handling of the cyst cavity.

The cyst distribution in our series (right lobe: 79%; left lobe: 25%; some overlap) aligns with previous reports and reflects the hemodynamic dominance of the right portal vein. Bilobar involvement (approximately 3-4%) posed additional challenges but was successfully managed with multiple cystectomies in a single session, avoiding major resections or liver transplantation. Emergency operations accounted for 9% of cases and were performed for acute complications such as rupture or cholangitis. Despite these risks, outcomes were excellent with no mortality, highlighting the importance of prompt and effective management. The predominance of elective surgeries (91%) underscores the value of early intervention to prevent cyst rupture or biliary complications. Postoperative recurrence was extremely low (~1.4%), far below the 5-30% reported in the literature. Key factors contributing to this success included careful intraoperative handling to prevent spillage, thorough evacuation of cyst contents, and adjuvant postoperative albendazole therapy administered to 18.8% of patients, particularly in high-risk cases. Notably, none of the albendazole-treated patients experienced recurrence, consistent with evidence that antiparasitic therapy significantly reduces recurrence risk.[14] Compared with other published series, our morbidity (2.1%) and mortality (0%) rates are notably lower, as many reports cite morbidity rates of 10-20% and mortality rates of 1-3%. While limitations exist, including the lack of randomization between surgical methods and incomplete long-term follow-up for all patients, the large sample size and consistent methodology strengthen the validity of our findings. Although open surgery predominated in our series, largely due to cyst characteristics and the study period, the potential role of laparoscopic hydatid surgery warrants consideration. Evidence suggests that laparoscopic partial cystectomy can be safe and effective in selected patients, offering reduced morbidity and a faster recovery. Gradual adoption of minimally invasive techniques, particularly for moderate-sized, anterior cysts, could further improve outcomes. Although splenic hydatid disease was rare in our cohort, management principles remain important. Splenectomy remains the treatment of choice for splenic hydatids, and synchronous surgery may be considered when cysts are present in both the liver and spleen.

Study Limitations

This study has several limitations that should be acknowledged. It was conducted retrospectively at a single tertiary surgical center. Although this design ensured uniformity in surgical approach, perioperative management, and follow-up protocols, it also limits the generalizability of our results. The favorable outcomes observed may reflect the experience of a specialized surgical team and the regional characteristics of the patient population. Moreover, retrospective data collection carries an inherent risk of selection bias, as only patients who underwent surgery were included, potentially excluding those managed conservatively or at other institutions. Future multicenter, prospective studies with larger and more diverse populations are warranted to validate these findings and assess their applicability to different clinical settings.

CONCLUSION

Hydatid cysts of the liver remains a significant surgical challenge in endemic areas, although excellent outcomes can be achieved with appropriate management. Our single-center, 13-year experience demonstrates that the Papadimitriou partial cystectomy is a highly effective first-line surgical approach for hepatic echinococcosis. This organ-preserving technique resulted in near-zero recurrence and minimal complications, confirming its role in conserving liver tissue without compromising cure rates. Hepatic resection is rarely necessary and should be reserved for selected cases where conservative surgery is impractical, such as giant or anatomically complex cysts. When indicated, limited resections can yield favorable results but carry greater operative risks and must be weighed carefully against potential benefits. Adjunctive perioperative albendazole therapy markedly improved outcomes in our cohort, with no recurrences observed among treated patients, supporting its routine use where not contraindicated. Our results provide a benchmark for conservative surgical management of hepatic echinococcosis. Surgeons in endemic regions should be proficient in performing partial cystectomy and should reserve liver resection for exceptional cases. A strategy combining organ-preserving surgery with targeted adjunctive therapy achieves high cure rates with low morbidity. This approach, exemplified by the Papadimitriou procedure with perioperative albendazole, offers patients the best chance of complete recovery while preserving maximal liver function. Future efforts should emphasize early detection programs and the broader adoption of minimally invasive techniques to further enhance outcomes. Overall, our findings reinforce the growing body of evidence that conservative, organ-sparing strategies should be considered the standard of care for hepatic hydatid disease.

MAIN POINTS

- Early elective intervention for sizable hydatid cysts simplifies surgery and reduces emergency presentations. Screening and early referral should be emphasized in endemic areas.
- The Papadimitriou partial cystectomy (cyst unroofing with cavity obliteration) is validated as an effective standard technique, achieving radical clearance of parasites while preserving liver function.
- Meticulous surgical technique, including preventing spillage, securing biliostasis, performing omentoplasty, and diligent postoperative care, including imaging follow-up and chemoprophylaxis, is critical to successful outcomes.
- Minimally invasive surgery represents a promising frontier, where expertise is available, laparoscopic management can further reduce patient morbidity and hospital costs, without deviating from the core principles of the open Papadimitriou method.

ETHICS

Ethics Committee Approval: The study was approved by the Ss. Cyril and Methodius University Faculty of Medicine for Human Research Ethics Committee (approval number: 03-4312/2, date: 14.07.2025).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: O.K., Concept: I.K., Design: I.K., Data Collection and/or Processing: O.K., I.K., Analysis and/or Interpretation: O.K., I.K., Literature Search: I.K., Writing: I.K.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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RESEARCH ARTICLE

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Genotoxic and Hemato-Biochemical Effects Induced By Anatase Titanium Dioxide Nano Particles in Sprague Dawley Rats

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Abstract

BACKGROUND/AIMS: This study aims to examine the adverse effects of titanium dioxide nanoparticles (TiO_2-NPs) on rats.

MATERIALS AND METHODS: Twenty-five rats, each weighing 200 to 220 grams, were acquired and later transported to the animal house for acclimatization. The rats were divided into five groups of five rats each (G1, G2, G3, G4, and G5). These groups were treated with varying amounts of TiO_2-NPs (mg/kg body weight): 0.00 (control), 0.9 (saline), 80, 120, and 160, every other day for 28 days. The rats' weight was examined regularly. After 28 days, blood samples were collected from the orbital sinuses into ethylenediaminetetraacetic acid -coated tubes, and serum was separated for biochemical analysis.

RESULTS: The data suggested that the rats' weight was substantially reduced compared with the control and saline groups. Hematological indicators, such as hemoglobin, hematocrit, red blood cells, mean corpuscular hemoglobin concentration, lymphocytes, and monocytes, significantly decreased ($p<0.05$), whereas mean corpuscular volume and white blood cells significantly increased. Biochemical indicators, including alanine transaminase, aspartate transaminase, alkaline phosphatase, urea, creatinine, lactate dehydrogenase, and total bilirubin, were markedly increased. Findings indicate increased oxidative stress and antioxidant enzyme activity in response to high-dose treatment. Genotoxic measurements demonstrated a large decrease in head length and head deoxyribonucleic acid (DNA) (%), but tail length, comet length, tail DNA (%), and tail moment increased markedly.

CONCLUSION: Anatase TiO_2-NPs induced significant genotoxic and hemato-biochemical effects in Sprague Dawley rats.

Keywords: Titanium dioxide nanoparticles, hemato-biochemical, genotoxicity, Sprague Dawley rats

INTRODUCTION

Nanotechnology is an emerging scientific field that focuses on the design and development of materials at the nanoscale (nanomaterials). These materials, which range in size from 1 to 100 nm, include metals such as copper, zinc, titanium (Ti), magnesium, gold, and silver. Nanomaterials have a wide range of applications from medicine and industrial products like fuel cells to everyday items such as cosmetics.[1] They are produced in large quantities because of their unique properties, which can enhance the performance of various products and processes, contribute to the development of smart medicines, and support sustainable development.[2]

Ti is the ninth most abundant element in the Earth's crust. Titanium dioxide nanoparticles (TiO_2-NPs) are white, odorless, and non-combustible powders that exhibit low solubility.[3] Due to their chemical

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stability, inertness, cost-effectiveness, and wide range of applications, TiO_2-NPs are extensively used in fields such as photocatalysis, catalysis, dye-sensitized solar cells, photovoltaics, and water-splitting technologies.[4] Of the two primary crystalline forms of TiO_2-NPs, anatase and rutile, anatase is generally considered more toxic than rutile. Both types are capable of generating reactive oxygen species (ROS), which are oxygen-containing reactive molecules naturally produced during cellular metabolism. Although ROS play vital roles in immune defense and cell signaling, their excessive buildup can result in oxidative stress. This study examines the toxicity of anatase TiO_2-NPs, drawing on findings from Zhang et al.[5], Wang et al.[6], and Vasantharaja et al.[7]

TiO_2-NPs, when present in rats, can translocate systemically and accumulate in various organs. This can lead to varying degrees of damage to organs sensitive to oxidative stress, such as the liver, lungs, kidneys, small intestine, testes, and brain. These nanoparticles induce oxidative stress, damage deoxyribonucleic acid (DNA), and alter enzymatic activity in the body.[8]

The genotoxic effects of nanoparticles have been extensively investigated, revealing a considerable increase in cancer risk associated with nanoparticle-induced toxicity in the context of modern technological advances. Studies on genotoxicity encompass various aspects such as DNA damage, gene mutations, and chromosomal alterations. According to the International Agency for Research on Cancer, TiO_2-NPs have been classified as possibly carcinogenic to humans Baan et al.[9], Wani et al.[10], Wen et al.[11] In this study, biochemical markers related to liver and kidney function-such as aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine transaminase (ALT), lactate dehydrogenase (LDH), urea, creatinine, and total bilirubin-were evaluated because of their significance in detecting physiological abnormalities.[12] Furthermore, the ratios of tissue weight to body weight for the brain and gonads were analyzed.[13]

Antioxidants play a crucial role in neutralizing harmful free radicals and protecting cells from oxidative damage. Maintaining an appropriate balance between oxidants and antioxidants is essential for preventing chronic diseases and promoting overall health.[14] Prolonged exposure to TiO_2-NPs can trigger excessive oxidative stress, causing biochemical and structural damage in vital tissues. These nanoparticles disturb the oxidant-antioxidant equilibrium, leading to lipid peroxidation, protein oxidation, and DNA damage Talas et al.[15], and Gulhan et al.[16]

This study focused primarily on investigating the genotoxic and hematobiochemical effects of TiO_2-NPs. The wide applications of different nanoparticles, specifically TiO_2-NPs, and their potential toxicity in living organisms, as reviewed by previous studies, focused our attention on exploring their adverse effects in Sprague Dawley rats.

MATERIALS AND METHODS

The details of the materials and procedures are explained in the following section.

Biological Material and Ethical Approval

Twenty-five post-weaning male Sprague Dawley rats, weighing 200-220 grams, were purchased and kept in the animal house for acclimatization. After an acclimation period, animals were randomly assigned to five groups of five animals each. The control group received regular food and water, while the saline group (S) received a 1 mL intravenous injection of normal saline to ensure equivalence of shock. Groups 3, 4, and 5 were injected subcutaneously with TiO_2-NPs at doses of 80, 120, and 160 mg/kg on alternate days for 28 days.

Ethical approval was obtained on Government College University Faisalabad Ethics Review Committee (approval number: GCUF/ERC/976, date: 24.09.2019), but due to coronavirus disease-2019 (COVID-19) the study commenced in 2021. The trial was conducted from March to April 2021, after which bioassays were completed.

Sample Collection

Blood samples were collected from all animals at the start of the experiment and after 28 days of treatment to assess their hematological and genetic health. At the end of the study, the animals were fasted overnight, anesthetized with chloroform the following day, and euthanized. Blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes for analysis. The brain and male gonads were carefully weighed and placed in a preservative solution for histological examination.

Material Characterization

TiO_2-NPs were characterized by X-ray diffraction (XRD), fourier transform infrared spectroscopy (FT-IR), and scanning electron microscopy (SEM).

Ti Accumulation

A 0.5-g piece of brain and gonad tissue was taken and digested in a digestion solution (5 mL nitric acid and 2.5 mL perchloric acid) in a heating digester (Velp Scientifica D-6). Initially, the fumes were yellow. The digestion process was continued until the fumes became colorless and the solution remained at 1 mL. Distilled water was added to the remaining digested solution to bring the volume to 25 mL, and the solution was filtered. The sample was analyzed using inductively coupled plasma mass spectrometry (ICP-MS, PerkinElmer).[17]

Measurement

Body and organ weight

Body weights were recorded weekly to track changes throughout the study. Organ weights, specifically the brain and testes, were measured after euthanasia. Male gonads were measured (L*W) by vernier caliper immediately after separating.

Blood and Serum Analysis

Blood was analyzed using the automated hematology analyzer X5-1000i, manufactured by Sysmax. Serum was analyzed using commercially available kits manufactured by Merck on the chemistry analyzer Microlab 300.

Oxidative Stress Analysis

Oxidative stress was assessed using the following biomarkers in the brains of Sprague Dawley rats: glutathione (GSH), malondialdehyde (MDA), catalase (CAT), and lipid peroxidase (LPO).[18]

0.25 g of tissue sample (Brain) was homogenized with 2.5 mL of 0.1 M tris-hydroxymethyl aminomethane hydrochloride (Tris-HCl) buffer in bullet blinder (Pro-900) while keeping the PH 7.4 at “4 °C” and crude tissue homogenate was then centrifuged at a speed of “10,000 rpm” for

20 min in centrifuge (Sigma) at "4 °C". The superanent was stored at "-20 °C" and processed for further oxidative stress biomarker.

Estimation of lipid peroxidation

The freshly prepared homogenate (0.1 mL) was treated with FOX reagent (0.9 mL), which was composed of butylated hydroxytoluene (88 mg), xylenol orange (7.6 mg), ammonium iron sulfate (9.8 mg) dissolved in methanol (90 mL) and sulphuric acid (10 mL). The mixture was incubated at 37 °C for 30 min. The color developed was measured at 560 nm, and LPO was expressed as mM/g of tissue.

Estimation of malondialdehyde

A mixture of 1 mL distilled water 5 mL of n-butanol and pyridine 4 mL of sample solution (0.2 mL tissue homogenate +0.2 mL sodium dodecyl sulfate +1.5 mL acetic acid +1.5 mL thiobarbituric acid was shaken on a vortex and centrifuged at 4000 rpm for 10 min and absorbance of upper organic layer was read at 532 nm using spectrophotometer. The amount of MDA was measured as nm/g of the respective tissue.

Estimation of catalase

CAT was estimated by mixing the sample with 1.90 mL of potassium phosphate buffer. 1 mL of hydrogen peroxide was added to initiate the reaction. The optical density was measured twice at 240 nm with a 30-sec interval between measurements. CAT activity was expressed as units per mL.

Estimation of glutathione

GSH was estimated by mixing the homogenate and sulfosalicylic acid in equal volumes and incubating the mixture at 4 °C. The mixture was centrifuged at 12000 rpm for 15 min. The supernatant (0.5 mL) was taken and mixed with 2.5 mL of potassium phosphate buffer; after adding 0.4 mL 5,5'-dithiobis (2-nitrobenzoic acid), the reaction was started, and absorbance was measured at 412 nm using a spectrophotometer; GSH was expressed as uM/g of tissue.

DNA Damage (Comet Assay)

Blood and organ samples (brain and gonads) were used to perform the comet test. The comet test was performed promptly after dissection. Frosted-end microscope slides were used for the comet assay. These slides were scorched with a blue flame to remove oil and dust particles, then soaked in methanol and cleaned with a gentle cloth. To protect them from dust, the slides were stored in a closed box and left overnight to dry. The humidity in the air was monitored because high moisture levels can prevent the slides from drying properly, which affects the electrophoresis process.

The day before starting the assay, slides were prepared by coating them with 1% normal melting point agarose. Once dry, a second layer of low-melting-point (LMP) agarose, mixed with the sample, was applied at 40 °C. In an Eppendorf tube, 100 µL of the sample and 300 µL of LMP agarose were gently mixed; 100 µL of this mixture was poured onto each slide. A coverslip was placed over each slide to evenly spread the sample, followed by cooling on an ice plate for 2-3 min. The coverslips were then gently removed.

Next, the slides were placed in a jar with a chilled lysis buffer (89 mL lysis solution, 18.62 g EDTA, 0.68 g Tris-HCl, 500 mL distilled water, 10 mL dimethyl sulfoxide, and 1 mL Triton X and kept in a dark box at 4 °C for 1.5 h. Electrophoresis was then performed using a horizontal electrophoresis unit (Wealtec) in a cold buffer (0.18 g EDTA, 6 g sodium hydroxide, and 250 mL distilled water) at 300 mA and 25 V for 20 min.

After electrophoresis, the slides were transferred to a stand and neutralized using a cold neutralizing buffer (24.22 g Tris-Base and 2.5 mL distilled water, pH 7.5). The neutralizing solution was left on the slide for 5 min before washing, which was performed under low-light conditions. Once dry, ethidium bromide (75 µL per slide) was applied and a coverslip was placed over each slide. After 20 min, the coverslips were removed, and the dried slides were examined under a fluorescence microscope (Nikon DS-Fi2) at 40× magnification. Fifty cells were analyzed on each slide. The comet assay showed comet-like structures in damaged cells, indicating varying degrees of DNA damage. Computer image analysis (using Casp software) was employed to measure parameters such as head length, tail length, comet length, head DNA, and tail DNA.

Statistical Analysis

Statistical significance was determined using Minitab software (Minitab 15). Body weights, organ weights, Ti accumulation, hematological parameters, oxidative stress analyses, and genotoxicity analyses were compared using the Duncan test. Group comparisons were made using a one-way analysis of variance, with significance tested at $p<0.05$.

RESULTS

The widespread industrial and medical applications of TiO_2-NPs-including their use in various consumer products, cosmetics, food items, and pharmaceuticals-have raised concerns about their potential adverse health effects. The extensive utilization of TiO_2-NPs has therefore prompted our research to focus on understanding their toxicological impacts.[19] These nanoparticles are known to target cellular mitochondria, disrupting oxidative phosphorylation and ultimately causing damage to cells and tissues.[20] The present study investigates TiO_2-NPs -induced toxicity in male Sprague Dawley rats. Variations in hematological, biochemical, genotoxic, cell-viability, and histopathological (brain and gonadal) parameters were evaluated. Additionally, nanoparticle characterization was performed using XRD, FT-IR, and SEM.

Characterization of TiO_2-NPs

Nanoparticles of TiO_2 were purchased from Sigma-Aldrich and analyzed using powder XRD, FT-IR, and SEM. The Graphical results of the XRD pattern shown in Figure 1 reveal the key peaks corresponding to specific diffraction angles (2θ) and their respective intensities. The highest peaks occur at 25.5°, 38°, 48.5°, and 54.5°, with intensities of 101, 112, 200, and 211, respectively. The prominent peaks were compared with Joint Committee on Powder Diffraction Standards data, and the peaks observed in the pattern coincide well with those reported in the literature. The 2θ peak at 25.5° confirms the anatase structure of TiO_2. Strong diffraction peaks at 25.5° indicate TiO_2-NPs in the anatase phase. FT-IR analysis provides functional characterization of TiO_2-NPs. The results were presented as a graph of % transmittance versus wavenumber Figure 2. The various bands observed in the FT-IR spectra of TiO_2-NPs confirm the presence of different functional groups in the molecule. Peaks were obtained in the range of 400-4000 cm-1. A broad absorption band observed at 3029 cm-1 corresponds to the hydroxyl

(O-H) stretching vibration mode of the TiO_2-NPs. The second band, observed at 1622.72 cm-1, corresponds to the characteristic bending mode of the H2O molecule. The morphology of TiO_2-NPs was analyzed by SEM. In Figure 3, results indicate spherical and rod-like agglomerates at different magnifications, ranging from 200 nm to 2 µm.

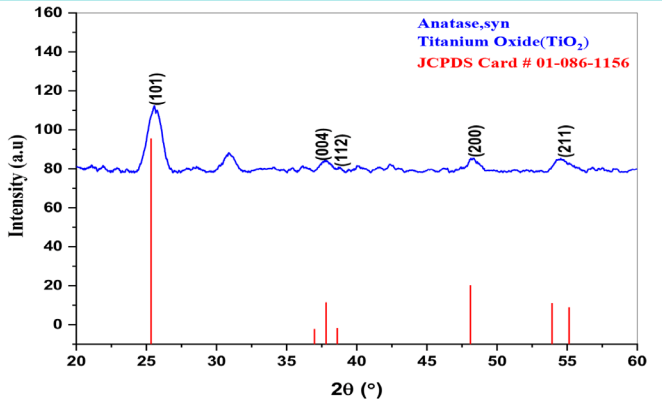

Figure 1. X-ray images of titanium dioxide nanoparticles (TiO_2-NPs) using powder diffraction (XRD).

XRD: X-ray diffraction.

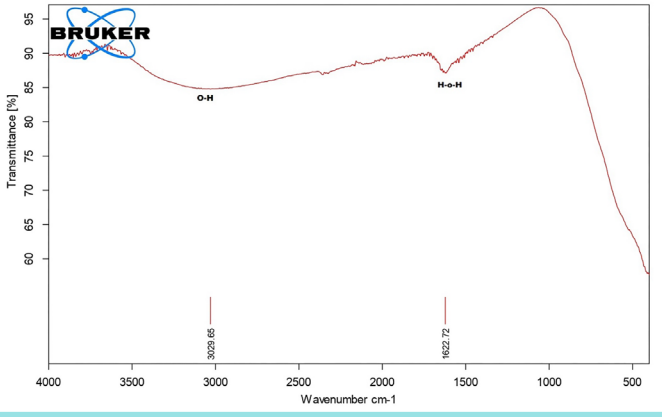

Figure 2. Fourier transform infrared images of (TiO_2-NPs).

TiO_2-NPs: Titanium dioxide nanoparticles.

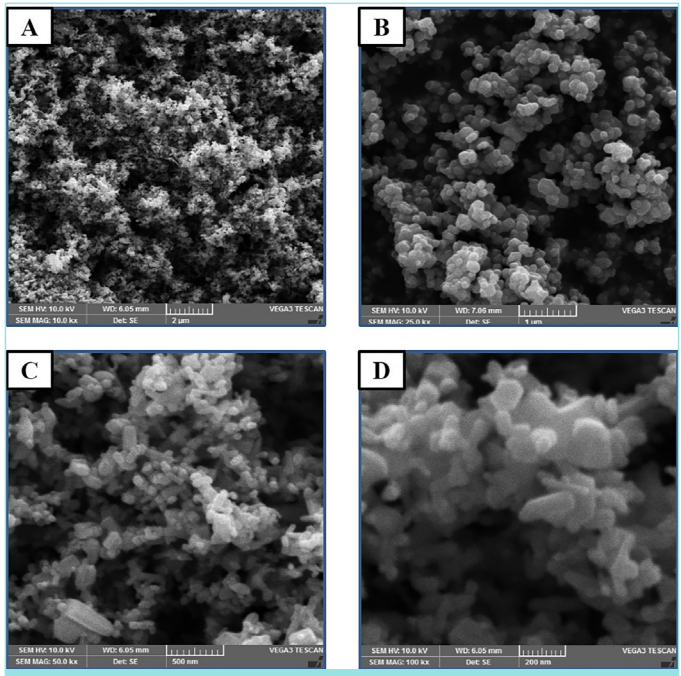

Figure 3. Scanning electron microscope images of (TiO_2-NPs). **(A)** 2 µm **(B)** 1µm **(C)** 500 nm **(D)** 200 nm.

TiO_2-NPs: Titanium dioxide nanoparticles.

Behavioral Changes

Significant behavioral changes, such as aggressiveness, piloerection (fluffiness), anorexia, cannibalism, and depression, were observed at the highest dose of TiO_2-NPs during the study (Table 1).

Weight Results

In our study, we observed a significant decrease in the body weight of rats treated with TiO_2-NPs (Table 2). Throughout our experiment, no fatalities occurred among rats treated with TiO_2-NPs at doses of 80, 120, and 160 mg/kg body weight. However, rats in the treatment groups (G3, G4, and G5) experienced a significant decrease in body weight over 28 days compared with the control group (G1) and the saline group (G2), indicating adverse effects of TiO_2-NPs (Table 2). Furthermore, in our study, both the absolute and relative weights of the brain and gonads were significantly lower in the high-dose treatment groups (G4 and G5), and measurements such as gonad length and width were also significantly reduced compared to the control and saline groups (Table 2).

Table 1. Behavioral alterations in rats after post-exposure to TiO_2-NPs

Parameters	Groups				
	G1	G2	G3	G4	G5
Aggressiveness	-	-	-	++	+++
Fluffiness	-	-	-	++	+++
Anorexia	-	-	-	+	++
Cannibalism	-	-	-	+	++
Depression	-	-	-	+	+++

TiO_2-NPs: Titanium dioxide nanoparticles.

Table 2. Weight Results

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	p-value
Start of experiment AW(g) ± SD	206.00±2.52[a]	206.20±1.85[a]	205.40±1.02[a]	205.80±1.77[a]	205.60±1.12[a]	p=0.269
End of experiment AW(g) ± SD	329.40±1.93[a]	332.80±1.96[a]	292.80±1.59[b]	275.80±2.55[c]	255.40±2.37[d]	p=0.000
End of experiment OW (Brain)(g) ± SD	0.01±0.09[a]	0.01±0.01[a]	3.39±0.10[b]	7.32±0.34[c]	13.84±0.33[d]	p=0.001
End of experiment OW (Testis)(g) ± SD	0.01±0.02[a]	0.01±0.14[a]	3.13±0.57[b]	11.89±0.38[c]	19.12±0.17[d]	p=0.000
Absolute weight (Brain) (g) ± SD	0.92±0.23[a]	0.91±0.02[a]	0.91±0.02[a]	0.86±0.01[b]	0.81±0.01[c]	p=0.000
Relative weight (Brain) (%) ± SD	0.28±0.01[a]	0.27±0.01[a]	0.27±0.02[b]	0.21±0.03[b]	0.14±0.04[c]	p=0.002
Absolute weight (Testis) (g) ± SD	1.30±0.01[a]	1.29±0.02[a]	1.28±0.02[a]	1.14±0.02[b]	1.07±0.02[c]	p=0.000
Relative weight (Testis) (%) ± SD	0.38±0.01[a]	0.38±0.01[a]	0.43±0.02[b]	0.40±0.05[c]	0.35±0.06[d]	p=0.000
Gonadal measurement (Length) (mm)	1.88±0.04[a]	1.86±0.02[a]	1.84±0.02[a]	1.42±0.09[b]	1.24±0.05[c]	p=0.000
Gonadal measurement (Diameter) (mm)	0.88±0.03[a]	0.86±0.02[a]	0.84±0.05[a]	0.68±0.03[c]	0.58±0.03[d]	p=0.000

[a]: Similar to each other, different from b and c, [b]: Different from a, similar within itself, [c]: Different from both a and b, [d]; Different from a, b and c.
AW: Animal weight, OW: Organ weight, SD: Standard deviation.

Ti Accumulation

Table 3 shows the dose-dependent accumulation of Ti in the brain and testes of Sprague Dawley rats. It appeared that the Ti concentration was significantly higher in the medium- and high-dose treated groups (G4 and G5) than in the low-dose (G3), control (G2), and placebo (G1) groups.

Hematological Parameter

In the present study, hematological parameters fluctuate due to chronic exposure to TiO_2-NPs. Red blood cells (RBC) count, hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), lymphocytes, and monocytes were significantly decreased (Table 4). In our study, MCV significantly increased at higher doses of TiO_2-NPs (Table 4). Similarly, the serological parameters ALT, AST, ALP, urea, creatinine, LDH, and total bilirubin are significantly increased with increasing dose compared with the control and saline groups (Table 5).

Table 3. Titanium accumulation in selected organ (brain and testis)

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	p-value
Ti accumulation in brain	0.01±0.09[a]	0.01±0.01[a]	3.39±0.10[b]	7.32±0.34[c]	13.84±0.33[d]	p=0.000
Ti accumulation in testis	0.01±0.02[a]	0.01±0.14[a]	3.13±0.57[b]	11.89±0.38[c]	19.12±0.17[d]	p=0.000

[a]: Similar to each other, different from b and c, [b]: Different from a, similar within itself, [c]: Different from both a and b, [d]: Different from a, b and c.
Ti: Titanium, SD: Standard deviation.

Table 4. Hematological parameters in rats by subcutaneous administration with anatase TiO_2-NPs for alternately "28 days"

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	p-value
Erythrocytes (1012/L)	8.65±0.32[a]	8.64±0.14[a]	7.64±0.17[ab]	6.62±0.26[b]	5.09±0.22[c]	p=0.000
Hemoglobin (g/dL)	13.28±0.23[a]	13.10±0.24[a]	12.52±0.20[a]	10.82±0.22[b]	8.86±0.19[c]	p=0.001
Hematocrit (%)	46.18±0.29[a]	46.76±0.41[a]	46.04±0.37[a]	42.09±0.52[b]	37.98±0.81[c]	p=0.000
Mean corpuscular volume (fL)	49.60±0.50[a]	50.00±0.70[a]	50.00±0.70[a]	45.60±0.67[b]	42.40±1.02[c]	p=0.002
Mean corpuscular hemoglobin (pg)	15.10±0.29[a]	14.70±0.40[a]	15.56±0.22[ab]	16.44±0.29[b]	19.50±0.54[c]	p=0.000
Mean corpuscular hemoglobin concentration (g/dL)	41.64±0.87[a]	41.47±0.67[a]	40.44±0.86[ab]	31.02±0.77[b]	29.50±0.87[c]	p=0.001
Leucocytes (109/l)	10.42±0.32[a]	10.64±0.55[a]	10.78±0.62[a]	13.34±0.34[b]	10.02±0.24[b]	p=0.000
Lymphocytes (%)	64.62±0.85[a]	64.52±0.67[a]	62.24±0.56[ab]	48.32±0.76[b]	40.96±0.97[c]	p=0.001
Monocytes (%)	7.06±0.19[a]	7.04±0.19[a]	6.18±0.38[a]	3.93±0.50[b]	2.83±0.24[b]	p=0.000

[a]: Similar to each other, different from b and c, [b]: Different from a, similar within itself, [c]: Different from both a and b, [ab]: Similar to both a and.
TiO_2-NPs: Titanium dioxide nanoparticles.

Table 5. Serological parameters in rats by subcutaneous administration with anatase TiO_2-NPs for alternately "28 days"

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	p-value
Alanine transaminase (IU/L)	41.40±2.90[a]	42.20±1.93[a]	53.80±2.67[b]	78.40±3.21[c]	82.00±2.55[d]	p=0.000
Aspartate transaminase (IU/L)	52.40±2.50[a]	52.80±2.41[a]	53.00±1.70[a]	61.00±1.94[b]	60.60±2.34[b]	p=0.002
Alanine phosphatase (IU/L)	266.80±4.35[a]	241.00±3.45[a]	249.30±4.24[b]	275.80±3.20[c]	307.80±4.21[d]	p=0.000
Urea	41.20±1.01[a]	42.40±0.87[a]	52.40±1.07[b]	58.40±0.92[c]	70.00±1.30[d]	p=0.002
Creatinine	0.84±0.01[a]	0.87±0.02[a]	0.91±0.01[b]	1.03±0.05[bc]	1.17±0.07[c]	p=0.005
Lectate dehydrogenase	177.40±1.93[a]	179.20±1.16[a]	188.00±1.22[b]	288.00±11.84[c]	332.20±16.71[d]	p=0.000
Total bilirubin	0.41±0.05[a]	0.41±0.06[a]	0.55±0.05[b]	0.87±0.04[c]	0.90±0.05[d]	p=0.000

[a]: Similar to each other, different from b and c, [b]: Different from a, similar within itself, [c]: Different from both a and b, [d]: Different from a, b and c, [bc]: Similar to both b and c.
TiO_2-NPs: Titanium dioxide nanoparticles.

Oxidative Stress Analysis

The values of LPO, MDA, CAT and GSH were significantly increased in the high-dose treated groups (G4 and G5) compared with the control and saline groups (G1 and G2) (Table 6).

Genotoxicity by Commet Assay

In our study, both blood and organ samples showed significant decreases in head length and head DNA percentage and significant increases in tail length, comet length, tail DNA percentage, and tail moment (Tables 7-9). These abnormalities were not significant in the low-dose-treated and normal-saline-treated groups compared with the control group, suggesting that they were caused by DNA breakage, lagging acentric chromosomes, and chromatid fragments induced by the toxicant (Figure 4).

Table 6. TiO_2-NPs induced alteration in oxidative stress enzymes of brain tissue after "28 days" of exposure

Parameters	Group 1	Group2	Group 3	Group 4	Group 5	p-value
Lipid peroxidase mm/100g	18.40±2.90[a]	18.20±1.93[a]	23.80±2.67[b]	53.40±3.21[c]	69.00±2.55[d]	p=0.001
Malondialdehyde nM/g	135.40±2.50[a]	134.80±2.41[a]	151.00±1.70[b]	176.00±1.94[c]	195.60±2.34[d]	p=0.000
Catalase (unit/mL)	348.80±4.35[a]	347.00±3.45[a]	384.30±4.24[b]	411.80±3.20[c]	474.80±4.21[d]	p=0.000
Glutathione (uM/g)	461.20±1.01[a]	460.40±0.87[a]	492.40±1.07[b]	540.40±0.92[c]	593.00±1.30[d]	p=0.001

[a]: Similar to each other, different from b and c, [b]: Different from a, similar within itself, [c]: Different from both a and b, [d]: Different from a, b and c.
TiO_2-NPs: Titanium dioxide nanoparticles.

Table 7. Genotoxic abnormalities in blood of rats treated with anatase TiO_2-NPs assessed by commet assay

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	p-value
Length head	41.40±1.36[a]	40.40±0.67[a]	39.80±2.74[a]	16.40±2.08[b]	7.60±1.93[c]	p=0.002
Length tail	7.60±1.43[a]	7.40±0.60[a]	8.00±0.89[a]	37.40±1.80[b]	49.00±1.76[c]	p=0.001
Length commet	46.60±0.87[a]	46.20±0.96[a]	46.59±0.65[a]	53.80±1.24[b]	56.80±1.29[c]	p=0.000
Head DNA (%)	84.98±0.76[a]	84.91±0.57[a]	81.19±0.59[a]	43.22±0.49[b]	11.84±0.41[c]	p=0.000
Tail DNA (%)	15.01±0.11[a]	15.08±0.16[a]	17.21±0.23[a]	56.83±0.56[b]	88.29±0.98[c]	p=0.001
Tail Moment	0.78±0.01[a]	0.76±0.05[a]	2.39±0.11[a]	14.20±0.90[b]	29.72±1.37[c]	p=0.000

[a]: Similar to each other, different from b and c, [b]: Different from a, similar within itself, [c]: Different from both a and b.
DNA: Deoxyribonucleic acid, TiO_2-NPs: Titanium dioxide nanoparticles.

Table 8. Genotoxic abnormalities in Brain of rats treated with anatase TiO_2-NPs assessed by commet assay

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	p-value
Length head	44.80±1.2[a]	43.80±0.8[a]	42.40±0.8[a]	21.80±0.5[b]	7.40±0.92[c]	p=0.001
Length tail	5.40±0.50[a]	6.40±0.50[a]	6.40±0.60[a]	35.00±1.5[b]	55.00±1.6[c]	p=0.000
Length commet	50.20±1.3[a]	50.30±0.8[a]	51.20±0.3[a]	56.80±1.3[b]	62.40±1.4[c]	p=0.000
Head DNA (%)	90.95±0.6[a]	90.77±0.6[a]	89.62±0.6[a]	61.01±0.4[b]	36.83±0.6[c]	p=0.001
Tail DNA (%)	8.83±0.54[a]	8.88±0.57[a]	8.99±0.22[a]	38.98±0.4[b]	63.16±0.6[c]	p=0.000
Tail moment	0.76±0.03[a]	0.79±0.02[a]	2.25±0.05[a]	11.43±0.1[b]	21.59±0.9[c]	p=0.000

[a]: Similar to each other, different from b and c, [b]: Different from a, similar within itself, [c]: Different from both a and b.
DNA: Deoxyribonucleic acid, TiO_2-NPs: Titanium dioxide nanoparticles.

Table 9. Genotoxic abnormalities in testis of rats treated with anatase TiO_2-NPs assessed by commet assay

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	p-value
Length head	45.20±0.71[a]	45.00±0.70[a]	44.20±1.21[a]	21.20±1.09[b]	9.00±0.70[c]	p=0.000
Length tail	5.00±0.70[a]	5.20±0.37[a]	5.80±0.37[a]	41.40±0.81[b]	62.20±1.23[c]	p=0.001
Length commet	50.20±0.42[a]	49.60±0.52[a]	50.80±0.61[a]	62.60±1.22[b]	71.20±1.24[c]	p=0.002
Head DNA (%)	90.39±0.22[a]	90.28±0.34[a]	89.50±0.62[a]	45.56±1.67[b]	27.13±0.72[c]	p=0.000
Tail DNA (%)	9.60±0.27[a]	9.72±0.32[a]	9.82±0.31[a]	54.43±1.62[b]	72.86±1.72[c]	p=0.000
Tail moment	0.48±0.01[a]	0.49±0.01[a]	1.95±0.30[a]	15.30±0.41[b]	43.48±1.14[c]	p=0.000

[a]: Similar to each other, different from b and c, [b]: Different from a, similar within itself, [c]: Different from both a and b.
DNA: Deoxyribonucleic acid, TiO_2-NPs: Titanium dioxide nanoparticles.

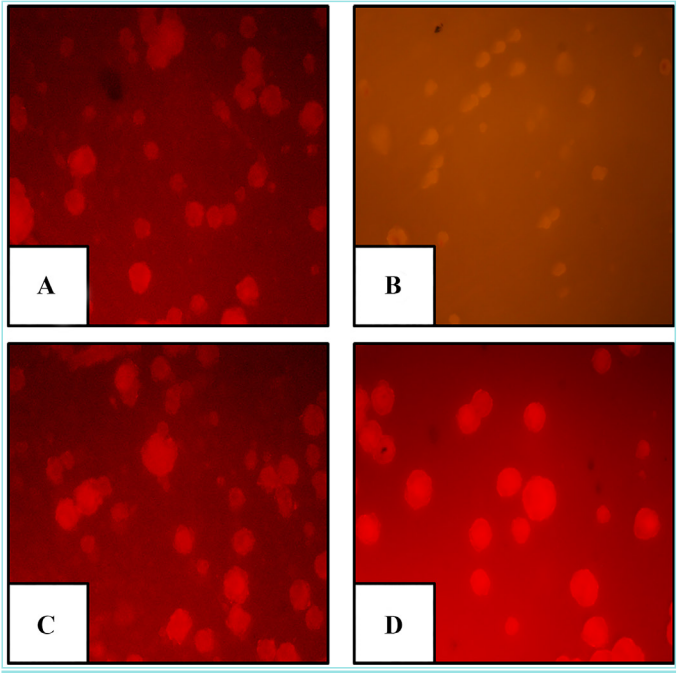

Figure 4. DNA damage induced by TiO_2-NPs in Sprague Dawley rats. **(A)** The control group shows normal nuclei with intact DNA and minimal or no comet tail formation, indicating no DNA damage. **(B)** Blood cells showing comet formation with visible DNA tail migration, representing TiO_2-NP-induced DNA strand breaks. **(C)** Gonadal cells exhibiting DNA damage as evidenced by increased comet tail length and head deformation, indicating significant genotoxic effects of TiO_2-NPs on reproductive tissues. **(D)** Brain cells showing pronounced comet tails, suggesting extensive DNA fragmentation and high levels of TiO_2-NP-induced DNA damage.

DNA: Deoxyribonucleic acid, TiO_2-NPs: Titanium dioxide nanoparticles.

DISCUSSION

The present study, entitled "Genotoxic and Hemato-biochemical effects induced by Anatase Titanium dioxide Nano Particles in Sprague Dawley rats", was conducted. The benefits of TiO_2-NPs are diverse in application, but vigorous use of TiO_2-NPs induces health abnormalities, such as behavioral changes, antioxidant imbalance, impairment of biochemical indices, cellular membrane damage, DNA damage, and reduced growth rate. The average size of TiO_2-NPs used in the current study was 25 nm, as confirmed by XRD and SEM. The findings of the current study are in good agreement with the study by[21], who used TiO_2-NPs of sizes 25 nm and 25-50 nm in albino rats and found that TiO_2-NPs of these sizes were toxic to their model organism. In the current study, TiO_2-NPs of 25 nm were toxic to Sprague Dawley rats. The anatase morphology of TiO_2-NPs is associated with greater toxicity than other polymorphs, such as rutile, due to its increased surface reactivity and its ability to generate ROS, which can lead to oxidative stress and cellular damage.[22]

Significant behavioral changes such as increased aggression, excessive grooming, piloerection (fluffed fur), anorexia, cannibalism, and signs of depression were observed in the group exposed to the highest dose of TiO_2-NPs. These alterations suggest a potential neurotoxic effect associated with high-level exposure. Such behaviors may indicate stress, neurological disruption, and systemic toxicity induced by nanoparticles. The presence of anorexia and cannibalism further reflects impaired physiological and social functioning. These findings highlight the need for careful evaluation of TiO_2-NPs safety, particularly at higher concentrations. This result is consistent with those reported by[23], who observed the same behavioral changes in rats due to TiO_2-NPs. Exposure nanoparticles have the potential to accumulate in sensitive organs such as the brain and testes, where they may disrupt normal physiological functions. This accumulation can result in neurotoxicity or reproductive toxicity by interfering with cellular mechanisms. Our study demonstrated a dose-dependent increase in Ti accumulation within the brain and testes, with significantly higher concentrations observed in the medium- and high-dose groups. These findings align with the results of El-Shafai et al.[24], Bermudez et al.[25], and Mahrousa[26], who also reported greater Ti accumulation at higher exposure levels. This consistency reinforces the evidence that Ti exhibits bioaccumulative properties in sensitive organs.

Exposure to TiO_2-NPs led to a significant reduction in body weight and the somatic indices of organs, suggesting potential toxic effects that may be linked to organ stress or damage. Such outcomes indicate that TiO_2-NPs can induce oxidative stress, inflammation, impaired organ function, and raise serious concerns about their safety under long-term exposure. Similar decreases in body weight following TiO_2-NP exposure have been documented in earlier studies by Geraets et al.[27], Faucher and Lespes[28], and Tian et al.[29] In the present study, no mortality was observed among rats treated with TiO_2-NPs at doses of 80, 120, and 160 mg/kg body weight.

However, animals in experimental groups (G3, G4, and G5) exhibited a significant decrease in body weight after 28 days compared with the control and saline-treated groups ($p<0.05$), indicating adverse effects of TiO_2-NPs (Table 3). These findings are consistent with reports by El-Sharkawy et al.[30] and El-Sheikh et al.[31] who also observed marked

reductions in body weight in rats exposed to TiO_2-NPs for 45 days. Likewise, other studies[32] have confirmed notable declines in body weight associated with TiO_2-NP exposure.

A reduction in both relative and absolute organ weights may indicate toxicity of TiO_2-NPs. Such alterations can disrupt normal organ function and may signify tissue damage or physiological stress, ultimately affecting overall health and homeostasis. In our study, the high-dose treatment groups (G4 and G5) showed a significant decrease in both absolute and relative weights of the brain and gonads. Additionally, gonad dimensions, including length and width, were markedly reduced compared to the control and saline groups. Similar findings have been reported in other studies involving rats exposed to TiO_2-NPs[33], reinforcing that prolonged exposure to excessive amounts of TiO_2 can lead to adverse effects.

Hematological parameters are essential for evaluating the overall health and detecting a wide range of disorders, such as anemia and infections. They help monitor disease progression and the effectiveness of treatments. In the present study hematological parameters fluctuate due to chronic exposure of TiO_2-NPs. RBC count, Hb, HCT, MCV, MCHC, lymphocytes, and monocytes were significantly decreased, and white blood cells (WBC) were significantly increased at higher doses of TiO_2-NPs.[34] Reported a decrease in RBC count and Hb in female mice after TiO_2-NPs, which is in accordance with our results. The observed decrease in these parameters indicates anemia. This decrease may be due to the production of immature RBCs, the suppressive impact of TiO_2-NPs on stem cells in the bone marrow, and malfunction of erythropoiesis.[35] The study is also similar to our study, but differs in the mode of administration of NPs. An experiment conducted by[36] reported fluctuations in hematological parameters induced by TiO_2-NPs in pregnant mice, consistent with our study.[37] Performed a similar study on male Sprague Dawley rats, and the results are consistent with our findings.[38] Also reported similar hematological findings in rats. WBC levels in our study increased significantly at higher doses of TiO_2 -NPs (Table 4). This increase is may be due to innate defense system activation.[39] A similar increase in WBC was also reported by Smith et al.[40], Shakeel et al.[41], and Heo et al.[42] in their studies on rats. In this study, lymphocyte and monocyte counts were significantly reduced (Table 4). The decrease in lymphocytes, also reported by[43] after oral administration of TiO_2-NPs, is in accordance with our results. The significant decrease in monocytes observed in our study is consistent with[44], who administered TiO_2-NPs to albino rats.

In this study, TiO_2-NPs doses of 80, 120, and 160 mg/kg were injected subcutaneously. Administered doses of 120 mg/kg and 160 mg/kg body weight alter serum parameters, as supported by[45], who orally administered TiO_2-NPs at 62.5 mg/kg, 125 mg/kg, and 250 mg/kg body weight and reported alterations at doses higher than 125 mg/kg body weight. In study[46], TiO_2-NPs doses of 10, 50, 100, and 200 mg/kg body weight were administered for 60 days. “100 mg/kg” and “200 mg/kg” showed significant fluctuations in serum biochemical parameters, supporting our dose-dependent study.

Toxic chemicals can cause hepatotoxicity. The liver protects the body from toxic chemicals and serves as an important site for detecting biological changes. The liver secretes its substances into bile, resulting in nanoparticle distribution throughout the animal body. In[47], hepatic enzymes (ALT, AST, and ALP) were significantly increased after 28 days of chronic exposure to TiO_2-NPs in high-dose groups. ALT, AST, and ALP are present in the liver, and fluctuations in their levels indicate liver injury. Similar studies related to AST, ALT, ALP fluctuations were supported by.[48]

In our study, a significant increase in hepatic enzymes (Table 6) may be due to liver injury, as supported by previous work of Sadiq et al.[49], Meena and Paulraj[50], Rizk et al.[51], and Relier et al.[52] Similar studies related to AST, ALT, ALP fluctuations were supported by Abu-Dief et al.[53], Sheydaei et al.[54], Chen et al.[55] and Yang et al.[56]

Following exposure to toxicants, LDH activity increased in various organs, including the liver, heart, and lungs. LDH is an isoenzyme that plays a key role in glycolysis and gluconeogenesis. Toxicant-induced injury causes LDH to be released from damaged cells or organs into the bloodstream, resulting in elevated serum LDH activity.[57] In the present study, LDH activity in rats increased in proportion to TiO_2 nanoparticle exposure (Table 6). Similar findings were reported in[58], who observed a significant increase in LDH activity after oral administration of TiO_2 nanoparticles to female mice, consistent with our results.

The kidneys are responsible for removing nitrogenous waste from the blood. TiO_2-NPs circulating in the bloodstream can lead to renal dysfunction and damage. In this study, rats treated with TiO_2-NPs exhibited renal impairment, as evidenced by significant increases in urea and creatinine levels (Table 5) compared with the control group, particularly at doses of 120 and 160 mg/kg body weight. The observed rise in urea and creatinine may be attributed to nephron damage, glomerular toxicity, congestion of renal tubules with protein-rich fluid, and glomerular swelling, all due to the accumulation of TiO_2-NPs in the kidneys. These findings are consistent with previous studies[59], which reported similar increases in urea and creatinine following exposure to TiO_2-NPs, and with studies[60] confirming renal toxicity. Additionally, total bilirubin levels in this study were significantly elevated in the high-dose groups G4 and G5 ($p<0.05$) (Table 6). This increase may result from TiO_2-NP-induced RBC destruction, potentially leading to anemia and liver dysfunction, a possibility further supported by fluctuations in hepatic enzyme levels observed in this study. The results for total bilirubin agree with earlier research[61], which reported similar increases following TiO_2-NPs exposure.

A key parameter for assessing the potential toxicity of nanoparticles is oxidative stress. Antioxidants play a crucial role in protecting the body from the harmful effects of free radicals and ROS, which can damage cells and tissues, by neutralizing them. Insufficient antioxidant levels increase vulnerability to oxidative stress and toxicity, highlighting their importance in mitigating these effects Selamoglu et al.[62], Adnan et al.[63] TiO_2-NPs can induce fenton-type reactions, leading to ROS generation, including oxyradicals, and modulate antioxidant defenses, resulting in altered CAT and GSH levels and increased oxidative stress markers such as LPO and MDA (Salam et al.[64], Ibrahim et al.[65] In the present study, levels of GSH, LPO, and MDA were significantly elevated in the medium- and high-dose-treated groups, indicating activation of the antioxidant defense system in response to nanoparticle-induced toxicity. These findings are consistent with previous reports on alterations in CAT, GSH, and LPO levels Ishak et al.[66], Ahmad et al.[67]

In our experiment, we investigated genotoxic abnormalities using the comet assay. Damage to bone marrow DNA can result in alterations in the number or types of cells present in the bloodstream. The comet assay evaluates DNA damage by producing images that reveal various

forms of damage, including characteristic comet-like structures in cells. We analyzed several parameters measured in this assay, including head length, tail length, comet length, percentage of DNA in the head and tail, and tail moment, in the blood, brain, and gonads of rats. Previous in vivo studies have demonstrated the genotoxic potential of TiO_2-NPs. For instance[68], conducted research in mice treated with the same toxicant, reporting significant DNA damage in the blood, although their results differ from ours. Our genotoxicity findings, particularly those from the comet assay, are consistent with studies by Chen et al.[69], and Valentini et al.[70] Moreover, studies evaluating DNA damage in the brains of mice, such as[71], reported results similar to our comet assay observations.

Study Limitations

This study provides valuable insights into the genotoxic and hemato-biochemical effects of anatase TiO_2-NPs. The study was limited by a small sample size and short exposure duration, but this study highlights the acute toxicological impacts of short-term exposure to small-sized anatase TiO_2-NPs in a limited population, enabling focused observation of dose-dependent effects.

CONCLUSION

In conclusion, our study shows that TiO_2-NPs are highly toxic at the moderate and high doses tested, causing genotoxic and hemato-biochemical effects in Sprague Dawley rats.

MAIN POINTS

- Titanium dioxide nanoparticles (TiO_2-NPs) showed significant accumulation in brain and gonads (especially in G4 and G5 groups) confirmed by inductively coupled plasma mass spectrometry.
- Body and organ weights were significantly decreased (brain and gonads) in the medium- to high-dose groups. Gonadal measurements (length and diameter) were also reduced significantly, indicating possible reproductive toxicity.
- Exposure to TiO_2-NPs caused decreases in red blood cells, hemoglobin, hematocrit, mean corpuscular hemoglobin concentration, lymphocytes, and monocytes. Liver and kidney function markers (alanine transaminase, aspartate aminotransferase, urea, creatinine, lactate dehydrogenase, bilirubin) were increased significantly in the high-dose groups, indicating systemic toxicity and organ stress.
- High-dose groups (G4 and G5) exhibited significant increases in oxidative stress markers, including lipid peroxidase, malondialdehyde, catalase, and glutathione. This suggests reactive oxygen species -mediated cellular damage as a key mechanism of toxicity.
- Comet assay results from blood, brain, and gonads showed increased deoxyribonucleic acid (DNA) fragmentation in high-dose groups, evidenced by longer tail lengths, higher tail DNA %, and increased tail moment.

ETHICS

Ethics Committee Approval: Ethical approval was obtained on Government College University Faisalabad Ethics Review Committee (approval number: GCUF/ERC/976, date: 24.09.2019), but due to COVID-19 the study commenced in 2021. The trial was conducted from March to April 2021, after which bioassays were completed.

Informed Consent: Patient approval has not been obtained as it is performed on animals.

Footnotes

Authorship Contributions

Concept: M.A.L., F.J., Design: M.A.L., F.J., Data Collection and/or Processing: M.A.L., Z.I., A.K., Analysis and/or Interpretation: M.A.L., F.J., Literature Search: M.A.L., Z.I., A.K., Writing: M.A.L., F.J.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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RESEARCH ARTICLE

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Retrospective Analysis of Anesthesia Approaches in Clavicle Surgery: Experience from A Single Center

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Abstract

BACKGROUND/AIMS: Due to the complex and variable innervation of the clavicle, clavicle fracture repair is usually performed under general anesthesia (GA). Ultrasound-guided regional anesthesia (UGRA), which targets multiple innervation zones, may offer an effective and resource-efficient alternative.

MATERIALS AND METHODS: This single-center retrospective cohort study by the Samsun University Non-Interventional Clinical Research Ethics Committee (approval number: 2025/10/26, date: 14.05.2025) examined adults aged 18-70 years [American Society of Anesthesiologists (ASA) I-III] who underwent clavicle surgery between January 1, 2022, and April 1, 2025. UGRA consisted of a predefined combination of modified interscalene, modified superficial cervical plexus, and clavipectoral fascial plane blocks, using 30 mL of local anesthetic (0.25% bupivacaine and 0.5% lidocaine). Outcomes included operating room (OR) time components, anesthetic and analgesic requirements, complications, hospital length of stay, and cost analysis modeled with a constant coefficient of 1 United States Dollar/min, using 2025 unit prices.

RESULTS: Twenty-six patients were analyzed: UGRA, (n=19) (73%), and GA, (n=7). The groups were similar in age, sex, ASA class, fracture type, surgical time, and time to discharge. All UGRA cases were completed without conversion; no block-related complications occurred. Two UGRA patients experienced mild intraoperative discomfort, which was managed with propofol infusion (1 mg/kg/h). Compared with GA, UGRA demonstrated significantly shorter OR occupancy and lower OR costs (both $p=0.032$), lower equipment and medication costs ($p<0.001$ and $p=0.004$, respectively), and lower total costs ($p=0.007$). Variable medication costs were observed only with GA.

CONCLUSION: Combined UGRA enabled safe awake clavicle surgery without conversion to GA and was associated with shorter OR occupancy and lower overall costs compared with GA. Larger prospective studies are needed to confirm efficacy, improve patient selection, and evaluate long-term outcomes.

Keywords: Regional anesthesia, clavicle fracture, awake surgery

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INTRODUCTION

Clavicle fractures account for approximately 2.6-4% of all adult fractures and represent about 44% of shoulder-girdle injuries. The midshaft is the most frequently affected region. Surgical treatment typically involves open reduction and internal fixation, necessitating effective anesthesia management, particularly with regard to postoperative analgesia.[1,2]

The clavicle has a complex and variable innervation pattern that is not yet fully elucidated, leading to an ongoing debate about the optimal regional anesthesia technique for surgical repair of the clavicle.[3] While the cutaneous innervation overlying the clavicle is clearly supplied by the supraclavicular nerve (a branch of the superficial cervical plexus), the source of deep sensory innervation is controversial. Some studies suggest the supraclavicular nerve also provides deep innervation, whereas others implicate contributions from the brachial plexus branches, including the subclavian nerve, long thoracic nerve, and suprascapular nerve.[4]

This anatomical complexity limits the effectiveness of single-site regional blocks and has led many centers to favor general anesthesia (GA). However, GA has drawbacks, including delayed postoperative recovery, increased analgesic requirements, and higher overall costs.[5]

The advent of ultrasound-guided regional anesthesia ((UGRA) has transformed clinical practice by enabling direct visualization of nerve structures and the safer, more effective administration of nerve blocks. Techniques such as interscalene brachial plexus block, superficial cervical plexus block, and the recently introduced clavipectoral fascial plane block have emerged as viable alternatives to GA for clavicle surgery.[5,6] Combining blocks that target multiple innervation zones has been shown to enhance both intraoperative and postoperative analgesia while reducing complication rates. Importantly, some of these techniques avoid phrenic nerve involvement, thereby preserving respiratory function-an advantage for patients with pulmonary compromise.[7] Furthermore, several studies have demonstrated that clavicle fracture surgery can be successfully performed under combined regional blocks in awake patients, providing adequate surgical anesthesia and excellent patient comfort.[8]

Beyond clinical outcomes, cost-effectiveness is also a critical factor in selecting anesthetic techniques. Regional anesthesia reduces intraoperative opioid use, shortens recovery times, and promotes more efficient use of healthcare resources, benefits that are especially relevant in resource-limited settings.[9]

The purpose of this study was to retrospectively evaluate our institutional experience in clavicle surgery and to compare GA and ultrasound-guided regional techniques in terms of efficacy, safety, resource use, and postoperative outcomes.

MATERIALS AND METHODS

Study Design and Ethical Approval

This retrospective cohort study was approved by the Samsun University Non-Interventional Clinical Research Ethics Committee (approval number: 2025/10/26, date: 14.05.2025). Medical records of patients aged 18-70 years with an American Society of Anesthesiologists (ASA) physical status of I-III who underwent clavicle surgery between January 1, 2022, and April 1, 2025, were reviewed. Patients were excluded if they had neurological deficits in the upper extremity; a history of neck surgery or radiotherapy; moderate or severe pulmonary disease; contraindications to peripheral nerve block (e.g., allergy to local anesthetics, coagulopathy, or infection at the injection site); preoperative opioid therapy; had psychiatric conditions likely to affect pain perception; or had incomplete clinical records.

Data Collection

Eligible patients were identified using the hospital information management system (FONET, v4.22.6.1). Data were extracted from electronic records and patient files. Variables recorded included demographic characteristics, ASA classification, comorbidities, anesthesia type, volume of local anesthetic administered, additional intraoperative anesthetic and analgesic requirements, surgical fracture type, duration of surgery, drug and material costs, operating room (OR) charges, length of hospital stay, and postoperative complaints.

Operating Room Time Components

OR utilization was assessed across four time intervals, each measured in minutes:

Anesthesia duration: From the start of induction to tracheal intubation and the start of mechanical ventilation,

Positioning time: The time required to properly position the patient for surgery,

Operating time: From skin incision to completion of surgery,

Recovery time: From the end of surgery to discharge from the OR (including extubation and recovery).

Total OR time was defined as the sum of these four components.

Costing Approach

Patient-level drug and consumable costs, excluding procedure-specific surgical materials (e.g., implants, plates/screws, staples), were calculated separately as unit price × quantity based on hospital purchasing records. For comparability, unit prices for devices and medications were fixed according to the 2025 price list. To standardize OR operating costs, a minute-based fixed coefficient (k), excluding personnel salaries, was used; in this study, k=1 US dollar per minute. When Turkish-lira-denominated results were converted to TL using the exchange rate prevailing on the reporting date.

Patients were categorized into two groups based on the type of anesthesia received:

Group UGRA: Ultrasound-guided regional anesthesia

Group GA: General anesthesia

Routine Ultrasound-Guided Regional Anesthesia Procedure in Our Clinic

Patients in the UGRA group were those who had routinely received, as part of standard care for clavicle fracture surgery, a predefined combination of UGRA blocks (modified interscalene, modified superficial cervical plexus, and clavipectoral fascial plane blocks). According to anesthesia records, all blocks were performed under aseptic conditions using a high-frequency linear ultrasound transducer, an in-plane approach, and a 22-gauge Stimuplex® needle. A total of 30 mL of local anesthetic (0.25% bupivacaine and 0.5% lidocaine) was

administered, distributed across three planes as follows: 7.5 mL to the interscalene level, 12.5 mL to the superficial cervical plexus level, and 10 mL to the clavipectoral fascial plane. The modified interscalene technique targeted the C5 nerve root with circumferential deposition, aiming to limit spread to adjacent cervical roots. The superficial cervical plexus block was performed through the same skin entry used for the interscalene block. After completing the deeper injection, the needle was redirected slightly upward into a more superficial layer just beneath the deep fascia of the sternocleidomastoid muscle, while the ultrasound probe remained steady. For the clavipectoral fascial plane block, two injections (one medial and one lateral to the fracture line) were performed, with caudal-to-cranial needle advancement to deposit local anesthetic within the clavipectoral fascia.

Standard monitoring (3-lead electrocardiogram, non-invasive blood pressure, and pulse oximetry) was applied. Procedural sedation/analgesia was provided according to departmental practice, using intravenous midazolam (0.01-0.02 mg/kg) and ketamine (0.15 mg/kg). Approximately 20 minutes after block placement, patients were transferred to the OR. The surgical field was assessed for sensory block, and surgery commenced when adequate anesthesia was documented.

General Anesthesia Management

In Group GA, GA was administered according to our clinic's routine protocol for clavicle fracture surgery. With standard monitoring in place, induction was achieved with propofol (1.5-2.5 mg/kg), fentanyl (1-2 µg/kg), lidocaine (1 mg/kg), and rocuronium (0.6 mg/kg). Maintenance was provided with sevoflurane or desflurane at minimum alveolar concentration. Neuromuscular blockade was reversed using atropine (0.02 mg/kg) and neostigmine (0.05 mg/kg). Patients who underwent alternative anesthesia approaches (e.g., non-standard or combined regional techniques, deep sedation without airway instrumentation) or whose anesthesia documentation was incomplete were excluded from the study. No additional regional anesthesia techniques were included in the GA group.

Statistical Analysis

Were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as mean ± standard deviation and compared using the Independent samples t-test or the Mann-Whitney U test, depending on the normality of the distribution. Categorical variables were analyzed using the chi-square test, with Yates' continuity correction applied when appropriate. A p-value of <0.05 was considered statistically significant.

RESULTS

Data from 26 patients who underwent clavicle surgery were included in the final analysis after excluding 14 patients who did not meet the inclusion criteria or had missing data. Patients were divided into two groups: UGRA (n=19) and GA (n=7) (Figure 1). There were no statistically significant differences between the groups in terms of age, gender, ASA classification, or fracture type. Although the difference in discharge time did not reach statistical significance (p=0.054), the observed trend toward earlier discharge in the UGRA group may indicate faster postoperative recovery. Surgical duration and discharge times are summarized in Table 1.

All patients in the UGRA group received the combined peripheral nerve block technique as previously described. All blocks were successful, and no complications were reported. Two patients experienced mild intraoperative discomfort and were managed with a propofol infusion at 1 mg/kg/h; neither required dose escalation nor conversion to GA. The discomfort was attributed to mild anxiety rather than inadequate sensory blockade, as ultrasound guidance showed no evidence of incomplete nerve coverage in either patient.

The comparison of cost parameters between the groups is presented in Table 2. OR usage time and fees were significantly lower in the UGRA group than in the GA group (p=0.032 for both). Equipment and medicine costs were also reduced in the UGRA group ($p<0.001$ and p=0.004, respectively). The gas cost, present only in the GA group, was included in the "Medicine cost" category rather than listed separately, as it represents a pharmacologic anesthetic expense calculated on the same cost line. Consequently, the total cost was significantly lower in the UGRA group (p=0.007). A detailed breakdown of materials and medications used per group is provided in Table 3.

DISCUSSION

This study showed that UGRA was the predominant anesthetic technique for clavicular surgeries at our clinic. Of the 26 patients evaluated, 73% underwent surgery under UGRA. The combination of modified interscalene block, superficial cervical plexus block, and clavipectoral fascial plane blocks provided effective anesthesia and analgesia without conversion to GA, and no complications were observed.

Regional anesthesia is well established for awake surgery in the arm and forearm, but its use in shoulder and clavicular procedures is still relatively uncommon.[10,11] In our series, we focused on clavicular surgery and found that these operations can be completed safely and effectively without GA. The approach we used proved technically feasible and was well tolerated by patients. Our results are consistent with those of Akyurt et al.[12], who reported similar benefits for awake shoulder surgery, including lower perioperative costs. In our study, a comparable reduction in cost was also observed for clavicular procedures.

The primary aim of this study was not to perform a detailed cost analysis but to demonstrate that awake clavicular surgery is feasible in routine practice. GA is often preferred in these cases, partly because of the clavicle's complex sensory innervation and concerns that regional techniques alone may not provide a complete surgical block.[13] This tendency may discourage attempts to perform awake surgery during such procedures.

Similar findings were reported by Kaciroğlu et al.[8], who successfully performed surgical repair of clavicle fractures using a combination of ultrasound-guided interscalene and superficial cervical plexus blocks in awake patients. Their results, which were similar to ours, demonstrated effective surgical anesthesia and excellent patient comfort without the need for GA. Building on these findings, Balci et al.[14] described a different combination-the clavipectoral plane and serratus posterior superior intercostal plane blocks-and reported satisfactory postoperative analgesia for clavicular surgery.

By sharing our clinical experience, we aim to challenge this perception. With appropriate patient selection and block technique, awake

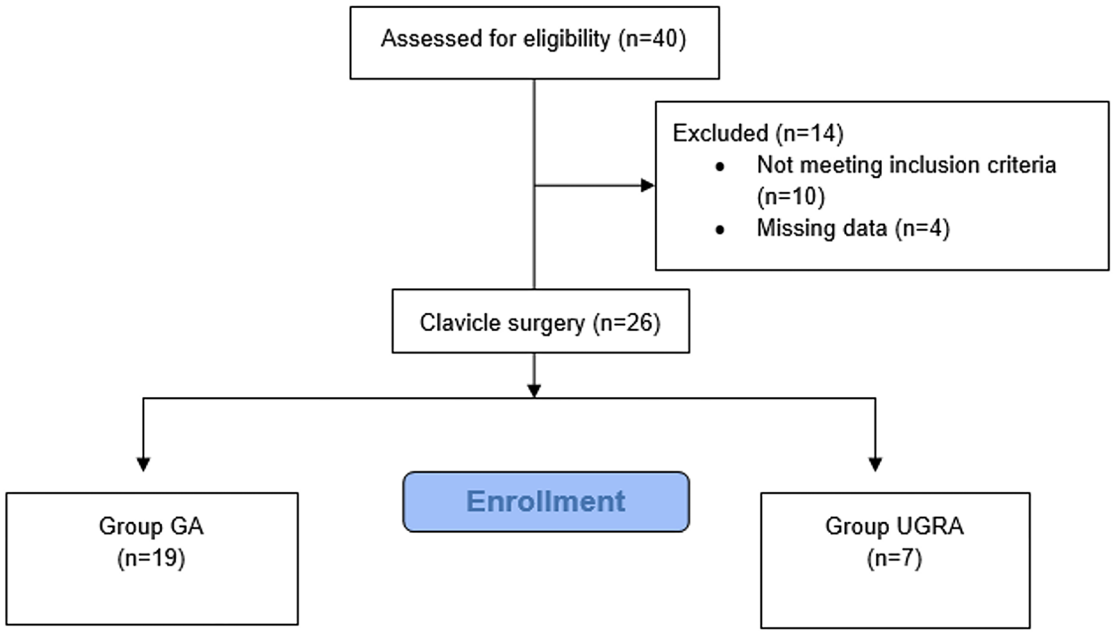

Figure 1. Flow chart of this study. A total of 40 patients were screened for eligibility; 14 were excluded (missing data, n=4; not meeting inclusion criteria, n=10). Finally, 26 patients were analyzed (UGRA=19, GA=7).

GA: General anesthesia, UGRA: Ultrasound-guided regional anesthesia.

Table 1. Patient demographic data and fracture type

	GA (n=7)	UGRA (n=19)	p
Age (years)	45.42±18.48	39.78±15.59	0.490
Gender (F/M)	1/6	5/14	0.904
Height	171.14±8.49	171.26±7.26	0.974
Weight	80.86±7.31	76.53±11.07	0.265
ASA* I/II/III	1/4/2	3/15/1	0.904
Fracture type (M/L)	5/2	17/2	0.604
Surgical time	89.85±26.99	72.10±26.78	0.165
Discharge time(<12hour/>12 hour)	2/5	15/4	0.054

Data are expressed as mean ± standard deviation or median (25th-75th percentiles). Normality of the data distribution was assessed using the Kolmogorov-Smirnov test. Continuous variables were compared using the Independent samples t-test for normally distributed data and the Mann-Whitney U test for non-normally distributed data. Categorical variables were analyzed using the chi-square test or Fisher's exact test, as appropriate.
F: Female, M: Male, ASA*: American Society of Anesthesiologists Classification, M: Middle, L: Lateral, GA: General anesthesia, UGRA: Ultrasound-guided regional anesthesia.

Table 2. Cost comparison by groups

	GA (n= 7)	UGRA (n=19)	p
Operating room usage time (minute)	114±27.56	84.05±26.46	0.032
Operating room usage fee (TL)	4689.96±1133.82	3457.82±1088.56	0.032
Equipment cost (TL)	378.14±31.76	189±3.74	<0.001
Medicine cost (TL)	408.88±120.57	177.36±29.30	0.001
Total cost (TL)	5476.97±1138.80	3.824.18±1.088.96	0.007

Data are expressed as mean ± standard deviation or median (25^{th}-75^{th} percentiles). Normality of data distribution was assessed using the Kolmogorov-Smirnov test. Continuous variables were compared using the Independent samples t-test for normally distributed data and the Mann-Whitney U test for non-normally distributed data. Categorical variables were analyzed using the chi-square test or Fisher's exact test, as appropriate. TL: Turkish lira, data are presented as mean ± standard deviation.
GA: General anesthesia, UGRA: Ultrasound-guided regional anesthesia.

clavicular surgery can be a practical alternative to GA. This study should be regarded as a proof-of-concept, demonstrating that such an approach is both feasible and beneficial in selected cases. Broader adoption could expand the role of regional anesthesia in upper-trunk surgery and optimize perioperative resource utilization.

Several factors may have influenced our results. Because this was a retrospective review, we could only use the information available in the records, and some variables could not be controlled. The number of awake clavicular surgery cases was small, which limits the strength of our statistical comparisons. Even so, the fact that most patients underwent the procedure while awake suggests that this technique can be applied in routine clinical practice. Cost figures should be viewed in context, as expenses vary widely between hospitals and countries. Ultimately, the real measure of success should be patient comfort and satisfaction rather than costs alone.

Study Limitations

This study has several limitations. Because it is retrospective, we relied on the accuracy and completeness of the medical records, and some potentially relevant clinical details were unavailable. The patient number was small and came from a single center with extensive experience in regional techniques; this may limit how well the findings can be applied to other settings. Cost estimates should also be

Table 3. Detailed list of drugs and supplies included in the cost analysis, by group

Equipment		Medicine/gas		
Group UGRA	**Group GA**	**Group UGRA**	**Group GA**	
EKG pallet	EKG pallet	Ketamine 1-2 mg/kg	Midazolam 0.03 mg/kg	Ephedrine 0.1 mg/kg**
İnjektor	İnjektor	Midazolam 0.03 mg/kg	Fentanyl 1-2 µg/kg	Paracetamol 1 g
İntravenous cannula	İntravenous cannula	Bupivacaine 0.5% 0.5-1 mg/kg (RA)	Lidocaine 2% 1 mg/kg (i.v.)	Ondansetron 4 mg
Fluid line	Fluid line	Lidocaine 2% 1-2 mg/kg (RA)	Propofol 1.5-2.5 mg/kg	Atropine 0.01-0.03 mg/kg
Oxygen mask	Anesthetic face mask	Fentanyl 1-2 µg/kg	Rocuronium bromide 0.6 mg/kg	Neostigmine 0.02-0.03 mg/kg
Peripheral nerve block needle	Ventilation line	Propofol 0.5-1 mg/kg	Remifentanil 0.05-0.2 µg/kg/ min	NSAID 50 mg
	Endotracheal tube	Saline 1,000 mL/h	Sugammadex 2 mg/kg	Sevoflurane 15 mL/h
	Bacteria filter	Ephedrine 0.1 mg/kg**	Tramadol 100 mg	Desflurane 35 mL/h
	Airway	Paracetamol 1 g	Saline 1,000 mL/h	

GA: General anesthesia, UGRA: Ultrasound-guided regional anesthesia, EKG: Elektrokardiyografi, NSAID: Non-steroidal anti-inflammatory drug, RA: Regional anesthesia.

interpreted with caution, as pricing structures vary among institutions. These factors should be considered when interpreting the results.

CONCLUSION

This retrospective study indicates that clavicular surgery can be carried out safely and at lower cost by combining regional anesthesia techniques. The results add to the growing body of evidence that awake surgery is a practical option even for procedures involving structures with complex innervation, such as the clavicle. While our experience supports its feasibility in everyday practice, these findings should be interpreted with caution given the study design and sample size. Larger observational and controlled trials are needed to validate these results, assess long-term outcomes, and better define patient selection criteria.

MAIN POINTS

- A predefined combined ultrasound-guided regional anesthesia (UGRA) protocol (modified interscalene, superficial cervical plexus, and clavipectoral fascial plane) enabled conversion-free, awake clavicle surgery without block-related complications.
- Compared with general anesthesia, UGRA was associated with shorter operating room utilization times, lower fees and reduced equipment and medication costs, yielding a lower total cost ($p=0.007$).
- Findings support UGRA as a feasible, resource-efficient alternative for clavicle fracture surgery; larger prospective studies are warranted.

ETHICS

Ethics Committee Approval: This retrospective cohort study was approved by the Samsun University Non-Interventional Clinical Research Ethics Committee (approval number: 2025/10/26, date: 14.05.2025)

Informed Consent: Since we only analyzed anonymized data from past patients, informed consent was not needed and was waived by the ethics committee. The study was conducted in line with the Declaration of Helsinki.

Footnotes

Authorship Contributions

Surgical and Medical Practices: G.R., A.Y., S.T., Concept: G.R., S.T., M.S., Design: G.R., S.T., Data Collection and/or Processing: G.R., H.Ş.Ç., A.Y., M.Y., Analysis and/or Interpretation: S.T., M.S., Literature Search: H.Ş.Ç., M.Y., Writing: G.R., S.T.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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RESEARCH ARTICLE

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"Behind Closed Doors…" The Loneliness Experience of Patients in the Adult Intensive Care Unit: A Qualitative Study

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Abstract

BACKGROUND/AIM: Staying in the intensive care unit (ICU) can be a very stressful experience for patients. This stressful experience can cause patients to be isolated and feel lonely. This study aimed to explore the loneliness experiences of ICU patients.

MATERIALS AND METHODS: This phenomenological study used thematic analysis to identify. The patients hospitalized in the ICU of a state hospital in the south of Türkiye between 01 January and 01 December 2020 were selected using purposive sampling . Data was collected from 29 patients through face-to-face individual in-depth interviews. The consolidated criteria for reporting qualitative studies checklist was used to ensure research reporting guidelines were met.

RESULTS: Two main themes were revealed: "Tides of loneliness" and "Whispers in the void". ICU patients suffered from loneliness faced with various psychosocial problems. The findings highlight ICU processes that influence patients' loneliness and their emotional, social, and physical well-being.

CONCLUSION: To further enrich our understanding of loneliness in the ICU, future research could include the perspectives of patients' families or caregivers. This would offer valuable insights into how family involvement, visitation policies, or the absence of support impact patients' emotional experiences in the ICU. A more holistic view of the loneliness phenomenon would also encompass the role of the patient's immediate circle. To facilitate this, ICUs should be designed to foster daily communication between healthcare professionals, patients, and their families, ensuring that care is truly centered around the individual and their support system.

Keywords: Intensive care unit, loneliness, nursing, patient experience, qualitative study

INTRODUCTION

The experience of critical illness is a life-altering event, often marked by physical, emotional, and psychological distress. Patients who are critically ill face not only the challenges of their medical conditions but also the overwhelming environment of the intensive care unit (ICU), a place where complex treatments, high levels of surveillance, and often life-threatening conditions intersect. While the ICU is designed to provide life-saving interventions, it is also a space where patients experience profound isolation, uncertainty, and vulnerability. For many, the physical environment of the ICU can amplify feelings of loneliness, as patients are cut-off from their loved ones, unable to engage in normal social interactions or find comfort in familiar surroundings. Loneliness is a distressing emotional state caused by perceived deficiencies in one's social connections, leading to feelings of isolation and distress.[1-3] The key aspect of this subjective experience is that loneliness is a painful

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and difficult feeling to cope with.[1,2] This intercultural, universal and psychosocial phenomenon makes loneliness inevitable, and people experience various degrees of loneliness, pain and distress in different periods of their lives.[1,3-5] As stated by Sha'ked and Rokach[2], "loneliness is intertwined with our being, just like joy, hunger, and self-actualization. People are born alone, often experience the horrors of loneliness at death and desperately try to avoid the loneliness in between".[2]

Critical illness, compounded by the ICU experience, can lead to emotional and psychological strain. Loneliness has emerged as a significant aspect of the ICU patient experience, impacting both their mental and physical well-being.[1-3] Emotional loneliness, stemming from a lack of close relationships and the absence of social support, is often heightened in the ICU due to the physical separation from family and friends. Social isolation further exacerbates feelings of abandonment and distress, with patients struggling to cope with the absence of personal connections in an environment dominated by medical staff and machines.[6]

Staying in the ICU can be a very stressful experience for patients.[4,5,7,8] This stressful experience can cause patients to be isolated from the environment they are used to, and to feel lonely.[5,8,9] Yildirim et al.[10] found in their study that cardiac ICU patients had moderate levels of loneliness and that this was associated with death anxiety.[10] It has been reported that patients feel lonely, especially at certain times of the day or night, because of the slow passage of time in the ICU.[5,9] Especially the mechanical noises coming from the complicated technological equipment used in the ICU, the voices of other patients and being unable to talk to their relatives, cause patients to feel lonely, scared, anxious and abandoned.[4,5,8,11,12] As a result of this experience, patients often feel lonely, and thus, they need love, respect, privacy, and psychological and spiritual support.[8] Psychological psychological care and spiritual interventions, for instance, the use of cognitive behavioral therapy for anxiety, mindfulness practices, or social support interventions (such as virtual visits), could be considered evidence-based approaches to address loneliness and improve patient outcomes.[13,14] Bulut et al.[15] found that the spiritual care intervention implemented in the ICU positively affected the loneliness levels of patients. Thus, understanding patients' experiences can help ICU nurses improve care by reducing psychological distress and loneliness.[6]

In the ICU, the nurse-patient interactions are important. Therapeutic communication techniques like active listening, empathetic responses, and presence (simply being there for the patient) are effective in mitigating loneliness in ICU patients. However, the therapeutic interactions between nurse and patient are affected by the potential challenges nurses face in providing emotional care, such as time constraints, the high acuity of patients, and institutional pressures that prioritize physical care. Understanding these challenges can provide a benefit on how to improve nurse-patient interactions. The studies highlight that loneliness can significantly affect patients' emotional and psychological well-being in the ICU, emphasizing the critical role of nurses and healthcare teams in addressing these needs.[6,16] In a recent systematic review, it is reported that the display of a positive attitude by health personnel towards ICU patients is of great importance.[17] However, there is inadequate evidence to support their effectiveness in the psychological recovery of ICU patients.[18] It is well known that with the holistic approach, both physiological and psychological care of the patients is met and that they receive high quality care.[7,19] Despite the growing recognition of loneliness as a significant concern for ICU patients, there remains a gap in the literature regarding the depth of this experience. While much is known about the medical and physiological aspects of critical illness and recovery, less is understood about how patients perceive and cope with the profound loneliness they experience in the ICU. Existing research often focuses on the clinical aspects of ICU care, with limited exploration of the emotional and psychological impacts on patients' well-being, particularly regarding their coping mechanisms and interactions with nursing staff. The aim of this study is to explore the lived experience of loneliness among adult patients in the ICU, shedding light on the emotional, social, and physical dimensions of isolation in this setting. Through a qualitative approach, the study seeks to deepen our understanding of the factors that contribute to ICU patients' loneliness and the ways in which they cope with this experience. Nurses can create better outcomes for patients and help them to have better ICU experiences through a better understanding of patients' experiences. Nursing protocols or patient care routines are developed to create a more compassionate, supportive environment. In addition, hospital policy changes (e.g., extending visitation hours, allowing family members to stay overnight) can play an important role in mitigating loneliness for ICU patients. Finally, this study's findings might contribute to improving patient care in ICUs globally. By addressing this gap in knowledge, the study aims to provide valuable insights into how nursing interventions and ICU environments can be optimized to support patients' emotional and psychological needs during their critical illness.

MATERIALS AND METHODS

Study Design

In this study, the phenomenological method, a qualitative descriptive approach, was used. Qualitative descriptive studies explore participants' detailed thoughts, experiences, social processes, and working styles and offer a comprehensive summary of an event.[20,21] The phenomenological research design focuses not on the measurement of facts but on individuals' beliefs, perceptions, feelings, and experiences about a phenomenon.[20] In this type of study, the researcher determines "what" individuals experience and "how" they experience it.[22,23] The reporting of this study was performed in line with the consolidated criteria for reporting qualitative studies.[24]

Sample and Setting

The purposive sampling method was used in the present study. The inclusion criteria of patients were as follows: being ≥18 years old, volunteering to participate in the study, being conscious, oriented, and cooperative, being a native speaker of Turkish, having no communication problems, having no mental health problems, and staying in the ICU for at least one week without any intervals. In determining the sample size in qualitative studies, researchers use an approach that requires them to continue collecting data until the saturation point is reached. Saturation is considered the cornerstone of rigor in determining sample sizes in qualitative research, such as a narrow range of interviews (9-17) or focus group discussions (4-8). In addition, the study goal, nature of the study population, sampling strategy used (i.e., inductive vs. deductive), type of data, and saturation goal affect this number.[20,21] In the present study, the researcher stopped conducting interviews once data saturation was achieved and no new data or codes emerged. The researchers announced the study to ICU patients through ICU charge nurses. ICU patients who met the inclusion criteria and volunteered to participate in the study were contacted verbally. In the ICU, interviews

were conducted face-to-face. Accordingly, 30 ICU patients participated in the study, but one was excluded because the patient was in delirium. The study was completed with 29 ICU patients.

Data Collection Tools

The study data was collected by the researchers using the personal information form and semi-structured individual in-depth interview form. The personal information form prepared by the researchers was in line with the current literature, and it made inquiries in terms of the participants' sociodemographic characteristics (gender, age, marital status, educational background, childbearing status).[3,6,7] Semi-structured individual in-depth interview form prepared by the researchers was in line with the current literature.[7,12,16,17] For the semi-structured individual in-depth interview form, the expert opinion was obtained from two independent faculty members who specialize in psychiatric nursing. Before the form was administered, it was revised based on the opinions, and the final version of the form has the following five questions (Table 1).

Data Collection

The research data were collected through face-to-face interviews using a qualitative study with semi-structured interviews between 1 January and 1 December 2020. To assess the applicability of the semi-structured questionnaire, a pilot interview was conducted with two patients who met the inclusion criteria. The patients in the pilot study were included in the study sample because the research questions were not revised. Before starting the interviews, detailed information about the purpose and methodology of the study was provided. In-depth interviews were conducted until no new information about the phenomenon was obtained. Data saturation was judged to have been reached when no new information emerged. After the 26 interviews, the researchers did not observe any new data on loneliness among ICU patients. However, three more verification interviews were conducted to confirm that no new data emerged.[25] The interviews were conducted face-to-face and lasted between 20 and 47 minutes (mean 29 minutes). Repeated interviews were not required. During the analysis process, the field notes recorded by the interviewer were considered. One of the researchers was working as a nurse in the same hospital during the research period. The data collection process, which was conducted by two researchers, was, carried out during the night shift when there was a gap in the care plans of the health care professionals to avoid disrupting health care services and affecting the interview. For the patient to express themselves more comfortably during the interview, the patient's bed was enclosed with a folding screen, creating an environment only the researcher and the patient would be present. To ensure consistency, the same interview method, form, and voice recorder were used in all the interviews. No participant objected to the use of the voice recorder. To ensure credibility, the opinions of the patients were presented with explanatory notes in the conclusion section. The patients participating in the study were coded as "Patient (P-1, P-2 and so forth)," and their names were kept confidential.

Table 1. Semi-structured interview form

Questions
What does staying in the ICU mean to you?
What are your thoughts about staying in the ICU?
How do you feel about staying in the ICU?
What does loneliness mean to you? Have you experienced loneliness in the ICU?
In your opinion, how can one cope with the feeling of loneliness?

ICU: Intensive care unit.

The study was conducted by four female researchers trained and experienced in qualitative research. Two of the researchers work as academicians at a state university, while the other two work as specialist nurses in different state hospitals.

Statistical Analysis

In the data evaluation process, all recorded interviews were transcribed verbatim by four researchers. Computerized algorithms were not used in the data analysis; the recordings were manually transcribed. Raw data were obtained by combining the transcripts with the observation notes. After transcription, interview texts were shared with all researchers for their feedback. Researcher conducted the analysis independently, considering the field notes during this process. Transcripts were returned to participants for comment or correction. Inductive thematic analysis was employed to analyze the ICU patients' experiences of loneliness, encompassing stages of open coding, category creation, and abstraction. The data obtained from the interview form were evaluated using thematic analysis, which included the following steps: (1) familiarization with the data, (2) generating initial codes, (3) searching for themes, (4) reviewing themes, (5) defining and naming themes, and (6) producing the report.[26]

Trustworthiness

The research team consisted of four female researchers (a Master of Science, two PhD candidates, and a PhD). All the researchers were trained in qualitative research techniques and had previously conducted research. Lincoln and Guba explained trustworthiness, credibility, transferability, dependability, and confirmability.[27] To improve the credibility of the findings, a methodological approach was adopted in the data analysis phase, and this process was explained in detail. The fact that the data were analyzed and interpreted by more than one researcher also reinforced reliability. In addition, participant triangulation was ensured by including participants from 6 ICUs. Thus, multidimensional information on the loneliness experiences of ICU patients was obtained. Credibility is ensured through patients' opinions in the findings section. Also, selection criteria were established to provide data diversity for the socioeconomic status of the patients, and interviews usually took a long time. The researchers addressed the potential cognitive limitations of ICU patients during interviews. For instance, they used simple language or allowed extra time for responses. They took steps to ensure patients' emotional readiness for the interview, especially if they were in a vulnerable or altered state. Two preliminary interviews were held to review the interview questions, and these interviews, which were included in the analysis, were evaluated by two associate professors in the field of psychiatric nursing who had experience in qualitative research. For transferability, purposeful sampling was used, and the purpose and technique of the research were explained to ICU patients in detail. For dependability, the same interview form and voice recorder were used in each of the interviews conducted.

Ethics Approval

Before the study was conducted, ethics committee approval was obtained from the relevant institution (approval number: 2018/432, date: 07.11.2018), and institutional permission was obtained from the institution where the study was to be conducted (approval number: 27868579-605.01). Before the interviews were started, the participants were informed about the scope of the study and confidentiality of the data to be obtained from them in detail, and they were told that the interviews would be recorded.

RESULTS

The mean age of the patients participating in the study was 54.55±9.12 years. Of them, 18 were women, 9 were primary school graduates, 22 were married, and 26 had children (Table 2). After the thematic analysis of the data, two main themes ("Tides of loneliness" and "Whispers in the void") and five sub-themes were synthesized.

Theme 1. Tides of loneliness

Emotional Isolation

Patients described their experiences of loneliness as worse than the fear of death, with the ICU environment exacerbating these feelings. According to the patients, the ICU connoted the following negative meanings: illness-death, prison, privacy, prejudice-tension, uneasiness, fear-uncertainty, worry, longing, difficulty, desolation, loneliness, suffering, torment, sadness, and distress. The patients attributed positive meanings to the ICU such as comfort, excitement, happiness, and joy. In addition, patients stated that they felt pity for the ICU nurses and other patients staying in the ICU. Some patients defined the ICU as a safe place where immediate treatment was given and severely ill patients were treated. The patients hospitalized in the ICU defined loneliness as desolation, not being with their loved ones, abandonment, nothingness, non-existence, being at loose ends, suffering, torment and difficulty.

"We do not have our clothes on here; we are only covered with sheets; this bothers me. We can't go to the toilet or something, we use diapers for toileting, which is a very bad situation, but unfortunately there is nothing we can do. I want to get well soon. You are alone for days in a room, it is like a prison life, there is nothing you can do" (Patient-5).

"Experiencing loneliness can be highly challenging, especially for a daughter. May God not leave anyone alone! It is difficult if you do not have relatives and children near you. You are away from them. Loneliness is worse than death. Loneliness means having no human being around" (Patient-20).

Table 2. Descriptive statistics for the participants (n=29)

Patient no	Gender	Age	Marital status	Educational background	Childbearing status
P-1	Female	41	Married	Graduate	Yes
P-2	Male	48	Married	Graduate	Yes
P-3	Female	55	Married	Illiterate	Yes
P-4	Female	64	Married	Primary school	Yes
P-5	Male	51	Married	Primary school	Yes
P-6	Male	63	Single	Secondary school	No
P-7	Female	68	Married	Primary school	Yes
P-8	Female	53	Married	Illiterate	Yes
P-9	Male	50	Married	High school	Yes
P-10	Female	49	Married	Primary school	Yes
P-11	Female	55	Married	High school	Yes
P-12	Female	39	Single	High school	No
P-13	Female	56	Divorced	Primary school	Yes
P-14	Male	69	Married	Secondary school	Yes
P-15	Female	55	Divorced	Secondary school	Yes
P-16	Male	29	Single	Graduate	No
P-17	Male	48	Married	High school	Yes
P-18	Female	45	Divorced	Secondary school	Yes
P-19	Male	69	Married	High school	Yes
P-20	Female	65	Divorced	Primary school	Yes
P-21	Female	57	Married	Illiterate	Yes
P-22	Male	52	Married	High school	Yes
P-23	Male	65	Married	Primary school	Yes
P-24	Female	51	Married	Secondary school	Yes
P-25	Male	64	Married	High school	Yes
P-26	Female	52	Married	Primary school	Yes
P-27	Female	55	Married	Illiterate	Yes
P-28	Female	63	Married	Primary school	Yes
P-29	Female	54	Married	Graduate	Yes

Social Isolation

Patients expressed a longing for human connection, which significantly affected their mental health. They wished for more communication from nurses and other staff. According to the patients, the ICU negatively affects their mental health. The patients stated that they would like to have a companion in the ICU, that if they were accompanied, they would feel less lonely, that their longing for their relatives would be relieved, that they would be happy, and that their mental health would improve. Moreover, patients wanted the nurses working in the ICU to be friendly and communicate effectively. They also stated that they wanted nurses to talk to patients at short intervals every day, and to approach them with respect and love. The patients stated that they wanted teams that would provide them with morale and psychological support in the ICU.

"The only thing I want them to do is to put themselves in place of patients and to tell patients" "You will get through this; you will regain your health and will be with your family, with your children again; you shouldn't worry" They can help us get rid of this negative psychology. They can provide psychological support" (Patient-22).

Physical Isolation

The patients stated that they were bored in the ICU and wanted some physical arrangements to be made. Patients were unable to engage in basic activities such as sleeping or reading because of environmental discomforts like noise and low temperatures. The patients stated that they would pass the time better if there were a television, radio, newspapers, magazines, and books in the ICU. Additionally, if there was a telephone, they could talk to their relatives, overcoming their longing; their morale would recover, and their time would pass more easily and efficiently. In addition, the patients stated that they could not sleep in the ICU due to sounds coming from machines, light, and cold.

"I want to leave here as soon as possible. We have had enough of noise from machines and other sounds, as well as disturbances from light and insomnia. I am no longer patient. This machine is always beeping. The nurse comes and checks for issues, but there is always a persistent noise. I can't sleep at night. Moreover, it is very cold here. I can't sleep in a cold environment. I like heat. I have no patience any longer, believe me." (Patient-23).

Theme 2. Whispers in the Void

This theme examines how patients cope with their loneliness; distinguishing between adaptive and maladaptive responses, and exploring the role of nurse-patient relationships.

Adaptive Responses to Loneliness

The patients stated that they coped with the loneliness they experienced during their stay in the ICU by turning to religion, praying and being patient. Moreover, the patients stated that they tried to cope with loneliness by thinking positively, such as considering the positive aspects in their lives, making suggestions for their own health, and hoping to reunite with their loved ones when they get better.

Positive interactions with nurses, such as compassionate care and communication, provide comfort and support. Patients appreciated the therapeutic relationship, even when nurses were busy with their duties. The patients stated that there was a therapeutic relationship between patients and nurses, the nurses gave good care to patients, fed them, chatted with them, came when they were called, and showed a very high level of interest. The patients also stated that the nurses working in the ICU did their best despite their heavy workload and that they appreciated them.

"In the ICU, the only thing I do is to be patient, I pray a lot, by God, I cannot do anything else" (Patient-15).

"They do their task. What else can they do? They cannot stop doing their task to come near us. Nonetheless, they still come and chat. They are interested. You can feel their presence that they are here" (Patient-1).

Unhealthy Escapes From Loneliness

Some patients resort to negative coping strategies due to a lack of care or attention from nurses. These behaviors include excessive stress, crying, and a sense of abandonment in patients when nurses fail to meet their basic needs. The patients stated that nurses lacked interest, caring, and empathy. Moreover, the patients stated that the nurses did not come when they were called, did not give water when they were asked for it, and did not take care of them. Some patients stated that they could not cope with the feeling of loneliness in the ICU and that they experienced stress, and cried thus.

"I had very severe pain. I called them, but they didn't come. When I'm cold, I call them to warm me up, but they don't come. I'm tired of calling them. For instance, if I were very sick, I would call them and shout at them, but no one would care." (Patient-4).

DISCUSSION

The results obtained from the present study are expected to make a significant contribution to ICU nurses' understanding of patients' thoughts about the ICU, their experiences of loneliness, and the strength of the nurses. Within the scope of the sub-themes of the first theme derived from the results obtained in this study, it is determined that individuals receiving treatment in intensive care experience loneliness, while emotional, social, and physical factors emerge prominently within the context of their loneliness. The participants hospitalized in the ICU defined loneliness as desolation, not being with their loved ones, abandonment, nothingness, non-existence, being at loose ends, suffering, torment, and difficulty. In a study, patients defined loneliness as the feeling of abandonment, being unnoticed, and experiencing unmet needs.[28] Within the scope of emotional isolation, according to the patients in the present study, the ICU evokes negative meanings such as illness, death, imprisonment, loss of privacy, prejudice, and tension. However, the participants also attribute positive meanings to the ICU such as comfort, excitement, happiness, and joy. Furthermore, the participants define the ICU as a safe place, which indicates their positive feelings. Examining their definitions and feelings, it becomes apparent that the participants have feelings of ambivalence towards the ICU. Literature was supporting this finding,[1,5,6,11,29,30] and it is seen that patients define intensive care in many ways.[30-32] Within this concept, the differing attribution of positive and negative meanings to the ICU is probably due to individual and cultural differences. The patients who participated in the study stated that they felt pity for the ICU nurses and other ICU patients. The patients' pity for nurses and other patients suggests that they focus on the positive aspects of the situation they are

in, by thinking that their own health and comfort areas are better, and thus they thank God. This sense of pity, associated with compassion-defined in the literature as "being sensitive to the suffering of others", can mean that loneliness, which is a common human experience, is welcomed.[14]

Within the scope of social isolation, the participants stated that they wished for companionship in the ICU, believing it would ease their loneliness and improve mental health. Moreover, the participants wanted nurses working in the ICU to smile, to communicate with patients, and to approach patients with respect and love. Similar to the findings of the study, Mattiussi et al.[33], patients stated that having relatives accompanying them in the ICU increased and motivated their life energy. They also reported that the most important factor improving the quality of care given by healthcare professionals is treating patients friendly and establishing a relationship of trust.[33] Previous studies have determined that receiving spiritual care positively affects the loneliness of patients.[15,34] In Özer and Akyil[35], it was determined that patients who were informed about intensive care preoperatively had lower rates of discomfort from loneliness compared to those who were not. Within this context, ICUs are settings where the use of therapeutic communication skills is important and patients are psychosocially encouraged to cope with their loneliness experiences. It is also very important to provide information on patients' experience of loneliness in intensive care. Furthermore, staffing levels and nurse-patient ratios they affect the quality of care, especially in terms of providing the emotional support patients need.

Within the scope of physical isolation, patients stated that they wanted some physical arrangements, so that their loneliness and distress would be relieved. A few patients stated that they could not sleep in the ICU due to sounds, light and cold. In the literature, as in our study, patients go through negative experiences and suffer from sleep problems due to physical conditions such as noise, cold, and lack of privacy, because men and women stay together.[6,7,17,19,30] Gunnels et al.[36] stated that the most disturbing factors for ICU patients were sleep problems due to pain and the presence of device-equipment cables and tubes , while the least discomforting factor was the absence of a telephone.[36] According to Soh et al.[37], ICU stressors perceived by patients are boredom, longing for spouses, and being in a very cold/hot environment.[37] In a meta-synthesis including seven qualitative studies, it was emphasized that the three main themes affecting the sleep quality of ICU patients were complex interactions with the environment, intense feelings, and care of a similar standard.[38] Environmental planning in the ICU will increase the quality of patient care and ensure that patients' psychosocial needs are met, and they are strengthened spiritually.

Within the scope of the second theme, the participants tried to cope with the loneliness they experienced during their stay in the ICU by engaging in positive thoughts such as hoping and turning to religion. Similarly, the patients in a study tried to cope with the negativities they experienced by turning to religion, such as talking to God and praying.[39] In the present study, the participants emphasized that the presence of nurses in the ICU had both positive and negative effects on them. Some patients stated that they resorted to negative coping strategies due to a lack of care from nurses. Studies stated that patients' dependence on health professionals and inability to communicate cause experiences of anxiety, fear, loneliness, and uncertainty in the ICU.[1,6,29,30] In a systematic review performed in Türkiye, it is stated that nurses express both positive and negative reflections regarding patient care.[17] In their study, Hophuis et al.[16] stated that the patients could not tell the nurses what they wanted to consult, due to their nurses' angry appearance, and nearly half of the patients described their ICU experience as bad or very bad.[16] Hintistan et al.[39] stated that the patients' satisfaction level with care in the ICU was moderate[39] while Adsay and Dedeli[19] stated that the patients' satisfaction was at a very high level compared to that in the literature. In Alexandersen et al.[40], it was determined that most of the participants felt safe due to factors such as healthcare professionals being kind, confident, and providing information. Some patients could not get enough information, and could not communicate with some healthcare professionals, and experienced loneliness.[40] These differences between the results of the studies may be due to ICUs not having a common standard; patients having different experiences of ICUs; and individual differences. ICU nurses should be able to empathize with ICU patients' experiences of loneliness and provide emotional and social support to patients, so that ICU nurses can improve patient care by eliminating traumatic and negative experiences.

Study Limitations

The study's findings may not be fully generalizable to other contexts or patient populations. In addition, this study highlights how the cultural context (e.g., Turkish norms around family care and hospital interaction) may shape how patients perceive loneliness and the availability of social support. ICU patients with different characteristics (e.g., marriage status, having a chronic disease, living in poor economic conditions) may have different stressors and coping styles. Thus, these different characteristics may affect ICU patients' loneliness experience.

CONCLUSION

In this study, it was determined that the participants experienced loneliness and wanted physical and social changes to be made, and that nurses pay attention to therapeutic communication techniques to reduce their loneliness experiences and increase their mental health during the treatment process in intensive care. In this context, it is recommended that nurses providing health care services in intensive care should receive training on therapeutic communication techniques, strengthen the social support systems of individuals by including the family in care plans, and make physical arrangements appropriate to the care conditions. Moreover, to better understand the loneliness experiences of individuals treated in intensive care, future studies can include families or involve qualitative research with a larger and different sample. To reduce the loneliness experiences of individuals receiving treatment in intensive care and to protect their mental health, it is recommended that quantitative studies measuring the functionality of evidence-based practices be planned, and the results obtained be transferred to clinical practices.

MAIN POINTS

- Intensive care unit (ICU) patients suffered from loneliness.
- ICU patients faced with various psychosocial problems.
- This study identifies ICU-related factors that shape how patients experience and cope with loneliness.

ETHICS

Ethics Committee Approval: Before the study was conducted, ethics committee approval was obtained from the relevant institution (approval number: 2018/432, date: 07.11.2018),and institutional

permission was obtained from the institution where the study was to be conducted (approval number: 27868579-605.01).

Informed Consent: Then their written consent was obtained from the participants.

FOOTNOTES

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Authorship Contributions

Concept: E.A., G.K.T., H.A.D., M.Y., Design: E.A., G.K.T., H.A.D., M.Y., Data Collection and/or Processing: G.K.T., H.A.D., Analysis and/or Interpretation: H.A.D., M.Y., Literature Search: E.A., H.A.D., Writing: E.A., G.K.T., H.A.D., M.Y.

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RESEARCH ARTICLE

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Evaluation of the Impact of Waist Circumference and Other Predictors on Shock Wave Lithotripsy Outcomes in Ureteral Calculi: A Retrospective Analysis

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Abstract

BACKGROUND/AIMS: In this study, our aim was to evaluate the impact of waist circumference (WC) along with other patient- and stone-related factors on the success of extracorporeal shock wave lithotripsy (SWL) in the treatment of ureteral calculi.

MATERIALS AND METHODS: A retrospective analysis was performed on patients who underwent SWL for a single-radio-opaque ureteral stone (5-15 mm) in our center. Stone-free (SF) status after the treatment was defined as having <4 mm residual fragment on radiography and/or ultrasonography after 4 weeks following the last SWL session. Patient- and stone-related factors were compared.

RESULTS: A total of 200 patients were included in this study. Compared to the SF group, body mass index (BMI) ($p<0.05$), WC ($p<0.05$), Hounsfield unit (HU) ($p<0.05$), stone volume (SV) ($p<0.05$), skin-stone distance ($p<0.05$), grade of hydronephrosis ($p<0.05$), and proximal ureteral diameter (PUD) ($p<0.05$) were higher in the non-SF group. During the evaluation of the parameters with respect to their impact on the prediction of SWL success, univariate analysis did show that BMI ($p<0.05$), WC ($p<0.05$), SV ($p<0.05$), HU ($p<0.05$), and PUD ($p<0.05$) were the significant factors in this aspect. On the other hand, in multivariate analysis, none of these factors have shown statistically significant importance for the development of non-SF status after SWL.

CONCLUSION: Patient- and stone-related factors such as BMI, HU, skin to stone distance, mean SV, grade of hydronephrosis, PUD, have been shown to be associated with SF rates after SWL in accordance with previous studies. On the other hand, according to our results, WC may be a novel predictor of SWL outcomes in ureteral stones.

Keywords: Ureteral calculi, extracorporeal shockwave lithotripsy, waist circumference, success

INTRODUCTION

Urolithiasis is a prevalent urological pathology, affecting approximately 4-20% of the population in developed countries.[1] Ureteral calculi form a significant portion of urinary stone disease and are one of the most common urological presentations in the emergency department. Nearly 65% of all ureteral stones have been shown to pass spontaneously; distal stones have a higher chance of passage compared to proximal ones.[2] While observation can be preferred in asymptomatic and small stones (especially <5 mm), active treatment may be needed in case of urinary obstruction, renal colic pain, or renal insufficiency.[3] Regarding the management of such stones, medical expulsive therapy, extracorporeal

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shock wave lithotripsy (SWL), and ureteroscopy are among the available treatment options. SWL is the recommended treatment option in both proximal and distal ureteral stones less than 10 mm.[4]

Although SWL offers the advantages of safety and non-invasiveness, its lower success rate compared to other treatment modalities remains a major limitation.[5] The identification of predictive factors for SWL outcomes is essential to optimize success rates and minimize complications. In previous studies, high body mass index (BMI), Hounsfield unit (HU), stone density, skin to stone distance (SSD), ureteral wall thickness (UWT), stone volume (SV)/area, proximal ureteral diameter (PUD), and hydronephrosis have been shown to be associated with SWL success in both renal and ureteral stones.[6-8] But, to our knowledge, no study has ever evaluated the effect of waist circumference (WC) on SWL for ureteral calculi. In this study, our aim was to demonstrate the patient- and stone-related factors affecting SWL success in ureteral calculi.

MATERIALS AND METHODS

This study was approved by University of Health Sciences Türkiye, Sancaktepe Şehit Prof.Dr. İlhan Varank Training and Research Hospital Ethics Committee (approval number: 138, date: 30.04.2025), data of the patients treated for single opaque ureteral stone with SWL from January 2023 to April 2025 in our clinic were retrospectively evaluated. A total of 285 patients were identified initially. The exclusion criteria were: patients who are <18 or >65 years old, active urinary infection, radiolucent stones, multiple stones, patients with indwelling ureteral stent, solitary kidney, inflammatory and/or malignant diseases, and patients with urinary anatomical disorders. Patient-related factors such as age, sex, BMI, WC, comorbidities, anticoagulant use, and previous treatment were recorded for each patient. Stone characteristics (stone side, level, volume, density, SSD) were derived by non-contrast computed tomography (NCCT) of the patients. SV was calculated by using the following formula: (long axis × short axis × depth × 0.52). UWT was measured at the stone site, and PUD was the diameter, which is the ureteral lumen right above the stone in the ureter. All measurements were in millimeters. A successful outcome was defined as being completely stone-free (SF) on radiography and ultrasonography at 4 weeks after the last SWL session. Success rates were evaluated in a comparative manner based on the measurement values assessed, as longest diameter and volume of the stone treated. Patients with residual fragments <4 mm were considered SF. After applying the exclusion criteria, a total of 200 patients were included in the final analysis (Figure 1).

The treatment was performed using the electromagnetic lithotripter, Modulith SLX- F2- FD21 (Storz Medical AG, Tägerwilen, Switzerland) under fluoroscopy in all patients. The standard pulse frequency was 60 shockwaves per minute, with a maximum of 3000 shocks applied at each session. All patients received oral anti-inflammatory therapy before every session for pain management. A minimum interval of one week was applied between consecutive SWL sessions. If the stone or residual fragments could not be identified in fluoroscopy, confirmatory radiography and ultrasonography were performed. Patients with no symptoms and residual stones were re-evaluated 4 weeks after the last SWL session with radiography and ultrasonography.

Statistical Analysis

Statistical analysis was performed with Jamovi (version 2.6.0, for Mac OS). Distribution of the variables was measured by Kolmogorov-Smirnov test. Mann-Whitney U test was used for continuous variables and chi-square test was used for categorical variables. Univariate analysis and multivariate analysis (stepwise logistic regression) were used to determine parameters that influence SF status. A p-value <0.05 was considered statistically significant.

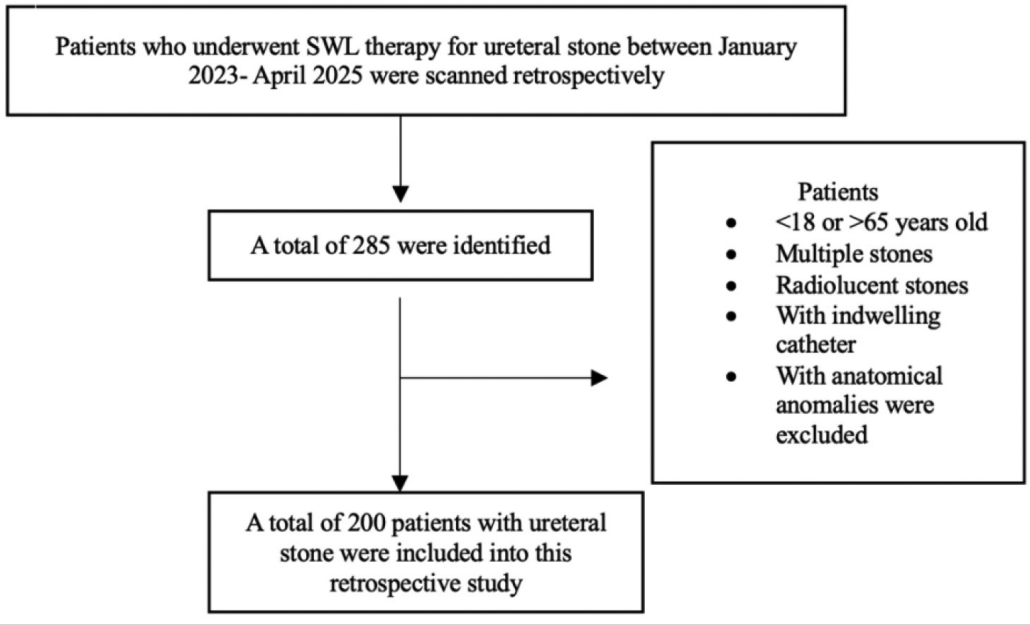

Figure 1. PRISMA diagram showing the selection of the patients.

SWL: Shock wave lithotripsy, PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

RESULTS

A total of 200 patients (male/female: 135/65) were included in this study. Median BMI and WC of the patients were 25.5 (18.3-38.06) and 94 (71-120) cm, respectively. While 149 had no comorbidity, 28 had diabetes mellitus and 23 had coronary artery disease (CAD). Although 148 patients had no previous stone treatment history, 20 patients had a history of SWL, 27 patients had undergone ureterorenoscopy; 3 patients had percutaneous nephrolithotomy, and 2 patients had undergone open surgery.

Our findings revealed that, while 139 patients (70%) were completely SF after SWL, 61 patients (30%) had residual fragments larger than 4 mm at four weeks. Patients in the non-SF group tended to have a higher median BMI (26.8 vs. 25.3, $p<0.05$) and WC (98 vs. 91, $p<0.05$) compared to those in the SF group. There was no statistically significant difference between the two groups regarding age, family history, and previous stone treatment history. No statistically significant difference was found also in stone side, stone level, and the mean UWT values between the two groups. On the other hand, PUD was significantly higher in the non-SF group (8.6 mm) compared to the SF group (7.4 mm) ($p<0.05$). Median SV was higher in non-SF group patients than in SF patients, with values of 149.6 mm^3 and 104.9 mm^3, respectively ($p<0.001$). HU ($p<0.001$), SSD ($p<0.05$), and hydronephrosis grade ($p<0.05$) were significantly higher in non-SF group cases compared to those in the SF group. Complication rate ($p<0.001$) and need for auxiliary intervention ($p<0.001$) were also found to be higher in the non-SF group than in the SF group. All of our findings were summarized in Table 1. Careful univariate analysis of the parameters with respect to their impact on the prediction of SWL success showed that BMI ($p<0.05$), WC ($p<0.05$), SV ($p<0.05$), HU ($p<0.05$), and PUD ($p<0.05$) were the significant parameters in this analysis. On the other hand, in multivariate analysis (logistic stepwise regression), we were not able to show statistical significance in any of these factors. Results of the univariate and multivariate analysis are shown in Table 2. In Figure 2, we illustrate the receiver operating characteristic curve of the multivariate logistic regression model used to predict SF status after SWL. The model yielded an area under the curve of 0.70, indicating moderate predictive accuracy.

DISCUSSION

SWL can be the treatment of choice in the majority of kidney and ureteral stones. Success rates after SWL in ureteral calculi have been reported to vary from 82-90% for proximal-mid and from 58-67% for distal stones.[9] To overcome this considerable range in SWL success rate, vigorous efforts have been made by endourologists to identify predictive factors that may influence the outcomes of SWL.

A proportion of patients fail to achieve SF status after SWL due to either suboptimal stone fragmentation, or impaired clearance of residual fragments. These treatment failures not only lead to repeated sessions, increased cumulative radiation exposure, and complication rates, but may also cause a higher financial burden on the healthcare system. As a result, the ability to accurately predict SWL success using objective and measurable parameters has become a key focus in the optimization of stone management strategies. Various radiological and anthropometric factors are now being incorporated into clinical decision-making algorithms to improve patient selection and success rates.

Both patient-related variables-including age, sex, BMI, WC, and comorbidities-and stone-related factors -including volume, density (HU), composition, anatomical location, and SSD- have been investigated for their potential roles in determining SWL efficacy.

In terms of stone-related factors, SV and HU are among the most extensively investigated parameters associated with the success rates of SWL. SV directly affects the likelihood of effective stone fragmentation; lower fragmentation rates and reduced stone-free rates (SFRs) following SWL.[10] The HU, which is a radiological measure of stone density assessed by NCCT, correlates closely with stone composition and predicts resistance to fragmentation. Stones with higher HU values typically exhibit decreased fragmentation efficiency, resulting in poorer SWL outcomes.[11,12] Our results are in accordance with the literature: higher SV and HU were detected in the non-SF group compared to the SF group, and univariate analysis also demonstrated the significant roles of these parameters.

In addition to SV and density, other factors have been implicated as important predictors of treatment success. The SSD has emerged as another critical factor influencing SWL success. Shock waves must pass multiple tissues (skin, muscle, and fat) before reaching the stone. With longer SSD, the distance covered by the shock waves increases and the final energy that reaches the stone decreases; longer SSD values negatively affect shock wave energy delivery, thereby diminishing fragmentation effectiveness and subsequent stone clearance.[13] In our study, SSD was found to be statistically higher in the non-SF group compared to the SF group.

The UWT, PUD, and grade of hydronephrosis, which often represent stone impaction and ureteral obstruction, have also been studied, particularly regarding ureteral calculi. Increased UWT, dilated PUD, and higher-grade hydronephrosis indicative of chronic obstruction or ureteral inflammation, have been linked to lower SFRs and decreased likelihood of spontaneous passage following SWL.[14,15] In our study, higher PUD and hydronephrosis are associated with lower SF rates after SWL.

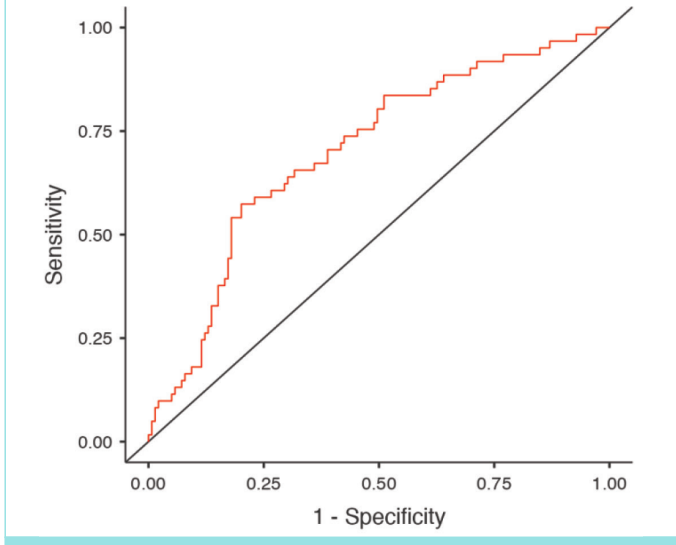

Figure 2. ROC curve of the multivariate logistic regression model predicting stone-free status after SWL.

ROC: Receiver operating characteristic, SWL: Shock wave lithotripsy.

Table 1. Patient- and stone- related characteristics of both groups

	All patients	Stone-free group	Non-stone-free group	p-value
Number	200	139 (70%)	61 (30%)	
Age (median, min-max)	40 (17-68)	39 (20-68)	41 (17-66)	0.132[a]
Sex				
Male	135	96	39	
Female	65	43	22	
BMI (median, min-max)	25.5 (18.3-38.06)	25.3 (18.3-35.6)	26.8 (19.5-38.06)	**0.01**[a]
Waist circumference (median, min-max) (centimeter)	94 (71-120)	91 (71-118)	98 (71-120)	**0.001**[a]
Anticoagulant use				
Yes	18	8	10	**0.01**[b]
No	182	131	51	
Comorbidity				
None	149	112	37	
Diabetes mellitus	28	12	16	**<0.005**[b]
Coronary artery disease	23	15	8	
Previous treatment				
None	148	99	49	
Shock wave therapy	20	17	3	
Ureteroscopy	27	21	6	0.2[b]
PNL	3	1	2	
Open surgery	2	1	1	
Ureteral localization				
Upper	98	62	36	
Middle	53	39	14	0.1[b]
Lower	49	38	11	
Side of ureter				
Right	86	63	23	0.3[b]
Left	114	76	38	
Hounsfield unit (median, min-max)	670 (260-1781)	650 (260-1781)	750 (300-1510)	**<0.001**[a]
Stone volume (median, min-max) (mm^3)	124.4 (33.4-542.04)	104.9 (33.4-440.3)	149.6 (59.5-542.04)	**<0.001**[a]
Skin-stone distance (median, min-max) (millimeters)	124 (55-175)	120 (72-175)	130 (55-174)	**0.003**[a]
Hydronephrosis				
Grade 1-2	133	99	34	**0.01**[b]
Grade 3-4	67	40	27	
Ureteral wall thickness (median, min-max)	2.7 (1.1-5.9)	2.6 (1.1-5.9)	2.8 (1.2-5.9)	0.4[a]
Proximal ureteral diameter (median, min-max)	7.8 (3.5-19.2)	7.4 (3.5-15.6)	8.6 (4.7-19.2)	**0.01**[a]
Number of SWL sessions				
Single	65	51	14	**0.05**[b]
Multiple	135	88	47	
Complications				
None	106	101	5	
Hematuria	11	9	2	**<0.001**[b]
Pain	63	22	41	
Steinstrasse	20	7	13	
Auxiliary intervention				
None	126	124	2	
Medical expulsive therapy	42	14	28	**<0.001**[b]
JJ insertion	3	1	2	
Ureteroscopy	29	0	29	

[a]Mann-Whitney U test.
[b]chi- square test.
BMI: Body mass index, min-max: Minimum-maximum, PNL: Percutaneous nephrolithotomy, SWL: Shock wave lithotripsy.

Table 2. Uni- and multi-variate analysis of the factors

	Univariate analysis			Multivariate analysis	
	95% Confidence interval	OR	p-value	OR	p-value
BMI	0.82-0.97	0.89	**0.007**	1.04	0.5
Waist circumference	0.92-0.98	0.95	**0.002**	1.04	0.1
Stone volume	0.99-0.99	0.99	**0.01**	1.00	0.5
Hounsfield unit	0.99-0.99	0.99	**0.005**	1.00	0.06
SSD	-0.01-0.02	1.01	0.247		
PUD	0.79-0.98	0.88	**0.02**	1.08	0.2

BMI: Body mass index, SSD: Skin to stone distance, PUD: Proximal ureteral diameter, OR: Odds ratio.

Additionally, patient-related factors also significantly impact the outcomes of SWL. Among these factors, BMI has been extensively studied and is consistently associated with reduced success rates following SWL. Elevated BMI often corresponds to greater SSD, potentially impairing shock wave energy transmission, stone fragmentation, and ultimately stone clearance.[16] In addition, the accuracy of stone localization via imaging techniques may be reduced in obese patients, further compromising SWL efficacy.[17] Nevertheless, BMI may not always serve as a reliable indicator for adiposity distribution or body composition. Individuals with similar BMI values can exhibit significantly different body types depending on their relative proportions of lean muscle mass and adipose tissue, leading to variations in treatment outcomes.

Consequently, WC, which specifically measures central obesity and visceral fat deposition, may provide a more clinically relevant assessment of patient-related factors influencing SWL outcomes. Elevated WC is strongly associated with various metabolic and cardiovascular comorbidities, including metabolic syndrome, diabetes mellitus, hypertension, and CAD.[18] Although there are currently limited data specifically investigating WC as a predictor of SWL success, the known associations between central obesity, increased SSD, and adverse clinical outcomes in related urological procedures strongly support the rationale for its evaluation in this context.[19] The potential for WC to more accurately reflect factors that negatively influence shock wave penetration and stone clearance underscores its utility in predicting SWL outcomes beyond conventional BMI measurements.

In our study, both BMI and WC were significantly higher in patients who failed to achieve SF status compared to those who did. This observation was supported by univariate analysis, suggesting a meaningful association between body composition and SWL outcomes. However, in multivariate analysis, these parameters did not retain statistical significance. This may be due to the limited sample size, retrospective design, or collinearity with other stone-related parameters such as SV, stone density, and SSD. Although our findings regarding WC are promising, they should not be interpreted as evidence of WC being an independent predictor of SWL success at this stage. Rather, WC may represent a parameter with potential predictive value that needs confirmation in larger prospective and multicenter studies.

Study Limitations

The small number of cases and the retrospective design are the major limitations of this study. In addition, its single-center nature may limit the generalizability of our findings. Measurements such as WC, SSD, and PUD were operator-dependent and subject to interobserver variability. Stone composition was not confirmed by stone analysis, but only inferred by stone density values. Moreover, SF status was evaluated after four weeks, and longer-term outcomes were not evaluated; longer-term outcomes were not considered. Finally, potential collinearity among predictive variables may have influenced the results of multivariate analysis.

CONCLUSION

With this study, we demonstrated the significance of stone-related parameters-including SV, stone density, SSD, PUD, and hydronephrosis grade- along with patient-related factors, including BMI and WC, in predicting SFRs following SWL. BMI has long been recognized as a relevant anthropometric predictor. Our findings suggest that WC, as a marker of central obesity, may also influence SWL success as a new serve as a predictor of SWL success. While WC was not an independent predictor in multivariate analysis, its association with poorer outcomes in univariate analysis indicates its potential value in patient selection and pre-treatment evaluation.

MAIN POINTS

- Stone volume, stone density, skin to stone distance, proximal ureteral diameter, and hydronephrosis were associated with shock wave lithotripsy (SWL) outcomes, but none were independent predictors in multivariate analysis.
- Waist circumference (WC) was associated with SWL outcomes in univariate analysis, but was not an independent predictor.
- WC may represent a potential predictive parameter for SWL outcomes in ureteral stones, but it requires validation in larger, prospective studies.

ETHICS

Ethics Committee Approval: This study was approved by University of Health Sciences Türkiye, Sancaktepe Şehit Prof.Dr. İlhan Varank Training and Research Hospital Ethics Committee (approval number: 138, date: 30.04.2025).

Informed Consent: This study was designed retrospectively, so no written consent was obtained from the patients.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Y.K., K.C.G., K.S., Concept: D.İ.K., K.S., Data Collection and/or Processing: Y.K., K.C.G., Analysis and/or

Interpretation: Y.K., D.İ.K., Literature Search: K.C.G., Writing: Y.K., D.İ.K., K.C.G., K.S.

DISCLOSURES

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CASE REPORT

CYPRUS
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Posterior Reversible Vasogenic Cerebral Edema Syndrome: Rethinking Atypical PRES Presentations Without Encephalopathy

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Abstract

Posterior reversible encephalopathy syndrome (PRES) is an acute neurological disorder characterized by reversible subcortical vasogenic edema, predominantly affecting the posterior regions of the brain, particularly the parieto-occipital lobes. Common clinical manifestations include headaches, seizures, visual disturbances, and altered mental status, collectively referred to as encephalopathy. The pathophysiology of PRES is not entirely understood but is believed to involve endothelial dysfunction and impaired cerebral autoregulation, leading to hyperperfusion and subsequent vasogenic edema. While encephalopathy is considered a hallmark feature of PRES, the syndrome exhibits a broad spectrum of clinical presentations. Notably, cases without encephalopathy are underreported, potentially due to the variability in clinical manifestations and the absence of altered mental status, which may lead to misdiagnosis or delayed recognition. Such atypical presentations pose diagnostic challenges, emphasizing the necessity for heightened clinical suspicion and comprehensive evaluation. This report contributes to the existing literature by detailing a unique case of PRES without encephalopathy, underscoring the importance of considering PRES in patients presenting with visual disturbances and headaches, even in the absence of altered mental status. Early recognition and appropriate management are crucial, as timely intervention can lead to complete recovery, whereas delayed diagnosis may result in irreversible neurological deficits.

Keywords: Atypical PRES, encephalopathy, headache, hypertensive retinopathy, vasogenic edema

INTRODUCTION

Posterior reversible encephalopathy syndrome (PRES) is an acute neurotoxic condition characterized by reversible cortical-subcortical vasogenic edema, predominantly affecting the posterior regions of the brain, especially the parieto-occipital lobes.[1] The syndrome is frequently associated with severe hypertension, eclampsia, renal dysfunction, or immunosuppressive therapy.[2] Clinically, PRES commonly manifests with headaches, seizures, visual disturbances, and altered mental status, the latter often categorized under the umbrella of encephalopathy.[3] The pathophysiological mechanisms underlying PRES are not fully elucidated but are thought to involve endothelial dysfunction and impaired cerebral autoregulation, resulting in hyperperfusion and subsequent vasogenic edema.[4]

Although encephalopathy is traditionally regarded as a hallmark feature of PRES, the clinical spectrum is notably broad.[5] Atypical presentations lacking altered mental status are uncommon and, therefore, may be underrecognized or misdiagnosed. Such variability presents a significant diagnostic challenge, as the absence of classical neurological findings can delay appropriate intervention, increasing the risk of permanent neurological damage.[6] Prompt diagnosis is critical to initiate timely management strategies that can prevent irreversible neurological deficits.

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In this context, we present a rare and diagnostically challenging case of PRES without encephalopathy, where the patient presented with severe visual disturbances and headaches but maintained normal cognitive function throughout the disease course. This case underscores the importance of considering PRES in the differential diagnosis of patients with visual symptoms and hypertensive emergencies, even in the absence of altered mental status. Furthermore, it emphasizes the critical need for timely diagnosis and management to ensure favorable clinical outcomes.

CASE REPORT

A 42-year-old female without a known history of chronic illness presented with mild generalized headache and severe blurred vision in both eyes that began abruptly. She sought medical attention the following day at the ophthalmology clinic, where findings of papilledema and macular edema prompted referral to the neurology department for further evaluation. Upon admission to the neurology department, her blood pressure was critically elevated at 240/160 mmHg. Other vital signs were within normal limits. Neurological examination revealed no focal deficits, altered consciousness, or seizure activity. However, the patient exhibited significant visual impairment, with visual acuity reduced to counting fingers at 1 meter in both eyes. She was diagnosed by the ophthalmological examination with hypertensive retinopathy stage 4, characterized by retinal hemorrhages, hard exudates, cotton-wool spots, increased vascular tortuosity, and papilledema. Brain magnetic resonance imaging (MRI) revealed vasogenic edema predominantly involving the brainstem (midbrain and pons), cerebellum, and temporo-parieto-occipital lobes (Figure 1A). These findings effectively excluded differential diagnoses such as stroke, malignancy, central nervous system (CNS) demyelinating disorders, and leukoencephalopathy, while being consistent with a diagnosis of PRES. Further imaging, including magnetic resonance venography (MRV) and magnetic resonance arteriography (MRA), ruled out cerebral venous sinus thrombosis and reversible cerebral vasoconstriction syndrome (Figure 1B). Laboratory studies revealed no significant abnormalities, effectively excluding differential diagnoses such as hepatic encephalopathy, encephalitis, and CNS vasculitis. The patient initially received parenteral antihypertensive therapy with

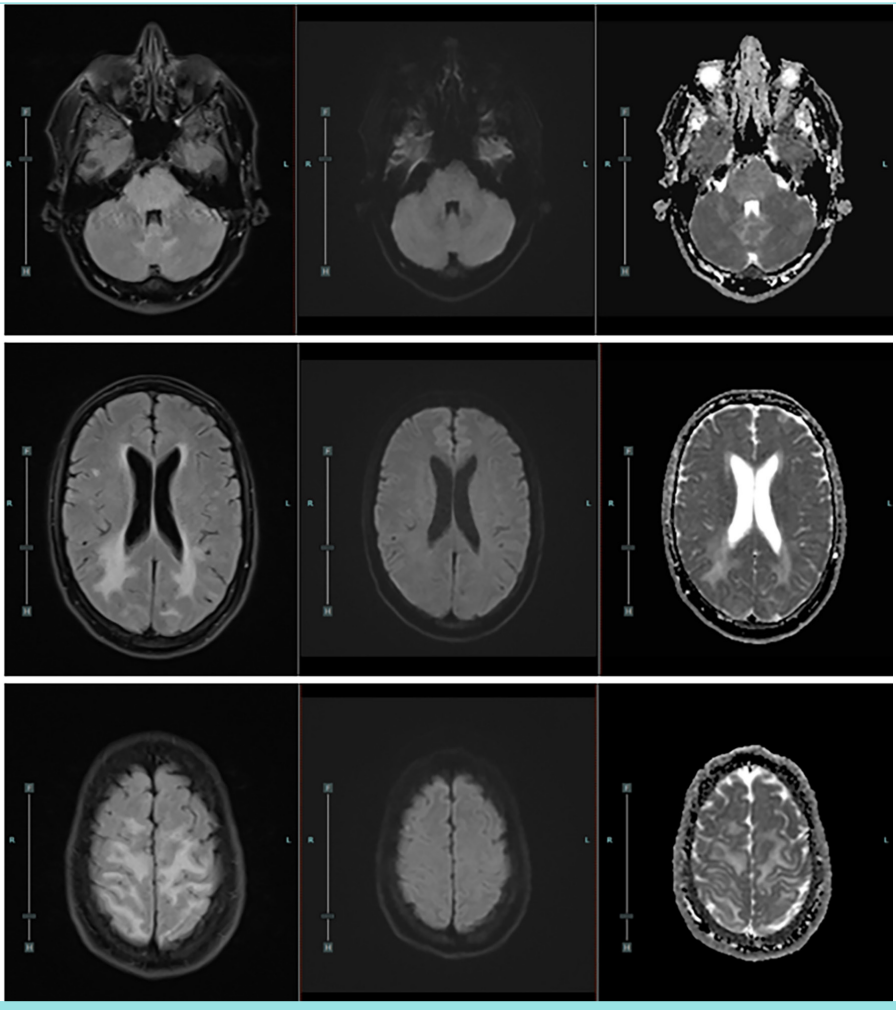

Figure 1A. The axial FLAIR, diffusion-weighted imaging (DWI), and apparent diffusion coefficient (ADC) sequences demonstrate vasogenic edema prominently involving the pons, cerebellum, and bilateral temporo-parieto-occipital lobes. The lesions appear hyperintense on FLAIR and DWI images, while corresponding ADC maps show no significant diffusion restriction, supporting the vasogenic rather than cytotoxic nature of the edema.

FLAIR: Fluid-attenuated inversion recovery

nimodipine (1 mcg/kg/hour) under close monitoring, followed by oral amlodipine 10 mg once daily for maintenance. Electroencephalography was performed and revealed normal findings. Levetiracetam (500 mg, twice daily) was initiated as a prophylactic measure, considering the high-risk of seizures associated with the observed vasogenic edema. No seizures occurred during the hospital stay. Echocardiography revealed mild left ventricular hypertrophy with an ejection fraction of 60%. Renal artery Doppler ultrasonography excluded renal artery stenosis. With antihypertensive therapy, the patient experienced gradual relief from headaches and a progressive improvement in visual acuity. By discharge on day four, visual acuity had improved to 20/25 with a reported 80% subjective recovery. At one-month follow-up, complete resolution of visual deficits and normalization of retinal findings were observed (Figure 2). On follow-up MRI, the vasogenic edema observed in prior imaging was no longer evident, indicating radiological resolution (Figure 3). Levetiracetam tapering was initiated in light of sustained clinical and radiological recovery. Written informed consent was obtained from the patient for publication of this report and accompanying images.

DISCUSSION

PRES is commonly characterized by the presence of encephalopathy, a key clinical feature that manifests in up to 94% of patients. This spectrum ranges from mild confusion and cognitive deficits to stupor and, in severe cases, coma.[7] The presented case of PRES is notable for its absence of encephalopathy, a finding that deviates from the classical clinical profile commonly described in the literature.[8] Such presentations are underreported and pose significant diagnostic challenges, as the absence of altered mental status may lead to misdiagnosis or delayed

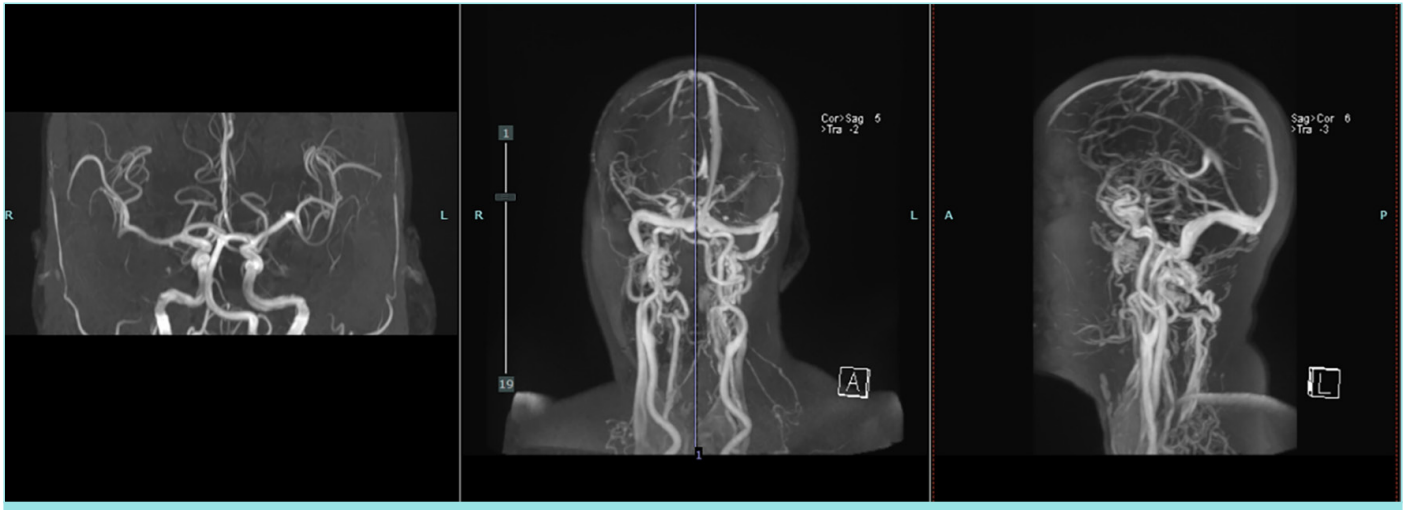

Figure 1B. The study involves displaying vascular imaging studies, including magnetic resonance arteriography (MRA) in the first image and magnetic resonance venography (MRV) in the subsequent two images. The MRA shows normal intracranial arterial structures without evidence of segmental narrowing or beading. Similarly, the MRV sequences demonstrate normal venous flow within the major dural sinuses.

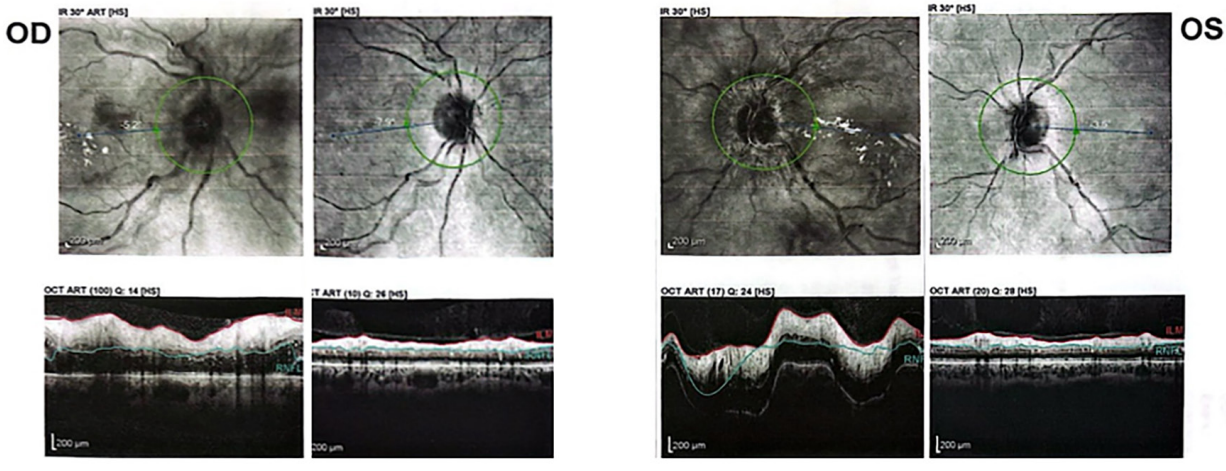

Figure 2. Comparing pre- and post-treatment fundus photographs and optical coherence tomography (OCT) of the retinal nerve fiber layer, (RNFL), images of both eyes involves an analysis of these data. Initial fundus findings include retinal hemorrhages, hard exudates, cotton-wool spots, vascular tortuosity, and papilledema, while OCT shows diffuse RNFL thickening consistent with optic disc edema. Post-treatment images demonstrate marked radiological and clinical improvement.

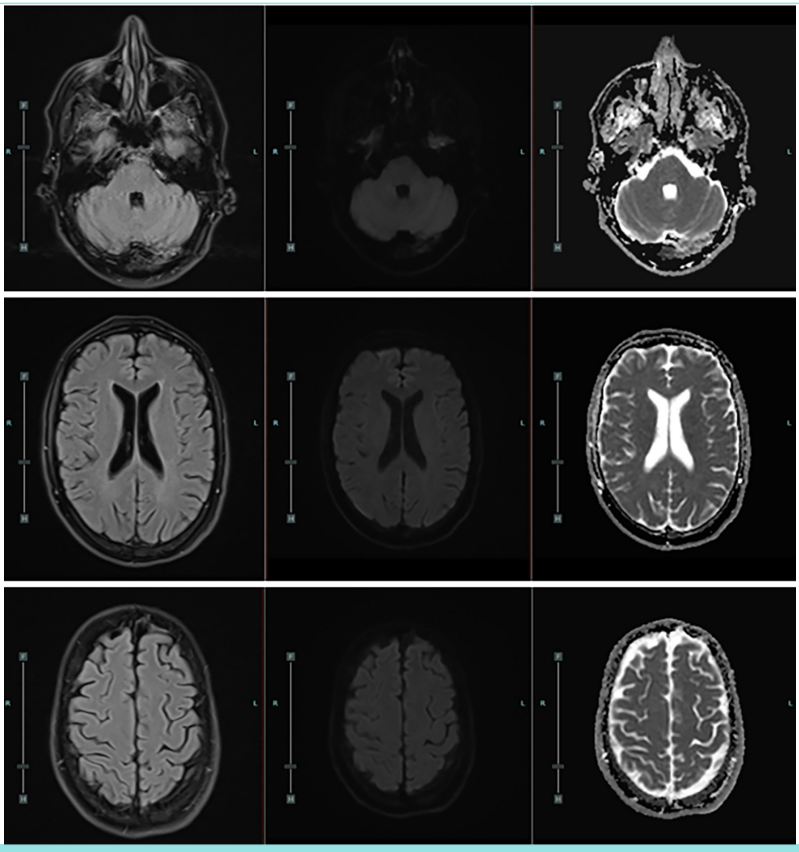

Figure 3. Demonstrating complete resolution of previously noted vasogenic edema in the brainstem, cerebellum, and temporo-parieto-occipital regions, indicating radiological improvement in follow-up MRI.

MRI: Magnetic resonance imaging

recognition.[9] Literature suggests that encephalopathy a hallmark feature of PRES and is present in the majority of cases. However, studies have highlighted that PRES is a spectrum disorder, and clinical manifestations may vary depending on the extent and location of cerebral involvement.[10] In particular, atypical presentations lacking encephalopathy are often overlooked, despite the presence of imaging findings consistent with vasogenic edema.[9] This case exemplifies such a diagnostic dilemma and emphasizes the necessity for heightened clinical vigilance when evaluating patients presenting with severe hypertension and visual disturbances.

The pathophysiology of PRES is believed to involve endothelial dysfunction and impaired cerebral autoregulation, resulting in hyperperfusion, blood-brain barrier disruption, and subsequent vasogenic edema.[2] The absence of encephalopathy in this case suggests possible individual variability in cerebral autoregulatory capacity or regional susceptibility to hypertensive injury. Sympathetic-mediated vasoconstriction tends to be more pronounced in the anterior circulation and relatively weaker in the posterior regions, potentially contributing to the predilection for vasogenic edema in posterior brain structures.[11] This mechanism could explain why the patient's clinical presentation was dominated by visual symptoms without altered mental status. However, further research is warranted to elucidate the mechanisms that may confer protection against altered mental status in certain patients.

The co-occurrence of hypertensive retinopathy with PRES further substantiates the concept of shared vascular pathology between the brain and retina. Both conditions are rooted in endothelial dysfunction and impaired autoregulatory mechanisms triggered by acute hypertensive insults. Retinal findings, such as papilledema, hemorrhages, and exudates, closely parallel the vasogenic edema observed in PRES, reflecting a common pathophysiological mechanism.[12] This relationship highlights the systemic nature of hypertensive crises and positions retinal changes as potential markers of CNS involvement. Such insights emphasize the importance of interdisciplinary diagnostic approaches and suggest that retinal assessments could facilitate early identification of CNS pathology, particularly in resource-limited settings.

Given the predominance of vasogenic edema in imaging findings, the term "Posterior Reversible Vasogenic Cerebral Edema syndrome"(PRVCES) has been informally proposed to highlight the central imaging and pathophysiological feature of vasogenic edema in PRES, offering conceptual clarity particularly in atypical cases without encephalopathy where traditional clinical markers may be absent.[13] While this terminology may provide a more descriptive and pathophysiologically accurate framework, it has not yet been incorporated into international classification systems or clinical practice guidelines. Accordingly, its use should be regarded as a descriptive and explanatory tool rather than as an established diagnostic category. Moreover, "PRES" remains the widely accepted and internationally recognized term in both clinical and academic literature, encompassing the broad clinical spectrum of

the syndrome, including both typical and atypical presentations.[14,15] Therefore, although PRVCES may contribute to enhanced understanding of unusual cases, its broader adoption would require validation in larger case series and consensus among experts. Until such recognition is achieved, the term should be applied with caution.

This case further underscores the critical role of neuroimaging in the diagnostic process, particularly in patients presenting with limited symptoms and signs. MRI findings of vasogenic edema involving the brainstem, cerebellum, and posterior cortical regions were pivotal in establishing the diagnosis of PRVCES. The integration of MRV and MRA facilitated comprehensive vascular assessment, effectively ruling out cerebral venous sinus thrombosis and reversible cerebral vasoconstriction syndrome-both important differential diagnoses in patients presenting with severe hypertension and neurological symptoms. The early use of these advanced imaging modalities not only ensured diagnostic accuracy but also guided therapeutic decisions. Timely imaging likely contributed to the favorable clinical outcome observed, while delayed or inadequate imaging in atypical presentations may risk diagnostic uncertainty, mismanagement, and irreversible neurological complications. The emphasis on vasogenic edema as the key diagnostic feature in PRVCES further underscores the importance of prioritizing imaging in cases with atypical presentations, where encephalopathy is absent.

Antiepileptic drugs (AEDs) play a crucial role in the management of patients with PRES, particularly those who experience seizures during the disease course. In cases with clinical seizure activity, AEDs are essential to stabilize the patient and prevent recurrent episodes. However, the use of AEDs in PRES patients without seizures remains controversial, and is often guided by the extent of vasogenic edema and the perceived seizure risk.[16] Prophylactic treatment, as seen in the presented case, is typically individualized and aims to mitigate potential complications in high-risk scenarios. Gradual discontinuation of AEDs is an essential consideration in the management process and should be based on a comprehensive assessment of both clinical and radiological findings. In this case, tapering was planned following evidence of radiological resolution and clinical stability. This approach minimizes unnecessary medication exposure while safeguarding against potential seizure recurrence. However, the optimal timing for AED withdrawal in PRES remains undefined and warrants further investigation.

CONCLUSION

PRES can present without encephalopathy, as demonstrated in this patient with hypertensive retinopathy and severe hypertension. Clinicians must maintain a high index of suspicion for PRVCES in atypical presentations of PRES, particularly in the absence of encephalopathy, to avoid diagnostic delays and mismanagement. This report contributes to the growing recognition of the diverse clinical spectrum of PRES and emphasizes the critical need for further research into its pathophysiological mechanisms, diagnostic strategies, and management approaches to optimize patient outcomes.

MAIN POINTS

- Posterior reversible encephalopathy syndrome (PRES) may present without altered mental status; the presence of isolated visual disturbances in the setting of a hypertensive emergency should raise clinical suspicion for this diagnosis, even in the absence of encephalopathy.
- The coexistence of advanced hypertensive retinopathy and radiologically confirmed vasogenic edema underscores the shared pathophysiological basis between the retinal and cerebral microvasculature, highlighting the diagnostic value of ophthalmological findings in neurovascular syndromes such as PRES.
- This case reinforces the indispensable role of advanced neuroimaging in the early identification of atypical PRES presentations and advocates for a broader diagnostic paradigm that accounts for regional cerebral vulnerability and interindividual variability in autoregulatory responses.

ETHICS

Informed Consent: Written informed consent was obtained from the patient for publication of this report and accompanying images.

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Footnotes

Authors Contributions

Surgical and Medical Practices: Ö.Ö., Ş.I., Concept: Ö.Ö., Design: Ş.I., Data Collection and/or Processing: Ö.Ö., Analysis and/ or Interpretation: Ö.Ö., Ş.I., Literature Search: Ş.I., Writing: Ö.Ö.

DISCLOSURES

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ERRATUM

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Published on page 71;

MATERIALS AND METHODS

Experimental Groups

Ethical approval for the research was received from İstanbul Bağcılar Training and Research Hospital Animal Experiments Local Ethics Committee (approval number: 2017/63, date: 30.05.2017).

Corrected page 71;

(The corrected parts are given in bold)

MATERIALS AND METHODS

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ETHICS

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Deren Çeker
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Ersoy Doğan
Ezgi Bağrıaçık
Ferda Selçuk
Ferdiye Taner
Funda Ekimci Deniz
Gezim Hodolli
Gökçe Akan
Gözde Kanmaz Kutman
Gülcan Dürüst Sakallı
Gülin Acar
Gülten Sucu Dağ
Gülten Tuncel
Hafize Seda Vatansever
Haitham Elfateh
Halil Yılmaz
Hammad Tufail Chaudhary
Hanife Özkayalar
Hasan Hüseyin Kazan
Hatice Özsoy
Hazel Şahin Tarım
Hilal Kabadayı
Hüsamettin Vatansev
Ibrar Alam
İlker Çakır
İlker Etikan
İsa Sincer
Itır Erkan
Kaya Suer
Konul Rahimli
Kübra Hamzaoğlu Canbolat
Louai Alsaloumi
Mehmet Baykara
Mehtap Tınazlı
Merve Berika Kadıoğlu
Merve Gözde Sezgin
Merve Mercan
Meryem Güvenir
Muhammed Abdulcelil
Muhammed Fevzi Kılınçkaya
Müjgan Fırıncıoğluları
Mustafa Kotmakçı
Nalan Ogan
Nazife Sultanoğlu
Nejlan Eratam
Neriman Çolakoğlu
Nermin Eroğlu
Neşe Akcan
Nevra Dursun Kepkep
Nilgün Mutlu Aksoy
Nilüfer Güzoğlu
Noyan Özlü
Nuri Yıldırım
Oğuz Han Edebal
Onur Yavuz
Osman Nuri Dilek
Özen Aşut
Özlem Bilik
Pınar Tulay
Palin Çiftçioğlu
Pınar Gelener
Pınar Özdemir Çetinkaya
Pınar Tunçbilek Özmanevra
Rahmi Onur
Remzi Tınazlı
Şahin Kılıç
Saliha Baykal
Seha Akduman
Seide Karasel
Selma Şengiz Erhan
Serkan Sayıner
Serpil Özsoy
Sevda Lafçı Fahrioğlu
Sevi Burçak Çehreli
Tevhide Ziver Sarp
Tolga Kencer
Tuğba Gürbüz
Tülin Fırat
Ufuk Atlıhan
Ulaş Öz
Ulaş Solmaz
Umut Mousa Marasuna
Volkan Öztekin
Yaprak Sarıgöl Ordin
Yönal Kırsal
Yusuf Bahap
Yusuf Bozkuş
Zeliha Selamoğlu
Zuhal Kaltuş