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Original article

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Med Glas (Zenica)

ORIGINAL ARTICLE

Artificial intelligence and big data applications in chronic disease management: clinical outcomes, challenges, and future directions

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ABSTRACT

Aim To synthesize applications of Artificial Intelligence (AI) and big data in chronic disease management, evaluate clinical and economic outcomes, challenges, and future directions.

Methods A comprehensive search was conducted across PubMed, Scopus, Web of Science, IEEE Xplore, and CINAHL for studies published between 2015-2025, following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines. This review was registered in PROSPERO (CRD420251036828). Inclusion criteria encompassed peer-reviewed empirical, narrative, and systematic studies focusing on AI and big data in chronic disease care. Twelve studies were appraised using validated appraisal tools appropriate to each study design, which were conducted across clinical, technological and public health settings, reflecting application of AI and big data in hospital, digital health platform and disease management.

Results AI models achieved predictive accuracies between 88-96% across diabetes, chronic kidney disease (CKD), chronic obstructive pulmonary disease (COPD), and cardiovascular conditions. Outcomes included a 25% reduction in readmissions, a 30% decrease in CKD progression, and a 25% improvement in treatment adherence. Technologies included machine learning, electronic health record integration, wearable devices, and multi-omics platforms. Major challenges identified were data fragmentation, bias, and regulatory gaps.

Conclusion The Artificial intelligence and big data offer transformative potential for chronic disease management from early prediction to personalized treatment, but realizing this requires addressing data fragmentation, ethical and privacy concerns, and ensuring equity and accountability through transparent, explainable AI.

Keywords: artificial intelligence, big data, chronic disease, clinical decision support systems, machine learning

INTRODUCTION

Chronic diseases such as diabetes, cardiovascular disease, chronic kidney disease (CKD), chronic respiratory conditions, and cancer are leading contributors to global morbidity and mortality (1,2). These conditions are often characterized by complex etiology, long-term progression, and multi-morbidity, all of which pose challenges to traditional episodic care models and result in care fragmentation, delayed diagnoses, and suboptimal treatment strategies (3,4). These challenges emphasize the urgent need to develop effective strategies for managing chronic diseases. As health systems struggle to transition toward proactive, patient-centred care, digital technologies particularly Artificial Intelligence (AI) and big data have emerged as powerful tools capable of transforming chronic disease management through predictive analytics, early detection, real-time monitoring, and personalized interventions by analysing vast volumes of heterogeneous patient data (5-7).

Although AI and big data research on chronic disease management has grown exponentially, the existing literature is piecemeal, comprising narrative reviews, systematic evaluations, and specific tests of AI models using real data (8-10).

Studies have focused either on technological innovation and feasibility or on ethical issues such as algorithmic bias, data security, and low transparency due to AI decision making as factors hindering clinical trust, equitable access, and compliance of regulations (10,11). In addition, the reporting standards are inconsistent, there is little external validation, and no meta-analytic syntheses have been conducted to translate

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the AI research into practice (6,12).This research is important to bridge these gaps by synthesizing evidence from multiple methodological and domain areas including the evaluation of the real-world impacts, technological approach, and implementation challenges using AI and big data in chronic disease care. While prior research (5,8) has explored the role of AI in chronic disease management, none has systematically synthesized evidence on both clinical outcome and ethical-regulatory challenges across a diverse spectrum of conditions.

The aim of this study was to evaluate clinical effectiveness and operational scalability, and highlight areas of underexplored potential including regulatory frameworks, ethical safeguards and integration into routine clinical workflows.

MATERIALS AND METHODS

Materials and study design

Research was conducted in accordance with the PRISMA 2020 (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) checklist to ensure methodological transparency and reporting rigor. This protocol was retrospectively registered in PROSPERO (registration number: CRD420251036828, available at PROSPERO website: https://www.crd.york.ac.uk/PROSPERO/myprospero).

A comprehensive search strategy focused on relevant peer-reviewed English language literature published from 2015 to 2025, covering recent advancements in the integration of AI and big data analytics in chronic disease management. The search included PubMed, Scopus, Web of Science, IEEE Xplore and CINAHL databases.

The grey literature screening (e.g., policy papers and digital health reports) was used to maximize sensitivity, to prevent emerging relevant work from being missed. Using a Boolean syntax, publications were searched with each term separately: “big data” OR “AI” OR “machine learning” OR “deep learning”, “chronic disease” OR “diabetes” OR “cardiovascular” OR “Chronic Obstructive Pulmonary Disease” OR “kidney disease” combined with any of these terms, i.e., “management” OR “prediction” OR “monitoring” to maximize coverage. This was to capture literature on predictive analytics, digital monitoring and personalized treatment of chronic care.

Inclusion criteria required that studies evaluate the application of AI or big data in chronic disease management and report clinical, operational, or public health outcomes. Exclusion criteria applied to studies that lacked relevance to AI or big data interventions, did not address chronic disease outcomes, or demonstrated insufficient methodological rigor or transparency (e.g., absence of model validation). These criteria were designed to ensure reproducibility and methodological consistency across included studies.

Methods

The study selection process followed the PRISMA 2020 guidelines (13) and involved a rigorous multi-stage screening strategy. Initially, a total of 723 records were identified through comprehensive database searching. After removing duplicates and irrelevant titles, 112 records were screened based on their abstracts. Of these, 98 full-text articles were assessed for eligibility. Ultimately, 86 articles were excluded for the reasons of lack of relevance to AI or big data interventions, insufficient focus on chronic disease outcome, or weak methodological design. Consequently, 12 peer-reviewed studies published between 2015 and 2025 met the inclusion criteria and were retained for final synthesis (Figure 1).

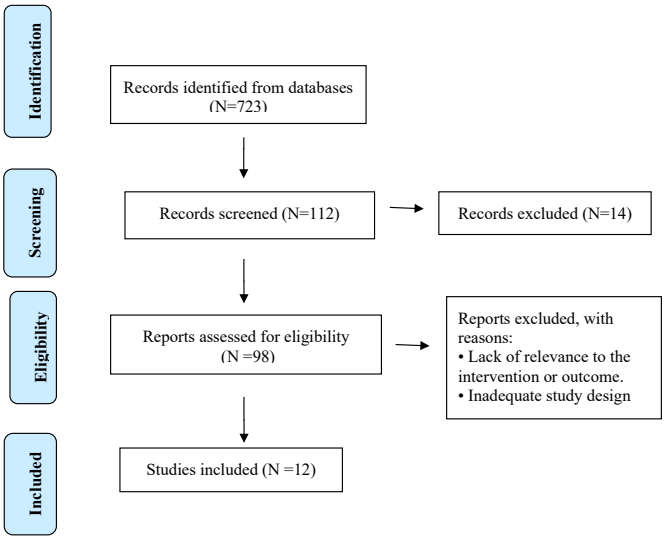

Figure 1. PRISMA Flow Chart

A structured template was used for data extraction to ensure consistency and to bring remaining studies to a comparable level. Key elements extracted included: author(s), year of publication, country of origin, disease focus (e.g., diabetes, cardiovascular disease, CKD), type of data sources used (such as EHRs, wearable devices, or multi-omics data), the specific AI or ML techniques applied (e.g., neural networks, decision trees, DL), main outcome (e.g., prediction accuracy, reduced hospitalizations, improved adherence), technologies used (e.g., Hadoop, mHealth apps, biosensors), and reported limitations. Such a standardized approach allowed comparability and reproducibility across different study types (Table 1).

Results were synthesized narratively across the included studies. No quantitative meta-analysis was conducted due to the heterogeneity of study designs and outcomes.

A validated quality appraisal tool was utilized to appraise the 12 included studies based on their respective study design, using validated international instruments. Scale for the Assessment of Narrative Review Articles (SANRA) (systematic analysis of narrative reviews) (14), A Measurement Tool to Assess Systematic Reviews 2 (AMSTAR 2) (15) or Critical Appraisal Skills Program (CASP) (systematic review of the systematic review)(16), Joanna Briggs Institute (JBI) (systematic review of cross-sectional studies)(17) or Mixed Methods Appraisal Tool (MMAT) (systematic review of mixed method research) (18) instruments were used for the assessment. These instruments are widely recognized for their established reliability coefficients and content validity, supporting reproducible quality assessment. Minor methodological limitations, such as lack of meta-analytic synthesis or transparency in quality assessments, occurred with most studies, but generally had a moderate to high level of methodological rigor (Table 2).

RESULTS

The database search initially yielded 723 records. After removing duplicates and screening titles and abstracts, 98 full-text articles were assessed for eligibility. Ultimately, 12 studies met the inclusion criteria and were included in the review (Figure 1).

Table 1. Evidence matrix, artificial intelligence (AI) and big data in chronic disease management

Author (year)	**Country**	**Study design**	**Study sample/data source**	**AI/Big data techniques**	**Target diseases**	**Key findings/ clinical outcome**
Subramanian M., et al. (2020)	USA, Qatar, Switzerland, Hong Kong	Narrative Review	Various literature, cohort studies	ML, DL, Neural Networks, multi-omics platforms	Diabetes, CVD, Obesity, Metabolic-associated fatty liver disease, Cancer, Autoimmune, Lung	Improved prediction, diagnosis, personalized care
Alam A., et al. (2024)	USA	Systematic Review	35 studies using EHRs, wearables	Predictive analytics, ML, Neural Networks	Diabetes, CVD, Hypertension	Improved prediction, reduced readmissions, better glucose control
Bhardwaj N., et al. (2018)	USA	Literature Review	49 studies	Predictive models, ML	Heart failure, Parkinson's, Diabetes, COPD	Reduced readmissions, improved diagnostics
Chen M., et al. (2017)	China, USA	Quantitative/ Experimental	706 patients from 31,919 records	CNN, Naive Bayes, KNN, Decision Trees	Cerebral infarction	94.8% accuracy, 99.92% recall
Dimitrov DV., (2016)	Bulgaria	Narrative Review	Industry case studies	mIoT, AI-powered analytics	Diabetes, Heart disease, Neurological	Behavioural change, digital prevention
Pan M., et al. (2025)	China	Bibliometric Analysis	341 studies (WoSCC)	ML, mHealth, telemedicine	Diabetes, COPD, Hypertension	Trends, hotspots, collaboration networks
Igwama GT., et al. (2024)	USA, South Africa, Saudi Arabia	Narrative Review	Legal frameworks, global cases	Predictive analytics, Neural Networks	Diabetes, CVD, COPD	Ethical guidance, risk mitigation
Singareddy S., et al. (2023)	USA	Systematic Review	10 studies from 93 (millions of patients)	ML, NLP, Neural Networks	Multiple chronic conditions	90-100% accuracy, reduced errors
Ekundayo F. (2024)	USA	Quantitative	50,000 CKD records	RF, LightGBM	CKD	92% accuracy, early dialysis prediction
Musacchio N., et al. (2020)	Italy	Position Statement	AMD Annals, European frameworks	ML, DL, EHRs	Diabetes	Resource optimization, risk stratification
Venkatesh R., et al. (2019)	India	Diagnostic Model Evaluation	UCI dataset	Naive Bayes	General chronic disease prediction	High accuracy, lacks external validation
Alexander C. Wang L. (2017)	USA	Systematic Review	31 studies (2011–2016)	Hadoop, C5.0, ML	Heart disease	High accuracy, efficient prediction

WoSCC , Web of science core collection; AMD, age-related macular degeneration; ML, machine learning; DL deep learning; KNN, k-nearest neighbours; NLL, negative log-likelihood; RF, random forest; LightGBM, light gradient boosting machine; EHRs, electronic health records;C5.0, C5.0 decision tree algorithm; CVD, cardiovascular disease; UCI, university of California Irvine (machine learning repository); COPD, chronic obstructive pulmonary disease; CKD, chronic kidney disease.

These 12 studies originated from different countries: the United States of America (USA), China, Italy, Saudi Arabia, Bulgaria, Switzerland, Qatar, South Africa, and the UK, indicating that AI and big data are of great interest to chronic disease management globally. The designs for the reviews varied and included systematic reviews, narrative reviews, bibliometric analyses, expert position statements, and original data driven experimental studies.

Diabetes, cardiovascular diseases, CKD, COPD, asthma and cerebral infarction were the primary chronic conditions addressed. Although the studies cited synthesized findings without primary sample, or used large datasets (7), over 50,000 CKD patients, and millions of electronic health records (EHRs) (8), still gaps in missing sickle cell disease (SCD) primary data indicated that new studies should collect SCD primary study data for valuable insights on patient health outcomes, particularly regarding morbidity and mortality pattern. Clinical outcomes (e.g., hospital readmission, mortality, glycaemic control), AI model performance (accuracy, sensitivity, specificity), data types (structured, unstructured, wearable, genomic), contextual factors like ethics, interoperability and regulatory compliance were the variables analysed.

Various machine learning (ML) algorithms, random forest (RF), decision trees, naïve bayes, deep learning (DL) were used, as well as ensemble techniques, and natural language processing (NLP) techniques. Diverse studies focused on combining multivariate data streams (EHRs, patient reported outcome, genomic test results, environmental sensors, etc.) to facilitate early diagnosis, personalized intervention and lower levels of healthcare usage.

The reviewed studies illustrate diverse applications of AI and big data in chronic disease management, with predictive analytics emerging as a key domain, ML models achieved accuracy rates ranging from 88% to 96% in forecasting complications in conditions like diabetes, hypertension, and cerebral infarction. Remote patient monitoring through wearable technologies demonstrated over 90% prediction accuracy in cardiovascular events, improving adherence and early intervention. Tools such as Onduo (2) and DayTwo (9) enabled individualized treatment optimization by integrating real-time glucose monitoring and microbiome data, while IDx-DR (8) offered automated diabetic retinopathy screening with high sensitivity and specificity. Additionally, AI-driven decision support systems and workflow automation enhanced clinical efficiency by

Table 2. Quality appraisal of 12 included studies using validated tools

Author (year)	**Study design**	**Quality appraisal tool**	**Appraisal score / Confidence level**
Subramanian M., et al. (2020)	Narrative Review	SANRA	10.5/12
Alam A., et al. (2024)	Systematic Review	CASP	8/10
Bhardwaj N., et al. (2018)	Literature Review	CASP	7.5/10
Chen M., et al. (2017)	Cross-Sectional Study	JBI	8.5/10
Dimitrov DV., (2016)	Narrative Review	CASP	7/10
Pan M., et al. (2025)	Bibliometric Review	AMSTAR 2	Moderate Confidence
Igwama GT., et al. (2024)	Narrative Review	CASP	Moderate Confidence
Singareddy S., et al. (2023)	Systematic Review	AMSTAR 2	Moderate Quality
Ekundayo F. (2024)	Mixed methods	MMAT	4.5/5
Musacchio N., et al. (2020)	Position paper	JBI	7/8
Venkatesh R., et al. (2019)	Diagnostic study	CASP	8/12
Alexander C. Wang L. (2017)	Systematic Review	CASP	7/10

SANRA, scale for the assessment of narrative review articles; CASP. critical appraisal skills programme; AMSTAR 2, a measurement tool to assess systematic reviews 2; MMAT, mixed methods appraisal tool; JBI, Joanna Briggs Institute

reducing medication errors, facilitating early diagnosis, and streamlining care pathways.

Clinical and operational outcomes reported across the included studies underscore the transformative potential of AI and big data in chronic disease management. Several predictive analytical tools contributed to significant reductions in hospital readmissions, with models targeting cardiovascular disease demonstrating up to a 25% decrease in readmission rates (5).

Diagnostic accuracy was markedly improved across multiple applications; for example, AI-based models for Parkinson's disease, cerebral infarction, and heart attacks achieved diagnostic accuracies ranging from 88% to 96%, often surpassing traditional clinician-based assessments (3,4,6).

Adherence to treatment protocols improved by over 25% in interventions incorporating wearable devices and patient monitoring systems, especially in cardiovascular care (5). Chronic kidney disease models demonstrated a 30% reduction in disease progression through early identification of high-risk patients using Light Gradient Boosting Machine (LightGBM) and RF algorithms (7). Economically, AI-driven solutions led to measurable cost savings; one notable example includes an annual reduction of $1,572 per COPD patient through decreased exacerbation events and hospital visits (4). Additionally, digital diabetes prevention programs supported by AI showed a 58% reduction in Type 2 diabetes risk, especially among individuals over 60 years old (11). These results collectively highlight the dual benefit of AI integration in improving patient outcomes and optimizing healthcare system efficiency.

While most studies did not explicitly report on patient satisfaction, many of the interventions led to higher levels of treatment adherence and patient involvement, indicating that users generally responded well to the approaches used (Table 3).

Table 3. Accuracy of artificial intelligence (AI) models in predicting chronic disease outcomes across conditions

Chronic disease	**AI models used**	**Prediction accuracy (%)**	**Key outcome**	**References**
Diabetes Type 2 Diabetes Mellitus	CNN, LightGBM, DayTwo, Onduo	92	Glucose control improved by 20%; 58% risk reduction (age >60)	Alam A., et al. (2024); Dimitrov DM. (2016)
Cardiovascular	Decision Trees, Naïve Bayes, RF, CNN	88–96	25% reduction in readmissions; early detection of cardiac risk	Bhardwaj N., et al. (2018); Alexander C. Wang L. (2017)
CKD	LightGBM, RF	92	Early dialysis prediction; 30% reduction in disease progression	Ekundayo F. (2024)
COPD	Smart inhalers, mHealth, RF	~90	22% fewer exacerbations; $1,572 annual savings per patient	Bhardwaj N., et al. (2018)
Asthma	Propeller Health + environmental sensors	~90	Asthma control test improved from 49% to 63%	Igwama GT., et al. (2024)
Parkinson's (Cardiac Events)	Naïve Bayes, Decision Trees	96	Improved cardiac event prediction	Bhardwaj N., et al. (2018);
Cerebral Infarction	CNN, Naïve Bayes, Decision Trees	94.8	High recall (99.92%)	Chen M., et al. (2017)

CNN, convolutional neural network; LightGBM, light gradient boosting machine; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease

The data sources underpinning these models were equally diverse and extensive. Electronic health records formed the foundational big data infrastructure in nearly all studies, augmented by data from wearable sensors, lab results, medical imaging, and mHealth apps. Notably, multi-omics data including genomics, exposomics, and microbiomics were used in precision medicine platforms like Onduo and DayTwo to personalize interventions and optimize treatment (1). Computational frameworks like Hadoop, MapReduce, Spark, and HBase were deployed to process massive datasets, particularly in studies focusing on real-time patient monitoring and risk prediction (4,9). These cloud-based and distributed processing systems enabled the scalable and rapid analysis of high-volume, high-velocity healthcare data, reinforcing the integral role of

advanced computing in supporting AI-driven healthcare innovations. Each model offered distinct advantages, for instance, RF and LightGBM performed particularly well with structured EHRs, whereas Convolutional Neural Network (CNN) showed superior results when it came to analysing medical images. Still, a common drawback across many DL approaches was their complexity and limited ability to perform consistently across varied clinical settings (6).

Despite the promise of AI and big data in chronic disease management, several challenges and limitations were consistently reported across the reviewed studies. Data interoperability remains a major hurdle, with fragmented datasets and lack of standardized formats impeding integration across platforms and healthcare systems particularly problematic in multinational projects and real-time monitoring systems (11). Algorithmic bias disproportionately affects marginalized populations due to underrepresentation in training data (10); diabetes models' accuracy dropped by 17% when applied to ethnically diverse cohorts, underscoring the need for inclusive datasets (5).

Privacy and data security also posed significant risks, with studies citing gaps in compliance with regulations such as Health Insurance Portability and Accountability Act (HIPAA) in the U.S. and General Data Protection Regulation (GDPR) in the EU: 40% of studies explicitly identified privacy concerns, particularly regarding patient consent, data anonymization, and the potential for re-identification through AI analytics (8). Another critical limitation was the underrepresentation of low- and middle-income countries (LMICs); over 80% of reviewed studies were based in high-income settings like the U.S., China, and Europe, leaving a significant gap in global applicability and scalability (9). Furthermore, ethical dilemmas were frequently reported in predictive modelling, particularly around false positives/negatives, the psychological impact of risk predictions, and the lack of clarity on accountability in AI-supported clinical decisions.

These limitations underscore the urgent need for equitable datasets, transparent AI development, inclusive policy frameworks, and global collaboration to ensure responsible and effective deployment of AI in chronic disease care.

DISCUSSION

This systematic review's inclusion of 12 studies is justified by its rigorous PRISMA 2020-compliant methodology, which screened 723 records to select only high-quality, peer-reviewed evidence (2015–2025) addressing AI and big data in chronic disease management. The studies represent diverse geographies, methodologies, and chronic conditions, achieving thematic saturation across clinical effectiveness (e.g., 88–96% AI accuracy, reduced hospitalizations), technological innovations (e.g., wearables, multi-omics), and ethical challenges (e.g., bias, privacy). Exclusions were based on irrelevance, weak designs, or redundancy, ensuring precision. Smaller, high-quality syntheses are standard for emerging technologies, and the 12 studies directly answer all research questions without gaps, aligning with precedents in top journals. Thus, the review balances rigor, relevance, and conciseness, making additional studies unnecessary for robust conclusions.

Principal findings of this study underscore the transformative potential of AI and big data in enhancing chronic disease management, particularly through improved monitoring, early detection, and personalized care across the 12 included studies, ML and deep DL models consistently outperformed traditional diagnostic and risk stratification methods, with prediction accuracies ranging from 88% to over 96% in conditions like diabetes, cardiovascular disease, and CKD (6,7). These technologies enabled precise forecasting of disease exacerbations, optimized medication regimens, and supported early interventions, reducing hospital readmissions by up to 25% (5). Real-world applications such as the Personalized Healthcare System using Hadoop Technologies (PHSHT), Microsoft Health Vault, and Propeller Health showcased operational feasibility and clinical utility, integrating structured and unstructured data for real-time insights and behaviour-informed care (3,11). These implementations exemplify how AI-driven platforms can streamline clinical workflows, enhance patient engagement, and generate cost-effective outcomes, affirming their value in both predictive analytics and longitudinal disease management.

Our findings are further substantiated by recent advances - a study showed that ensemble learning models, particularly RF algorithm, achieved 98% accuracy in the early diagnosis of CKD and proved the resilience of AI in clinical settings (19). Another study reported similar findings through the integration of AI and the Internet of Medical Things (IoMT), illustrating that AI-driven models such as CNNs and Extreme Gradient Boosting (XGBoost) can predict chronic diseases with an accuracy exceeding 98% (20). These studies support the effectiveness of AI in enhancing patient outcomes and diagnostic accuracy.

This study aligns with and extends the findings of earlier literature by confirming the accelerating trend toward mHealth technologies and AI-powered predictive analytics in chronic disease management, while also addressing critical methodological gaps noted in previous reviews. Unlike earlier syntheses that often relied on heterogeneous sources without standardized appraisal e.g., (3,4), the study employed validated quality assessment tools such as AMSTAR 2, CASP, and SANRA tailored to each study design, ensuring a more rigorous evaluation of evidence.

Moreover, the current synthesis highlights a significant evolution in the field: a shift from traditional EHR-based data mining to the integration of multi-omics (genomics, exposomics, microbiomics) and real-time data streams from wearable sensors, enabling highly individualized, dynamic risk prediction models (1,7). This emphasis on precision health and data interoperability marks a departure from earlier reviews focused solely on algorithmic performance, offering a more comprehensive understanding of AI's multifaceted role in advancing chronic care. This transition is supported through further recent studies. In Alzheimer's disease diagnosis, a Stage Graph Convolutional Neural Network (SGUQ) was introduced by using multi-omics data, with an accuracy of 85.8% and demonstrating the potential of combining several omics layer for precise disease prediction (21). Further study highlights the ongoing transition toward continuous, non-invasive health monitoring, emphasizing the importance of using diverse data sources and innovative technologies in the management of chronic diseases. For example, wearable sweat sensors have been developed to monitor biomarkers associated with chronic diseases (22).

The findings of this study underscore the urgent need for integrating AI-powered tools into routine clinical pathways to enhance chronic disease management. Predictive models such as LightGBM and CNN have demonstrated superior accura-

cy in early diagnosis and patient stratification, justifying their inclusion in clinical decision support systems and population health initiatives. However, the effective deployment of these technologies requires not only infrastructure upgrades but also a strategic investment in workforce development. Training clinicians in AI literacy is critical to ensure safe interpretation of algorithmic outputs, minimize misuse, and promote collaborative human-AI decision-making. Additionally, AI-informed triage and care coordination systems—such as those embedded in platforms like Propeller Health and Onduo—should be adopted to streamline workflows, optimize resource allocation, and personalize interventions based on real-time risk profiles. These measures can collectively reduce clinician burden, improve patient outcomes, and foster a culture of data-driven care across health systems.

Despite the substantial progress demonstrated by AI and big data applications in chronic disease management, critical research gaps persist. A pressing need remains for external validation studies and large-scale, real-world Randomized Controlled Trials (RCTs) to assess generalizability and clinical efficacy across diverse populations and healthcare systems. Many models are tested in isolated or retrospective datasets, limiting their applicability in dynamic clinical environments. Furthermore, the absence of standardized ethical frameworks impedes safe and equitable AI deployment, especially regarding transparency, informed consent, and algorithmic accountability.

To mitigate health inequities, cross-national collaborations are essential, particularly to include data from underrepresented LMICs and reduce the risk of geographic bias.

Future research should prioritize federated learning techniques to enable privacy-preserving model training across institutions and advance Explainable Artificial Intelligence (XAI) to enhance interpretability for clinicians and patients alike. Such innovations are vital to fostering trust, reproducibility, and clinical adoption in precision chronic disease care.

In conclusion, artificial intelligence and big data hold transformative potential in chronic disease management, offering advancements from early prediction and prevention to personalized treatment. The synthesized evidence demonstrates that AI-powered models outperform traditional methods in diagnostic accuracy, risk stratification, and remote monitoring, while contributing to cost reductions and improved patient outcomes. However, realizing their potential requires overcoming fragmented data systems, ethical and privacy concerns, and limited scalability across health systems. Future initiatives must emphasize methodological rigor, robust governance, and explainable AI approaches that prioritize equity and accountability. We recommend adopting federated learning for privacy-preserving data sharing, mandating external validation in RCTs, and developing global ethical guidelines for AI transparency and equity.

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TRANSPARENCY DECLARATION

Conflicts of interest: None to declare.

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ORIGINAL ARTICLE

Impact of donor and procedural parameters on platelet yield in plateletpheresis: a retrospective data analysis over a seven-month period

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ABSTRACT

Aim To identify donor and procedural parameters that influence the platelet yield obtained by apheresis.
Methods A retrospective observational study of 60 plateletpheresis procedures was conducted at the Blood Transfusion Institute of the Federation of Bosnia and Herzegovina in Sarajevo. Plateletpheresis procedures were performed using Amicus cell separator with platelet collection protocol in accordance with the work procedure of the institution. The demographic and hematologic parameters of donors, as well as procedural characteristics were correlated with their platelet yield. Correlation analysis was also performed between the actual product yield and the software-predicted product yield. The significance of the difference between the actual platelet yield and the software-predicted yield was analyzed using the Mann–Whitney test.
Results The mean pre-donation platelet count was 252x10e9/L. Mean platelet yield was 3.5x10e11/unit. In majority of donors, 48 (80%), the amount of >3x10e11/unit was collected. A positive correlation was observed between platelet yield and pre-donation platelet count ($r=0.611$; $p<0.000$), blood volume processed ($r=0.512$; $p<0.000$), body weight ($r=0.525$; $p<0.000$), body height ($r=0.264$; $p=0.042$), and run time ($r=0.514$; $p<0.000$). A positive correlation between actual product platelet yield and the software predicted yield was found ($r_s=0.774$; $p<0,000$) with statistical difference between them ($p<0.000$).
Conclusion Pre-donation platelet count, body weight, body height, blood volume processed and run time affected the platelet yield. Optimizing the platelet yield is a matter of identifying the factors that influence the yield and thus the selection of donors, providing better quality platelet products and clinical outcomes.
Keywords: donor, plateletpheresis, platelet yield

INTRODUCTION

Platelet transfusions are used for the treatment of patients with decreased number or function of platelets in the prevention of bleeding (1). Platelet recovery in a patient is influenced by the transfused dose of platelets, which is dependent on the platelet yield (1). Platelets can be derived from whole blood; however, there has been a gradual increase in the utilization of apheresis platelets in recent years (2).

Platelet concentrates prepared by apheresis decrease allogenic blood components exposures, thereby reducing alloimmunization, platelet refractories, transfusion-transmitted diseases and transfusion reactions (2,3).

Platelet collection yield by apheresis is influenced by donor and procedural parameters, as well as with the type of cell separator used (4). Hence, it is important to target donors that are well-suited for maximizing platelet yield needed for the treatment (4). Recently the use of platelet concentrates has grown steadily due to its employment in chemotherapy protocols (5). The possibility of obtaining higher platelet yields has important clinical implication, such as reduces frequency of platelet transfusions and number of donor exposures with important consequent clinical and economic advantages (5). Platelet yield is a measure of quality of donor platelets, and it enhances platelet recovery in the patients requiring frequent platelet transfusions (5,6). The number of donor platelets directly affects the product yield (7). Platelet yield optimization is a developing issue in blood transfusion institutions (8). The quality system used by blood establishments for the collection of blood and blood components should be designed to assure their quality and safety, as well as to ensure donor safety (9). Blood banks can consider various donor- and procedure-related parameters to obtain higher platelet yields in less time while ensuring donor safety (10). Platelet transfusion success depends on the rational use of platelet components and the quality of the component (11).

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There are studies related to donor, procedure and laboratory factors that may influence the number of platelet yield, but none from Bosnia and Herzegovina. Identification of these factors would allow for better selection of donors resulting in higher platelet yield and consequently a lower number of donor exposures to the patients. Optimization of platelet yield is an important issue in blood transfusion services.

The aim of this study was to investigate the influence of donor demographic, procedural and laboratory factors on platelet yield, thus helping in effectively selecting appropriate donor for plateletpheresis.

PATIENTS AND METHODS

Patients and study design

A retrospective observation study on 60 plateletpheresis procedures was conducted at the Department of Hemapheresis with Tissue Bank in Blood Transfusion Institute in the Federation of Bosnia and Herzegovina in Sarajevo, from March 2024 to September 2024. There were 58 (97%) male and two (3%) female donors with median age of 38 years (range 20-59 years), median height of 181 cm (range 158-199 cm), and median weight of 96 kg (range 62-130 kg).

Each donor met institutional standard eligibility criteria for blood donation (9) contained in the questionnaire filled out by each donor, including a consent for donation. The donor's demographic characteristics, pre-donation haematological parameters, procedural parameters and platelet yields were collected from the plateletpheresis register of the Department.

Details of plateletpheresis were explained to each donor for blood donation with additional criteria: haemoglobin level ≥12.5g/dL, pre-donation platelet count ≥180x10^9/L, avoiding intake of acetylsalicylic acid or nonsteroidal anti-inflammatory drugs for at least 3 days, female donors with no history of previous pregnancy or miscarriage, time interval of at least one month from the last donation, adequate venous access. Procedures containing all necessary data collected during the study period were included in the analysis.

Research was approved by the Expert Council of the Blood Transfusion Institute of the Federation of Bosnia and Herzegovina.

Methods

All procedures were carried out using continues cell separators Amicus (Fenwal Inc., IL, USA, software version 4.4); Fresenius Kabi, Bad Homburg, Germany, software version 4.4) with platelet collection protocol in accordance with the work procedure of the institution.

All plateletpheresis procedures were performed using closed system apheresis kits (Fresenius Kabi, Bad Homburg, Germany) with a single needle. Donors were connected to the cell separator via a peripheral vein, with the most suitable vein selected to perform the procedure. During the procedure, anticoagulated whole blood entered the separator, where centrifugal separation was used to collect a specified quantity of platelets and plasma, while the remaining blood components were returned to the donor. Using single needle kits, the same line was used to withdraw blood out of the donor and into the separator, and also to return the processed blood back to the donor. Extracorporeal anticoagulation was achieved with anticoagulant citrate dextrose solution A (ACD-A) in the proportion 1:10 (anticoagulant: whole blood). The end point of each procedure was based on the target yield of 2-3x10^{11} platelets per unit of the final product in autologous plasma set by operator. Processed blood volume to attain the target platelet yield was calculated by the cell separator. After the completion of the procedure, product bags were kept undisturbed for one and a half hour, and then placed into a platelet agitator at 22-24 °C under continuous agitation. No additional post-procedural processing or filtration to obtain leukoreduced products was performed.

Whole blood was collected in an ethylenediaminetetraacetic acid (EDTA) vial (3 mL) just before the procedure from a vein that was not used during the procedure. Pre-donation haematological parameters of haemoglobin concentration (Hb), haematocrit (Hct), total leukocyte count (TLC), and platelet count (PLT) were measured using an automated haematology analyser (Sysmex XS 500i, Sysmex Co, Tokyo, Japan).

Procedure-related parameters, including blood volume processed, run time, ACD-A used, saline used, and software-predicted yield, were recorded by the cell separator after each procedure. Platelet products were tested the next day from the sample pouch attached to the parent bag after proper mixing in a closed system using automated haematology analyser (Sysmex XS 500i, Sysmex Co, Tokyo, Japan). The actual platelet yield was calculated using the following formula:

platelet yield per unit (x10^{11}) = product volume (mL) x product count (platelets/L)/100.000.

Statistical analysis

The distribution of data was tested for normality. Data were expressed as arithmetic means±SD (standard deviation) or medians with a range depending on the data distribution. Demographic and hematologic parameters of donors, as well as procedural characteristics were correlated with their platelet yield. Correlation was also done between actual product yield and the software-predicted product yield. The significance of the difference between actual product platelet yield and the software predicted yield was analysed using the nonparametric Mann-Whitney test. For all comparison, the level of statistical significance was $p<0.05$.

RESULTS

During the study period, a total of 60 donors underwent plateletpheresis procedures. The mean blood volume processed during plateletpheresis procedures was 2031 mL (1499-2531 mL) in the median time duration of 40 min. (36-43 min.).

The mean pre-donation platelet count of donors was 252x10^9/L (187-351x10^9/L). The mean platelet yield distribution among plateletpheresis donors was 3.5x10^{11}/unit (2-4.9x10^{11}/unit) (Table 1).

Twenty-three donors had a pre-donation platelet count of 180-239x10^9/L and it was observed that the mean yield of the product prepared from these donors was 3x10^{11}/unit (2-4.1x10^{11}/unit). The pre-donation platelet count was 240-299x10^9/L in 32 donors and the product prepared had mean yield of 3.7x10^{11}/unit (2.8-4.9x10^{11}/unit). In five donors with pre-donation platelet count of 300-359x10^9/L, the mean yield of product prepared was 3.9x10^{11}/unit (3.5-4.6x10^{11}/unit) (Table 2).

In the majority of donors, 48 (80%), amount of >3x10^{11}/unit was collected (Table 3).

A positive correlation was observed between platelet yield and pre-donation platelet count ($r=0.611$; $p<0.000$), blood

Table 1. Donor's and procedural characteristics of plateletpheresis (N=60)

Parameter	**Mean±SD** (range/median)
Age	38.2±8.3 (20-59)
Height	181.8±8.9 (158-199)
Weight	96.1±16.5 (62-130)
TLC	5.7-7.6 (median 6.5)
Hb	14.5-15.8 (median 15.2)
Hct	43.1±2.6 (37.1-52.4)
Platelet count	252±36.2 (187-351)
Platelet yield	3.5±0.6 (2-4.9)
Blood volume processed	2031±238 (1499-2531)
Run time	39±4.7 (28-49)
ACD-A used	249±26.1 (178-292)
Saline used	436±16.5 (404-467)

SD, standard deviation; TLC, total leucocyte count; Hb, haemoglobin concentration; Hct, haematocrit

Table 2. Effect of pre-donation platelet count on platelet yield

Pre-donation platelet count ($x10^9$/L)	**No of donors**	**Mean platelet yield ±SD (range)**
180-239	23	3.0±0.6 (2-4.1)
240-299	32	3.7±0.5 (2.8-4.9)
300-359	5	3.9±0.5 (3.5-4.6)

SD, standard deviation

Table 3. Distribution of platelet yield among donors

Platelet yield ($x10^{11}$/unit)	**No of donors**
2.0-2.49	5
2.5-2.99	7
3.0-3.49	21
3.5-3.99	16
>3.99	11

Table 4. Correlation between various parameters and platelet yield

Parameter	**r**	**p**
Height	0.264	0.042*
Weight	0.525	<0.000*
TLC	0.207	0.112
Hb	0.129	0.325
Hct	0.003	0.981
Platelet count	0.611	<0.000*
Blood volume processed	0.512	<0.000*
Run time	0.514	<0.000*
Software predicted yield	0.774	<0.000*

*significant correlation;
r, Pearson/Spearman coefficient; TLC, total leucocyte count; Hb, haemoglobin concentration; Hct, haematocrit

volume processed (r=0.512; p<0.000), and run time (r=0.514; p<0.000). Also, height and weight had significant correlation with platelet yield with values of (r=0.264; p=0.042) and (r=0.525; p<0.000), respectively.

No significant correlation was found between platelet yield and other donor parameters (TLC, Hb, and Hct). Also, no significant correlation was found between donor parameters (TLC, Hb, and Hct) and procedural parameters (blood volume processed, and run time).

Positive correlation between actual product platelet yield and the software predicted yield (r_s=0.774; p<0.000) with statistical difference between them (p<0.000) was found (Table 4).

The effect of gender on platelet yield was not studied, as only two donors were female.

DISCUSSION

Platelets are essential for the maintenance of haemostasis (5). Platelet transfusions are needed for either prophylactic or therapeutic purposes in thrombocytopenic patient (5).

The present study evaluated the effect of the donor's demographic and haematological characteristics, as well as procedural parameters on the platelet yield.

The mean body weight of our donors was 96 kg (range: 62–130 kg), while the mean body height was 181 cm (range: 158–199 cm). Our study found a significant positive correlation between body height and weight and platelet yield. Several previous studies have also observed a positive correlation between BMI (or height and weight) and platelet yield (3,4,6–8). It has been reported that individuals with higher body weight have a greater blood volume available for processing, and therefore achieve a higher platelet yield (6). On the other side some other studies found no significant correlation between them (5,10-13).

Analysis of other donor-related parameters on platelet yield revealed that pre-donation platelet count has a significant positive correlation with platelet yield. Donors having pre-donation platelet count in the higher range yielded products with higher platelet count. In the 60 procedures, the mean yield obtained was 3.5 x 10^{11}/unit. In the majority of donors, 48 (80%), amount of >3x10^{11}/unit was collected. According to the FDA (14) and AABB criteria (15), 75% of the plateletpheresis products prepared must contain ≥3x10^{11} platelets per unit, while the European guidelines recommend platelet count of ≥2x10^{11} platelets per unit (9). This will give an increment of 30.000 to 60.000/μL (5). Other authors (1,3–8,10–13,16–21) have also reported a positive correlation between them, meaning that the higher the donor's platelet count, the higher the platelet yield.

In present study we found no statistical correlation between platelet yield and haemoglobin, nor between platelet yield and haematocrit and total leucocyte count. No correlation between Hb and Hct and platelet yield was reported (1,3,5). Some studies reported significant positive correlation of pre-apheresis Hb with yield (6,19). On the other side, inverse relationship between pre-donation haemoglobin and yield was reported, i.e., the lower the haemoglobin concentrations, the higher the platelet yield (17). The inverse relationship between haemoglobin and platelet yield is likely due to the higher plasma volume processed in donors with low haemoglobin concentration (11). Reportedly, a negative correlation with donor pre-apheresis Hb and platelet yield was found (12,18). Some studies reported that the total leucocytes count of donors correlated positively with platelet yield (3,6,7), while others found no statistically significant impact of pre-donation total leucocyte count on platelet yield (18).

The present study also found a significant correlation between total blood volume processed and yield similar to other studies (10,20,21).

Run time or processing time is an important parameter not only for yield but also for donor comfort and retention (10). Longer

run time is associated with more anticoagulant infusion in donors and having more chances of citrate-related adverse reactions (10). The mean run time for plateletpheresis in our study was 39 minutes; also, a significant correlation between run time and platelet yield was found. Run time was not correlated with yield in some studies (10,20,21), while others revealed a significant positive correlation between them (6).

In our study, no significant correlation was found between donor parameters (TLC, Hb, and Hct) and procedural parameters. However, another study reported that the donor's total leukocyte count correlated positively with blood volume processed, while TLC correlated negatively with processing time and total blood volume processed (7). Studies also reported that haemoglobin correlated significantly with processing time and blood volume processed (6,7).

We could not evaluate the effect of gender on platelet yield as only two donors were female in the present study. However, some previous studies found that females had higher yield because of more prevalence of iron deficiency anaemia among females with the consequent rise in platelet count and thus platelet yield (10). Hormonal influence could also play a role (11).

The present study observed a statistically positive correlation between the actual product platelet yield and the software-predicted yield (r_s=0.774, p<0.000) with statistical difference between them (p<0.000). Some other authors (4,22) described similar results as well. Considering that in plateletpheresis we are mostly guided by the software-predicted platelet yield, a statistically significant difference between them represents important information for us to pay attention to the amount of platelets we collect from donors and to look for the causes that lead to this. This would aim to obtain the most accurate amount of platelets in the products and, above all, to protect the donor from possible thrombocytopenia.

By examining the factors that can affect the platelet yield we could get information that helps us select donors in order to provide the highest quality product and thus support patients more efficiently.

The limitation of the study was that we conducted our study on 60 data. A greater sample size would provide a more representative picture and would be required to evaluate the effects of all these factors on platelet yield for better selection of donors.

Pre-donation platelet count, body weight, body height, blood volume processed and run time affected platelet yield. Optimizing the platelet yield is a matter of identifying the factors that influence the yield and thus the selection of donors, providing better quality platelet products and clinical outcomes. The impact of different parameters on platelet yield has to be studied more closely.

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TRANSPARENCY DECLARATION

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ORIGINAL ARTICLE

Risk factors for red blood cell alloimmunization in polytransfused patients

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ABSTRACT

Aim Alloimmunization depends on the type of the erythrocyte antigen, as well as the number of transfused doses of blood and recipient factors reflected in genetic and immunological status, comorbidities and therapy. The aim was to determine the frequency and type of antierythrocyte antibodies, to examine the correlation between the number of transfused doses and the frequency of antibodies, as well as the influence of recipient factors on the prevalence of antierythrocyte antibodies in polytransfused patients.

Methods A retrospective study was conducted in the Department of Pretransfusion Testing and Blood Product Therapy at the Polyclinic for Transfusion Medicine of the University Clinical Centre Tuzla. The data of patients who received two or more doses of red blood cell concentrate, the frequency of antierythrocyte antibodies, as well as a dose and patient factors including age, sex, comorbidity and therapy that influenced alloimmunization were analysed.

Results The highest percentage of patients developed the anti-E (23%), anti-C (11%) and anti-K (17%) antibodies. The recipient factors that have shown to be positive predictors for the development of antierythrocyte antibodies were: surgical bleeding (OR=3.74) and autoimmune diseases (OR=2.3). The probability that patients will not develop antierythrocyte antibodies was more pronounced in oncological patients (1/OR=7.14), as well as in patients with iron supplementation (1/OR=2.22) and antihypertensive therapy (1/OR=2.32).

Conclusion The results emphasize the importance of pretransfusion testing, illuminate the positive and negative effects of frequent transfusions, and propose strategies for improving transfusion therapy with a focus on the benefits of phenotyping erythrocytes for Rh and Kell antigens.

Keywords: antierythrocyte antibodies, pretransfusion testing, transfusion

INTRODUCTION

Alloimmunization develops after the transfusion of blood products, pregnancy, or organ, cell, and tissue transplantation in circumstances where the recipient does not have the antigen expressed on the surface of the donor's red blood cells (1,2). The frequency of alloimmunized patients in the general hospital population ranges from 1 to 3%, and in groups frequently treated with red blood cell transfusions it can be as high as 59% (3-5). Researches have shown that the prevalence of immunization to red cell antigens in multitransfused adults ranges even from 1.5% to 38%, and 1-2% in children (6,7).

The speed and strength of alloimmunization depend on the type and strength of the erythrocyte antigen, as well as the number of transfused doses of erythrocyte concentrates. It was shown in their research that several factors play an important role in the development of immunization to erythrocyte antigens: antigen factors, dose factors of erythrocyte concentrate, and biological factors of the donor and recipient (8). Immunogenicity and density of antigens are defined as factors of erythrocyte antigens and are presented with the number of foreign antigens on erythrocytes (9).

Donor factors are determined by ethnic background, presence of inflammatory diseases, and the individual response of erythrocytes to actual storage conditions. The dosage factors of erythrocyte concentrates are defined by the length and method of storage of the blood products, their modification, and cell damage during storage. Recipient factors that can contribute to alloimmunization include: age and ethnic background, antigen recognition, immune system, and previous exposure to the antigen (8-10). Despite the phenotyping of erythrocyte concentrates in the Rh and Kell system, the possibility of sensitizing patients to erythrocyte antigens that are not included in

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the routine phenotyping procedure during pretransfusion blood processing still persists (11,12).

Recipient factors include various genetic causes, belonging to certain ethnic groups, the medical and immunological condition of the patient, the presence of infection, therapy, and many other causes which can lead to an increased tendency to produce antierythrocyte antibodies (13,14). In a large number of patients, associated antibodies can be detected, which can further complicate the search for an adequate blood dose for the patient (15). It has been proven that women are more prone to alloimmunization than men, and that various chronic inflammatory diseases and autoimmune diseases also favour the production of antibodies (15,16).

The number of scientific studies from the domain of alloimmunization is limited in Bosnia and Herzegovina and the region of South-East Europe. It is a result of inadequate systematic immunohematological monitoring, insufficient registry structures, and the lack of research infrastructure necessary for comprehensive, long-term investigations.

The aim of this study was to determine the frequency and specificity of antierythrocyte antibodies in polytransfused patients, as well as the influence of dose and recipient factors to the occurrence of antierythrocyte antibodies in polytransfused patients.

PATIENTS AND METHODS

Patients and study design

A retrospective study was conducted during the period between 1 January 2024 and 30 June 2024, at the Department of Pretransfusion Testing and Blood Product Therapy at the Polyclinic for Transfusion Medicine of the University Clinical Center Tuzla, Bosnia and Herzegovina.

Data were analysed from patients who were polytransfused and who received two or more doses of red blood cell concentrates. The source of data was the Renovatio RGB transfusion information system, and through the analysis of electronic medical records, data were collected of patients, as well as data of the blood products and the referred immunohematological testing: ABO blood type, Rh-factor, screening for the presence of antierythrocyte antibodies.

The analysis included age, sex, diagnoses of the underlying disease, diagnoses that required patient transfusions, presence of comorbidities and therapy, previous transfusions during life and the number of transfusions received, development of alloimmunization and the occurrence of specific antierythrocyte antibodies. The amounts, methods of storage of erythrocyte concentrates, and the type of preservative used in storage were analysed. The exclusion criteria included polytransfused patients with detected positive antierythrocyte antibody screening, where the specificity of immune antibodies in serum could not be precisely determined (false positive results due to therapy and pathological proteins) and women whose alloimmunization was exclusively the result of previous pregnancy.

All polytransfused patients during the specified period, based on whether they developed anti-erythrocyte antibodies or not, were classified into two groups. The study group consisted of polytransfused patients who developed antierythrocyte antibodies, while the control group consisted of polytransfused patients (with similar characteristics in terms of age, gender, present morbidity, and comorbidities) who did not develop antierythrocyte antibodies.

Methods

The identification of antierythrocyte antibodies was performed using a microgel method on ID DiaPanel cards. The procedure was carried out in 3 environments: NaCl medium, Liss-Coombs medium, and enzyme medium with the addition of bromelain. Each panel of cards for the identification of antierythrocyte antibodies in a specific medium consists of 12 microtubes (11 microtubes and one autocontrol).

The procedure was carried out by adding 50 μL of 0.8% red blood cell suspension from the ID-DiaPanel into an 11-cell identification panel. In microtubes for autoconfirmation, 50 μL of the patient's 0.8% red blood cell suspension was added. After that, 25 μL of the patient's serum was added to each microtube, and the microcards were incubated for 15 minutes at 37 °C, then centrifuged for 10 minutes. The NaCl medium cards were immediately centrifuged for 10 minutes in the Dia-Med Centrifuge (Bio-Rad/DiaMed, Cressier, Switzerland) after the addition was completed. For the enzymatic medium cards, 25 μL of bromelain enzyme was also added. The identification result was interpreted based on the presence of agglutination in the microtubules. LISS/Coombs ID cards contain polyspecific AHG and were used for antibody screening and identification, and the presence of agglutination was compared with the probability of the presence of a specific antibody in the identification panel.

Statistical analysis

In the statistical processing of data, standard methods of descriptive statistics were used (median, minimum, maximum). To test the statistical significance of differences among samples, both parametric and nonparametric significance tests were used. In analysing the impact of predictors on the occurrence of antierythrocyte antibodies, the principles of hierarchical logistic regression were used, which showed the contribution of each predictor to the occurrence of antierythrocyte antibodies in polytransfused patients. The contribution of each predictor was evaluated based on the z-test and the odds ratio (OR). Values of $p<0.05$ were considered statistically significant.

RESULTS

During the study period a total of 10,950 transfused patients were recorded, of which 2,160 were polytransfused. Patients were divided into 2 groups: the study group of 65 polytransfused patients who developed antierythrocyte antibodies and 65 polytransfused patients in the control group who did not develop antierythrocyte antibodies. Of the total number of transfused patients (N=10,950), 103 (1%) developed antierythrocyte antibodies. In the total number of polytransfused patients, a total of 65 (2%) patients were recorded with detected antierythrocyte antibodies. Forty-six patients (35%) included men, while 84 patients (65%) were women. In average, the patients were 63 years old (M = 62.88, SD=15.98, Min=22, Max=94). The majority of patients belonged to blood group O+, followed by blood groups A+, B+, O-, and AB+.

The largest number of patients suffered from anaemia, 48 (37%) and oncological diseases, 33 (25%). Patients used various types of medications and therapies depending on their primary illness, predominantly iron supplements, 30 (23%) and antihypertensive therapy, 30 (23%) (Table 1).

In the test group, the highest percentage of patients developed the anti-E antibody, 15 (23%), followed by patients with an-

Table 1. Frequency of patients according to sex, blood group, disease, therapy, and membership in the test (developed antibodies) or control (without antibodies) group

Variable	No (%) of patients	Cumulative %
Sex		
Man	46 (35)	35
Female	84 (65)	100
Blood type		
A+	39 (30)	30
A-	3 (2)	32
AB +	8 (6)	38
AB -	1 (1)	39
B +	21 (16)	55
B -	2 (2)	57
0 +	42 (32)	89
0 -	14 (11)	100
Disease		
Anaemia	48 (37)	37
Autoimmune diseases	13 (10)	47
Depressive disorder	1 (1)	48
Surgical bleeding	28 (22)	70
Urinary tract infection	6 (5)	75
Myeloproliferative disorder (JAK2+ mutation)	1 (1)	75
Oncological diseases	33 (25)	100
Therapy		
Anticoagulants	1 (1)	1
Antibiotics	18 (14)	1
Antidepressants	1 (1)	15
Antidiabetics	5 (4)	19
Antihypertensive therapy	30 (23)	42
Cytotherapy	21 (16)	58
Immunotherapy	1 (1)	59
Cardiotonic drugs	8 (6)	65
Corticosteroids	8 (6)	72
Iron products	30 (23)	95
Substitutive therapy with thyroid hormones	7 (5)	100
Group		
Test (appearance of antibodies)	65 (50)	50
Control (without antibodies)	65 (100)	100

ti-K, 11 (17%) and anti-C, 5 (11%). Among patients who developed two or more antibodies, the most common combinations were anti-E and anti-C, 8 (12%) (Table 2).

Patients were divided into two groups: patients who developed one antibody and patients who developed two or more antibodies. A $\chi 2$ test was conducted to determine whether the frequency of patients with one versus patients with two or more antierythrocyte antibodies statistically differed. The test was statistically significant, $\chi 2\ (1)=16.75$; $p < 0.001$, indicating that patients with one antibody were significantly more represented than patients with two or more antibodies (Table 2).

A total of 46 patients (70.77%) developed single antierythrocyte antibody and 19 patients (29.33%) patients were detected with multiple antibodies. It is important to emphasize that one of the detected antibodies in combination always belonged to the Rh-system.

In the test and control group, the blood product factors (number of transfused doses and type of preservative used during

Table 2. Frequency of patients with antierythrocyte antibodies by antibody specificity

Antibody type	No (%) of patients	Cumulative (%)
Single antibodies		
Anti E	15 (23)	43
Anti K	11 (17)	91
Anti C	7 (11)	11
Anti Fy a	5 (8)	65
Anti Jk a	4 (6)	72
Anti M	2 (3)	98
Anti Jk b	1 (2)	66
Anti Lu a	1 (2)	95
Anti Le b	1 (2)	100
Multiple antibodies		
Anti E, Anti c	8 (12)	55
Anti C, Anti E	2 (3)	14
Anti D, Anti C	2 (3)	20
Anti E, Anti Fy a	1 (2)	57
Anti E, Anti C, Anti K, Anti Jk a	1 (2)	74
Anti E, Anti K	1 (2)	92
Anti K, Anti Fy a	1 (2)	94

product preparation and storage) and recipient factors (primary diagnoses and type of medication applied) were analysed. Patients were divided into 4 subgroups depending on the number of doses of erythrocyte concentrate they received before the ap-

Table 3. Results of hierarchical regression analysis considering dose and recipient factors

Predictors	Model 1				Model 2			
	b	OR	Z	p	b	OR	z	p
Segment	0.15	1.16	0.52	0.600	1.38	3.98	1.58	0.115
Doses with CPDA-1	0.02	1.02	0.18	0.857	0.11	1.12	0.81	0.417
Doses with CPDA-SAGM	0.09	0.91	1.18	0.237	0.10	0.90	1.19	0.234
Age of patients					0.02	0.98	1.43	0.154
Sex (male vs. female)					0.09	1.10	0.21	0.831
Surgical diseases vs. all diseases					1.32	3.74	2.55	0.011
Autoimmune diseases vs. all diseases					0.83	2.30	1.20	0.232
Anaemia vs. all diseases					0.36	1.44	0.74	0.461
Iron therapy vs. all medicaments					0.80	0.45	1.54	0.123
Cytotherapy vs. all medicaments					1.92	6.85	2.07	0.039
Antihypertensive therapy vs. all medicaments					0.85	0.43	1.76	0.079
Antibiotics vs. all medicaments					0.09	1.09	0.13	0.898
	AIC = 184.73 AUC = 0.55				AIC = 182.72 AUC =0.55			

Model 1 includes: number of doses conserved with CPDA-1 and CPDA-SAGM; Model 2 includes: number of doses conserved with CPDA-1 and CPDA-SAGM, age of patients, sex, diseases and therapy,
b, non-standardized regression coefficient; OR, odds ratio.z, value of z-test which determines the significance of individual contribution of the predictor. AIC, Aikake's information criterion; AUC, The area under the Roc curve.

pearance of antierythrocyte antibodies. A total of 33 (50.77%) patients received 2 doses, 24 (36.92%) patients received 3-5 doses; four (6.15%) and 10 (6.15%) and more than 10 doses.

The applied preservative in the blood bag as a dose factor has not proven to be a reliable predictor in the development of alloimmunization in polytransfused patients, regardless of whether it involved blood preservation with CPDA-1 or CPDA-SAGM preservative (OR=1.1; OR=0.9).

The analysed recipient factors that were found to be positive predictors for the development of antierythrocyte antibodies were: surgical bleeding (OR=3.74), autoimmune diseases (OR=2.3), anaemia (OR=1.44), and cytostatic therapy (OR=6.85). The probability that patients will not develop antierythrocyte antibodies was more pronounced in patients with oncological diseases (1/OR=7.14), as well as in patients who used iron supplementation (1/OR=2.22) and antihypertensive therapy (1/OR=2.32) (Table 3).

DISCUSSION

The risk of the erythrocyte alloimmunization in transfused patients depends on a number of factors including the number and frequency of transfusion procedures, previous pregnancies, immunogenicity of erythrocyte antigens in the blood dose, and the immune response of the recipient and donor. The frequency of antierythrocyte antibodies in the studied population of polytransfused patients was 2.0%, which was higher than the results of other studies, where the frequency of antibody occurrence ranged from 0.9-1.1% (1,3).

The research results showed a higher proportion of the female population in the category of alloimmunized individuals, which is consistent with the findings of all conducted studies published on this topic (1, 8-13). This result is explained by greater exposure to erythrocyte antigens during gestational periods in life, which also leads to higher vulnerability of women regarding alloimmunization (17,18). Other studies, on the other hand, did not show a statistically significant difference in the incidence of antierythrocyte antibodies among polytransfused patients based on sex (19,22).

The most common antibodies detected as a result of alloimmunization to erythrocyte antigens are anti-E, anti-C, anti-K, and anti-Fya, which are also the clinically most significant antibodies due to their potency in causing posttransfusion haemolytic reactions and haemolytic disease of the newborn (23,24).

The great significance of our results lies in the fact that at the Polyclinic for Transfusion Medicine of the University Clinical Centre in Tuzla, all blood doses are regularly phenotyped only if they belong to RhD-negative blood groups, while RhD-positive doses and all other doses are phenotyped in the Rh and Kell system only occasionally and depending on the need for blood and blood products within the hospital. Therefore, it is not surprising that the largest number of antibodies comes from these two very extensive blood group systems.

Our research has also shown that the frequency of transfusions affects the rate of occurrence of antierythrocyte antibodies, as repeated blood transfusions lead to greater exposure to antigens, thus increasing the risk of alloimmunization (25-27).

The type of substance used during blood conservation has not been shown to be a reliable factor in predicting the occurrence of antierythrocyte antibodies, and such scientific analysis has not been found in the available literature.

The results of the research showed that the patient's factors have a much more significant impact on the frequency of the occurrence of antierythrocyte antibodies in polytransfused patients, primarily the influence of existing diseases, pathological conditions, and the therapy being applied. The analysis was quite complicated, as all patients, in addition to their primary disease, have many additional comorbidities that complicate the statistical analysis of data. Therefore, the results were presented as the probability of antibody occurrence in patients with a certain disease compared to all other patients in the observed group. Thus, patients suffering from oncological diseases had a 7.14 times greater chance of not developing antibodies after transfusion due to the immunosuppressive effect of therapy.

Patients who were transfused during various surgical operations had a higher risk of developing antibodies, with the explanation that massive surgical operations, especially solid organ surgeries with complications, involve the transfusion of more blood doses in a shorter time span (26). From the perspective of therapy usage, patients who underwent cytostatic therapy had a higher risk of developing antibodies, which can be explained by the fact that cytostatic therapy lowers blood count values, thereby increasing the need for frequent transfusions of red blood cell concentrates (27).

An interesting fact was that patients with autoimmune diseases predominately Hashimoto's thyroiditis, rheumatoid arthritis, and ulcerative colitis had 2.3 times greater chances of developing alloimmunization, which is a result consistent with all available scientific achievements that have examined the inflammatory risk for diseases in transfused patients (27, 28).

None of the patient factors (age, sex, comorbidity and therapy) can be taken as a single predictive factor for the development of alloimmunization, but in combination with other factors and laboratory findings that are regularly monitored alongside the primary disease, one can assume the risk and dynamics of the development of antierythrocyte antibodies.

During the study, donor factors were not taken into account, although from a scientific point of view, various enzymatic and immunological mechanisms in the blood may likely influence the probability of the occurrence of antierythrocyte antibodies to some extent.

The limitation of this research is the inability to completely analyse certain patient factors such as genetic predisposition and the immunocompromised state of the patient. This should be included as a potential topic for some future analyses. The very occurrence of autoimmunity and the immune reactions that arise at that time certainly contribute significantly to the emergence of antierythrocyte antibodies, but due to the lack of laboratory equipment, we were unable to investigate this in more detail.

In conclusion, the results of this study emphasize the importance of pretransfusion immunohematological testing of blood donors and recipients of blood and blood products, highlighting the positive and negative effects of frequent blood transfusions, and proposing potential strategies for improving transfusion therapy with an emphasis on the benefits of phenotyping erythrocyte antigens of the Rh and Kell systems in order to minimize immunohematological complications in polytransfused patients.

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Competing interest: None to declare.

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ORIGINAL ARTICLE

Comparative evaluation of HbA1c in point-of-care testing (POCT) devices: enzymatic and immunoassay methods compared to ion-exchange high-performance liquid chromatography (IE-HPLC)

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ABSTRACT

Aim To evaluate 3 point-of-care testing (POCT) devices in testing HbA1c: the Simplex TAS™101 (Tascom Co. Ltd., South Korea), which utilizes an enzymatic method, and two immunoassay-based devices, the Zybio Q8 Pro and Zybio EXR 110 (Zybio Inc., China).
Methods A total of 40 whole blood samples with EDTA anticoagulant were collected from patients undergoing HbA1c testing at an outpatient department. The samples were analysed using 3 POCT devices and one reference method.
Results Passing-Bablok regression revealed no significant fixed or proportional bias. Strong correlations with the gold standard were observed for Simplex TAS™101 (r=0.944), Zybio Q8 Pro (r=0.910), and Zybio EXR 110 (r=0.839), all with p<0.001. Bland-Altman analysis confirmed that the majority of measurements fell within an acceptable clinical range**.**
Conclusion This study demonstrated that the three POCT devices employing enzymatic and immunoassay methods served as reliable alternatives to ion exchange-high-performance liquid chromatography (IE-HPLC) for HbA1c measurement in clinical practice.
Keywords: diabetes, laboratory, point-of care testing

INTRODUCTION

Glycated haemoglobin (HbA1c) is widely recognized as a critical biomarker for assessing long-term glycaemic control in patients with diabetes (1). This parameter provides an accurate reflection of average blood glucose levels over the preceding two to three months, making it a reliable indicator for evaluating the risk of diabetes-related complications (1,2). In addition to its role in monitoring, HbA1c is also utilized as a diagnostic criterion for diabetes (2). Both the American Diabetes Association (ADA) (3) and the World Health Organization (WHO) (4) have approved HbA1c testing as a standard diagnostic tool, with a recommended threshold of 6.5% (5).

HbA1c levels are commonly measured through the collection of a venous blood sample, which is subsequently sent to a laboratory for analysis. While this method is widely used, it is often associated with considerable time and cost implications (6). Point-of-Care Testing (POCT) HbA1c devices allow testing to be conducted in diverse settings, such as doctors' offices, outpatient clinics, pharmacies, and nursing facilities. These devices require smaller sample volume and primarily utilize capillary blood obtained through a finger stick, though certain models also accommodate venous blood samples (7). POCT technology has significantly advanced clinical laboratory practices by providing reliable and user-friendly equipment with demonstrated clinical benefits. Its accessibility and ease of use, supported by low-maintenance analysers and visually interpretable kits, offer an effective solution for conducting laboratory tests directly at or near the patient's location (8). The adoption of POCT for HbA1c has been linked to shorter turnaround times, improved glycaemic control in patients, timely delivery of test results with corresponding treatment adjustments, enhanced decision-making processes, reduced need for multiple patient visits, and improved communication between patients and medical professionals (6).

High-performance liquid chromatography (HPLC) has been the primary method for HbA1c analysis, offering precise measurements with high resolution. In recent years, alternative methods such as immunoassays and enzymatic assays have

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been introduced, enabling HbA1c measurement through a variety of methodologies (9). Studies on method evaluation and comparison indicate varying performance among different HbA1c POCT devices. Ensuring accurate HbA1c measurements and reliable results across diverse clinical settings is essential in the current era of POCT (10).

The aim of this study was to evaluate the performance of POCT devices utilizing enzymatic and immunoassay methods in comparison with IE-HPLC, the reference method used in the central laboratory settings.

PATIENT AND METHODS

Patients and study design

This study was conducted from August 2025 to September 2025 at the Central Laboratory of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. A total of 40 fresh blood samples were collected from patients undergoing HbA1c testing at the outpatient department. Exclusion criteria included patients <18 years of age, pregnant women, and patients with anaemia. For each patient, 5 mL of whole blood was drawn using EDTA as an anticoagulant.

A total of 40 samples were analysed using four devices, including three point-of-care testing (POCT) devices such as Simplex TAS™101 (Tascom Co. Ltd., South Korea), Zybio Q8 Pro (Zybio Inc., China), and Zybio EXR 110 (Zybio Inc., China), and a gold-standard examination using BioRad D-10 (Bio-Rad Laboratories, USA). We chose 40 samples, as it is the minimum samples required for validation study (11). The results from the POCT devices were systematically compared with those obtained from the gold-standard device for evaluation.

A written informed consent was obtained from each patient or their representative after a thorough explanation. This study was approved by the Health Research Ethics Committee of Dr. Soetomo General Academic Hospital, Surabaya (approval letter number: 0744/KEPK/VIII/2023).

Methods

Three HbA1c POCT devices were evaluated: the Simplex TAS™101 utilizes an enzymatic method, and two immunoassay-based devices, the Zybio Q8 Pro and Zybio EXR 110. The enzymatic HbA1c assay is based on the enzymatic digestion of lysed whole blood samples by proteases, which break down haemoglobin beta chains into amino acids, including glycated dipeptides. These glycated dipeptides act as substrates for fructosyl peptide oxidase (FPOX), an enzyme that specifically cleaves N-terminal valines, generating hydrogen peroxide as a byproduct. The resulting hydrogen peroxide is subsequently quantified through a reaction catalysed by horseradish peroxidase (POD) in the presence of an appropriate chromogenic substrate. The immunoassay-based methods used immunofluorescence chromatography and a double-antibody sandwich method for rapid, quantitative detection of HbA1c in whole blood. The test line, coated with a mouse anti-human HbA1c monoclonal antibody, captures an antigen-antibody complex formed between sample HbA1c, Hb, and a fluorescently labelled antibody. The complex migrates via capillary action, producing a fluorescent band. An immunofluorescence analyser quantifies HbA1c by analysing the fluorescence signal (12).

The BioRad D-10 was utilized as a reference method for HbA1c measurement. This device operates on the principle of ion-exchange high-performance liquid chromatography (IE-HPLC) using whole blood samples anticoagulated with ethylenediaminetetraacetic acid (EDTA). The method enables the separation of HbA1c and HbA0 based on differences in their isoelectric points, leveraging variations in ionic interactions. The separated haemoglobin fractions are subsequently passed through a flow cell equipped with a photometric filter and measured based on absorbance changes at a wavelength of 415 nm. The HPLC method is widely recognized for its ability to eliminate labile components, such as pre-A1c or Schiff base. These components originate from the initial phase of haemoglobin (HbA) glycation, a rapid and reversible process that is highly dependent on plasma glucose concentration (13).

Two concentrations of Quality Control (QC) materials (Level 1 and Level 2) were used in this study. Precision verification was conducted by analysing five replicates of each QC level daily over a period of five days across the three POCT devices. Imprecision was assessed by calculating the total %CV for Within-Laboratory conditions (14).

Statistical analysis

The normality of data distribution was assessed using the Shapiro-Wilk test. Passing-Bablok regression analysis was applied to evaluate the linear relationship and potential systematic bias between the measurement methods. Spearman's rank correlation test (r) was conducted to determine the strength and direction of the association between the two methods, with statistical significance set at $p<0.05$. Finally, method agreement was assessed using Bland-Altman analysis to define the limits of agreement for HbA1c level variations across different measurement devices.

RESULTS

This study analysed data from 40 patient samples, consisting of 18 males and 22 females aged between 32 and 69 years. HbA1c levels were measured in all participants (Table 1).

Table 1. Characteristics of HbA1c from 40 patients for each device

Device	Median of HbA1c (min – max)
BioRad D-10	6.40% (4.70–12.00)
Simplex TAS™101	6.20% (4.50–10.70)
Zybio Q8 Pro	6.89% (5.00–12.37)
Zybio EXR 110	6.54% (5.00–11.25)

Precision testing with 25 repetitions of level 1 and Level 2 controls demonstrated that the lowest %CV values were observed for the Simplex TAS™101 (L1=2.8%; L2=1.4%), whereas the highest %CV values were measured for the Zybio Q8 Pro (L1=3.26%; L2=4.8%) (Table 2).

The correlation analysis results of HbA1c measurements from 40 patient samples were assessed using four different devices (Table 3).

The statistical analysis comparing SimplexTAS™ and BioRad D-10 (N=40) demonstrated a strong agreement and correlation between the two methods. The Passing–Bablok regression analysis between Simplex TAS™101 and BioRad D-10 (N=40) yielded an intercept of −0.1326 (95% CI: −0.6252-0.3588) and a slope of 0.9565 (95% CI: 0.8824-1.028), indicating no

Table 2. Total precision testing result of HbA1c for each device

Device	Control level 1			Control Level 2		
	Mean	SD	%CV	Mean	SD	%CV
Simplex TAS™101	5.95	0.16	2.8	8.7	0.12	1.4
Zybio EXR 110	5.28	0.14	2.8	9.01	0.43	4.7
Zybio Q8 Pro	5.32	0.17	3.2	9.07	0.43	4.8
BioRad D-10	5.32	0.13	2.4	10.32	0.16	1.6

SD, standard deviation; CV, coefficient of variation

significant fixed or proportional bias (Table 3). Spearman's correlation analysis showed a very strong positive correlation (r=0.944; p<0.001), confirming the consistency between the two methods (Figure 1A). Bland-Altman analysis revealed a mean bias of -0.45, indicating that SimplexTAS™ tended to measure slightly lower HbA1c levels than BioRad D-10. The 95% limits of agreement ranged from −1.29 to 0.38, with the majority of data points falling within these limits (Figure 2A). The statistical analysis comparing Zybio EXR 110 and BioRad

Table 3. Correlation analysis of HbA1c level between different point-of-care testing (POCT) SimplexTASTM101, Zybio EXR 110, and Zybio Q8 Pro with BioRad D-10

POCT device	r Spearman	Slope	Intercept	Mean bias	p value
Simplex TAS™101 vs. BioRad D-10	0.944	0.9565	–0.1326	–0.45	<0.001
Zybio EXR 110 vs. BioRad D-10	0.839	0.9515	0.1561	–0.04	<0.001
Zybio Q8 Pro vs. BioRad D-10	0.910	1.011	0.059	0.2	<0.001

r Spearman, correlation coefficient; Slope, a slope of 1 indicates perfect association; Intercept, a point where a line crosses y-axis; Mean bias, tendency of underestimate or overestimate.

D-10 (N=40) demonstrated a strong correlation and overall agreement between the two methods. The Passing–Bablok regression analysis between Zybio EXR 110 and BioRad D-10 (N=40) yielded an intercept of 0.1561 (95% CI: −0.6624-0.9975) and a slope of 0.9515 (95% CI: 0.8211-1.072), in-

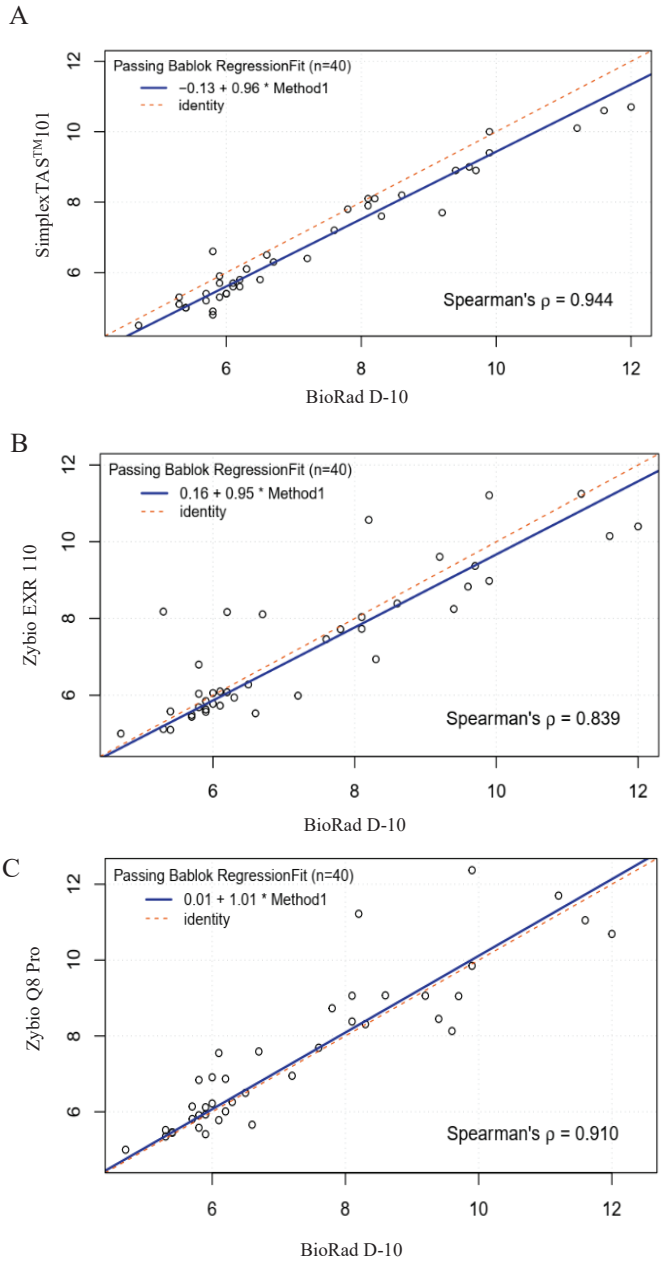

Figure 1. Scatterplot of HbA1c level for: A) the SimplexTASTM101 and BioRad D-10; B) Zybio EXR 110 and and BioRad D-10; C) Zybio Q8 Pro and BioRad D-10

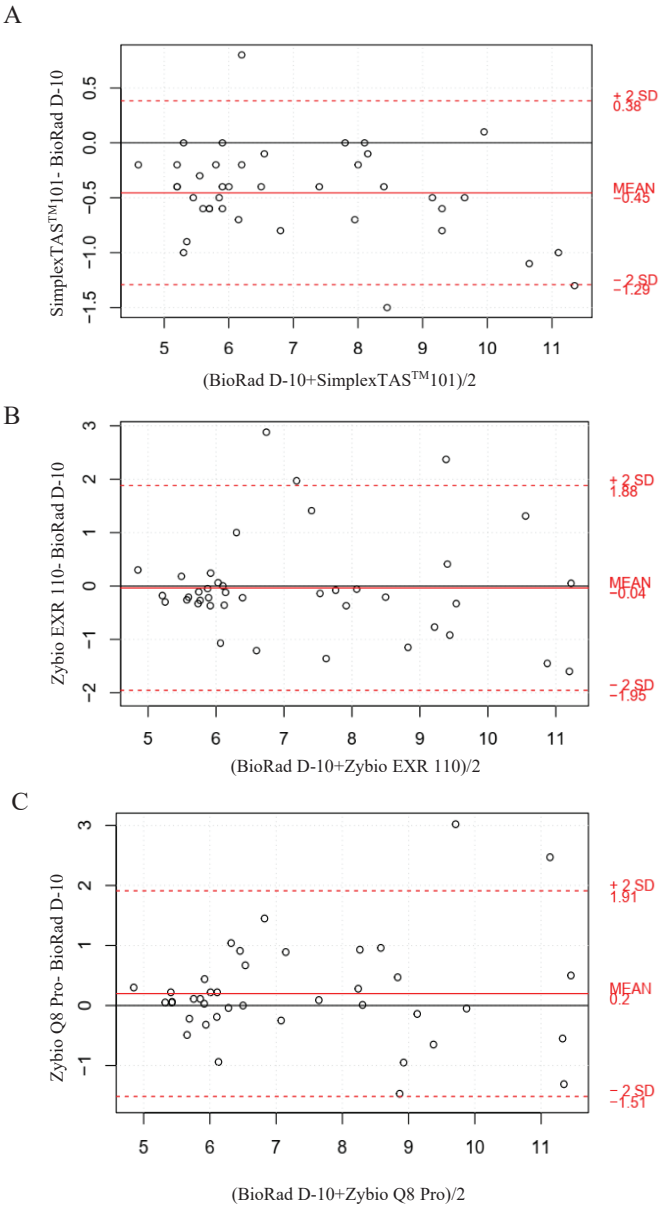

Figure 2. Blant Altman analysis of HbA1c between: A) the SimplexTASTM101 and BioRad D-10; B) Zybio EXR 110 and and BioRad D-10; C) Zybio Q8 Pro and BioRad D-10

dicating no significant fixed or proportional bias (Table 3). Spearman's correlation analysis revealed a strong positive correlation (r=0.839; p<0.001), confirming a consistent relationship between the two methods despite some variability (Figure 1B). Bland–Altman analysis revealed a mean bias of -0.04, suggesting that Zybio EXR 110 reported slightly lower HbA1c values compared to BioRad D-10. The 95% limits of agreement ranged from −1.95 to 1.88, with most data points falling within this interval, suggesting acceptable agreement between the two methods (Figure 2B).

The statistical analysis comparing Zybio Q8 Pro and BioRad D-10 (N=40) demonstrated a strong correlation and overall agreement between the two methods. The Passing–Bablok regression analysis between Zybio Q8 Pro and BioRad D-10 (N=40) yielded an intercept of 0.0059 (95% CI: −1.078-0.784) and a slope of 1.011 (95% CI: 0.8952-1.183), indicating no significant fixed or proportional bias (Table 3). Spearman's correlation analysis revealed a very strong positive correlation (r=0.910; p<0.001), confirming a high level of agreement between the two methods (Figure 1C). Bland–Altman analysis revealed a mean bias of 0.20, indicating that Zybio Q8 Pro tended to slightly overestimate HbA1c values compared to the BioRad D-10. The 95% limits of agreement ranged from −1.51 to 1.91, with most data points falling within this interval, suggesting that the level of disagreement remains within an acceptable clinical range (Figure 2C).

DISCUSSION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycaemia, which, if left uncontrolled, can lead to severe long-term degenerative complications. Therefore, the diagnosis, management, and monitoring of diabetic patients require comprehensive laboratory testing for assessing glycaemic control and guiding appropriate therapeutic interventions (15–17). HbA1c provides a cumulative representation of blood glucose levels over the preceding two to three months, making it a more reliable indicator of chronic hyperglycaemia. HbA1c measurement does not require fasting conditions or specific timing for blood sample collection, thereby enhancing its practicality and convenience in routine clinical monitoring and diabetes management (18)

The three devices used in this study are widely available in Asia, but they have not yet undergone validation, making this study the first to validate all of these devices. POCT-based HbA1c analysers are now widely integrated into routine clinical practice, offering rapid and reliable results while minimizing the need for centralized laboratory testing. These devices have proven particularly beneficial in resource-limited settings or situations where conventional laboratory assessments are too time-consuming. For instance, paediatric patients, individuals with poorly controlled diabetes, and those requiring frequent monitoring can greatly benefit from near-patient HbA1c testing. Furthermore, POCT devices enable real-time decision-making by healthcare providers, allowing for immediate adjustments in diabetes treatment regimens (19).

A key challenge in HbA1c measurement lies in the standardization and reliability of different analytical methods. Variability in measurement techniques can lead to inconsistent results, potentially affecting clinical decision-making. Therefore, numerous studies have been conducted to evaluate the analytical performance of HbA1c assays, comparing them with established reference methods to ensure accuracy, precision, and reproducibility (14,20). In this study, Simplex TAS™ 101 exhibited the lowest total coefficient of variation (%CV) among the three evaluated devices, indicating superior precision. The comparative analysis of SimplexTAS™101, Zybio EXR 110, and Zybio Q8 Pro against BioRad D-10 demonstrated a very strong correlation and overall agreement, with slight variations in bias. SimplexTAS™101 showed the highest association with BioRad D-10, followed by Zybio Q8 Pro and Zybio EXR 110. The Spearman test showed a very strong correlation for all three devices, indicating that the HbA1c results from these devices can be considered a reliable alternative (21).

Passing-Bablok regression analysis indicated no significant fixed or proportional bias, as all confidence intervals encompassed zero and the slopes were close to one. Bland-Altman analysis further confirmed that while minor differences existed between the methods, the majority of measurements fell within an acceptable clinical range. Despite these variations, all three devices demonstrated high comparability with BioRad D-10, making them viable alternatives for HbA1c measurement. However, the observed biases should be taken into account when interpreting clinical results.

Haemoglobin variants may interfere with HbA1c measurements due to various factors. The reference method meets the requirements for high throughput, accuracy, and reliability in HbA1c testing. It is also capable of detecting haemoglobin variants, which can be considered both an advantage and a disadvantage (22). Unlike reference methods, our POCT devices utilized chemical approaches such as enzymatic and immunoassay techniques, which are easily implemented on standard chemistry analysers. These methods do not detect haemoglobin variants and do not interfere with HbA1c measurements, offering a more streamlined approach but at the cost of lacking variant detection capabilities (23).

The limitations of this study include a relatively small sample size although it meets the criteria of the Clinical Laboratory Improvement Amendments (CLIA), the fact that it does not take into account potential interference factors, such as hemoglobinopathies that may be present in certain populations, and last, the study was conducted at only one study centre. Further validation studies involving larger, multicentre, and more diverse sample populations are recommended to refine clinical thresholds and confirm the generalizability of these findings.

In conclusion, enzymatic and immunoassay methods, which strongly correlate with HPLC, enhance accuracy and reliability in clinical practice. This study confirms the precision of the three POCT devices, demonstrating their strong correlation with reference methods. POCT-based HbA1c analysers are valuable for diabetes management, particularly in settings requiring immediate clinical decisions.

AUTHOR CONTRIBUTIONS

Conceptualization, methodology, validation, formal analysis, data curation: WRA, YP, and FRM; Software: WRA and FRM; Investigation, resources, visualization, supervision: YP. Data curation, writing, project administration: WRA and YP; Funding acquisition: YP and FRM.

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TRANSPARENCY DECLARATION

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Med Glas (Zenica)

ORIGINAL ARTICLE

Stem cells and secretome in aplastic anaemia: a bibliometric analysis of global research trends (2018–2023) and future directions for regenerative therapies

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ABSTRACT

Aim This study analysed research trends related to stem cells and secretome in aplastic anemia between 2018 and 2023 using a bibliometric approach to understand developments and research distribution in this field.

Methods The Scopus database was used to search for relevant articles on aplastic anaemia and its relationship to stem cells or secretome, collecting publication and citation data found in article titles, abstracts, and keywords. VOSviewer and Biblioshiny bibliometric software were used to visualize author, country, journal, and keyword networks. Visualization maps aimed to highlight the emergence of words and phrases in titles and abstracts.

Results Based on information from 1,405 articles identified from 2018 to 2023, publication numbers experienced significant fluctuations. Research showed that publications on aplastic anaemia, stem cells, or secretome increased by an average of 2.52% annually over the past five years. The most productive country was China with 321 documents, while the most productive institution was the Chinese Academy of Medical Sciences & Peking Union Medical College with 53 documents. We identified four cluster groups: "severe aplastic anaemia", "allogeneic stem cell transplantation", "bone marrow failure", and "hematopoietic stem cell transplantation".

Conclusion This study provides a comprehensive overview of research trends in aplastic anaemia related to stem cells and secretome from 2018 to 2023, highlighting steady growth and key contributors like China. Future research will likely focus on secretome applications and international collaborations to advance regenerative therapies for aplastic anaemia.

Keywords: aplastic anaemia, bibliometrics, secretome, stem cells, mesenchymal stem cells

INTRODUCTION

Aplastic anaemia is a medical condition characterized by chronic primary hematopoietic failure caused by injury, resulting in reduced or absent hematopoietic precursors in the bone marrow, accompanied by pancytopenia (1). Multiple factors can influence this condition, including genetic predisposition, environmental exposures, infections, autoimmune mechanisms, and idiopathic causes. Understanding these factors affecting aplastic anaemia is crucial for developing more effective treatment strategies (2). Over the past several decades, using stem cells and secretome as regenerative therapeutic approaches has garnered significant attention in medicine. Hematopoietic stem cell transplantation represents one of the primary therapeutic options for patients with severe aplastic anaemia, especially for those without matched donors (2). Mesenchymal stem cells (MSCs) and their secretome are potential therapies for aplastic anaemia, where the bone marrow fails to produce sufficient blood cells. The secretome from MSCs contains various growth factors and cytokines that can support blood cell regeneration and repair the bone marrow microenvironment (3). Bioinformatic analyses have revealed several key molecular pathways through which the MSC secretome influences haematopoiesis in aplastic anaemia (4). Transcriptomic profiling has identified differential expression of genes involved in cell cycle regulation, apoptosis, and immune modulation in CD34+

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progenitor cells exposed to MSC-derived extracellular vesicles (EVs) (5). Specifically, MSC secretome upregulates C-X-C motif chemokine ligand 12 (CXCL12), stem cell factor (SCF), thrombopoietin (TPO), and FMS-like tyrosine kinase 3 ligand (FLT3L) expression in bone marrow stromal cells, which are crucial for hematopoietic stem cell (HSC) maintenance and proliferation (6,7). Proteomic analyses of MSC-derived EVs have identified over 200 proteins involved in cell adhesion, migration, proliferation, and immune regulation, with particular enrichment in factors like interleukin-6 (IL-6), leukaemia inhibitory factor (LIF), stromal cell-derived factor 1 (SDF-1), and hepatocyte growth factor (HGF) that directly support haematopoiesis (8,9). Single-cell RNA sequencing studies have demonstrated that MSC secretome modulates the expression of key transcription factors like GATA binding protein 1 (GATA1), GATA binding protein 2 (GATA2), and purine-rich box-1 (SPI1) in HSCs, redirecting differentiation toward erythroid and myeloid lineages (10, 11). Furthermore, pathway analysis through gene ontology and KEGG has revealed significant enrichment of phosphoinositide 3-kinase/protein kinase B (PI3K/AKT), janus kinase/signal transducer and activator of transcription (JAK/STAT), and mitogen-activated protein kinase (MAPK) signalling pathways in HSCs treated with MSC secretome, all of which are critical for hematopoietic cell survival and proliferation (12–14).

In aplastic anaemia patients, genetic variants in human leukocyte antigen-DR15 (HLA-DR15), anti-thymocyte globulin (ATG), runt-related transcription factor 1 (RUNX1), and additional sex combs-like 1 (ASXL1) have been identified as predisposing factors that interact with MSC secretome response pathways (15,16). Polymorphisms in cytokine receptor genes like interleukin-17 receptor (IL-17R), interleukin-2 receptor (IL-2R), and interferon gamma receptor (IFN-γR) affect the response to immunomodulatory factors in MSC secretome, potentially explaining variable therapeutic outcomes (17). Epigenetic profiling has also revealed that MSC secretome induces hypomethylation of promoter regions controlling genes involved in haematopoiesis, such as homebox B4 (HOXB4) and GATA2, potentially restoring normal HSC function (18). Recent studies have highlighted the role of secretome in reducing cytotoxic effects and enhancing hematopoietic cell regeneration without significant immunosuppression risks (19). Bioinformatics network analyses have identified miRNA-mRNA interaction networks, with miR-146a, miR-155, and miR-223 from MSC-EVs targeting genes involved in T-cell activation and pro-inflammatory cytokine production, thereby mitigating immune-mediated destruction of HSCs (20). The pathophysiology of aplastic anaemia involves complex interactions between genetic susceptibilities and environmental factors. The disease typically manifests through three primary mechanisms: intrinsic stem cell defects, immune-mediated destruction of hematopoietic stem cells, and alterations in the bone marrow microenvironment (1). Approximately 70-80% of aplastic anaemia cases are attributed to autoimmune mechanisms, where T-cell-mediated attacks target CD34+ hematopoietic progenitor cells, leading to their destruction and impaired proliferation (21).

Spatial transcriptomics and multi-omics integration approaches have recently mapped the bone marrow niche alterations in aplastic anaemia, revealing dysregulation of the CXCL12-CXCR4 axis and N-cadherin-mediated cell adhesion pathways that are directly modulated by MSC secretome components (6,22). Machine learning algorithms applied to multi-omics data have identified biomarker signatures predicting response to MSC secretome therapy, with elevated expression of CD55, CD59, and reduced NLRP3 inflammasome activation correlating with favourable outcomes (22,23). This understanding of the underlying pathophysiology has led to the development of various therapeutic approaches, including stem cell transplantation and immunosuppressive therapies.

There is growing interest in using stem cells and their secretomes to treat aplastic anaemia. As a result, conducting a bibliometric analysis of the relevant scientific literature from the past five years has become essential. Bibliometric analysis allows us to understand research trends, geographical distribution, researcher collaborations, and the influence of this topic in the scientific community.

This study has focused on publications discussing stem cell or secretome applications in aplastic anaemia between 2018 and 2023 to map scientific developments, challenges, and future opportunities.

MATERIALS AND METHODS

Materials and study design

Data for this bibliometric analysis were extracted from the Scopus database in September 2024. The initial search targeted article titles, abstracts, and keywords using the following formula: (“anemia” OR “anaemia”) AND aplastic AND (“stem cell” OR “secretome”). The complete search strategy was TITLE-ABS-KEY (“anemia” OR “anaemia”) AND aplastic AND (“stem cell” OR “secretome”) AND PUBYEAR > 2018 AND PUBYEAR < 2024 AND (LIMIT-TO(EXACTKEYWORD, “Human”) AND (LIMIT-TO(LANGUAGE, “English”). The analysis was limited to articles published from 2018 to 2023 and written in English. In total, 1,405 articles were identified and exported as CSV files for further analysis (Figure 1).

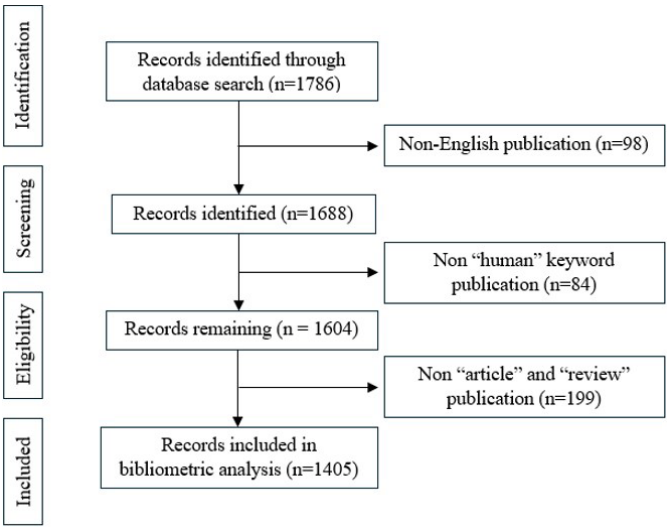

Figure 1. Literature search process flow diagram

Methods

To ensure a comprehensive and unbiased bibliometric analysis, a multi-step methodology was employed. Scopus was selected as the database due to its extensive coverage of biomedical literature and robust citation tracking capabilities. The search strategy was carefully refined through pilot searches to

maximize the retrieval of relevant articles while minimizing irrelevant results. Following data extraction, the exported comma-separated values (CSV) files were systematically cleaned to remove duplicates and correct inconsistencies in author names, affiliations, and keywords. The bibliometric analysis encompassed various parameters, including publication output by year and document type, source metrics such as journals and impact factors, author productivity, collaboration networks, contributions from institutions and countries, citation metrics highlighting the most cited papers and citation patterns, as well as keyword and thematic analyses involving co-occurrence networks and thematic evolution. For visualization, VOSviewer 1.6.19 was utilized to create network visualizations covering co-authorship, co-citation, and keyword co-occurrence, while Biblioshiny supplemented these with additional analyses and visualizations of research themes and trends.

Statistical analysis

Descriptive statistics were used to summarize publication growth rates. Bradford's law was applied to identify core journals in the research field, supporting the bibliometric findings and enhancing the robustness of the analysis.

RESULTS

A total of 1,405 documents were identified consisting of 226 reviews (16.1%) and 1,179 articles (83.9%). Results showed that publications about stem cells or secretome in aplastic anaemia increased by an average of 2.52% annually, indicating considerable interest in researching this topic. Publication trends on stem cells or secretome in aplastic anaemia experienced significant fluctuations from 2018 to 2023. Two thousand nineteen research trends experienced a surge, reaching their peak in 2021, followed by a gradual decline in 2022 and 2023. Various factors, including the COVID-19 pandemic and increased research funding opportunities in this field, may have influenced the increase from 2019 to 2021. The decline from 2022 to 2023 could be attributed to several factors, including uneven technological development across countries, limited research funding, and various other factors (Figure 2A). Leading journals included Biology of Blood and Marrow Transplantation (73 documents), Transplantation and Cellular Therapy (69), and Frontiers in Immunology (40), reflecting the multidisciplinary nature encompassing haematology, immunology, and transplantation (Figure 2B). The United States (341 documents), China (326), and Japan (118) dominated research output, influenced by strong infrastructure and investments, while Southeast Asian countries lagged behind, pointing to regional research gaps and opportunities for collaboration (Figure 2C). International collaborations remain limited (15.02% co-authorship), highlighting potential for enhancing global research networks (Figure 2D).

Highly cited works included Young NS et al.'s 2018 comprehensive review (447 citations) and D'Souza et al.'s 2020 study on hematopoietic cell transplantation trends (347 citations), underscoring pivotal insights into aplastic anaemia pathophysiology and treatments (Table 1). Thematic clustering identified four major areas: clinical management of severe aplastic anaemia; allogeneic stem cell transplantation and its adaptation during COVID-19; bone marrow failure with mesenchymal stem cell therapies; and pharmaceutical approaches including eltrombopag and immunosuppressive therapy (Figure 3A, B, Table 2).

Cluster analysis identified four main research areas: Cluster 1 focuses on severe aplastic anaemia, including clinical management, conditioning regimens, complications, and survival; Cluster 2 covers allogeneic stem cell transplantation, emphasizing special populations like children and the impact of COVID-19; Cluster 3 centres on bone marrow failure, linking aplastic anaemia with related disorders and mesenchymal stem cell therapies; Cluster 4 highlights hematopoietic stem cell transplantation alongside pharmaceutical treatments such as eltrombopag and immunosuppressive therapy. This reflects an evolving, interconnected research landscape that recognizes disease heterogeneity and the need for personalized therapies.

Keyword analysis showed "aplastic anaemia", "hematopoietic stem cell transplantation", and "male" as the most frequent, while "mortality", "chronic myeloid leukaemia", and "human cell" were less studied, indicating research gaps (Figure 3C-D). Temporal trends reveal a shift from foundational treatment

Table 1. Ten most valuable publications based on citation count

No	Title	Year	Source	Citations
1	Aplastic Anemia	2018	N England J Med	447
2	Current Use of and Trends in Hematopoietic Cell Transplantation in the United States	2020	Biol Blood Marrow Transplant	347
3	Clonal Hematopoiesis and Evolution to Hematopoietic Malignancies	2018	Cell Stem Cell	325
4	Indications for haematopoietic stem cell transplantation for haematological diseases, solid tumours and immune disorders: current practice in Europe, 2019	2019	Bone Marrow Transplant	257
5	Cancer in the National Cancer Institute inherited bone marrow failure syndrome cohort after fifteen years of follow-up	2018	Haematologica	230
6	The consensus on indications, conditioning regimen, and donor selection of allogeneic hematopoietic cell transplantation for hematological diseases in China—recommendations from the Chinese Society of Hematology	2018	J Hematol Oncol	219
7	Diagnostic utility of telomere length testing in a hospital-based setting	2018	Proc Natl Acad Sci USA	153
8	The consensus from The Chinese Society of Hematology on indications, conditioning regimens and donor selection for allogeneic hematopoietic stem cell transplantation: 2021 update	2021	J Hematol Oncol	150
9	The EBMT activity survey on hematopoietic-cell transplantation and cellular therapy 2018: CAR-T's come into focus	2020	Bone Marrow Transplant	150
10	Ribosomal proteins and human diseases: molecular mechanisms and targeted therapy	2021	Signal Transduct Target Ther	149

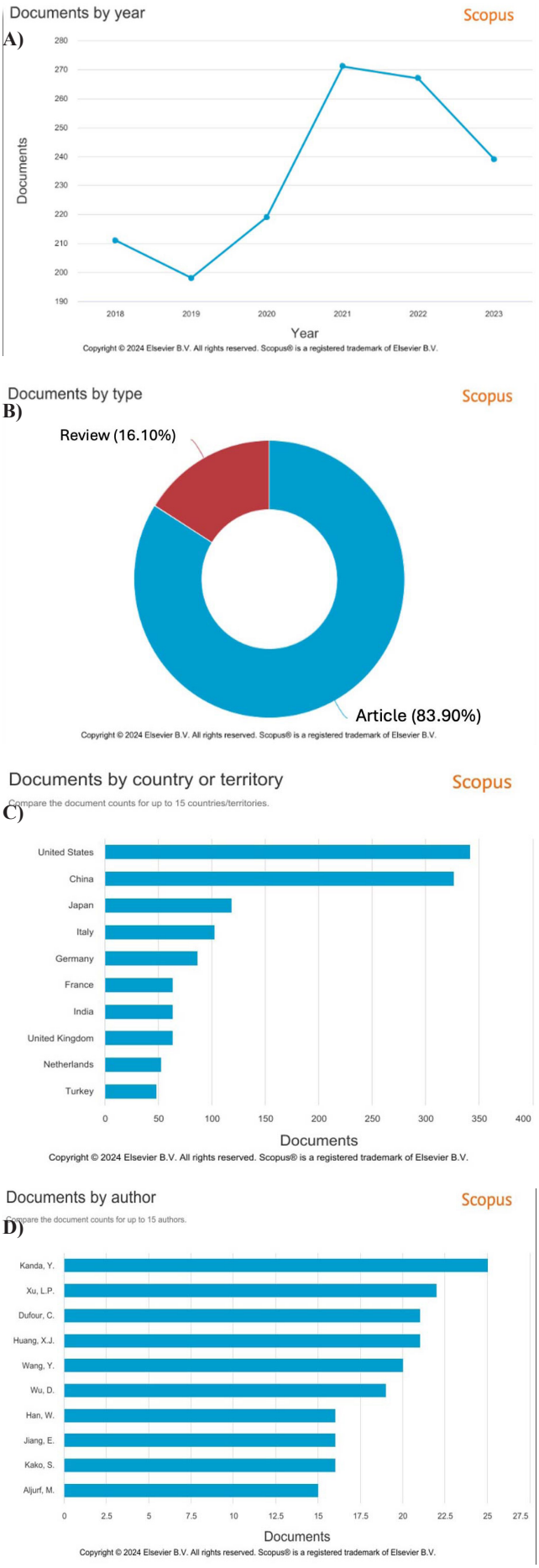

Figure 2. Publication types and publication development. A) Document by year; B) Document by type. Countries and authors with the most publications on stem cells or secretome in aplastic anaemia: C) Document by country or territory; D) Document by author

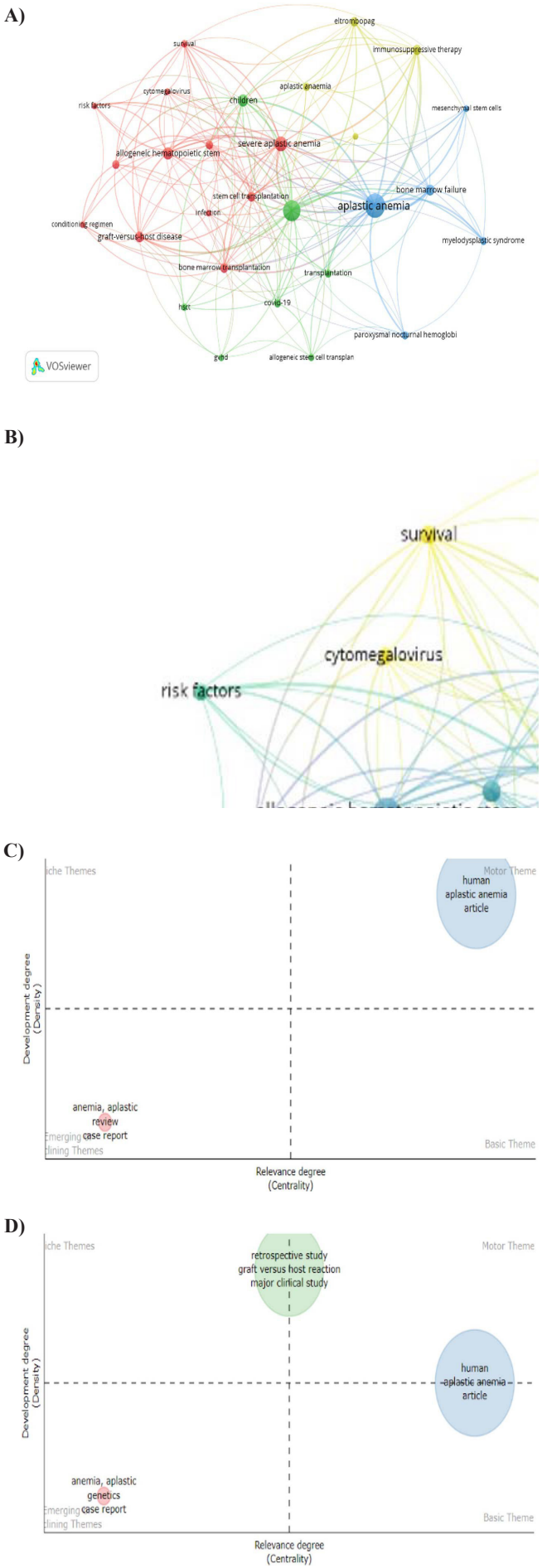

Figure 3. Network Visualization. A) Overlay Visualization; B) using VOSviewer; C) themes in 2018-2021; D) themes in 2022-2023

Table 2. Co-occurrence clusters of keywords based on authors

No	Cluster 1 (12 items)	Cluster 2 (7 items)	Cluster 3 (5 items)	Cluster 4 (4 items)
1	Allogenic hematopoietic stem cell transplantation	Allogeneic stem cell transplantation	Aplastic anaemia	Aplastic anaemia
2	Bone marrow transplantation	Children	Bone marrow failure	Eltrombopag
3	Conditioning regimen	Covid-19	Mesenchymal stem cells	Hematopoietic stem cell transplantation
4	Cytomegalovirus	Graft-versus-host-disease (GVHD)	Myelodysplastic syndrome	Immunosuppressive therapy
5	Graft-versus-host disease	Hematopoietic stem cell transplantation		
6	Hematopoietic cell transplantation	Hematopoietic stem cell transplantation		
7	Infection	Transplantation		
8	Paediatric			
9	Risk factors			
10	Severe aplastic anemia			
11	Stem cell transplantation			
12	Survival			

and disease mechanism focus (2018–2021) to specialized approaches including novel drugs, genetics, and outcome optimization (2022–2023). Emerging interests include secretome therapies, COVID-19's influence, and thrombopoietin receptor agonists like eltrombopag, suggesting promising directions for future aplastic anaemia management.

DISCUSSION

Aplastic anaemia is a chronic failure of the bone marrow to produce blood cells, often resulting in pancytopenia (1). Multiple factors, including genetics, environment, infections, and autoimmunity, contribute to its onset, highlighting the need for improved treatments. Our bibliometric analysis of 1,405 articles from 2018 to 2023 showed continued research interest, especially in countries with advanced healthcare infrastructure like the United States, China, and Japan.

Molecular studies reveal that the mesenchymal stem cell (MSC) secretome plays a significant role in supporting haematopoiesis and bone marrow function (24–26). Thematic clustering in our analysis puts "hematopoietic stem cell transplantation" and "bone marrow failure" at the forefront, underscoring the importance of stem cell therapies. MSC secretome components help restore the bone marrow niche and influence key signalling molecules (6), while pharmaceutical strategies such as eltrombopag target specific molecular pathways like JAK/STAT and MAPK to boost stem cell activity (13,19). In addition, stem cell therapy, particularly hematopoietic stem cell transplantation (HSCT), has emerged as a cornerstone in managing severe aplastic anaemia. This therapy offers the potential for a cure by replacing the dysfunctional or depleted hematopoietic system with healthy progenitor cells from a compatible donor, restoring normal blood cell production. HSCT is widely recognized as the treatment of choice for younger patients with severe disease who have a suitable donor. This molecular convergence explains the synergistic effects of combination therapies and supports the evolving research trends identified in our temporal analysis.

Research interest has also grown in immunosuppressive therapies, paralleling new findings that MSC secretomes carry miRNAs able to modulate T-cell activity and inflammatory cytokines, potentially protecting hematopoietic stem cells from immune-mediated damage (27,28). The intersection with COVID-19 research emphasizes shared inflammatory mechanisms and highlights opportunities for cross-applications in bone marrow failure treatment (29).

Genetic and multi-omics studies remain underrepresented but show immense promise for future research. Advances in CRISPR screening and proteomics have identified new molecular targets, paving the way for more personalized therapies (30–32). Highly cited works in the field, such as Young's "Aplastic Anemia," reflect the ongoing evolution of molecular understanding and the emergence of proteomic biomarkers that could refine treatment selection (15,21). Specifically, proteomic analyses have identified over 200 proteins in MSC-derived extracellular vesicles involved in cell adhesion (33,34), migration, proliferation, and immune regulation, with particular enrichment in factors like IL-6, LIF, SDF-1, and HGF that directly support haematopoiesis (35-37).

Top journals in the field demonstrated its multidisciplinary nature, while spatial transcriptomics revealed how MSC secretomes modulate critical cellular interactions within the bone marrow niche (6). The field's progression from basic research to targeted therapies indicates maturing scientific strategies. Increased international collaboration and the integration of genetic and secretome-based approaches will be essential for advancing precision medicine in aplastic anaemia.

In conclusion, current research trends, molecular insights, and evolving therapies for aplastic anaemia illustrate a dynamic landscape with exciting prospects for effective and personalized treatment strategies.

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TRANSPARENCY DECLARATION

Conflict of interest: None to declare.

AUTHOR CONTRIBUTIONS STATEMENT

Conceptualization, supervision, and validation: CPM, HS, YLRD; Methodology, formal analysis, and data curation: CPM; Writing original draft, analysis, and visualization: CPM and AP; Review draft and project administration: BS and AGM.

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ORIGINAL ARTICLE

Metabolic and renal predictors of coronary artery calcification: the independent role of the uric acid/estimated glomerular filtration rate (UA/eGFR) ratio and Castelli indices

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ABSTRACT

Aim To examine the association between metabolic parameters and novel cardiometabolic indices with the coronary artery calcium score (CACS).

Methods This retrospective cross-sectional study included 130 patients who underwent coronary computed tomography angiography (CCTA) at the Radiology Clinic of the Clinical Centre of the University of Sarajevo between January and June 2024. Patients were classified into two groups: those with CACS ≤100 and those with CACS >100. Platelet count, mean platelet volume (MPV), estimated glomerular filtration rate (eGFR), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), uric acid (UA), and novel cardiometabolic indices, including Castelli risk index I and II (CRI-I and CRI-II), non-high-density lipoprotein cholesterol (non-HDL-C), were compared between the groups.

Results Patients with CACS >100 had significantly higher MPV, TC, LDL-C, UA, non-HDL-C, CRI-I, CRI-II, and the UA/eGFR ratio. Older age, increased platelet activity, dyslipidaemia, hyperuricemia, and the higher UA/eGFR ratio correlated positively with CACS, whereas eGFR correlated negatively. In multivariate regression analysis, the UA/eGFR ratio emerged as an independent predictor of higher CACS (OR=2.37; 95% CI 1.18–4.78; p=0.017).

Conclusion Elevated UA level and adverse cardiometabolic indices were associated with greater coronary artery calcification. The UA/eGFR ratio independently predicts higher CACS, highlighting its potential prognostic value.

Keywords: coronary angiography, glomerular filtration rate, uric acid, vascular calcification

INTRODUCTION

Coronary artery calcification (CAC) is a well-established marker of atherosclerosis that reflects calcium deposition within the coronary arterial wall. Although it does not identify soft, non-calcified plaques, the overall calcium burden remains a strong predictor of future cardiovascular events (1). The coronary artery calcium score (CACS) (Agatston score), is the most widely used measure of CAC. It is traditionally derived from non-contrast cardiac CT scans; however, with advances in imaging technology, contrast-enhanced coronary computed tomography angiography (CCTA) can also provide reliable calcium quantification and simultaneous visualisation of both calcified and non-calcified plaques (2).

CACS helps clinicians stratify cardiovascular risk; a score above 100 suggests an intermediate to high risk of CAD. It is often used to guide preventive strategies such as starting statins or aspirin. The primary limitation of CACS is that it is not particularly useful in symptomatic patients with established CAD; instead, it is primarily applied in asymptomatic patients or those with uncertain risk profiles (3). Since CACS does not capture non-calcified plaques and may not fully reflect the metabolic activity of atherosclerosis, incorporating metabolic parameters can enhance its reliability and improve early detection and risk stratification in cardiovascular disease. Metabolic parameters such as total cholesterol (TC), low-den-

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sity lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), and serum uric acid (UA) levels are significantly associated with the development of atherosclerosis and cardiovascular risk. LDL-C is a significant factor in plaque formation, whereas HDL-C exerts a protective effect through its involvement in reverse cholesterol transport (4). To complement conventional lipid measurements, several cardiometabolic indices have been explored to provide a more integrated assessment of lipid-related cardiovascular risk.

The atherogenic index of plasma (AIP) indicates the balance between protective and atherogenic lipids, with higher values reflecting greater cardiovascular risk. Non-HDL cholesterol (non-HDL-C) reflects the total concentration of atherogenic lipoproteins and is considered a stronger predictor of atherosclerotic risk than LDL-C alone. The Castelli indices, defined as the ratio of TC and HDL-C (Castelli I) and LDL-C to HDL-C (Castelli II), provide additional insight into lipid balance, with increased ratios reflecting a more atherogenic profile (5).

Elevated UA is associated with increased CAC, most likely due to pro-inflammatory and pro-oxidative mechanisms that promote endothelial dysfunction and vascular injury (6). Similarly, a reduced estimated glomerular filtration rate (eGFR), reflecting impaired kidney function, is associated with increased CAC risk due to disruptions in mineral metabolism and shared cardiovascular risk factors. The UA to eGFR ratio (UA/eGFR) has recently emerged as a composite biomarker that integrates metabolic and renal components. Higher values of this ratio correlate with greater atherosclerotic burden and CAC, even among patients with normal UA levels, suggesting superior predictive potential for cardiovascular risk (7-9). This interplay between UA metabolism, renal function, and vascular calcifications highlights the importance of managing metabolic and renal health to lower the cardiovascular risk (10).

Although previous studies have examined the impact of individual metabolic parameters on cardiovascular risk, data regarding their combined indices and direct links to CAC remain limited. Moreover, evidence from Bosnia and Herzegovina is particularly scarce, and no prior studies have specifically addressed this topic, underscoring the importance of filling this research gap.

The aim of this study was to investigate the role of metabolic and renal markers, including UA, lipid indices, and the UA/eGFR ratio, in severity of CAC and to identify metabolic determinants of coronary calcification to improve cardiovascular risk prediction.

PATIENTS AND METHODS

Patients and study design

A retrospective cross-sectional study included 130 patients who underwent CCTA at the Clinic for Radiology of the Clinical Centre of the University of Sarajevo between January 2024 and June 2024. Due to the retrospective nature of the study, the sample size was defined by the number of eligible patients available during the study period, and a formal sample size or power calculation was not performed. The inclusion criteria encompassed patients aged between 18 and 75 with complete medical history data, laboratory findings before CCTA, and a CCTA report with a recorded CACS. Patients who did not meet age criteria, patients with incomplete medical records, active oncological or haematological disease, previously known CAD, including prior myocardial infarction, percutaneous or surgical intervention, were not included in the study. Patients receiving medications that could significantly affect lipid profile or uric acid levels (statins, fibrates, allopurinol) were also excluded.

The Ethics Committee of the Clinical Centre of the University of Sarajevo approved the study.

Methods

Calcium score values were obtained from CCTA results performed on the Aquilion Prime CT scanner (Canon Medical Systems Corporation, Tochigi, Japan), using an iobitridol contrast agent. The area of each calcification was measured in mm^2 and multiplied by the calcium density within the calcification. Individual results for all arterial segments were summed to obtain the CACS (11). A CACS threshold of 100 Agatston units was used to stratify patients into groups, as initially proposed by Agatston et al. (12) and subsequently validated in extensive population studies such as the Multi-Ethnic Study of Atherosclerosis (MESA) (13).

The first group included patients with measured CACS≤100, while the second group comprised patients with CACS>100. Patients with CACS≤100 have a low to moderate risk of CAD, whereas those with CACS>100 have a high risk of CAD (11,3).

Demographic and relevant clinical data were collected through a detailed analysis of medical records. The following parameters were extracted: age, sex, platelet count (PTL), mean platelet volume (MPV), serum creatinine, TC, LDL-C, HDL-C, TG, and UA. eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula, which considers serum creatinine levels, sex, age, and race (14).

Using the collected laboratory parameters, cardiometabolic indices were calculated according to established formulas, including the atherogenic index of plasma ($AIP=\log_{10}[TG/HDL\text{-}C]$), non-HDL cholesterol (non-HDL-C = TC – HDL-C), Castelli risk index I (CRI-I = TC/HDL-C), Castelli risk index II (CRI-II = LDL-C/HDL-C), and the uric acid to estimated glomerular filtration rate ratio (UA/eGFR = UA/eGFR).

Laboratory parameter values and cardiometabolic indices were compared between the two patient groups.

Statistical analysis

The results are presented in tabular form using the number of cases, arithmetic mean (M) with standard deviation (SD), or median and interquartile range (IQR), depending on the data type. The normality of continuous variables was tested using the Shapiro-Wilk test. Variables with non-normal distribution were analysed using nonparametric tests (Mann-Whitney and Spearman correlation); log transformation was not applied. Differences between the groups were assessed using the Student's t-test (t score) or Mann-Whitney U test (Z score), as appropriate. The association between CACS and individual laboratory parameters and cardiometabolic indices was analysed using Spearman's rank correlation coefficient (r). To identify independent predictors of higher CACS, a model using bivariate logistic regression analysis, incorporating all predefined predictors from the study framework. The results of all tests were considered statistically significant at a confidence level of 95% (<0.05).

RESULTS

The study included 130 patients, with a significantly higher prevalence of women, 90 (60%) (p=0.029). Patients with CACS>100 were older than those with CACS≤100, with a median age of 69 years (p=0.001).

Regarding haematological parameters, the platelet count (PTL) did not differ significantly between the groups (p=0.351). However, the mean platelet volume (MPV) was considerably higher in the CACS >100 group, with a median value of 8.80 fL (p=0.022).

In terms of kidney function and renal parameters, patients in the CACS>100 group had lower estimated glomerular filtration rates (eGFR) (p=0.038) and higher level of uric acid (UA) with a median of 334.5 µmol/L (p=0.0001).

The analysis of lipid profiles demonstrated that total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels were significantly higher in the CACS>100 group, with median values of 5.3 mmol/L (p=0.0001) and 3.7 mmol/L (p=0.0001), respectively. However, no significant differences were found in high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) between the groups (p=0.830, p=0.639, respectively).

Significant differences in cardiometabolic indices were observed between the groups. The non-HDL-C (p=0.0001), Castelli risk indices I (CRI-I) (p=0.0001), and II (CRI-II) (p=0.001) were significantly higher in the CACS>100 group. Furthermore, the UA/eGFR ratio was notably higher in this group (p=0.0001) (Table 1).

Table 1. Comparison od atherogenic and renal indices between patients with CACS≤100 and CACS>100

Variable	CACS≤100	CACS > 100	Test statistic*	p
AIP (Mean±SD)	0.13 ± 0.23	0.15 ± 0.60	t = -0.503	0.613
		Median (IQR)		
Non-HDL-C (mmol/L)	2.99 (2.70–3.64)	4.20 (3.70–5.00)	Z= -5.353	<0.001
CRI-I	3.72 (3.20–4.12)	4.64 (3.53–5.98)	Z= -3.902	<0.001
CRI-II	2.15 (1.55–2.48)	3.17 (2.10–3.88)	Z= -4.551	0.001
UA/eGFR ratio	3.49 (3.01–4.30)	4.71 (3.80–6.19)	Z= -5.189	<0.001

*t=test statistics for independent samples t-test; Z=test statistics for Mann-Whitney U test.
HDL-C, high-density lipoprotein; AIP, atherogenic index of plasma; CRI-I, Castelli risk index I; CRI-II, Castelli risk index II; UA/eGFR ratio, uric acid/estimated glomerular filtration rate ratio; IQR, interquartile range; SD, standard deviation; CACS, coronary artery calcium score

Correlation analysis revealed several parameters that were significantly associated with CACS. Age (r=0.405; p=0.0001), uric acid (r=0.439; p=0.0001), total cholesterol (r=0.449; p=0.001), and LDL-C (r=0.413; p=0.001) had moderate to strong positive correlations with CACS, indicating that older patients and those with higher levels of uric acid, TC, and LDL-C were more likely to have higher CACS. In contrast, eGFR showed a weak negative correlation with CACS (r=0.238; p=0.008), suggesting that patients with higher eGFR had a lower likelihood of having significant coronary artery calcification.

Further analysis of cardiometabolic indices revealed a strong positive correlation between the UA/eGFR ratio (r=0.471; p=0.0001) and CACS, suggesting that a higher UA/eGFR ratio was associated with a higher probability of increased coronary artery calcification. Additionally, non-HDL-C (r=0.425; p=0.0001) and Castelli risk indices (CRI-I and CRI-II) showed moderate positive correlations with CACS, further supporting their role as predictors (Table 2).

Table 2. Correlation analysis of the impact of parameters on coronary artery calcium score (CACS)

Variable	r*	p
Sex	-0.123	0.163
Age	0.405	<0.001
eGFR	-0.238	0.008
PTL	-0.024	0.786
MPV	0.221	0.012
TC	0.449	<0.001
HDL-C	0.102	0.250
LDL-C	0.431	<0.001
TG	-0.013	0.881
UA	0.439	<0.001
AIP	-0.055	0.534
Non-HDL-C	0.425	<0.001
CRI-I	0.247	0.005
CRI-II	0.319	<0.001
UA/eGFR ratio	0.471	<0.001

* r=Pearsons correlation coefficient.
eGFR, estimated glomerular filtration rate; PTL, platelet count; MPV, mean platelet volume; TC, total cholesterol; HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein; TG, triglycerides; UA, uric acid; AIP, atherogenic index of plasma; CRI-I, Castelli risk index I; CRI-II, Castelli risk index II; UA/eGFR ratio, uric acid/estimated glomerular filtration rate ratio.

Table 3. Logistic regression analysis of predictors of higher coronary artery calcium score (CACS) (N=130)

Variable	Coefficient (β)	Wald (t)	p	OR (95% CI)*
PTL	-0.073	-0.802	0.424	–
MPV	0.130	1.440	0.153	–
HDL-C	-0.078	-0.329	0.743	–
LDL-C	-0.371	-0.539	0.591	–
TG	0.092	0.321	0.749	–
UA	-0.160	-1.065	0.289	–
AIP	-0.275	-0.795	0.428	–
Non-HDL-C	0.338	0.564	0.574	–
CRI-I	-0.444	-0.602	0.548	–
CRI-II	0.543	0.705	0.482	–
UA/eGFR ratio	0.864	2.413	0.017	2.37 (95% CI 1.18–4.78)
Sex	-0.077	-0.839	0.403	–
Age	0.122	1.252	0.213	–

Model fit: Nagelkerke pseudo-R^2 = 0.470
*Odds ratio (OR) with 95% CI is shown only for the significant predictor (UA/eGFR ratio).
* Coefficient (β) - regression coefficient; a positive β indicates an increase, while a negative β indicates a decrease in the likelihood of higher CACS.
* Wald (t) - test of the significance of an individual regression coefficient (β); higher values indicate a greater contribution to the model
PTL, platelet count; MPV, mean platelet volume; HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein; TG, triglycerides; UA, uric acid; AIP, atherogenic index of plasma; CRI-I, Castelli risk index I; CRI-II, Castelli risk index II; UA/eGFR ratio, uric acid/estimated glomerular filtration rate ratio; N, number;

Regression analysis identified the UA/eGFR ratio as the only independent predictor of having CACS>100 (OR 2.37, 95% CI 1.18–4.78; p=0.017) (Table 3). The overall model explained 47.0% of the variance in CACS classification (pseudo-R^2, Nagelkerke = 0.470).

DISCUSSION

In our study, the UA/eGFR ratio emerged as an independent predictor of CACS>100, remaining significant after adjustment for traditional cardiovascular and metabolic risk factors. Secondary findings suggest that dyslipidaemia and increased platelet activity are strongly associated with a higher CACS score. Renal function is inversely related to coronary calcification.

Older age and the predominance of women in the high CACS group may be explained by the fact that women generally live longer than men, resulting in a higher proportion of elderly females in the study population. In addition, coronary calcium scores in women tend to rise sharply after menopause due to the loss of estrogen-mediated vascular protection (15,16). Consequently, the combination of greater longevity and postmenopausal acceleration of calcification likely accounts for the predominance of older women with higher CACS values observed in our cohort.

Patients with higher CACS values also demonstrated increased MPV, which correlated positively with the severity of coronary calcification. An elevated MPV indicates enhanced platelet reactivity, potentially leading to an increased risk of thrombosis. This effect, in addition to plaque instability, is particularly significant in atherosclerosis and cardiovascular disease. It may be a potential predictor of coronary heart disease, with higher MPV values associated with an elevated risk of cardiovascular events (17,18).

Patients with higher CACS values had lower eGFR and higher UA concentrations, suggesting that impaired kidney function and elevated uric acid jointly contribute to the progression of coronary calcification. These factors promote atherosclerosis through endothelial dysfunction, oxidative stress, systemic inflammation, and disturbances in mineral-bone metabolism (19-21). Moreover, elevated serum uric acid may accumulate in the arterial wall, increasing vascular rigidity and reducing elasticity (22).

In our study, neither UA nor eGFR independently predicted higher CACS, whereas the UA/eGFR ratio demonstrated a consistent association. This supports the concept that integrating metabolic and renal components provides a more sensitive indicator of subclinical atherosclerosis and cardiovascular risk (23). Similar results have been reported in Japanese and European cohorts, where higher UA/eGFR ratios were associated with increased coronary calcification and vascular stiffness, even among patients with normal uric acid levels (24,25).

Patients with CACS >100 showed markedly higher levels of TC, LDL-C, non-HDL-C, CRI-I, and CRI-II, confirming a strong relationship between dyslipidaemia and coronary calcification (26–29). In contrast, HDL-C, triglycerides, and AIP did not differ significantly between the groups, suggesting that these parameters may be less relevant in later stages of atherosclerosis (30-32). Moderate positive correlations between TC and LDL-C with CACS further underscore the contribution of atherogenic lipid fractions to plaque calcification. These findings align with previous reports demonstrating that non-HDL-C and Castelli indices are useful markers of overall lipid-related cardiovascular risk and may enhance risk stratification beyond conventional lipid parameters (27–29).

Study limitations include a relatively small sample size and a retrospective, single-centre, cross-sectional design, which may introduce selection bias and limit causal interpretation. The absence of a power analysis may limit the detection of smaller effects. Data on all medications were not available, which could have influenced results. Renal markers may fluctuate with hydration or metabolic changes, and there is a residual confounding by unmeasured comorbidities such as hypertension or obesity that cannot be excluded. The lack of longitudinal follow-up also limits the assessment of temporal associations. Nevertheless, our results offer essential insight into how metabolic and renal factors relate to coronary calcification and may have clinical implications for simple, cheap, and widely accessible cardiovascular risk evaluation.

In conclusion, this study demonstrates that the UA/eGFR ratio is independently associated with the extent of coronary artery calcification, highlighting the interplay between metabolic, renal, and lipid pathways in the continuum of vascular injury leading to calcification. Future multicentric, prospective studies with larger and more diverse populations are needed to validate these results and to explore whether integrating UA/eGFR into existing CAC-based risk models can enhance predictive accuracy and guide preventive strategies.

AUTHOR CONTRIBUTIONS

Statement: Conceptualization, Z.B.J. and M.B.; Methodology, Z.B.J., S.P. and M.B.; Software, M.B., and A.B.; Validation, E.B., M.B., S.P. and F.Z.; Formal analysis, Z.B.J. and M.B.; Investigation, Z.B.J., M.B., E.B., S.P., F.Z., A.B.; Resources, Z.B.J and M.B.; Data curation Z.B.J. and M.B.; Writing—original draft preparation, Z.B.J., M.B. and M.B.; Writing— review and editing, E.B., M.B., S.P., F.Z., and A.B.; Visualization, M.B.; Supervision, S.P., and F.Z.; Project administration, funding acquisition, Z.B.J and M.B.. All authors have read and agreed to the published version of the manuscript.

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TRANSPARENCY DECLARATION

Conflicts of interest: None to declare.

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Med Glas (Zenica)

ORIGINAL ARTICLE

Enhanced myocardial protection in failing hearts: comparative outcomes of single-dose Del Nido and conventional blood cardioplegia in emergency low-ejection fraction coronary artery bypass grafting (CABG)

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ABSTRACT

Background: Optimal myocardial protection remains challenging in emergency coronary revascularization, especially in patients with severely reduced left ventricular function. Contemporary cardioplegia strategies aim to limit ischemia–reperfusion injury and enhance postoperative ventricular recovery. The aim of this study was to compare operative parameters, early postoperative outcomes, and myocardial functional recovery between single-dose Del Nido cardioplegia and conventional blood cardioplegia in adults with left ventricular ejection fraction (LVEF) ≤40% undergoing emergency isolated coronary artery bypass grafting (CABG).

Methods: This retrospective study included 150 consecutive patients with left ventricular ejection fraction (LVEF) ≤40% who underwent emergency isolated CABG between 2022 and 2024. Patients were assigned to either the Del Nido group (n=80) or the conventional blood cardioplegia group (n=70). Demographics, operative variables, postoperative complications, ventricular function changes, and short-term mortality were analysed. Myocardial recovery was assessed using ΔEF (postoperative minus preoperative LVEF).

Results: The Del Nido group demonstrated significantly shorter aortic cross-clamp and cardiopulmonary bypass times and required less intraoperative defibrillation. The incidence of postoperative atrial fibrillation was lower in the Del Nido group (18%) compared with conventional blood cardioplegia (29%). Improvement in ventricular function was greater with Del Nido (ΔEF +5.8±2.1%) than with blood cardioplegia (+3.2±2.4%). Rates of stroke, perioperative myocardial infarction, and early mortality were comparable between the groups.

Conclusion: Single-dose Del Nido cardioplegia provides effective and safe myocardial protection in emergency low-EF CABG, offering improved operative efficiency and superior early ventricular recovery without increasing perioperative complications.

Keywords: cardiopulmonary bypass, cardioplegic solutions, coronary artery bypass, heart ventricles, myocardial reperfusion injury

INTRODUCTION

Myocardial protection remains a central determinant of outcomes in coronary artery bypass grafting (CABG), particularly in patients with severely reduced left ventricular ejection fraction (LVEF). Despite advances in myocardial preservation and surgical techniques, patients with low LVEF continue to face increased perioperative morbidity and mortality due to ischemia–reperfusion injury and poor ventricular recovery (1,4). The optimal cardioplegia strategy in this high-risk population, especially in emergency CABG, remains a subject of ongoing debate. Del Nido (DN) cardioplegia, originally developed for paediatric cardiac surgery, has recently gained wide application in adults because of its simplicity, single-dose administration, and prolonged myocardial protection duration (5,6,7). Current evidence suggests that DN may offer comparable or superior myocardial protection compared with conventional blood cardioplegia (BC), potentially reducing cross-clamp time, postoperative arrhythmias, and myocardial injury markers (8-9). However, most available studies are limited to elective procedures, and data in emergency settings or among patients with severely depressed ventricular function remain scarce (10-12).

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Recent investigations have highlighted that improving myocardial protection in this subgroup could directly influence early ventricular recovery and postoperative outcomes (13,14). Given the ongoing evolution of myocardial protection strategies, evaluating the effectiveness of single-dose DN cardioplegia in emergency CABG for low LVEF patients is both clinically and scientifically relevant. Initially developed for paediatric cardiac surgery, Del Nido (DN) cardioplegia has become increasingly popular in adults due to its single-dose administration, extended arrest duration (up to 90 minutes), and ability to mitigate calcium overload. However, most evidence concerns elective, lower-risk populations, leaving uncertainty about its safety and efficacy in emergency cases and failing myocardium (15-16). Furthermore, prolonged cross-clamp duration is independently associated with adverse outcomes, emphasizing the need for efficient myocardial protection (17).

Patients undergoing emergency coronary artery bypass grafting (CABG) with severely reduced left ventricular ejection fraction (LVEF) represent one of the highest-risk groups in contemporary cardiac surgery, with myocardial ischemia–reperfusion injury and impaired contractile reserve contributing to significant perioperative morbidity (18). Therefore, selecting an effective cardioplegia strategy is essential for optimizing myocardial protection and postoperative recovery in this vulnerable population. Recent evidence has increasingly focused on optimizing myocardial protection strategies in high-risk coronary bypass populations. Several contemporary meta-analyses and large cohort studies have demonstrated that Del Nido cardioplegia provides comparable or superior myocardial protection compared with conventional blood cardioplegia in adult cardiac surgery, including CABG (19-21). Del Nido's extended arrest duration, lower calcium load, and simplified single-dose administration have been associated with reduced ischemia–reperfusion injury and improved early myocardial recovery, particularly in patients with ventricular dysfunction (18,19). Moreover, a comprehensive 2025 narrative review confirmed the safety and expanding use of Del Nido cardioplegia in adults, including those with low EF and complex surgical profiles (21). Despite these advances, most of the current evidence is derived from elective or mixed surgical cohorts, and the applicability of Del Nido cardioplegia in urgent settings with severely impaired ventricular function remains uncertain. A notable gap persists in the literature regarding its effectiveness in emergency CABG among patients with low LVEF, a subgroup in whom ischemic tolerance is significantly reduced and operative risk elevated (11).

The aim of this study was to compare intraoperative characteristics, myocardial recovery, and early postoperative outcomes between single-dose Del Nido and conventional blood cardioplegia in adults undergoing emergency CABG with reduced LVEF.

MATERIAL AND METHODS

Patients and study design

This retrospective single-centre study included 150 consecutive patients who underwent emergency isolated coronary artery bypass grafting (CABG) at the Trabzon Ahi Evren Thoracic and Cardiovascular Surgery Training and Research Hospital (Turkey) between October 2022 and September 2024. All patients had preoperative left ventricular ejection fraction (LVEF) ≤40% and required urgent surgical revascularization for acute coronary syndromes (11–13). Patients with previous cardiac surgery, combined valve or aortic procedures, or incomplete perioperative data were excluded.

Demographic variables, comorbidities, preoperative LVEF, intraoperative parameters (cross-clamp time, CPB duration, number of grafts, defibrillation requirement), and postoperative outcomes (atrial fibrillation, stroke, myocardial infarction, ICU stay, hospital stay, and 30-day mortality) were collected from institutional databases. Myocardial recovery was assessed by calculating the change in LVEF (ΔEF = postoperative – preoperative).

Patients were divided into two groups according to the cardioplegia strategy used during surgery: the Del Nido group (DN group, n=80), who received single-dose Del Nido cardioplegia, and the blood cardioplegia group (BC group, n=70), who received conventional multidose blood cardioplegia.

Written informed consent was obtained from all participants or their legal representatives prior to surgery.

Ethical approval for the study was obtained from the relevant ethics committee (approval number: 2022/54).

Methods

Surgical technique and cardioplegia protocol. All procedures were performed via median sternotomy under moderate hypothermia (32–34 °C) using standard cardiopulmonary bypass (CPB) with a membrane oxygenator and roller pump (6,7). Distal anastomoses were completed during aortic cross-clamping, followed by proximal anastomoses performed under partial clamping. In the Del Nido (DN) group, the cardioplegic solution was prepared with a 1:4 blood-to-crystalloid ratio using Plasma-Lyte A as a base, administered as a single 1200 mL dose at 4 °C. Supplemental doses (200–300 mL) were given only if cross-clamp time exceeded 90 minutes. In the blood cardioplegia (BC) group, a 4:1 blood-to-crystalloid mixture was used (2.6) (initial 1000 mL, then 200 mL every 15–20 minutes) at 4–8 °C. Cardioplegia was delivered antegrade through the aortic root in all cases. Myocardial temperature, arrest time, and hemodynamic stability were monitored continuously. Rewarming and weaning from cardiopulmonary bypass (CPB) were performed under standardized institutional protocols.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation and compared using Student's t-test or Mann–Whitney U-test, depending on distribution. Categorical variables were compared using Chi-square or Fisher's exact test. Two-tailed p-values <0.05 were considered statistically significant.

RESULTS

A total of 150 patients were included: 80 received DN cardioplegia and 70 received BC. Baseline demographic and clinical characteristics were comparable between the two groups, including age, sex distribution, prevalence of diabetes and hypertension, chronic kidney disease, and preoperative LVEF (Table 1).

Intraoperative Outcomes: Del Nido cardioplegia was associated with shorter aortic cross-clamp duration (81.8±17.3 vs.

Table 1. Baseline demographic and preoperative clinical characteristics of patients undergoing isolated CABG receiving Del Nido versus blood cardioplegia

Variable	Del Nido (n=80)	Blood Cardioplegia (n=70)	p-value
Age (years)	64.8 ± 9.1	65.3 ± 8.7	0.72
Male sex (%)	62 (77.5%)	52 (74.2%)	0.64
Hypertension (%)	49 (61.3%)	45 (64.2%)	0.71
Diabetes mellitus (%)	32 (40.0%)	30 (42.8%)	0.75
Chronic kidney disease (%)	12 (15.0%)	10 (14.2%)	0.89
Preoperative LVEF (%)	32.1 ± 5.2	32.4 ± 4.9	0.78

94.2±19.1 minutes) and shorter cardiopulmonary bypass time (111.9 ± 22.8 vs. 128.3 ± 25.6 minutes). The need for intraoperative defibrillation was also lower in the Del Nido group compared with conventional blood cardioplegia (11.3% vs. 27.1%) (Table 2, Figure 1).

Table 2. Intraoperative outcomes of patients undergoing isolated CABG receiving Del Nido versus blood cardioplegia

Outcome	Del Nido (n=80)	Blood Cardioplegia (n=70)	p-value
Cross-clamp time (min)	81.8 ± 17.3	94.2 ± 19.1	0.001
CPB time (min)	111.9 ± 22.8	128.3 ± 25.6	0.002
Number of grafts	3.1 ± 0.9	3.2 ± 1.0	0.54
Need for defibrillation (%)	9 (11.3%)	19 (27.1%)	0.01

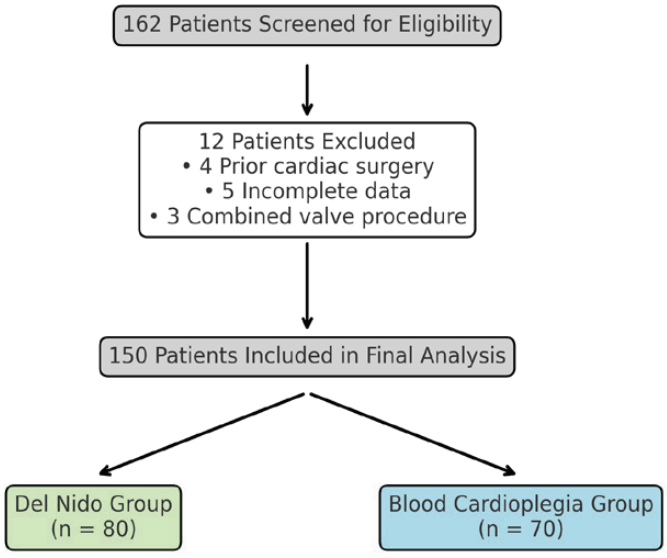

Figure 1. Flow diagram of patient selection and group allocation

Patients in the DN group demonstrated higher postoperative LVEF at discharge (41.0±4.8% vs. 37.8±5.1%; p=0.012). The incidence of postoperative AF was significantly lower with DN (18% vs. 29%; p=0.046). Rates of stroke and perioperative myocardial infarction were low and did not differ significantly between the groups. Thirty-day mortality was 3.8% in the DN group and 5.7% in the BC group (p=0.38). ICU stay was shorter in the DN group (3.1±1.8 vs. 3.9±2.1 days; p=0.048), while total hospital stay showed a non-significant trend toward reduction (6.8±2.7 vs. 7.4±3.1 days; p=0.09) (Table 3, Figure 2). Improvement in ventricular function was greater in the Del Nido group, with ΔEF calculated as +5.8±2.1% compared with +3.2±2.4% in the blood cardioplegia group. Postoperative atrial fibrillation occurred less frequently in the Del Nido cohort (18% vs. 29%).

Table 3. Postoperative outcomes of patients undergoing isolated CABG receiving Del Nido versus blood cardioplegia

Outcome	Del Nido (n=80)	Blood Cardioplegia (n=70)	p-value
ΔEF (%)	+5.8 ± 2.1	+3.2 ± 2.4	0.009
Atrial fibrillation (%)	14 (18%)	20 (29%)	0.04
Stroke (%)	1 (1.3%)	2 (2.8%)	0.52
Perioperative MI (%)	1 (1.3%)	2 (2.8%)	0.52
ICU stay (days)	3.1 ± 1.8	3.9 ± 2.1	0.03
Hospital stay (days)	7.8 ± 3.2	8.4 ± 3.6	0.18
30-day mortality (%)	3 (3.8%)	4 (5.7%)	0.61

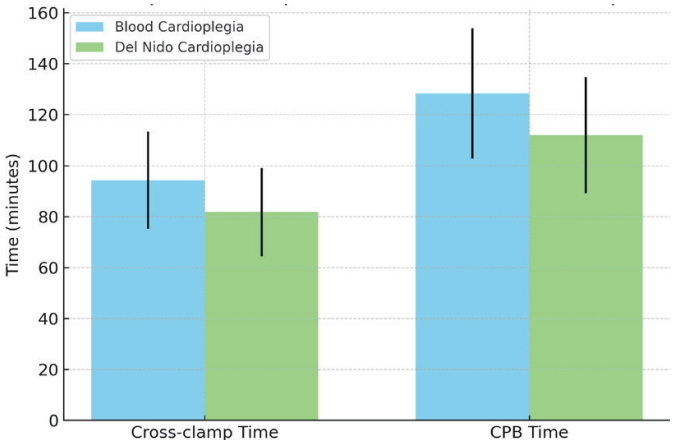

Figure 2: Comparison of Aortic Cross-Clamp and Cardiopulmonary Bypass Durations Between Cardioplegia Strategies

DISCUSSION

This study indicates that Del Nido cardioplegia provides effective myocardial protection in patients with low LVEF undergoing emergency CABG. Compared with conventional BC, DN significantly reduced operative times, improved postoperative ventricular function, and decreased atrial fibrillation without increasing mortality or neurological events. These findings align with prior studies supporting DN's safety and efficacy in adult cardiac surgery (3–9). The trends further demonstrate DN's advantages in myocardial recovery and rhythm stability. The advantages of DN likely stem from its composition: lidocaine and magnesium stabilize cellular membranes and reduce calcium influx, while mannitol mitigates oxidative injury (2,4,6). The single-dose approach minimizes interruptions, reducing ischemia–reperfusion injury, which is particularly critical in compromised myocardium. Reduced postoperative atrial fibrillation may reflect improved ionic homeostasis. Recent meta-analyses confirm DN as a superior or equivalent myocardial protection method in high-risk cardiac surgery (1,2,10,14). The present study demonstrates that single-dose Del Nido cardioplegia provides safe and effective myocardial protection in adults undergoing emergency coronary artery bypass grafting (CABG) with reduced left ventricular ejection fraction (LVEF). In this high-risk clinical setting, Del Nido was associated with shorter cross-clamp and cardiopulmonary bypass durations, reduced need for intraoperative defibrillation, and su-

perior early ventricular recovery, as reflected by a significantly higher ΔEF compared with conventional blood cardioplegia. These findings align with contemporary evidence supporting the increasing use of Del Nido cardioplegia in adult cardiac surgery (15–17).

Several recent meta-analyses and large cohort studies have shown that Del Nido offers myocardial protection equivalent or superior to blood cardioplegia, particularly by prolonging arrest duration, reducing calcium influx, and enhancing metabolic stability during ischemia–reperfusion events (15,16). Its lower calcium load and lidocaine-enriched composition have been proposed to mitigate intracellular calcium overload and arrhythmogenic potential, contributing to improved early postoperative myocardial function (18,19). The results of our study, demonstrating greater improvement in postoperative ejection fraction, support these mechanistic insights.

In high-complexity adult cardiac operations, especially those involving prolonged ischemic times, Del Nido cardioplegia has been shown to maintain adequate myocardial protection while reducing interruptions for repeated dosing (17). Our findings of shorter cross-clamp and bypass times are consistent with this benefit and may partly explain the improved ΔEF and reduced arrhythmia rates observed in the Del Nido group. Lower incidence in the Del Nido group (AF14 (18%) of postoperative atrial fibrillation in our cohort similarly agrees with recent comparative studies reporting enhanced electrophysiological recovery with Del Nido (18–20).

Importantly, the safety profile of Del Nido cardioplegia observed in our study, evidenced by comparable rates of stroke, perioperative myocardial infarction, and early mortality between groups, is supported by a 2025 narrative review summarizing extensive adult surgical experience (21). This confirms that Del Nido cardioplegia does not increase adverse neurological or ischemic complications, even in patients with significantly compromised ventricular reserve.

Despite advances in the literature, limited data exist specifically for emergency CABG patients with low LVEF—a particularly vulnerable subgroup with diminished ischemic tolerance. Most previously published studies have evaluated elective or mixed surgical cohorts (11). Our study contributes important evidence supporting the use of Del Nido in this context, demonstrating superior early myocardial recovery without increasing perioperative risk.

This study is limited by its retrospective design and the absence of postoperative biochemical markers (e.g., troponin, CK-MB), which were not routinely available for all emergency CABG patients. Future prospective studies with standardized biomarker evaluation and long-term ventricular function follow-up are warranted. Nevertheless, the consistency of our findings with recent high-quality meta-analyses, cohort studies, and narrative reviews strengthens the clinical relevance of our results.

In conclusion, Del Nido cardioplegia offers a simple, efficient, and reproducible myocardial protection strategy suitable even in high-risk emergency CABG cases.

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Conflicts of interest: None to declare.

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ORIGINAL ARTICLE

Predictive value of admission biomarkers for mortality and rehospitalization in hypertensive crisis

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ABSTRACT

Aim To identify predictors of all-cause mortality and 6-month rehospitalisation in patients with hypertensive crisis, focusing on inflammatory indices, metabolic markers measured at admission, and antihypertensive treatment profiles.

Methods This prospective observational study included 210 adult patients with hypertensive crisis. Demographic, clinical, and therapeutic data were collected, including data on comorbidities, antihypertensive drug use, and treatment adherence. Laboratory parameters obtained at admission included neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic immune-inflammation index (SII), pan-immune-inflammation value (PIV), homocysteine, and uric acid. Patients were followed for 12 months. Multivariate logistic regression and receiver operating characteristic (ROC) curve analyses were conducted to identify independent predictors.

Results Mortality occurred in 10.9% of patients, and 27.1% were rehospitalised within 6 months. Deceased patients exhibited significantly higher levels of PLR (p=0.0329), SII (p=0.0355), homocysteine (p=0.0488), and uric acid (p=0.021). In multivariate analysis, homocysteine (OR=3.55; p<0.001), uric acid (OR=1.03; p=0.007), PLR (OR=1.04; p=0.047), and SII (OR=1.01; p=0.030) remained independently associated with mortality. Chronic kidney disease (OR=2.15, p=0.012) and poor treatment adherence (OR=1.92; p=0.017) were also significant predictors. ROC analysis demonstrated moderate discriminative power, with AUC values of 0.68 for PLR, 0.66 for SII, 0.65 for homocysteine, and 0.63 for uric acid.

Conclusion Elevated inflammatory indices and metabolic markers, particularly homocysteine and uric acid, were independently associated with increased mortality risk. Additionally, chronic kidney disease and suboptimal adherence to antihypertensive therapy significantly contributed to adverse outcomes. These findings underscore the importance of comprehensive risk assessment and personalised management in this high-risk population.

Keywords: homocysteine, hypertensive crisis, inflammation, medication adherence, prognosis, uric acid

INTRODUCTION

Hypertensive crisis represents a severe elevation in blood pressure requiring urgent clinical attention (1). It is broadly categorized into two distinct entities: hypertensive urgency, characterized by the absence of acute target organ damage, and hypertensive emergency, marked by evidence of organ injury such as myocardial infarction, stroke, acute pulmonary oedema, or acute kidney injury (AKI) (2). Although less frequent than chronic hypertension, hypertensive crisis carries a disproportionately high risk of morbidity and mortality (3). It is estimated that up to 1–2% of individuals with hypertension will experience a hypertensive crisis during their lifetime, most often in the context of uncontrolled or untreated disease (4).

Recently, increasing attention has been directed towards identifying prognostic factors associated with short- and long-term outcomes in patients presenting with hypertensive crisis (5). Traditional risk markers, such as advanced age, renal impairment, and established cardiovascular comorbidities, have been extensively studied (6). However, these alone often fail to capture the full spectrum of systemic pathophysiological burden. Emerging evidence suggests that inflammatory and metabolic markers, including neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic immune-inflammation index (SII), pan-immune-inflammation value (PIV), homocysteine, and uric acid, may reflect ongoing vascular stress and en-

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dothelial dysfunction. They may provide additional prognostic insight beyond conventional parameters (7,8). These markers are particularly attractive in clinical practice because they are widely available, inexpensive, and can be calculated from routine laboratory tests, making them suitable for use in both tertiary and resource-limited healthcare settings (9).

Moreover, the influence of antihypertensive treatment patterns and patient adherence remains underexplored in this clinical setting (10). Complex medication regimens and suboptimal compliance may contribute not only to the onset of a crisis but also to poorer outcomes during follow-up. Early identification of high-risk individuals may inform the development of targeted follow-up strategies and customised therapeutic approaches (11).

While prior studies have frequently examined individual biomarkers or organ-specific endpoints, few have assessed the combined predictive value of biochemical, inflammatory, and treatment-related factors (12). Furthermore, regional data from Southeast Europe, including Bosnia and Herzegovina, remain limited.

Although guidelines emphasise blood pressure control, there remains limited consensus regarding which clinical or laboratory parameters should be prioritised for early risk stratification in hypertensive crisis (13). Integrating inflammatory, metabolic, and therapeutic dimensions into a unified prognostic framework may improve clinical decision-making and patient outcomes (14). By identifying simple, reproducible predictors of mortality and rehospitalisation, clinicians may be better equipped to implement early interventions and optimise follow-up care (15).

This study aimed to evaluate multimodal predictors of adverse outcomes in patients with hypertensive crisis, with a particular focus on inflammatory indices, homocysteine and uric acid levels, comorbid conditions such as chronic kidney disease, and patterns of antihypertensive treatment. The study further sought to examine the relationship between these parameters and the risk of all-cause mortality and six-month rehospitalisation. We hypothesised that inflammatory and metabolic markers measured at admission, together with chronic kidney disease and poor antihypertensive adherence, independently predict mortality and rehospitalisation in patients with hypertensive crisis.

SUBJECTS AND METHODS

Patients and study design

This prospective observational cohort study was conducted at the Clinic for Internal Medicine, University Clinical Centre Tuzla, over a period of six months in 2024. The study included 210 adult patients admitted with a confirmed diagnosis of hypertensive crisis, further classified as either hypertensive urgency or hypertensive emergency. Patients with hypertensive urgency and hypertensive emergency were analysed separately in all outcome analyses.

Inclusion criteria were age 18 years or older, systolic blood pressure of 180 mmHg or higher, and diastolic blood pressure of 110 mmHg or higher on admission, and clinical features consistent with hypertensive crisis, following the current European Society of Cardiology (ESC) guidelines (16). Patients were excluded if they had secondary hypertension of endocrine origin, pregnancy-related hypertension, active malignancy, or incomplete laboratory data. All patients, including those who experienced in-hospital mortality within the hypertensive urgency group, were included in the final analysis.

Upon admission, data were collected on demographics, comorbidities (including diabetes mellitus and chronic kidney disease), smoking status, family history of cardiovascular disease, current antihypertensive medications, and adherence to prescribed therapy. Antihypertensive regimens were reviewed, and any changes made during hospitalisation were documented. All patients underwent laboratory testing, which included complete blood count and hemogram-derived indices such as NLR, PLR, SII, and PIV. In addition, metabolic and biochemical markers were analysed, including serum homocysteine, uric acid, cystatin C, electrolytes, and renal function parameters.

Patients were prospectively followed for 6 months. The primary outcomes were all-cause mortality and rehospitalisation due to cardiovascular or hypertension-related events. In-hospital complications were also recorded, including acute pulmonary oedema, non-ST elevation myocardial infarction (NSTEMI), ischaemic stroke, haemorrhagic stroke, and AKI.

All patients provided written informed consent prior to inclusion.

The study protocol was approved by the Ethics Committee of the University Clinical Centre Tuzla and was conducted following the ethical standards set forth in the Declaration of Helsinki.

Methods

Demographic, clinical, and therapeutic data were collected for all included patients using a structured evaluation protocol upon hospital admission. This encompassed a detailed medical history, physical examination, review of prior diagnoses, and confirmation of antihypertensive medication use through patient interviews and prescription records. Comorbidities such as diabetes mellitus (DM type 2) and chronic kidney disease (CKD) were identified based on documented medical history and supporting laboratory findings. Tobacco use and family history of cardiovascular disease were also recorded. Blood pressure was measured using a calibrated automated sphygmomanometer, with values confirmed by repeated measurements. Therapeutic adherence was assessed using a standardised questionnaire administered to attending physicians, evaluating both the regularity and consistency of antihypertensive drug use. The assessment was based on patient self-report and prescription verification over the preceding 30 days. Poor adherence was defined as missing ≥20% of prescribed doses or complete therapy interruption prior to admission. Changes to therapy during hospitalisation were documented. Patients were stratified according to the number and type of antihypertensive agents used on admission, including angiotensin-converting enzyme inhibitors, angiotensin receptor blockers (ARBs), beta-blockers (BBs), calcium channel blockers (CCBs), and diuretics.

Venous blood samples were collected within 24 hours of admission and analysed in the hospital's central laboratory. A complete blood count (CBC) was performed, and haematological indices including NLR, PLR, SII, and PIV were calculated. Serum biochemical parameters were measured, including homocysteine, uric acid, electrolytes, liver transaminases, cystatin C, albumin, and creatinine, as part of the comprehensive renal panel. Inflammatory markers such as C-reactive protein (CRP) and ferritin were also assessed. Haematological analyses were conducted using the Sysmex XN-1000 automated haematology analyser (Sysmex Corporation, Japan), with samples collected in K2EDTA tubes. Patients were monitored

throughout hospitalisation for the development of complications, including acute pulmonary oedema, NSTEMI, ischaemic stroke, haemorrhagic stroke, and AKI. These were confirmed through clinical examination, electrocardiography, chest radiography, echocardiography, brain imaging, and laboratory tests, as appropriate.

Following discharge, patients were prospectively followed for six months. Follow-up data were obtained via outpatient clinic visits, structured telephone interviews, and a review of hospital records to document all-cause mortality and cardiovascular-related rehospitalizations. The final follow-up assessment was completed at the end of the six-month period.

Statistical analysis

Categorical variables were presented as absolute frequencies (N) and percentages (%), while continuous variables were expressed as means with standard deviations (SD) or as medians with interquartile ranges (IQR), depending on the distribution. Data normality was assessed using the Kolmogorov–Smirnov test. Group comparisons were performed using Pearson's χ^2 test for categorical variables and either the Student's t-test or the Mann–Whitney U test for continuous variables, as appropriate. Variables with $p<0.10$ in the univariate analysis, along with clinically relevant parameters, were included in a multivariate logistic regression model to identify independent predictors of all-cause mortality and 6-month rehospitalisation. Adjusted odds ratios (ORs) with corresponding 95% confidence intervals (CI) were reported. Model fit was assessed using the Wald χ^2 statistic. The diagnostic performance of selected laboratory markers, including PLR, SII, homocysteine, and uric acid, was evaluated through receiver operating characteristic (ROC) curve analysis. Predictive accuracy was quantified as the area under the receiver operating characteristic (ROC) curve (AUC) for each biomarker. Optimal cut-off values were determined using the Youden index, and corresponding sensitivity and specificity were reported. To account for the potential confounding effect of chronic kidney disease, additional stratified ROC analyses were performed according to the CKD status. A two-tailed $p<0.05$ was considered statistically significant.

RESULTS

This prospective study included 210 patients diagnosed with hypertensive crisis, of whom 52.9% were male. The mean age was 65.7 years, and the average body mass index (BMI) was 28.1 kg/m². Hypertensive emergency was observed in 50.5% of patients, while 49.5% presented with hypertensive urgency. Among comorbidities, DM type 2 was presented in 30.0% of patients, CKD in 25.7%, and 44.8% had a positive family history of cardiovascular disease. Tobacco use was reported in 32.4% of cases. During the 6-month follow-up, 57 patients (27.1%) were rehospitalised, and 23 (10.9%) died.

When outcomes were stratified according to hypertensive crisis type, mortality and rehospitalisation occurred more frequently in patients with hypertensive emergency compared to those with hypertensive urgency. Mortality occurred in 20 (18.9%) patients with hypertensive emergency and three (2.9%) patients with hypertensive urgency ($p<0.001$). Rehospitalisation was observed in 38 (35.8%) patients with hypertensive emergency and 19 (18.3%) with hypertensive urgency ($p=0.006$) (Table 1)

Initial blood pressure values were 198±18 mmHg systolic and 110±10 mmHg diastolic. The most frequently used antihypertensive drug classes at admission were BBs (59.5%), ACE inhibitors (54.8%), diuretics (49.5%), and CCBs (45.2%). ARBs were used in 30.5% of patients. A total of 73.3% were receiving at least two antihypertensive agents on admission. Good therapeutic adherence was documented in 70.0% of cases, and therapy modifications were made during hospitalization in 35.0% of cases.

Table 1. Mortality and rehospitalisation according to hypertensive crisis type

Outcome	No (%) of patients		p
	Hypertensive emergency (N=106)	**Hypertensive urgency** (N=104)	
Mortality	20 (18.9)	3 (2.9)	<0.001
Rehospitalisation	38 (35.8)	19 (18.3)	0.006

Among laboratory parameters, deceased patients had significantly higher values of PLR (120±45 vs. 160±50; p=0.0329), SII (480±130 vs. 610±140; p=0.0355), homocysteine (13.5±4.0 vs. 19.2±5.1 μmol/L; p=0.0488), and uric acid (350±70 vs. 410±85 μmol/L; p=0.021). Although NLR and PIV were also elevated in deceased patients, these differences did not reach statistical significance. Deceased patients were more likely to have CKD (41.3% vs. 25.7%; p=0.027) and lower adherence to therapy (60.9% vs. 74.6%; p=0.022). Parameters such as RDW, MPV, and antihypertensive drug class usage were not significantly associated with mortality. However, patients treated with a combination of ACE inhibitors or ARBs and CCBs showed numerically lower mortality (26.1% vs. 36.1%), although the difference was not statistically significant (p=0.076) (Table 2).

ROC curve analysis demonstrated moderate discriminatory capacity for PLR, SII, homocysteine, and uric acid in predicting mortality, with AUC values of 0.68, 0.66, 0.65, and 0.63, respectively (Figure 1). When ROC analyses were additional-

Table 2. Clinical, laboratory and therapeutic characteristics by mortality status

Variable	Survived	Deceased	p
	Mean±SD		
PLR	120±45	160±50	0.0329
SII	480±130	610±140	0.0355
Homocysteine (μmol/L)	13.5±4.0	19.2±5.1	0.0488
Uric Acid (μmol/L)	350±70	410±85	0.021
NLR	2.4±0.9	3.1±1.2	0.076
PIV	400±100	460±120	0.11
RDW (%)	13.4±0.9	13.6±1.0	0.33
MPV (fL)	10.1±0.6	10.2±0.7	0.48
	No (%) of patients		
CKD	25.7	41.3	0.027
Therapy adherence	74.6	60.9	0.022
Beta-blocker use	58.2	60.9	0.36
CCB use	47.2	39.1	0.31
ACEI/ARBs + CCBs combo	36.1	26.1	0.076

PLR, platelet-to-lymphocyte ratio; SII, systemic immune-inflammation index; NLR, neutrophil-to-lymphocyte ratio; PIV, pan-immune-inflammation value; RDW, red cell distribution width; MPV, mean platelet volume; CKD, chronic kidney disease; CCB, calcium channel blockers; ACEI/ARBs + CCBs combo, combined use of ACE inhibitors or angiotensin receptor blockers with calcium channel blockers

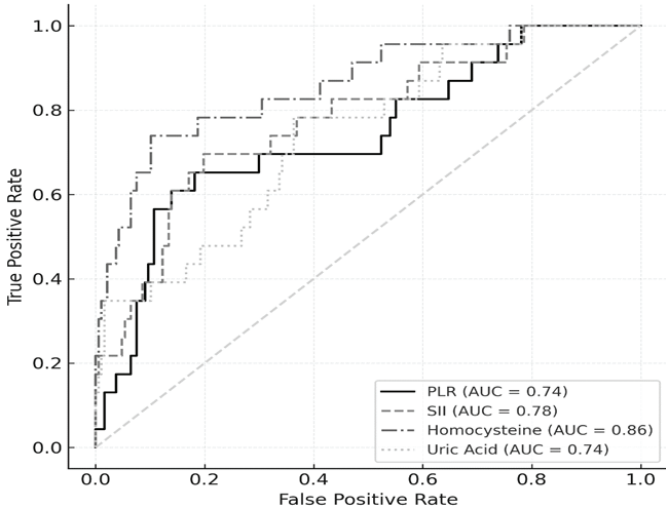

Figure 1. Receiver operating characteristic (ROC) curve analysis demonstrated modest but relevant predictive power of selected biomarkers for all-cause mortality. Area under the curve (AUC) values were: PLR=0.68, SII=0.66, Homocysteine=0.65, and uric acid=0.63. These findings support the prognostic utility of these parameters, especially when interpreted in the context of comorbidities and therapy

ly stratified by chronic kidney disease status, all biomarkers retained comparable discriminatory performance, indicating preserved prognostic value independent of renal impairment.

Regarding rehospitalisation, multivariate logistic regression analysis identified chronic kidney disease and poor therapeutic adherence as independent predictors of six-month readmission. Patients with CKD had a twofold increased risk of rehospitalisation (OR=2.01, 95% CI: 1.11–3.64; p=0.015), while poor adherence was associated with an 84% higher risk (OR=1.84, 95% CI: 1.03–3.27; p=0.034). No individual antihypertensive drug class was independently associated with rehospitalisation, although patients treated with a combination of ACE inhibitors and calcium channel blockers showed a non-significant trend toward reduced risk (OR=0.66; p=0.13) (Table 3).

Table 3. Multivariate predictors of rehospitalisation at 6 months

Variable	OR (95% CI)	p
CKD	2.01 (1.11–3.64)	0.015
Poor therapy adherence	1.84 (1.03–3.27)	0.034
ARBs at baseline	0.78 (0.42–1.47)	0.32
Diuretic use	1.22 (0.69–2.14)	0.41
ACEI + CCBs combination	0.66 (0.36–1.21)	0.13

CKD, chronic kidney disease; ARBs at baseline, use of angiotensin receptor blockers at the time of hospital admission; Diuretic use, administration of diuretic medications at baseline; ACEI+CCB combination, combined use of angiotensin-converting enzyme inhibitors and calcium channel blockers.

In a multivariate logistic regression model adjusted for age, homocysteine emerged as the strongest independent predictor of all-cause mortality (OR=3.55, 95% CI: 1.84–6.85; p<0.001), followed by uric acid (OR=1.03, 95% CI: 1.01–1.06; p=0.007), PLR (OR=1.04, 95% CI: 1.00–1.08; p=0.047), and SII (OR=1.01, 95% CI: 1.00–1.02; p=0.030). Chronic kidney disease (OR=2.15, 95% CI: 1.19–3.87; p=0.012) and poor therapeutic adherence (OR=1.92, 95% CI: 1.10–3.36; p=0.017) also remained independently associated with mortality (Table 4).

Among in-hospital complications, acute pulmonary oedema during the index hospitalisation was significantly associated with increased mortality (OR=1.89, 95% CI: 1.02–3.52; p=0.041). NSTEMI showed a non-significant trend toward higher mortality risk (OR=1.77; p=0.081) (Table 4).

Table 4. Multivariate predictors of all-cause mortality and in-hospital complications

Variable	OR (95% CI)	p
Homocysteine	3.55 (1.84–6.85)	<0.001
Uric Acid	1.03 (1.01–1.06)	0.007
PLR	1.04 (1.00–1.08)	0.047
SII	1.01 (1.00–1.02)	0.030
CKD	2.15 (1.19–3.87)	0.012
Poor therapy adherence	1.92 (1.10–3.36)	0.017
Complication: acute pulmonary oedema	1.89 (1.02–3.52)	0.041
Complication: NSTEMI	1.77 (0.91–3.42)	0.081

PLR, platelet-to-lymphocyte ratio; SII, systemic immune-inflammation index; CKD, chronic kidney disease; NSTEMI, non–ST elevation myocardial infarction during index hospitalization

In-hospital complications included acute pulmonary oedema (14.8%), NSTEMI (13.3%), ischaemic stroke (7.6%), haemorrhagic stroke (3.8%), and acute kidney injury (10.0%). The most frequent immediate causes of death were acute pulmonary oedema with respiratory failure, acute coronary syndromes, and severe ischaemic or haemorrhagic stroke. At the same time, a smaller proportion of patients died due to progressive multiorgan failure during hospitalization.

DISCUSSION

The results of this prospective cohort study confirm that inflammatory indices and metabolic markers, particularly PLR, SII, homocysteine, and uric acid, are independently associated with all-cause mortality in patients presenting with hypertensive crisis. Additionally, CKD and poor therapeutic adherence were significant predictors of both mortality and six-month rehospitalisation (17). These findings indicate that adverse outcomes in hypertensive crisis are influenced not only by the severity of blood pressure elevation but also by systemic inflammation, metabolic abnormalities, renal impairment, and long-term management (18). When outcomes were further stratified by hypertensive crisis type, patients with hypertensive emergency exhibited significantly higher mortality and rehospitalisation rates than those with hypertensive urgency. This finding confirms a more malignant clinical course of hypertensive emergency, and supports the need for more intensive in-hospital monitoring and structured post-discharge follow-up in this patient group.

Our results are consistent with other studies, which demonstrated that elevated SII and PLR were independent predictors of in-hospital mortality among patients with hypertensive emergencies (19). A large NHANES-based study also confirmed that high SII levels were associated with increased risk of stroke, regardless of traditional risk factors (20). These findings support our observation that platelet-leukocyte interactions and systemic inflammatory activity are essential contributors to vascular instability in acute hypertension (20).

Although NLR and PIV values were elevated among deceased

patients, these differences did not reach statistical significance. Similar findings were reported in cross-sectional studies involving individuals with elevated blood pressure, in which temporal fluctuations in inflammatory markers, such as NLR, were shown to reduce their reliability as stable prognostic indicators in acute clinical settings (21).

Homocysteine emerged as the strongest independent predictor of mortality in our cohort, with an odds ratio of 3.55. A recent cohort study using data from the NHANES database also identified elevated homocysteine as a significant predictor of cardiovascular mortality, reinforcing its relevance as a high-risk biomarker in hypertensive populations (22). Elevated uric acid levels also remained significantly associated with adverse outcomes. This observation agrees with results from recent meta-analyses that identified hyperuricemia as an independent risk factor for cardiovascular mortality among patients with hypertension (23).

CKD was another consistent predictor of mortality and rehospitalisation. These findings are supported by recent NHANES data showing that renal dysfunction significantly worsens prognosis in hypertensive populations (20). They highlight the importance of including renal function in initial patient assessments and follow-up planning (24). Importantly, additional stratified ROC analyses demonstrated that the prognostic performance of inflammatory and metabolic biomarkers remained preserved regardless of CKD status, indicating that their discriminatory value is not driven solely by renal impairment.

Poor adherence to antihypertensive therapy also showed a clear association with adverse outcomes. This observation is supported by previous analyses indicating that non-adherence significantly increases cardiovascular morbidity and mortality in patients with hypertension, highlighting its critical impact even during acute hypertensive presentations (25). Strategies aimed at improving medication compliance should be prioritised for high-risk groups, particularly those with comorbid CKD (26).

Although no specific antihypertensive drug class was independently associated with outcomes, patients treated with a combination of ACE inhibitors or ARBs and CCBs showed a trend toward lower mortality and rehospitalisation (27). Earlier randomized trials, including ASCOT-BPLA, support this therapeutic strategy. However, the lack of statistical significance in our cohort may reflect variability in dosing, therapy duration, or patient compliance (28).

Receiver operating characteristic curve analysis confirmed moderate predictive accuracy for PLR, SII, homocysteine, and uric acid, with area under the curve values ranging from 0.63 to 0.68. Although these markers are not sufficient on their own for diagnostic use, their combined interpretation with clinical parameters may improve early risk stratification (21). Their accessibility and affordability make them especially useful in routine emergency care.

In-hospital complications such as acute pulmonary oedema, NSTEMI, ischaemic stroke, haemorrhagic stroke, and acute kidney injury further underscore the systemic burden of hypertensive crisis. Among these, acute pulmonary oedema showed an independent association with increased mortality in our cohort, corroborating recent reports that highlight pulmonary complications as strong contributors to early adverse outcomes in hypertensive emergencies (29, 30). In line with this observation, most fatal outcomes in our cohort were attributable to acute pulmonary oedema with respiratory failure, acute coronary syndromes, and severe cerebrovascular events, reflecting the dominant cardiorespiratory and neurovascular mechanisms of early death in hypertensive crisis.

This study has several implications for clinical practice. It emphasises the value of multimodal risk assessments using basic inflammatory and metabolic markers, which are often overlooked in acute care (31). From a guideline perspective, our findings support consideration of simple inflammatory and metabolic biomarkers at hospital admission as adjunct tools for early risk stratification in patients with hypertensive crisis. It also underlines the importance of renal function and treatment adherence, both of which are modifiable risk factors.

However, some limitations must be acknowledged. This was a single-centred study, which may affect the generalisability of the findings. The sample size was sufficient for the primary analyses but insufficient for detailed subgroup analyses. Inflammatory biomarkers were measured only once at admission, so trends over time could not be assessed. Residual confounding is also possible, despite statistical adjustments.

In conclusion, our findings suggest that a combination of inflammatory and metabolic markers, renal function, and therapy adherence influences short-term outcomes in hypertensive crisis. Incorporating parameters such as PLR, SII, homocysteine, and uric acid in risk stratification models could improve the identification of high-risk patients and guide personalised treatment strategies (32).

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TRANSPARENCY DECLARATION

Conflicts of interest: None to declare.

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ORIGINAL ARTICLE

Molecular docking analysis of thymoquinone as a potential inhibitor of C-reactive protein and transforming growth factor β (TGF-β): an *in-silico* study for myocardial infarction therapeutics

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ABSTRACT

Aim Myocardial infarction (MI) is a cardiovascular disease that is the leading cause of death at all ages. Inflammation and oxidation processes constitute the basic pathophysiology of MI development. C-reactive protein (CRP) and transforming growth factor β (TGF-β) are markers that are often used to evaluate the level of inflammation, especially in MI. This study aimed to evaluate the anti-inflammatory potential of thymoquinone (TQ), the major bioactive compound of *Nigella sativa*, by assessing its binding affinity through molecular docking, in which TQ exhibited more favourable binding energies compared to the native ligand.
Methods Using the VegaZZ, PyMOL, and BIOVIA Discovery Studio tools, AutoDock Vina software was used for in silico research to test the active molecule TQ and produce visual profiles of native CRP and TGF-β ligands. Using the pkCSM method, pharmacokinetic predictions were carried out.
Results Thymoquinone (2-methyl-5-propan-2-ylcyclohexa-2,5-diene-1,4-dione) showed favourable binding affinity to both CRP and TGF-β, with docking scores of –3.60 and –4.15 kcal/mol, respectively, which are more favourable than those of the native ligands (-2.39 and –2.73 kcal/mol) and comparable to enalapril (–4.84 and –6.13 kcal/mol). The root mean square deviation (RMSD) value for CRP was 1.421 Å, while the value for TGF-β was 0.253 Å, indicating excellent structural alignment and validating the docking approach.
Conclusion These in silico findings suggest that TQ warrants further investigation in vitro and in vivo as a potential modulator of inflammatory pathways in MI.
Keywords: anti-inflammatory agents, Molecular Docking Simulation, myocardial infarction, *Nigella sativa*, thymoquinone

INTRODUCTION

Myocardial infarction (MI) is a leading cause of death worldwide, with an estimated 32% of the population experiencing death (1). Despite major advances in interventional and pharmacological therapy, the global prevalence of MI continues to rise, particularly in developing countries, due to lifestyle-related risk factors such as obesity, diabetes, and hypertension (2,3) The underlying pathophysiology of MI involves prolonged inflammation and oxidative stress, which cause endothelial dysfunction, resulting in the necrosis of cardiomyocytes and the development of MI. If left untreated, MI can cause further complications, namely, ventricular remodelling and heart failure [2]. Therefore, understanding and controlling the inflammatory process after MI remains a key focus in reducing post-infarction complications.

C-reactive protein (CRP) is an acute phase protein that is often used as a marker of inflammation in various disease conditions, one of which is MI. A study reported that high CRP levels are associated with the risk of expanding the necrotic area in MI (4). In addition to CRP, transforming growth factor β (TGF-β) is another significant factor involved in the pathophysiology of MI. TGF-β contributes to the development of myocyte hypertrophy, which is a key component in the process of ventricular remodelling after MI. In an in vivo study, the overexpression of TGF-β was associated with cardiac fibrosis and hypertrophy (5,6). Owing to their important roles, CRP and TGF-β levels may be promising therapeutic targets for patients with MI as cardioprotective agents (7).

Cardioprotective agents with both anti-inflammatory and antioxidant properties are therefore of high interest. Among current pharmacological options, angiotensin-converting enzyme inhibitors (ACEIs) such as enalapril are widely used in the

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management of MI and heart failure due to their systemic cardioprotective effects. Enalapril functions primarily by inhibiting the conversion of angiotensin I to angiotensin II, thereby reducing vascular resistance and mitigating inflammatory signalling (8). One example of an ACE-I drug that is widely used as a cardioprotective agent in patients with MI and heart failure is enalapril. Although ACEIs provide substantial clinical benefit, concerns related to long-term toxicity and side effects such as hyperkalaemia remain (9). This study mentioned enalapril as an established therapeutic relevance in MI treatment. However, it is important to note that enalapril does not directly bind to or inhibit CRP or TGF-β at their active sites (9). Therefore, it is not used as a structural positive control ligand in this docking simulation, but rather to provide a comparative context for the binding performance of thymoquinone (TQ).

Thymoquinone (TQ), also known as 2-isopropyl-5-methylbenzo-1,4-quinone, is a biochemical compound that has anti-inflammatory effects. TQ is one of the main phytochemical compounds that is abundant in black cumin (*Nigella sativa*). Previous studies have reported that the TQ compound present in *Nigella sativa* has cardioprotective effects by suppressing inflammation and oxidants (10). TQ compounds also offer other beneficial properties, including anticancer, antioxidant and hepatoprotective effects (10–12). In addition, TQ has a relaxing effect on heart muscle and vasodilators by inhibiting the entry of Ca2+ ions that mediate voltage-gated Ca2+ channels. TQ has demonstrated potential in preventing myocardial reperfusion injury and ischemia-induced arrhythmias. Additionally, TQ is associated with anti-inflammatory and antioxidant properties that may positively influence the progression of various inflammation-related conditions (13,14).

Molecular docking is a widely used computational approach to simulate and predict the interaction between bioactive molecules and protein targets. It offers valuable insights into drug discovery by evaluating binding affinity and interaction profiles (14)

However, despite extensive evidence regarding thymoquinone's antioxidant and anti-inflammatory properties, there is limited understanding of its direct molecular interaction with key inflammatory mediators such as CRP and TGF-β that play crucial roles in myocardial infarction. Previous studies have mainly focused on its biochemical or in vivo effects without clarifying the molecular binding mechanisms involved (1).

The aim of this study was to assess the binding potential of TQ to CRP and TGF-β through molecular docking analysis and to compare its in silico performance with that of enalapril as a clinical benchmark. Clinically, this study hypothesizes that thymoquinone, by targeting CRP and TGF-β signalling pathways, could contribute to reducing post-infarction inflammation and myocardial remodelling, thereby offering a potential adjunct therapeutic strategy for patients with myocardial infarction.

MATERIALS AND METHODS

Materials and study design

This was an in-silico experimental study designed to evaluate the molecular interaction of thymoquinone (TQ) with C-reactive protein (CRP) and transforming growth factor-β (TGF-β) as therapeutic targets relevant to myocardial infarction. The study was conducted at the Department of Cardiovascular Medicine, Faculty of Medicine, Sultan Agung Islamic University, Semarang, Indonesia, from March to May 2024. No human or animal subjects were involved. All computational analyses were performed under identical docking conditions to ensure reproducibility, using standardized protein structures obtained from the RCSB Protein Data Bank (CRP: PDB ID 1B09; TGF-β: PDB ID 1TGJ).

Methods

Applications and Software. AutoDock Tools (v1.5.6) was used for the preparation of proteins and ligands, while docking simulations were performed using AutoDock Vina. Visualization and analysis of ligand–protein interactions were conducted with BIOVIA Discovery Studio Visualizer 2021. The 3D structure of thymoquinone (TQ) was retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) in structure data file (SDF) format and converted to protein data bank (PDB) using Open Babel. The molecular geometry of TQ was optimized using Vega ZZ software with the MMFF94 force field, applying an energy minimization threshold of 0.01 kcal/mol to ensure a stable conformation. Protein structures of C-reactive protein (CRP, PDB ID: 1B09) and transforming growth factor beta (TGF-β, PDB ID: 1TGJ) were obtained from the RCSB Protein Data Bank (https://www.rcsb.org).

Proteins and ligand preparation. Protein preparation involved the removal of water molecules and native ligands, addition of polar hydrogen atoms, and assignment of Gasteiger charges using BIOVIA Discovery Studio. Protonation states were set to reflect physiological pH (7.4), and all histidine residues were manually checked and adjusted to the appropriate tautomeric forms using the same software. Grid box coordinates were defined separately for each protein based on their native ligand binding sites, and docking was performed via command-line execution. Pharmacokinetic properties of TQ were predicted using the SwissADME web server (https://www.swissadme.ch), which includes evaluation of absorption, distribution, metabolism, excretion (ADME), and drug-likeness according to Lipinski's Rule of Five. Toxicity profiles were assessed using the pkCSM platform (http://biosig.unimelb.edu.au/pkcsm), which estimates endpoints such as AMES mutagenicity, hepatotoxicity, hERG inhibition, and both acute and chronic oral toxicity in rodents. These computational predictions served to provide a preliminary evaluation of TQ's pharmacological and toxicological characteristics (14)

Validation of docking parameters. A measure of the mean distance that relates to the docking zone's size is the root mean square deviation (RMSD). Achieving an RMSD value of less than 2 Å is the aim. If the RMSD is less than 2 Å, the docking method is valid. In this study, three independent docking measurements were performed for each protein–ligand complex (CRP–TQ and TGF-β–TQ) to ensure reproducibility of the results. All measurements were conducted using the same protein structures and ligand samples under identical docking parameters, with the protein and ligand docking regions configured using the grid panel and grid submenu (15).

Molecular docking test. Molecular docking simulations were conducted using AutoDock Vina v1.5.6 (http://autodock.scripps.edu). Docking inputs were prepared via configuration files (conf.txt) and executed using the Windows command prompt with the command vina.exe -config conf.txt -log log.txt. Each configuration file specified the receptor (.pdbqt) and ligand (.pdbqt) files, along with grid box coordinates and dimensions. The output included binding affinities, multiple li-

gand conformations, and root mean square deviation (RMSD) values for pose validation.

Docking validation was performed by redocking the native ligands of each target protein to evaluate the accuracy of the docking protocol. For C-reactive protein (CRP; PDB ID: 1B09, DOI: https://doi.org/10.2210/pdb1B09/pdb), phosphocholine was used as the reference ligand and produced an RMSD of 1.421 Å. For transforming growth factor β (TGF-β; PDB ID: 1TGJ, DOI: https://doi.org/10.2210/pdb1TGJ/pdb), the redocking of the co-crystallized ligand yielded an RMSD of 0.253 Å. Both values are within the generally accepted threshold (<2 Å), indicating reliable docking performance. RMSD was calculated using PyMOL's alignment function, comparing heavy atoms between docked and crystallographic poses. Distinct grid box centres were defined for each protein: CRP at x=139.030, y=171.723, z=34.896 and TGF-β at x=37.455, y=39.338, z=63.313, with uniform dimensions of 40×40×40 Å to ensure proper coverage of the binding site (16,17).

Data visualization for molecular docking. The goal of molecular docking data visualization is to determine the spatial configuration and three-dimensional visual depiction of protein–ligand interactions. For every ligand that is evaluated, the position and visual representation of protein binding are ascertained by analysing the visualization data to evaluate compound interactions. To determine the conformation of the TQ molecule and its binders (hydrogen and nonhydrogen bonds), the resulting molecular interactions are utilized. Biovia DS Visualizer 2021 (v21.1) software was used to visualize molecular docking data. The pdbqt file format is used to hold the visualization data (18).

Pharmacokinetic analysis and toxicity analysis. Physicochemical properties and ADME parameters of thymoquinone (TQ), such as absorption, distribution, metabolism, and excretion, were evaluated using the SwissADME platform (https://www.swissadme.ch). These analyses included gastrointestinal absorption, bioavailability score, and drug-likeness based on Lipinski's Rule of Five. In parallel, toxicity predictions were conducted using two tools: the pkCSM web server (http://biosig.unimelb.edu.au/pkcsm) and the Toxtree application. The pkCSM platform was used to assess endpoints including AMES toxicity, maximum tolerated human dose, hERG I and II inhibition, acute and chronic oral toxicity in rodents, hepatotoxicity, and skin sensitization. Meanwhile, Toxtree was used to evaluate Tetrahymena pyriformis toxicity and other relevant toxicological profiles. These computational tools provided a comprehensive preliminary evaluation of TQ's pharmacokinetic behaviour and toxicological safety(19,20).

Statistical analysis

Binding affinity (kcal/mol) and RMSD values were expressed as mean ± standard deviation from three independent docking runs per ligand–protein complex. Comparative descriptive analysis was performed between thymoquinone, native ligands, and enalapril to evaluate relative docking performance. No inferential statistics were applied because the study was computational.

RESULTS

The binding affinities of thymoquinone (TQ) toward C-reactive protein (CRP) and transforming growth factor β (TGF-β) were analysed through molecular docking simulations. Grid box dimensions were set at 40×40×40 Å³, with centre coordinates adjusted based on each protein's native ligand binding site. For CRP (PDB ID: 1B09), the grid box centre was x=139.030, y=171.723, z=34.896. For TGF-β (PDB ID: 1TGJ), the centre was x=37.455, y=39.338, z=63.313. The native ligands, phosphocholine for CRP and DIO for TGF-β, were redocked to validate the docking protocol. The root mean square deviation (RMSD) values between docked and crystallographic poses were 1.421 Å for CRP and 0.253 Å for TGF-β, both of which are within the acceptable range (<2 Å), confirming the reliability of the docking setup.

Table 1. Affinity value of the interactions between ligands and C-reactive protein (CRP) and ligands and transforming growth factor β (TGF-β)

Ligand	Affinity value	Ligand type	RMSD
Ligands and CRP			
Phosphocholine	-2.39 Kcal/mol	Native ligand	
Thymoquinone	-3.60 Kcal/mol	Test ligand	1.421 Å
Enalapril	-4.56 Kcal/mol	Clinical references	
Ligands and TGF-β			
1,4 Diethylene Dioxide (DIO)	-2.73 kcal/mol	Native ligand	
Thymoquinone	-4.15 kcal/mol	Test ligand	0.253 Å
Enalapril	-6.16 kcal/mol	Clinical references	

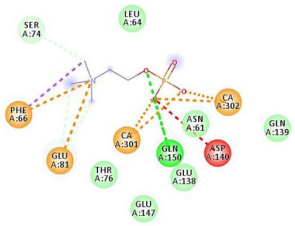
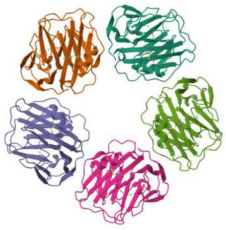
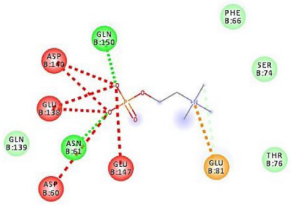
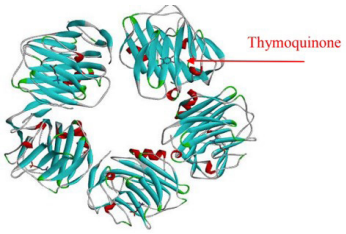
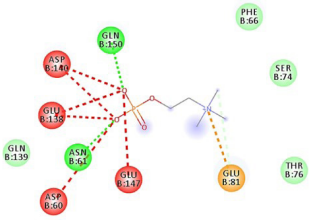
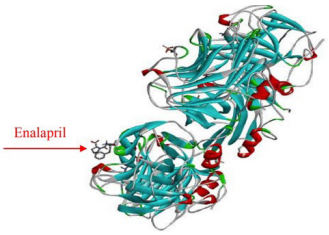
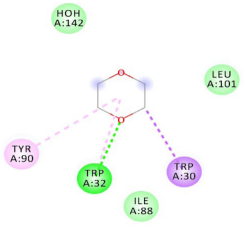
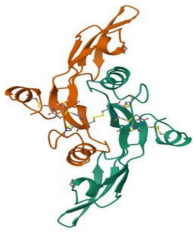
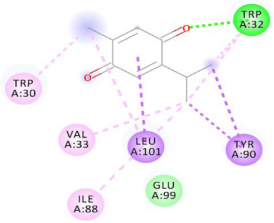
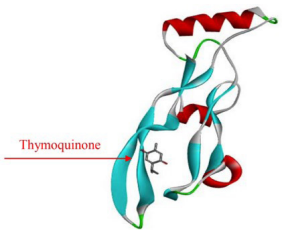
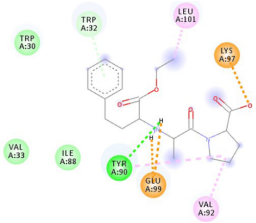
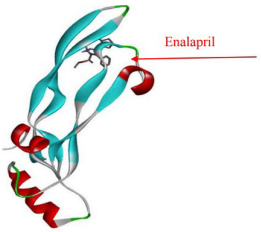
RMSD, root of mean standard deviation

For CRP, the native ligand (phosphocholine) had a binding affinity of –2.39 kcal/mol, while TQ showed a stronger affinity of –3.60 kcal/mol. For TGF-β, the native ligand (DIO) yielded a binding affinity of –2.73 kcal/mol, and TQ demonstrated a more favourable interaction with –4.15 kcal/mol. Although enalapril is not known to bind directly to the active sites of CRP or TGF-β, it was included for contextual comparison; its docking yielded binding affinities of –4.56 kcal/mol with CRP and –6.16 kcal/mol with TGF-β. These docking scores are presented solely for reference, as enalapril primarily acts through angiotensin-converting enzyme inhibition in clinical practice and does not serve as a structural positive control in this simulation.

List the amino acid residues involved in the molecular interactions between thymoquinone (TQ) and the target proteins CRP and TGF-β, as detailed in the following interaction analysis. These interactions were further visualized using BIOVIA Discovery Studio to generate 2D and 3D interaction diagrams, shown in Figures 1 to 4. TQ exhibited hydrogen bonding with Gln150 (2.8 Å) and Thr76 (3.1 Å) on CRP, alongside hydrophobic contacts with Phe66, Ser74, and Glu81. For TGF-β, TQ formed hydrogen bonds with Trp32 (3.0 Å) and interacted hydrophobically with Leu101 and Tyr90. These interactions contribute to the stable binding conformation of TQ within the active pockets of CRP and TGF-β, supporting its predicted anti-inflammatory potential. The visual evidence and specific residue contacts reinforce the molecular docking results and highlight TQ's capability to engage with key residues relevant to inflammatory regulation (Table 2).

The pharmacokinetic parameters of TQ absorption, distribution, metabolism, and excretion were predicted via the SwissADME tool. In addition, physicochemical tests using the pkCSM strategy were performed to predict drug similarity to the test ligand. The Lipinski 5 rules refer to the physicochemical properties. This is because every parameter's value is a multiple of five, meaning that the molecular weight, octa-

Table 2. Ligand - C-reactive protein (CRP) and Ligand - transforming growth factor β (TGF-β) interaction of molecular docking visualized in three dimensions (3D)

No	Ligand	Docking Interaction Ligand and Protein	3D Visualization
Ligands and CRP			
1	Phosphocholine (PC)		
2	Thymoquinone		
3	Enalapril		
Ligands and TGF-β			
1	1,4 Diethylene Dioxide		
2	Thymoquinone		
3	Enalapril		

nol–water partition coefficient (LogP), H-bond acceptor, and H-bond donors must all be less than 500 daltons, 10, and 5, respectively (5). Compounds that fulfil Lipinski's rule can be used as active oral drugs (10,13). The physicochemical analysis showed that thymoquinone (TQ) fulfilled all parameters of Lipinski's Rule of Five, with a molecular weight of 164.2 g/mol, two hydrogen bond acceptors, no hydrogen bond donors, and a logP value of 1.6. These properties indicate good lipophilicity and potential oral bioavailability, supporting its suitability as a drug-like molecule (Table 3).

The results of the ADME prediction test on the TQ compound showed the compound had a high absorption capacity in the digestive tract, which strengthens the use of the TQ compound as an oral drug (Table 4).

Table 3. The TQ's physicochemical properties

Formula	Molecular weight	H-Bond Acceptor	H-Bond Donors	Log P
$C_{10}H_{12}O_2$	164.2 g/mol	2	0	1.6

Table 4. ADME (Absorption, Distribution, Metabolism, and Excretion) predictions of TQ (Thymoquinone) predictions of Thymoquinone (TQ)

ADME	Parameter	Outcome/remark
A (Absorption)	GI Absorption	High
	TPSA	34,14
	Bioavailability Score	0,55
D (Distribution)	BBB	YES
	P-GP Substrate	NO
M (Metabolism)	CYP1A2 Inhibitor	NO
	CYP2C19 Inhibitor	NO
	CYPC9 Inhibitor	NO
	CYP2D6 Inhibitor	NO
	CYP3A4 Inhibitor	NO
E (Excretion)	LogKp	-5.74 cm/s

GI, gastrointestinal absorption; TPSA, topological polar surface area; BBB, blood–brain barrier; CYP, cytochrome P450 enzyme.

Toxicity predictions were performed using the pkCSM platform (http://biosig.unimelb.edu.au/pkcsm). TQ was not predicted to be mutagenic in the AMES test and did not exhibit hERG I or II inhibition, suggesting a low risk of cardiotoxicity. The predicted maximum tolerated dose in humans was 0.89 log mg/kg/day, and the highest tolerated dose in rats was estimated at 1.743 mol/kg. However, TQ was predicted to be hepatotoxic and a potential skin sensitizer. Chronic oral toxicity analysis estimated a dose of 2.378 log mg/kg that could result in liver damage. Additionally, the *Tetrahymena pyriformis* toxicity value was predicted to be 1.758 log mM. While these results suggest that TQ does not pose significant cardiac toxicity, the predicted hepatotoxicity represents a substantial limitation for its potential clinical use. Further experimental validation, particularly through in vitro and in vivo studies, is necessary to clarify its safety profile, especially regarding hepatic effects (Table 5).

Table 5. Predicted toxicity of TQ (Thymoquinone) compounds from *Nigella sativa*

No	Model name	Predicted value
1	AMES toxicity	NO
2	Hepatotoxicity	YES
3	Minnow oxicity	1.758 log mM
4	Acute oral toxicity in rats	1.743 mol/kg
5	Chronic oral toxicity in rats	2.378 mol/kg
6	hERG I inhibitor	NO
7	hERG II inhibitor	NO
8	Max. tolerable dose in humans	0.89 log mg/kg/day
9	Skin sensitization	YES
10	*T. pyriformis* toxicity	0.138 log ug/L

AMES, Ame's mutagenicity test; hERG, human ether-à-go-go-related gene potassium channel;
T. pyriformis, *Tetrahymena pyriformis* toxicity.

DISCUSSION

The present study provides computational evidence supporting the potential of thymoquinone (TQ) as a cardioprotective compound through molecular interaction with inflammatory and fibrotic mediators such as C-reactive protein (CRP) and transforming growth factor-β (TGF-β). These findings strengthen the growing body of literature suggesting that TQ plays a significant role in modulating post-infarction inflammation and myocardial remodelling.

Several recent investigations have demonstrated that TQ effectively downregulates pro-inflammatory cytokines and oxidative stress markers associated with myocardial injury. It was reported that TQ improved cardiac histology and reduced TNF-α and IL-6 expression in isoproterenol-induced myocardial infarction in rats (14). Likewise, a previous study showed that TQ attenuated fibrosis and reduced TGF-β expression in cardiac tissue was confirmed, supporting its role in inhibiting remodelling-related pathways (5). These *in vivo* data are consistent with our computational prediction that TQ interacts with residues critical for TGF-β signalling, potentially explaining its observed antifibrotic activity.

The anti-inflammatory potential of TQ through CRP modulation also aligns with previous studies demonstrating that TQ suppresses oxidative and inflammatory responses by inhibiting NF-κB and reducing CRP levels in cardiovascular and hepatic models (10,13). Such activity underscores the hypothesis that TQ may contribute to cardioprotection by interfering with CRP-mediated inflammatory cascades. Furthermore, accumulating evidence suggests that elevated CRP level serves not only as a marker but also as an active participant in the amplification of oxidative stress and tissue inflammation during myocardial injury (21). Previous investigations have revealed a significant association between CRP and uric acid concentrations in acute coronary syndrome, indicating that increased oxidative burden parallels heightened inflammatory signalling within cardiomyocytes (22). These insights reinforce the present findings and support the concept that targeting CRP-regulated pathways may represent a promising molecular mechanism underlying the cardioprotective potential of thymoquinone.

In addition to its molecular interactions, TQ exhibits favourable pharmacokinetic characteristics that support its therapeutic viability. Recent studies indicated that TQ has good oral bioavailability, high gastrointestinal absorption, and adherence to Lipinski's Rule of Five (23,24). Consistent with these findings, the present computational evaluation indicated good absorption potential and absence of mutagenicity or cardiotoxicity, further supporting TQ's suitability as a drug-like molecule. Nonetheless, possible hepatotoxicity predicted in silico should be interpreted cautiously, as mild hepatic enzyme elevation has been observed with chronic TQ exposure (24)

Overall, these results reinforce that TQ possesses dual anti-inflammatory and antifibrotic properties that could be beneficial in limiting post-infarction remodelling. The combined literature evidence from recent in vivo studies supports the molecular docking predictions, suggesting that TQ could serve as a complementary therapeutic candidate targeting CRP- and TGF-β–mediated pathways (25–27). However, because this study is purely computational, further experimental validation through *in vitro* binding assays and *in vivo* myocardial infarction models is essential to confirm the pharmacological relevance of these interactions.

This *in-silico* study suggests that thymoquinone (TQ), the major bioactive compound of *Nigella sativa*, has potential modulatory effects on key inflammatory mediators involved in myocardial infarction, namely C-reactive protein (CRP) and transforming growth factor β (TGF-β). The findings indicate that TQ may contribute to cardioprotection by targeting inflammation-related pathways. Although these computational

results are promising, experimental validation through *in vitro* and *in vivo* studies is essential to confirm its biological activity, therapeutic potential, and safety profile.

It must be emphasized that molecular docking assesses binding potential but does not provide evidence of biological activity or functional outcomes such as suppression of CRP or TGF-β levels. Moreover, this study lacked comprehensive visual representations of ligand–protein interactions, limiting the interpretive strength of the docking findings. Therefore, while the data suggest that TQ could serve as a candidate for further exploration, extensive *in vitro* and *in vivo* validation is essential to confirm its therapeutic efficacy and safety.

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TRANSPARENCY DECLARATION

Conflicts of interest: None to declare.

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ORIGINAL ARTICLE

Laboratory parameters, clinical characteristics and hemogram-derived indices in adult patients with measles at tertiary hospital admission

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ABSTRACT

Aim To describe clinical and laboratory findings, as well as to examine the significance of novel hemogram-derived inflammatory indices in adult patients with measles admitted to a tertiary hospital.

Methods This prospective, descriptive, and analytical study included adult patients over 18 years of age with clinical signs of measles, admitted at the Clinic for Infectious Diseases, Clinical Centre of Sarajevo. Upon admission, medical history, physical examination, and laboratory analyses were conducted from February 2024 to 31 December 2024.

Results The study included 124 patients, 52 men and 72 were women with a mean age of 38.33±13.18 years. The most common symptoms in most patients were rash (95.97%) and cough (69.36%). Neutrophilia (63.7%) and lymphopenia (74.19%) were the main haematological features along with elevated C-reactive protein (CRP) (95.17%) and transaminase (75%) in the vast majority of patients. The results of laboratory analyses also indicated hyponatremia and hypokalaemia, with elevated values of the enzymes creatine kinase (CK) and lactate dehydrogenase (LDH). The CRP, as the most commonly elevated laboratory parameter, showed a significant positive correlation with the neutrophil-to-lymphocyte ratio (NLR) and the systemic inflammation response index (SIRI) ($p < 0.05$), as well as with the systemic immune–inflammation index (SII) and the aggregate index of systemic inflammation (AISI) ($p < 0.01$).

Conclusion Adult measles was associated with significant inflammation, hematologic changes, and organ involvement, with CRP showing strong correlations with hemogram-derived indices, suggesting that these indices may be useful in the diagnostics of measles.

Key words: biomarkers, infectious disease, inflammation, viremia

INTRODUCTION

The causative agent of measles is an RNA virus, from the family *Paramyxoviridae*, genus *Morbillivirus*. The virus is resistant in the external environment, and is found in nasopharyngeal secretions, urine and blood 2-3 days before the appearance of the disease symptoms and one day after the appearance of symptoms of the disease (1). Measles is a cosmopolitan disease and occurs sporadically or epidemically in all parts of the world. Newborns are protected in the first 6 months of life by passive, transplacental immunity transferred from the mother, if she had measles (2). The reservoir of infection are humans, the source of infection is the nasopharyngeal secretion of an infected person. The route of spread of the infection is droplet transmission, and the entrance door of the infection is the mucous membrane of the conjunctiva and the upper parts of the respiratory tract. It occurs in an endemoepidemic form most often in children of preschool and early school age (3).

The incubation period for measles is 10-11 days. It proceeds without symptoms, and in the last third of the incubation period the patient is infectious. The symptoms that occur are cough, coryza, Koplik's spots and conjunctivitis. Patients go through a catarrhal, rash and convalescent stage of the disease (2). Measles can also have complications, the most common of which are pneumonia, diarrhea and ear infections (4).

Hemogram-derived indices (HDI) are new parameters obtained by simple mathematical calculation of inflammation indicators: the number of leukocytes, lymphocytes, neutrophils, monocytes, platelets, C-reactive protein (CRP), etc. (5).

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HDIs have a role in disease diagnostics, prediction of patient condition, disease and outcome prognosis and assessment of therapy success (6). A large number of studies have so far shown the usefulness of HDIs in respiratory infections such as COVID-19, respiratory syncytial virus (RSV) infection and *Mycobacterium pneumoniae* pneumonia (7,8). Given their demonstrated diagnostic potential in the viral infections, it would be worth testing them in measles as well.

The aim of this study was to describe clinical and laboratory findings and to examine the significance of novel HDIs in adult patients with measles admitted to a tertiary hospital.

PATIENTS AND METHODS

Patients and study design

This prospective, descriptive and analytical study included adult patients over the age of 18 who presented with symptoms of measles infection at the Clinic for Infectious Diseases, Clinical Centre University of Sarajevo, Bosnia and Herzegovina. Upon admission of patients with a referral diagnosis of measles, a medical history was obtained, a physical examination was performed, and blood samples were collected for laboratory analysis. Exclusion criteria included inadequate medical documentation, immunodeficiency, and autoimmune diseases. There were no pregnant women among the patients.

From February until the end of 2024, data on patients meeting the inclusion criteria were collected prospectively. Data on age, sex, symptoms, vaccination status and laboratory analysis values were obtained from the Hospital and Laboratory Information System. To ensure complete accuracy and completeness of patient data, two authors reviewed the database twice.

The study was conducted with the approval of the Ethics Committee of the Clinical Center of the University of Sarajevo (No. 06-04-9-92174).

Methods

A venous blood sample was taken from all patients for biochemical, haematological, coagulation and serological analyses. Blood samples were collected from the cubital vein by venipuncture. Complete and differential blood counts were analysed from whole blood samples, basic biochemical and serological analyses from serum, and coagulation analyses from plasma. A DxH 900 haematology analyser (Beckman Coulter, USA) was used for haematological analyses, an Alinity biochemical analyser (Abbott, USA) for biochemical analyses, and a BCS CP (Siemens, Germany) for coagulation analyses. The reference interval for sodium (Na) was 135-145 mmol/l, potassium (K) 3.5-5.2 mmol/l, CRP 0-5 mg/l, aspartate aminotransferase (AST) 0-39 U/l, alanine aminotransferase (ALT) 0-37 U/l, creatine kinase (CK) 29-285 U/l and lactate dehydrogenase (LDH) 120-240 U/l. Serological analyses were performed using an ELISA (enzyme-linked immunosorbent assay) test to determine IgM (SERION ELISA classic Masern/Measles Virus IgM assay, Serion Diagnostics).

The neutrophil-to-lymphocyte ratio was calculated by dividing the absolute neutrophil count by the lymphocyte count (NLR = Neutrophils/Lymphocytes). The platelet-to-lymphocyte ratio was obtained by dividing the platelet count by the lymphocyte count (PLR = Platelets/Lymphocytes). The systemic immune-inflammation index was calculated as SII = (Platelets × Neutrophils)/Lymphocytes. The aggregate index of systemic inflammation is calculated as AISI = (Neutrophils × Monocytes × Platelets)/Lymphocytes. The systemic inflammation response index was calculated as SIRI = (Neutrophils × Monocytes)/Lymphocytes.

Statistical analysis

Continuous variables were presented as arithmetic means and standard deviations (SD), and categorical variables as absolute and relative numbers. Data analysis was focused on descriptive statistics and correlation testing. The correlation between HDI and the inflammatory parameter CRP was tested by Pearson's correlation test. The statistical significance threshold was set at $p<0.05$.

RESULTS

The study initially included 270 patients and after the application of exclusion criteria 124 adult (>18 years) patients with clinically and serologically confirmed measles infection over a period of 11 months were included. Of the total number of patients, 52 (41.94%) were men and 72 (58.06%) were women with a mean age of 38.33±13.18 years. The most common symptoms in most patients were rash (95.97%) and cough (69.36%). CRP had mean value 70.1 mg/L and it was elevated in 95.17% of patients. Liver transaminases (AST and ALT) were elevated in 75% of patients (Table 1).

Table 1. Demographic and clinical characteristics of adult patients with measles infection

Variables	
Age (mean±SD) (years)	38.33±13.18
	No (%) of patients
Gender	
Male	52 (41.94)
Female	72 (58.06)
Symptoms	
Rash	119 (95.97)
Cough	86 (69.36)
Koplik spot	80 (64,51)
Coryza	31 (25)
Conjuctivitis	20 (16.13)
Diarrhea	19 (15.32)
Vaccinated	
YES	34 (27.4)
NO	90 (27.4)
Laboratory findings (reference value)	
Leukopenia (<4 x10^9/l)	16 (12.9)
Neutrophilia (>7 x 10^9/l)	79 (63.7)
Lymphopenia (<1 x 10^9/l)	92 (74.19)
Thrombocytopenia (<150 x 10^9/l)	49 (39.52)
Elevated transaminases (>50 U/l)	93 (75)
Elevated CRP (>5 mg/l)	118 (95.17)
IgM (mean±SD) (U/l)	451.25±345.83

CRP, C-reactive protein; IgM, immunoglobulin M

The results of laboratory analyses indicated hyponatremia and hypokalaemia, with elevated values of CRP, transaminases, and the enzymes CK and LDH (Table 2). Mean sodium and potassium levels of 135.71 mmol/L and 3.83 mmol/L were observed.

Table 2. Overview of main laboratory findings and hemogram-derived and inflammatory indices of adult patients with measles

Parameter (reference value)	**Mean±SD**
Na (135-145 mmol/L)	135.71±3.3
K (3.5-5.2 mmol/ L)	3.83±0.48
CRP (0-5 mg/l)	70.1±58.07
AST (8-34 U/l)	148.53±119.87
ALT (4-37 U/l)	201.16±189.56
AST/ALT ratio	1.05±1.12
CK (30-150 U/L for females and 40-250 U/L for males)	417.27±582.37
LDH (140-280 U/l)	503.68±253.99
NLR (0.78 to 3.53)	8.13±7.81
PLR (100-200)	278.95±185.17
SII (161-701)	1452.16±1754.98
AISI	929.09±1787,49
SIRI	4.58±8.32

CRP, C-reactive protein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AST/ALT ratio, De Ritis ratio; CK, creatine kinase; LDH, lactate dehydrogenase; NLR, neutrophils/lymphocytes ratio; PLR, platelet/lymphocytes ratio; SII, systemic immune-inflammation index; AISI, aggregate index of systemic inflammation; SIRI, systemic inflammatory response index

CRP had positive, significant correlations with NLR, SIRI ($p<0.05$) and with SII and AISI ($p<0.01$) (Table 3). AST/ALT and PLR did not show a significant correlation.

Table 3. Correlation of hemogram-derived and inflammatory indices with C-reactive protein (CRP) in adults patients with measles

CRP	**AST/ALT**	**NLR**	**PLR**	**SII**	**AISI**	**SIRI**
rho	0.027	0.205*	0.183	0.397†	0.433†	0.250†
p	0.775	0.028	0.052	0.000	0.000	0.007

*p<0.05; †p<0.01
AST/ALT ratio, De Ritis ratio; NLR, neutrophils/lymphocytes ratio; PLR, platelet/lymphocytes ratio; SII, systemic immune-inflammation index; AISI, aggregate index of systemic inflammation; SIRI, systemic inflammatory response index

DISCUSSION

Bosnia and Herzegovina previously experienced two major measles epidemics: 1997–1999 and 2014–2015, with over 10,000 patients. In 2019, more than 700 cases were recorded, of which 92.5% were unvaccinated (9). The COVID-19 pandemic had a negative impact on routine vaccination programs. In the period from 2020 to 2022, DTP3 vaccine coverage decreased by 17.6%, while MMR1 coverage decreased by 26.7% (10). During the measles epidemic in 2024, a total of 7,477 cases were reported in the Federation of Bosnia and Herzegovina, of which 4,514 were reported in Sarajevo Canton (11). Therefore, the purpose of our study was to provide a comprehensive analysis of adult patients with confirmed measles infection at hospital admission, highlighting key clinical presentations, laboratory results, and the utility of determining hemogram-derived inflammatory indices.

The vaccine against measles is a basic preventive measure, which protects against the onset of infection or, in case of illness, the clinical picture is mild (1). However, even in immunized adults, it has been proven that its effectiveness and the concentration of IgG antibodies decrease over the years, which may to some extent explain the emergence of this highly contagious viral infection, and thus increase the risk of an epidemic (12).

The vast majority of our patients exhibited classic measles symptoms, particularly rash and cough, aligning with the standard clinical presentation of measles in adults (13). Koplik's spots, were present in more than half of the cases, which is similar to findings from China (14, 15).

Neutrophilia and lymphopenia were prominent hematologic findings, consistent with the known immunosuppressive effects of measles virus on lymphocyte populations (16). Casasoprana A et al also reported that 95% of patients had lymphopenia and 49 had thrombocytopenia in their study of 134 measles patients (17). Elevated CRP and liver transaminases were also observed, indicating a robust systemic inflammatory response and hepatic involvement. Similar hepatic abnormalities have been presented in other adult measles studies, suggesting that transient hepatitis may be a common complication (18,19).

Additionally, elevated levels of CK and LDH point toward muscle involvement and generalized cellular injury, possibly due to viral cytopathic effects or immune-mediated damage (20). Hyponatremia and hypokalaemia were also observed. These findings may be attributed to gastrointestinal symptoms (diarrhea and vomiting) or syndrome of inappropriate antidiuretic hormone secretion, both of which have been reported in adult viral infections (21).

This study evaluated novel hemogram-derived inflammatory indices - NLR, PLR, SII, AISI, and SIRI, all of which were markedly elevated. Their altered values, in this case elevated, were the result of changes in the differential blood count (6). These indices showed significant positive correlation with CRP, suggesting their potential role as biomarkers for the laboratory diagnostics of measles infection in adult patients. For comparison, in a study involving 71 patients with a moderate clinical picture, NLR had a mean value of 5.74 against our study where NLR was 8.13 among 124 patients (18). Previous studies have validated these markers in other viral and inflammatory diseases, emphasizing their prognostic relevance (6). Their use could assist clinicians in early identification of patients at risk for complications, especially in resource-limited settings (22). Therefore, these results are the first to describe the diagnostic potential of hemogram-derived indices in adult patients with measles.

The study has several important strengths and limitations. According to the available literature, our study included a relatively large number of adult patients with comprehensive clinical and laboratory data. Also, to our knowledge, this is the first study to examine the significance of using new hemogram-derived indices in measles diagnosis. Limitations of the study include its single-centre design, which may affect external validity. Additionally, data on IgG results, clinical outcomes, and long-term sequelae were not available this time and they should be studied further.

In conclusion, adult measles infection is associated with a pronounced systemic inflammatory response, as evidenced by frequent haematological abnormalities, elevated CRP, liver enzyme disturbances, and electrolyte imbalances. The strong correlation between CRP and hemogram-derived inflammatory indices such as NLR, SII, AISI, and SIRI suggests that these readily available markers may serve as useful parameters for adult patients at hospital admission. Early identification of patients with symptoms through simple laboratory indices could improve the management and outcomes in adult measles cases. Further research is

needed to validate the prognostic value of these inflammatory markers in larger and more diverse populations.

AUTHOR CONTRIBUTIONS

Conceptualization, B.H., A.M., S.H.; methodology, B.H.; validation, B.H., L.A., and E.H.H.; formal analysis, B.H., and E.Š.; data curation, B.H., A.P., A.M., and A.S.; writing—original draft preparation, A.M., S.H., and B.H.; writing - review and editing, B.H.; visualization, E.Š.; supervision, B.H., L.A., E.H.H., and A.P.; project administration, B.H. All authors have read and agreed to the published version of the manuscript.

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Med Glas (Zenica)

ORIGINAL ARTICLE

Seroprevalence of *Helicobacter pylori* in Bosnia and Herzegovina: a single-center cross-sectional study

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ABSTRACT

Aim Many studies have demonstrated that more than half of the world's population is infected with *Helicobacter pylori* (*H. pylori*). To evaluate the current *H. pylori* seroprevalence in Bosnia and Herzegovina (B&H), *H. pylori* antibodies (immunoglobulin G, IgG) from patients with suspected presence were analysed.
Methods In total, 201/471 (42.7%) males and 270/471 (57.3%) females were enrolled between June 2024 and July 2024. They were tested using the enzyme-linked immunosorbent assay (ELISA) method.
Results The overall seroprevalence of *H. pylori* infection was 214 (out of 471; 45.4%) and did not differ in relation to sex. The seroprevalence rate of *H. pylori* was highest in the 50–69 age group, 81 (out of 137; 59.1%; 95% CI: 1.9–5.9), followed by ≥70 age group, 17 (out of 31; 54.8%; CI: 1.5–6.2) and the 30–49 age group, 101 (out of 219; 46.1%; 95% CI: 1.5–4.6), he lowest seroprevalence rate was in the younger age group (≤29) with 15 (out of 84; 17.9%). Older age groups were more likely to be *H. pylori* positive and equivocal, compared to younger age groups.
Conclusion This single-center study is the first study providing information on the *H. pylori* seroprevalence in the B&H population and investigating its association with age and sex. Further research is needed to explore other risk factors and to develop effective ways to reduce the burden of this infection.
Keywords: age distribution, cross-sectional studies, enzyme-linked immunosorbent assay, immunoglobulin G, prevalence

INTRODUCTION

Helicobacter pylori (*H. pylori*) infection is one of the most prevalent bacterial infections worldwide (1), with nearly half of the global population estimated to be infected (2,3). The infection is particularly common in low- and middle-income countries (4).

Recent global meta-analyses showed that the prevalence of *H. pylori* infection declined from 52.6% before 1990 to 43.9% between 2015 and 2022, mainly due to decreases in the Western Pacific, Southeast Asian, and African regions. However, infection rates remain high in other parts of the world, especially among children and adolescents (5).

In addition to these differences in prevalence, the growing problem of antibiotic resistance, particularly to clarithromycin (6), has become a major obstacle to successful *H. pylori* eradication (7). Studies report that resistance rates frequently exceed 15% in the Balkans region, reaching around 28% in B&H (8), 24% in Serbia (9), and 34.6% in Croatia (10). In Slovenia, however, the resistance rate has declined from 16% during 2013–2018 to 13.5% in 2019–2023 (11,12). Given these resistance rates, current international guidelines emphasize the need to adapt eradication therapy to local resistance patterns. According to the Maastricht VI guidelines, when clarithromycin resistance exceeds 15% in a population, standard triple therapy is no longer considered adequate (13). In such circumstances, bismuth quadruple therapy (BQT) is recommended because it avoids clarithromycin resistance and effectively overcomes metronidazole resistance, maintaining eradication rates typically above 90% (14,15). This makes BQT the preferred first-line regimen in areas with elevated antibiotic resistance (13,16).

In this context, there is a clear need for current, locally generated data on *H. pylori* seroprevalence to support evidence-based management and prevention efforts.

Therefore, the aim of this study was to conduct a preliminary study on *H. pylori* seroprevalence in B&H and explore its variation across different age groups and between sexes to establish a basis for future population-based studies.

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PATIENTS AND METHODS

Study design and patients

This single-center cross-sectional study was conducted in the Eurofarm Polyclinic in Sarajevo. A consecutive sampling method was employed, including all patients who came to the laboratory for *H. pylori* testing and consented to participate between 8 June and 25 July 2024. Out of 500 patients, 471 (201 males and 270 females) were included in the study. The remaining 29 were excluded because of missing data or insufficient sample quality (Figure 1). No further exclusion and inclusion criteria were applied. Since the study was anonymous, the only data of recruited patients available for statistical analyses were age and sex.

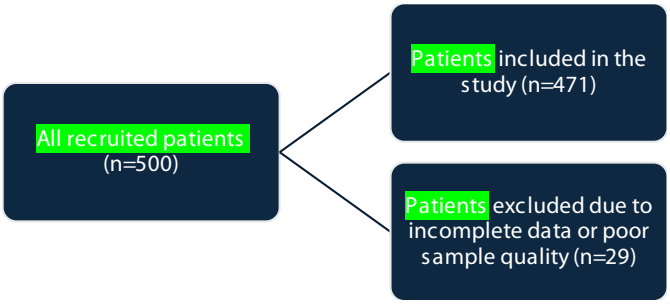

Figure 1. Patient flow diagram

The study was approved by the Ethical Committee of the Faculty of Engineering and Natural Sciences, International Burch University, Sarajevo, B&H. All patients older than 18 signed a written informed consent prior to the study, whereas parents or legal guardians provided consent for patients who were less than 18 years old.

Methods

Blood samples (3 mL) were obtained from a peripheral vein using standard venipuncture technique. Samples were kept frozen at -30°C until analysis, and each was subjected to a single freeze-thaw cycle prior to testing. *H. pylori* immunoglobulin G (IgG) was detected using commercial kits for enzyme-linked immunosorbent assay (ELISA, Euroimmun, Medizinische Laboratordiagnostika AG, Germany).

According to Euroimmun protocol (17), intra-assay coefficients of variation, determined from 20 replicates, ranged from 3.1% to 3.2%. Inter-assay coefficients of variation, based on four replicates across six runs, ranged from 3.4% to 4.6%. Sensitivity and specificity of 100% were reported based on 59 clinically characterized samples, excluding borderline results. No sensitivity or specificity data specific to the Balkan population are available.

The laboratory did not participate in external quality assessment programs during the study. Testing was performed according to the manufacturer's instructions following sample incubation, washing, conjugate incubation, washing, substrate incubation, and stopping the reaction. Afterwards, the colour intensity of samples was measured at a wavelength of 450 nm on ELISA plate reader.

Patients' *H. pylori* status was classified according to Euroimmun guidelines (17) as positive (ratio ≥1.1), equivocal (ratio ≥0.8 to <1.1), or negative (ratio <0.8). Equivocal results were not retested and were considered negative.

A post-hoc precision calculation for observed prevalence of 45.4% with a 95% confidence level (α=0.05) and sample size (n=471) yielded a confidence interval of (40.9%–49.9%), corresponding to a precision of ±4.5%.

Statistical analysis

To summarize the data, descriptive statistics were employed. The study employed the $\chi 2$ test to assess the association between categorical variables and Cramer's V to measure the strength of the association. The association for Cramer's V value was considered weak (<0.1), moderate (0.11–0.31), or strong (>0.31).

Log-binomial regression was conducted to estimate the prevalence ratio (PR) with 95% confidence intervals (CI). A $p<0.05$ was considered statistically significant.

RESULTS

In total, serum samples from 471 patients aged 3–88 years were collected. Of included patients, 214 tested positive for *H. pylori*, resulting in a seroprevalence of *H. pylori* infection of 45.4% in the study population. A higher number of samples was obtained from females, 270 (57.3%), than from males, 201 (42.7%) (Table 1).

The seroprevalence rates of anti-*H. pylori* IgG in females and males were similar, 126 (46.7%) and 88 (43.8%), respectively, without significant association between sex and *H. pylori* seroprevalence (p=0.788; Cramer's V=0.032). Log-binomial regression analysis showed that males had approximately 6% lower prevalence compared to females (PR=0.94; 95% CI: 0.7–1.2), but this difference was not statistically significant (p=0.646) (Table 1).

Most of tested patients were middle-aged, between 30–49 years, 219 (46.5%) (Table 2).

The seroprevalence rate of anti-*H. pylori* IgG was lowest in the youngest patients (≤29 years), 15 (17.9%), serving as the reference group. Prevalence increased substantially in older age groups: 46.1% among those aged 30–49 (PR=2.6; 95% CI: 1.5–4.6), 59.1% in the 50–69 group (PR=3.3; 95% CI: 1.9–5.9), and 54.8% in those aged ≥70 (PR=3.1; 95% CI: 1.5–6.2)

Table 1. Association between sex and *H. pylori* seroprevalence

Sex (No of patients)	No (%) of patients				PR (95% CI)*	p (regression)†	Cramer's V‡	χ^2 (p)§
	Positive	Equivocal	Negative	Total				
Female (270)	126 (46.7)	17 (6.3)	127 (47.0)	270 (57.3)	1 (Reference)	—	0.032	0.477 (0.788)
Male (201)	88 (43.8)	12 (6.0)	101 (50.2)	201 (42.7)	0.94 (0.7–1.2)	0.646		
Total (471)	214 (45.4)	29 (6.2)	228 (48.4)	471 (100)	—	—	—	—

*Prevalence Ratio (PR), ratio of prevalence between males and females (females as reference group); †p, statistical significance of log-binomial regression with 'Positive' as outcome and Female' as reference group; ‡Cramer's V test (ranges from 0 to 1; 0.032 indicates a weak association); §χ^2 test, association between sex and *H. pylori* status

Table 2. Association between age and *H. pylori* seroprevalence

Age group (No of patients) (years)	No (%) of patients				PR (95% CI)*	p (regression)†	Cramer's V‡	χ^2 (p)δ
	Positive	Equivocal	Negative	Total				
≤29 (84)	15 (17.9)	1 (1.2)	68 (81.0)	84 (17.8)	1 (Reference)	—		
30–49 (219)	101 (46.1)	14 (6.4)	104 (47.5)	219 (46.5)	2.6 (1.5–4.6)	<0.001	0.319	55.215 (<0.0001)
50–69 (137)	81 (59.1)	9 (6.6)	47 (34.3)	137 (29.1)	3.3 (1.9–5.9)	<0.001		
≥70 (31)	17 (54.8)	5 (16.1)	9 (29.0)	31 (6.6)	3.1 (1.5–6.7)	<0.001		
Total	214 (45.4)	29 (6.2)	228 (48.4)	471 (100)	—	—	—	—

*Prevalence Ratio (PR), ratio of prevalence between different age groups (<29 as reference group); †p, statistical significance of log-binomial regression with 'Positive' as outcome and '≤29 years' as reference group); ‡Cramer's V test (ranges from 0 to 1; 0.242 indicates a moderate association); δχ^2 test, association between age and *H. pylori* status)

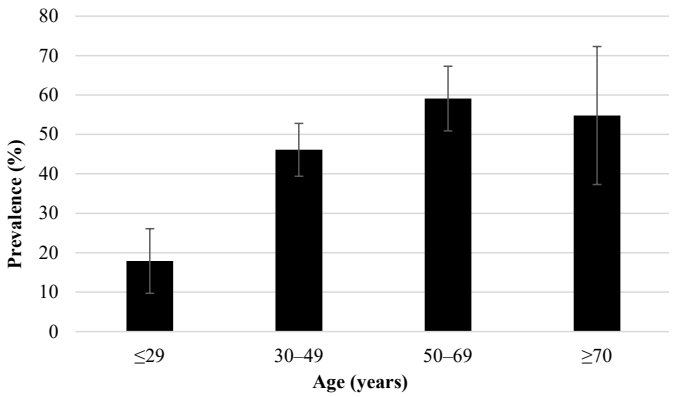

Figure 2. Age-stratified bar plot (with 95% confidence interval)

(Figure 2). A significant association was found between age group and infection status (p<0.0001), with the strength of this relationship assessed by Cramer's V at 0.242, indicating a moderate association (Table 2).

DISCUSSION

In this single-center cross-sectional study conducted in B&H, 45.4% of all tested patients were serologically positive to *H. pylori*, 6.2% were equivocal, and 48.4% were negative. These results indicate a substantial presence of *H. pylori* infection within the sampled population. When analysing demographic factors, no significant association was found between sex and *H. pylori* infection status. However, age was strongly associated with infection status, with older patients showing substantially higher prevalence rates compared to younger patients. The lowest prevalence was seen in the youngest age group (≤29 years), while infection rates increased progressively with age. This trend likely reflects cumulative lifetime exposure, variations in living conditions, and differences in health behaviours across age groups.

A recent global seroprevalence study confirmed the trend of high seroprevalence rates in the Balkans region with 13.3% in Croatia, 38.5% in Serbia and even 63.0% in Albania (5). When compared with these data from neighbouring countries, our findings indicate a higher seroprevalence in B&H compared to Croatia and Serbia, but still lower than the prevalence reported in Albania. This suggests that B&H fits within the regional pattern of a higher seroprevalence rate of *H. pylori*, although differences in socioeconomic status, sanitation, and healthcare access may influence variations between the countries.

Both the high seroprevalence rate of *H. pylori*, particularly among older populations, and the high clarithromycin resistance earlier reported to be 28% (8) underscore the need for targeted public health interventions and localized treatment strategies in B&H. According to GLOBOCAN 2022 data, B&H reported 687 new cases of stomach cancer in 2022, representing roughly 4.8% of all cancer diagnoses in the country (18). Effective *H. pylori* eradication is also a key strategy for gastric cancer prevention, given that persistent *H. pylori* infection is a major risk factor for malignancy (19).

Although this study represents an important first step in defining the seroepidemiology of *H. pylori* in B&H, it had several limitations that need to be considered. First, as the study was conducted at a single laboratory with the relatively small number of samples, the results may be affected by referral bias, potentially leading to an overestimation of the true seroprevalence in the general population and may affect the generalizability of our findings. Consequently, the findings should be interpreted as preliminary, providing a basis for further, larger-scale population studies in B&H. Second, the other relevant factors known to influence the risk of *H. pylori* infection, such as educational level, living conditions and sanitation, the type of residence (urban or rural), smoking, dietary habits, body mass index, were not included in our analysis. Inclusion of these variables could provide deeper insights into the epidemiological patterns and risk factors associated with *H. pylori* infection. Third, we did not collect data on patients' symptomatic status, clinical indications for testing (e.g., dyspepsia or post-therapy evaluation), prior *Helicobacter pylori* eradication treatments, recent systemic antibiotic use, or current proton pump inhibitor therapy (13). The absence of these data limits the ability to stratify prevalence accordingly and may have introduced the bias affecting the accuracy of our seroprevalence estimates. Fourth, the enrolment period in our study was relatively short, which may have resulted in seasonal or temporal biases. A longer enrolment period would allow for the capture of more comprehensive data, reducing potential variability and providing more accurate reflection of the seroprevalence rates over time.

Even though serological analysis is one of the most accessible and inexpensive methods for detecting *H. pylori* infection, it has important limitations that need to be acknowledged. IgG antibodies cannot distinguish active from past infection and remain detectable for months or even years after eradication, meaning that serology cannot be used to confirm cure (20). Moreover, because antibody levels decline slowly after successful treatment, studies based on serological diagnostic methods may lead to overestimation of prevalence, particularly in studies based on symptomatic populations (21).

In our study, age and sex were analysed as key demographic factors in relation to *H. pylori* seroprevalence. Although the influence on infection appears limited, both may affect host susceptibility and immune response. Age can modulate the

gastric inflammatory pattern during infection, while hormonal and behavioural differences between sexes may partly explain variations in infection rates (22).

A probability-based, multicentric follow-up study considering the relevant determinants mentioned in the limitations of the study is therefore recommended to provide a more comprehensive understanding of *H. pylori* epidemiology and to improve future public health strategies.

In conclusion, this is the first study to provide data on *H. pylori* seroprevalence in B&H. While limited by its single-canter cross-sectional design, the findings highlight the presence of a substantial burden of infection in the population. Age and sex, as key demographic factors with a negligible role in *H. pylori* infection, were included to assess their association within the studied population and to provide a basis for future research exploring additional risk factors and guiding public health strategies to reduce infection rates.

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Conflicts of interest: None to declare

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ORIGINAL ARTICLE

Infectious spondylodiscitis treated at the Department of Infectious Diseases, Cantonal Hospital Zenica, during the period from 2022 to 2023

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ABSTRACT

Aim Infectious spondylodiscitis is a rare but serious spinal infection that often presents with nonspecific symptoms like fever and back pain, causing delayed diagnosis. This study aimed to describe the clinical features, identify etiological agents, and outline treatment approach in patients treated at the Department of Infectious Diseases, Cantonal Hospital Zenica, Bosnia and Herzegovina, from January 2022 to December 2023.
Methods This retrospective descriptive study included 60 patients diagnosed with infectious spondylodiscitis based on clinical symptoms (fever and/or spinal pain) and confirmed by magnetic resonance imaging (MRI) or scintigraphy. Data were collected from medical records and included demographic information, clinical presentation, laboratory results: haemoglobin, leukocytes, C-reactive protein (CRP), liver enzymes), microbiological findings (blood cultures and serology), imaging, and treatment details.
Results Median time from symptom onset to hospital admission was 30 days. Blood culture was positive in 31 (51.6%) patients, while etiology was identified in 57 (95%) cases. *Brucella* species was the most common pathogen, confirmed serologically in 46 (90.2%) brucella cases. Most patients had a history of unpasteurized dairy consumption or animal contact. The MRI was performed in 58 (96.7%) patients, confirming vertebral inflammation. Complications such as abscesses, epidural collections, or empyema occurred in 35 (58.3%) patients.
Conclusion Due to its nonspecific presentation, infectious spondylodiscitis is often diagnosed late. In endemic regions, brucellosis should be considered as a potential cause. Blood cultures and serology are key for etiological confirmation, while MRI remains the diagnostic gold standard. Targeted antimicrobial therapy is the mainstay of treatment, with surgery indicated in complicated cases.
Keywords: antibiotics, brucellosis, magnetic resonance imaging (MRI), vertebral osteomyelitis

INTRODUCTION

Infectious spondylodiscitis (vertebral osteomyelitis) is an infection that affects the intervertebral discs and adjacent vertebrae, causing inflammation and destruction of the affected structures. Although rare, spondylodiscitis carries a high risk of severe complications, including irreversible sequelae if not recognized and treated promptly (1). Spondylodiscitis is often referred to as a chameleon among infectious diseases due to the lack of specific symptoms, which often leads to delayed diagnosis (2). Infectious spondylodiscitis may be caused by bacterial, fungal or parasitic organisms, with bacteria being the most common source of infection encountered in clinical practice (3). *Staphylococcus aureus* was reported as the predominant pathogen for bacterial spondylodiscitis (4). Tuberculous spondylodiscitis, also known as Pott's disease, remains a major public health issue, particularly in regions with high tuberculosis prevalence (5).

Brucellar spondylodiscitis is most commonly seen in countries where brucellosis remains endemic, including Bosnia and Herzegovina (6). Compared with pyogenic spondylodiscitis, brucellar spondylodiscitis has a slower symptoms progression with a long and recurrent course (6). Fungal infections are extremely rare, mostly responsible for opportunistic infections in immunocompromised patients. The most common causative agents are *Candida spp.* and *Aspergillus spp*. (7).

This study was conducted after an increasing number of hospitalized patients with spondylodiscitis was observed in our Department. Our aim was to analyse the etiological spectrum, leading clinical manifestations, radiological findings, treatment modalities, and treatment duration in order to improve patient management in the future. While international literature often emphasizes surgical approaches and CT-guided microbiological diagnostics, our study highlights the predom-

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inance and effectiveness of conservative treatment in our setting, where invasive diagnostic procedures are not routinely performed and a prevalence of cases remains etiologically unresolved. There are several studies in our country and region, mostly from infectious disease specialists, regarding this topic, that have examined etiology and treatment duration (8,9). Presented research reflects local epidemiological characteristics, therapeutic limitations and outcome.

The aim of this study was to investigate demographic, epidemiological, clinical, and laboratory characteristics, etiology, treatment and outcomes of patients with infectious spondylodiscitis treated at the Department of Infectious Diseases, Cantonal Hospital Zenica, over the period of two years.

PATIENTS AND METHODS

Patients and study design

This retrospective descriptive study analysed medical histories of 60 patients diagnosed with infectious spondylodiscitis who received treatment at Department of Infectious Diseases in the Cantonal Hospital Zenica, Bosnia and Herzegovina, from 1 January 2022 to 31 December 2023.

The Cantonal Hospital Zenica has 858 beds, with 60 beds in the Department of Infectious Diseases. Each year, 9,000 to 10,000 patients pass through the emergency admissions unit at our Department, and around 1,300 are hospitalized. The hospital provides medical care for the Zenica-Doboj Canton and Central Bosnia Canton, with around 300,000 and 250,000 inhabitants, respectively.

The diagnosis of spondylodiscitis was based on the presence of fever and/or spinal pain including changes verified on spine MRI scan or on a scintigraphic examination.

The study was approved by the Ethics Committee of the Cantonal Hospital Zenica (No: 00-03-35-18-6/25). It was conducted in compliance with the Ethical Principles for Medical Research Involving Human Subjects outlined in the Helsinki Declaration.

Methods

The data used for this study were obtained from the patients' medical records, including socio-demographic and epidemiological characteristics (age, gender, urban or rural residence, contact with domestic animals, consumption of unpasteurized dairy products), symptoms prior to hospitalization, clinical manifestation (back pain, fever, weakness, loss of weight, loss of appetite, night sweats), laboratory findings (haemoglobin level, erythrocytes, leukocytes, neutrophil granulocytes and platelets count, urea, creatinine, level of C-reactive protein - CRP, as well as aspartate aminotransferase and alanine aminotransferase level), blood cultures, serology tests (Rose Bengal test and enzyme-linked immunosorbent assay - ELISA for detection of anti-Brucella antibodies), contrast enhanced magnetic resonance imaging (MRI) findings, duration and type of antibiotic treatment.

Statistical analysis

Categorical variables are described using frequencies and percentages, while numerical variables are presented with medians and ranges.

RESULTS

A total of 60 patients treated at the Department of Infectious Diseases of the Cantonal Hospital Zenica with a diagnosis of infectious spondylodiscitis were included in the study. Of these, 42 (70.0%) were male and 18 (30%) were female; median age was 67 years (range 13–87 years).

Thirty-nine (65.0%) patients were from Zenica-Doboj Canton, while 21 patients (35.0%) were from Central Bosnia Canton; 54 (90.0 %) patients lived in rural, six (10.0%) patients in urban area. Among brucellosis spondylodiscitis, 33 (out of.51; 64.7%%) had a history of both consumption of the raw milk and close animal (sheep, goat or cow) contact. Other patients, 18 (35.2%), with brucellar spondylodiscitis had negative or unclear epidemiological histories. Patients with spondylodiscitis of other etiologies had endogenous infection routes (dental caries, urinary infections, otitis), except for one patient whose definitive causative agent was not identified.

Overall median time from symptom onset to hospitalization was 30 (range 2 – 365) days. During the initial examination, the predominant clinical presentations were back pain and fever (Table 1), accompanied by moderately elevated CRP level (Table 2).

Table 1. Main clinical manifestations in 60 patients with spondylodiscitis

Symptom	No (%) of patients
Back pain	53 (88.3)
Fever	50 (83.3)
Sweating	46 (76.7)
Malaise	40 (66.7)
Loss of appetite	40 (66.7)
Weight loss	21 (35.0)

Table 2. Laboratory parameters in 60 patients with infectious spondylodiscitis

Laboratory parameter (reference value)	Median (range)
Leukocytes (3.4–10.0) (x 10^9/L)	8.0 (2.7-29.3)
Erythrocytes (3.9–5.1) (x 10^{12}/L)	4.2 (3.2– 5.6)
Hemoglobin (119.0–157.0) (g/L)	119.0 (98.0–162.0)
Neutrophils (2.1–6.5) (x 10^9/L)	3.7 (0.4–26.3)
Platelets (150.0–400.0) (x 10^9/L)	260.0 (0.4–26.3)
CRP (0.0–10.0) (mg/L)	33.1 (0.1–239.0)
AST (10.0–34.0) (U/L)	36.5 (9.0–128.0)
ALT (10.0–49.0) (U/L)	42.9 (6.0–185.0)
Urea (3.2–8.2) (mmol/L)	5.5 (2.5–27.1)
Creatinine (44.2–97.2) (μmol/L)	65.0 (37.0–246.0)

CRP, C-reactive protein; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase

Brucella species (spp.) was the most common causative agent in patients with spondylodiscitis, identified in 51 (85%) cases, whereas among patients with brucellar spondylodiscitis, the disease was serologically confirmed in 46 (90.2%) patients. (Table 3.)

Positive blood culture was found in 31 (51.7%) patients: *Brucella* spp. in 25 (80.6%), *Staphylococcus aureus* in four (12.9%), *Escherichia coli* in one (3.2%), and *Proteus mirabilis* in one (3.2%) patient.

Table 3. Etiological agents identified in blood cultures

Microorganism isolated	Number (%) of patients
Brucella species	25 (80.6)
Staphylococcus aureus	4 (12.9)
Escherichia coli	1 (3.2)
Proteus mirabilis	1 (3.2)
Unknown	3 (9.7)

The MRI scan was performed in 58 (96.7%) patients revealing inflammatory disorders of the vertebral column in all cases. Bone scintigraphy was used in two (3.3%) patients as MRI could not be performed due to the presence of metallic foreign bodies. The scitigraphy showed increased radiopharmaceutical accumulation in the thoracic segments of the spine (the data are not shown).

Our data showed that 25 (41.7%) patents had isolated spondylodiscitis, while 35 (58.3%) had additional complications such as abscesses, epidural collections, or empyema. Affected areas included the psoas muscle (often bilateral or ipsilateral), paravertebral muscles (edema, cellulitis, or fluid collections), sacroiliac joints (sacroiliitis), and gluteal muscles (edema or fluid collections). Among the 35 patients with additional complications, 28 (80.0%%) had brucella spondylodiscitis, and seven (20%) had pyogenic spondylodiscitis (four caused by *Staphylococcus aureus*, one caused by *Proteus mirabilis*, one by *Escherichia coli*, and one by an unknown agent (the data are not shown).

Spinal segments were affected by spondylodiscitis in 82 patients, lumbosacral in 67, cervical in five, and thoracic region in 10 patients. In 48 patients, only one spinal segment was affected, and 12 patients had changes in 34 spinal segments; 10 of 12 patients from the last group had brucellar spondylodiscitis, one had staphylococcal spondylodiscitis, and one had spondylodiscitis of unknown etiology (the data are not shown).

Patients with spondylodiscitis were treated with combined antibiotic therapy involving two or three antibiotics, depending on the etiology. Spondylodiscitis caused by *Brucella* infection were treated with doxycycline 2x100 mg and gentamicin 2x80 mg or 2x120 mg (depending on the age of the patient and creatinine level), for three weeks, followed by doxycycline and rifampicin 3x300 mg for three, six, or nine months (depending on clinical presentation and MRI findings). The average duration of therapy for brucellar spondylodiscitis was six months (the data are not shown).

For the treatment of patients with non-brucellar spondylodiscitis, the most commonly used antibiotic combinations included (daily dose) cloxacillin 6x2 g with gentamicin 2x80 mg or 2x120 mg or meropenem 3x2 g with vancomycin 2x1 g for three to six months, as well as piperacillin and tazobactam 4x4.5 g together with metronidazole 3x500 mg or clindamycin 3x600 mg for four to six months. Despite the therapy, one patient with spondylodiscitis died during the hospital stay due to sepsis caused by *Staphylococcus aureus* (the data are not shown).

Post-treatment care and patients' follow-up involved regular clinical assessments and imaging diagnostics. No significant sequelae were observed, except for occasional mild to moderate pain in the affected spinal segments. There were no cases of relapse or reinfection of the primary disease.

Follow-up MRIs of the spine were performed after completing antibiotic therapy. Successful treatment outcomes were observed, with regression of spinal lesions following the conclusion of therapy (Figures 1, 2).

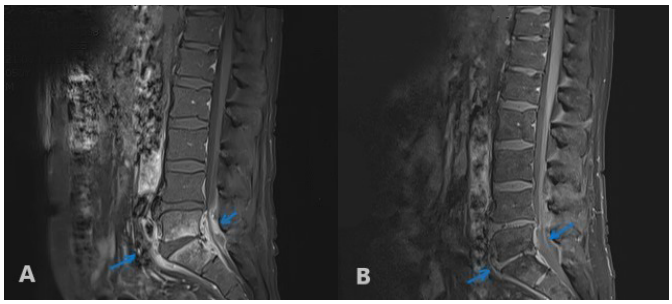

Figure 1. A) Inflammatory changes at the L5/S1 level with fluid collections compressing the dural sac, affecting the pre- and paravertebral musculature on the right side (arrow) (MRI) (Cantonal Hospital Zenica, 2024, 2023)**; B) Complete regression of previously observed inflammatory changes (arrow) (MRI)** (Cantonal Hospital Zenica, 2024)

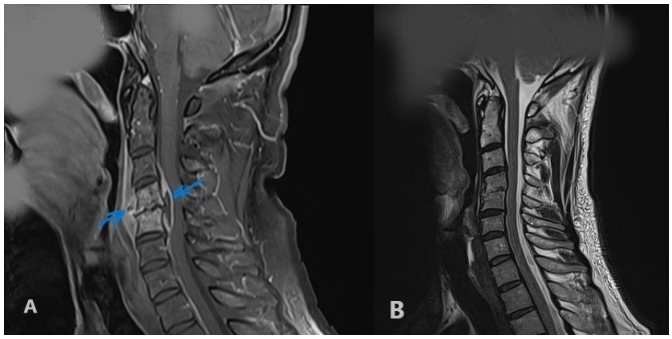

Figure 2. A) Pronounced inflammatory changes at the C4/C5 level with a reduction in intervertebral space width, erosions of both disc endplates, inflammatory changes in the prevertebral area, and within the spinal canal at the same level, and a fluid collection with a compressive effect on the spinal cord (arrow) (MRI) (Cantonal Hospital Zenica, 2023)**; B) Complete regression of inflammatory changes in the prevertebral area and spinal canal, along with post-inflammatory changes at the C4/C5 disc level (MRI)** (Cantonal Hospital Zenica, 2024)

DISCUSSION

Infectious spondylodiscitis presents a significant diagnostic and therapeutic challenge. Diagnosis is often delayed or missed due to the rarity of the disease and nonspecific symptomatology. The annual incidence in Europe has been estimated to be from 4–24 cases per million (10). Our findings demonstrated that the male/female spondylodiscitis rate complies with global data. It has been reported that the infectious spondylodiscitis is more common in females (male - female ratio 1.5–2:1) (11,12). Some studies showed that 56.7% of cases involved male patients (13). The reasons for male predominance in infectious spondylodiscitis remain unclear; reportedly a male predominance related mainly to gender-related differences in risk factor distribution, such as intravenous drug use, comorbidities, and prior interventions (14).

Spondylodiscitis has an increasing incidence by age, and with regard to all age groups, it occurs most frequently in patients >75 years of age (15). According to our study, the average age of patients with spondylodiscitis was lower than the average age presented in global data (15), which might be explained by

the predominance of brucellar spondylodiscitis in our cohort, a condition that more commonly affected younger individuals. Spondylodiscitis is characterized by nonspecific clinical symptoms in its early stage, such as mild fever, general malaise, weakness and weight loss, while pain and neurological symptoms typically appear later, also presented in our study. Similar to our results, available literature reports the lack of specificity of the symptoms in the initial phase of the disease, often with a delay of several weeks and up to six months in its diagnosis (2,15,16).

Laboratory findings indicating inflammation vary depending on the causative agent, which explains the wide range of the symptoms observed in our patients, comparable to findings in other studies (17). Spondylodiscitis Diagnosis and Treatment (SponDT) score, a tool for diagnosing spondylodiscitis, incorporates CRP level, pain level according to a numeric rating scale (NRS), and MRI stage of the disease according to Flamme. Based on the SponDT score, spondylodiscitis can be classified as severe, moderate and light/healed (18). The SponDT score was not utilized in our institution and thus not included in this study. Its potential application as a clinical tool may enable faster assessment of disease severity and stage, as well as better treatment planning in the future. Due to its simplicity, the SponDT score could serve as a valuable guide in future research (18).

Accurate etiological diagnosis is crucial for targeted antibiotic therapy. In our study, the etiological diagnosis was established in most cases. The most common causative agent in our sample was *Brucella species*, reflecting the endemic presence of brucellosis, which accounts for 21–48% of spinal infections in endemic areas (19)

Blood culture and serology are critical diagnostic techniques in infectious spondylodiscitis, as they enable precise identification of pathogens and detection of specific antibodies or antigens, allowing for adequate and targeted treatment. Pathogens can be detected in 40–70% of patients who have not previously undergone antibiotic treatment (20). Our results showed 51.7% of patients had positive blood cultures, which is consistent with previously published data.

The MRI is a preferable method for the diagnosis of spondylodiscitis. The sensitivity and specificity of MRI are 96% and 92%, respectively (21). The MRI remains the gold standard for diagnosing spondylodiscitis, although it does not aid in determining the exact etiology (22). The MRI was used not only for the diagnosis but also for monitoring therapeutic response. Cases involving adjacent structures, such as epidural, paravertebral, and psoas abscesses, were observed in this study and have been similarly reported in the literature (5,23,24).

Studies highlight the use of various antimicrobial drug classes for spondylodiscitis treatment based on blood culture and serology results, with surgical interventions performed in indicated cases (25,26). The length and course of conservative treatment for brucellar bone complications depend on both clinical monitoring and repeated MRI evaluations (9). Treatment optimization remains a challenge, and minimally invasive surgery should be considered alongside antibiotic treatment. Early surgical intervention is associated with reduced relapse and mortality risks (27). Indications for surgical treatment include failure of conservative therapy, mechanical instability, compression of neurological structures, and worsening of neurological deficits (28). In our study, no patients underwent surgical treatment or invasive diagnostic procedures.

Our study demonstrated one death related to sepsis. It is estimated that the global mortality rate for in-hospital-treated acute spondylodiscitis with sepsis (systemic inflammatory response syndrome – SIRS) is approximately 17%, while in other types of subacute/chronic spondylodiscitis it is up to 2% (29).

The limitations of this study are its retrospective nature and the small number of patients included. Only nine patients had pyogenic spondylodiscitis, and due to early antibiotic therapy in septic patients, fewer metastatic lesions were documented. Our study did not include patients with tuberculous spondylitis, as their diagnosis and treatment were not conducted in our Department, but was instead under the supervision of a pulmonologist. Despite these limitations, our findings show a favourable rate of recovery and support the effectiveness of conservative management in our patients.

In line with our aim, this study provides a comprehensive description of the clinical spectrum, etiological profile, and treatment approaches of infectious spondylodiscitis in a Bosnian cohort. The scientific novelty of our research is highlighting the predominance and outcomes of conservative treatment in a setting where invasive diagnostic procedures are limited showing brucellosis as an important etiological factor. Unlike many international studies that focus on surgical management and advanced microbiological diagnostics, our findings emphasize that careful clinical assessment, imaging, and tailored antimicrobial therapy can achieve good results even in resource-limited contexts.

In conclusion, infectious spondylodiscitis is often diagnosed late due to its nonspecific symptomatology. The endemic presence of brucellosis in certain regions highlights the epidemiological link between spondylodiscitis and this infection, which should be considered during diagnostic evaluations. Laboratory findings are less specific indicators in diagnosing spondylodiscitis, while blood culture and serological tests are critical for etiological diagnosis. The MRI remains the gold standard for diagnosing and monitoring spondylodiscitis. Treatment of infectious spondylodiscitis should primarily rely on antimicrobial drugs based on the etiological diagnosis, supplemented with surgical procedures in selected cases.

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TRANSPARENCY DECLARATION

Conflict of interest: None to declare.

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ORIGINAL ARTICLE

Prophylactic use of rectal diclofenac in mitigating post-endoscopic retrograde cholangiopancreatography pancreatitis risk and severity: low- vs high-dose efficacy and timing strategies— a meta-analysis

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ABSTRACT

Aim Post endoscopic retrograde cholangiopancreatography (ERCP) pancreatitis (PEP) is the most prevalent adverse event with significant morbidity following ERCP. Several guidelines have suggested the use of rectal diclofenac as a promising option to prevent PEP. Nonetheless, the recommended optimal dose and timing of administration remain unclear. The aim of this study was to evaluate the efficacy of different doses and timing of rectal diclofenac administration in preventing PEP.

Methods Literature search was conducted on 3 databases: PubMed/MEDLINE, Cochrane Library, and ScienceDirect. The included studies were evaluated for the method and reported data quality. Data were summarized, and statistical analyses were performed using RevMan 5.4.1 software.

Results Thirteen eligible studies with a total of 8602 patients undergoing ERCP were included. A significantly lower moderate-severe PEP prevalence in patients treated with rectal diclofenac compared to control (RR 0.67; 95% CI: 0.51-0.89; p=0.006) was found. High-dose rectal diclofenac significantly reduced the prevalence of moderate to severe PEP (RR 0.63; 95% CI: 0.450.89; p=0.008). Administering rectal diclofenac before procedure significantly lowered the prevalence of moderate to severe PEP (RR 0.66; 95% CI: 0.47-0.92; p=0.01). Adverse events related to ERCP procedure were comparable in both groups. No adverse event related to rectal diclofenac administration was reported across all studies.

Conclusion High-dose rectal diclofenac administered before ERCP procedure is effective in reducing the prevalence and severity of PEP, and is well tolerated with favourable safety profile.

Keywords: anti-inflammatory agents, non-steroids, pancreatitis, prevention, suppository

INTRODUCTION

Endoscopic retrograde cholangiopancreatography (ERCP) is an interventional procedure that involves upper gastrointestinal endoscopy and x-rays, indicated for the diagnosis and management of hepatobiliary and pancreatic system disorders (1). The prevalent adverse event following this extensively utilized procedure is acute pancreatitis. Post-ERCP acute pancreatitis (PEP) is common, occurring in 3 - 10% of patients, and is frequently linked to considerable morbidity and mortality (2). Therefore, the implementation of adequate prophylactic measures is crucial to avoid the development of PEP.

Current strategies for preventing post-ERCP pancreatitis (PEP) include pancreatic duct stent placement and the use of medications. However, most pharmacological agents, for instance nitroglycerine, somatostatin, and octreotide, assessed in clinical trials have shown limited effectiveness against PEP prevention (3). Several guidelines have suggested the use of rectal diclofenac as a promising option to prevent PEP (4-7). Despite this, the recommended optimal dose and timing of administration in relation to the ERCP procedure remain unclear. The European Society of Gastrointestinal Endoscopy (ESGE) recommends administering a high-dose (100 mg) rectal diclofenac before ERCP procedure (6). The Japanese Society of Hepato-Biliary-Pancreatic Surgery (JHBPS) recommends a low-dose (25–50 mg) rectal diclofenac administered immediately after the ERCP procedure (7). Thus, it remains controversial whether rectal diclofenac effectively reduces the incidence and severity of PEP.

The aim of this study was to assess the efficacy of 100 mg vs 25–50 mg of rectal diclofenac, administered before vs after ERCP procedure in preventing PEP compared to a control group of patients without any prophylaxes.

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MATERIALS AND METHODS

Materials and study design

This systematic review was done by conducting comprehensive literature search on three databases: PubMed/MEDLINE, ScienceDirect, and the Cochrane Library, to identify studies up to 2025. The keywords "rectal diclofenac", "pancreatitis", and "prophylaxis" were utilized for literature search. The keywords were entered into the advanced search forms of the databases, incorporating the Boolean operator 'OR' to obtain synonym results in accordance with the MeSH Term. Concurrently, the Boolean operator 'AND' was applied to the keywords between each term to yield results that encompass all specified keywords. The search terms were used as follows: ("rectal diclofenac" OR "diclofenac" OR "nsaid") AND ("post-ERCP pancreatitis" OR "pancreatitis" OR "endoscopic retrograde cholangiopancreatography" "ERCP") AND ("prophylaxis" OR "prevention").

The eligibility criteria included studies with a population of patients undergoing ERCP who received rectal diclofenac as the intervention and had a control group receiving either no treatment or a placebo as comparison. The studies must have reported outcomes including prevalence and severity of PEP as primary endpoint, alongside adverse events to assess the safety profile. Eligible studies were required to be published in English with full-text access available.

The excluded studies comprised those that included participants younger than 18 years of age, pregnant and/or lactating women, patients undergoing ERCP for biliary stent removal, or studies with NSAIDs as additional therapy to other preventions. Studies with insufficient data such as missing baseline or follow-up numerical data were excluded, as were animal studies, reviews, case reports, editorials, and duplicates.

The protocol for this review was registered on 18 June 2025 with the International Prospective Register of Systematic Reviews (PROSPERO) under identification number CRD420251076066.

Methods

This study was performed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (8). After comprehensive search was completed, duplicates were removed. The identified studies were screened based on title and abstract manually by two independent authors (ML and PL). Each article deemed relevant and within the scope of this systematic review by at least one author was selected for further evaluation. Each author would review the selected studies that passed the initial screening for their eligibility. Disagreements between authors were reviewed and settled by a third author (TS). Each accessible full-text article was independently rated by two authors. Third author's opinion settled the disagreement between authors' judgements.

The studies that fulfilled the inclusion criteria were analysed. Data extracted from the studies included the following: the name of the first author, year and site of publication, sample sizes, inclusion and exclusion criteria, number of patients treated with rectal diclofenac or assigned to the control group, patient outcomes for both groups, and baseline characteristics of the samples. The primary outcome was the prevalence of post-ERCP pancreatitis (PEP), defined as abdominal pain accompanied by a serum amylase level ≥3 times the normal value within 24 hours after ERCP (9). The second outcomes anticipated were the severity of PEP classified using the Revised Atlanta Classification (RAC) as mild (needed 2 - 3 days of hospital stay), moderate (needed 4 - 10 days of hospital stay), or severe PEP (needed > 10 days of hospital stay) (10); and adverse events.

Statistical analyses

The Cochrane risk of bias 2.0 tool (RoB-2) was utilized to assess the quality of the included randomized controlled trials. The tool is structured into five domains through which bias might be categorized as 'low risk', 'high risk', or 'some concerns' of bias (11). The Risk of Bias in Non-Randomized Studies – of Interventions (ROBINS-I) were used to assess all included non-randomized studies (12).

The statistical analyses were carried out utilizing RevMan 5.4.1 software. Outcomes of the studies were pooled as prevalence with 95% confidence intervals (CI). Subgroup analyses were performed to investigate the variability among trials regarding various rectal diclofenac doses and timing of administration. Risk ratio (RR) was used to compare the probability of an event occurring in the intervention group to that in the control group across studies. The I^2 statistic was used to determine the degree of heterogeneity among the studies included in this meta-analysis (13). The I^2 value indicates the percentage of variability attributable to differences in study populations, interventions, methodologies, or outcome measurements (14). The outcomes with $p<0.05$ were considered to have statistical significance.

RESULTS

PRISMA flowchart diagram (Figure 1) depicted the study selection process. After completed search in 3 scientific databases, a total of 963 studies were obtained: 510 from ScienceDirect, 317 from PubMed, and 136 from the Cochrane Library. Forty-eight duplicates were identified and removed, resulting in 915 studies for screening. After screening the remaining studies, 868 were excluded for not meeting the inclusion criteria, having irrelevant titles, containing inappropriate abstracts, and being published in languages other than English. The final 47 studies were assessed for eligibility, and only 36 were

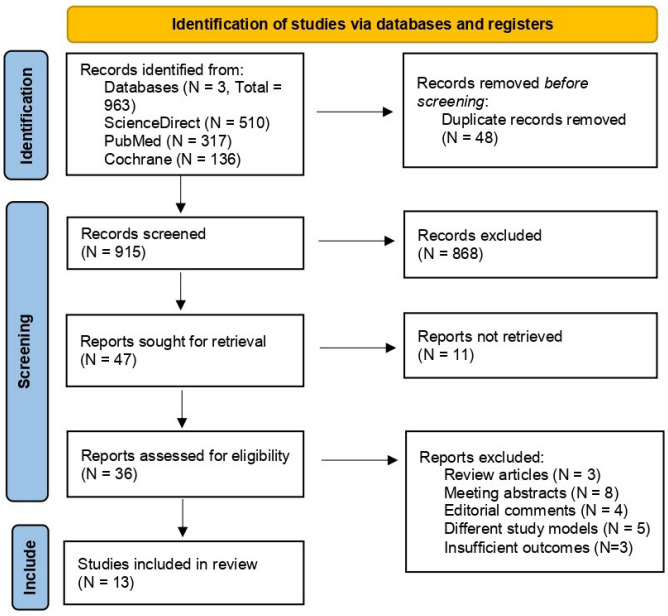

Figure 1. PRISMA flowchart

Table 1. Baseline demographic and clinical characteristics of the studies

Authors, Year, Country	Sample size	Baseline characteristics of participants				Rectal Diclofenac	
		Age (mean±SD) (years)	Sex, F/M (N)	BMI (mean±SD)	History of pancreatitis (N)	Dosage	Timing
Sakai et al., 2023 (15) Japan	Total = 367 D=187 C=180	D: 74.8±12.3 C: 74.8±13.0	D: 80/107 C: 84/96	D: 22.1±3.6 C: 21.9±3.9	D: 1 C: 2	25 – 50 mg	30 min before ERCP
Maeda et al., 2021 (16) Japan	Total =276 D=83 C=193	D: 84±2 C: 82±2.25	D: 49/34 C: 113/80	D: 22±0.75 C: 22±1.25	D: 4 C: 3	25 mg	30 min before ERCP
Takaori et al., 2021 (17) Japan	Total = 515 D=246 C=269	N/I	D: 104/142 C: 101/168	N/I	D: 7 C: 12	25 – 50 mg	30 min before ERCP
Tomoda et al., 2021 (18) Japan	Total =132 D=66 C=66	D: 74 ± 4.5 C: 70±3.75	D: 54/12 C: 56/10	N/I	D: 1 C: 7	25 – 50 mg	before ERCP
Katoh et al., 2020 (19) Japan	Total=297 D=147 C=150	D: 74.3±11.8 C: 74.0±12.7	D: 65/82 C: 55/95	N/I	D: 8 C: 8	25 – 50 mg	30 min before ERCP
Koskensalo et al., 2020 (20) Finland	Total=2000 D=1000 C=1000	D: 40± 14.25 C: 39±15.75	D: 370/630 C: 401/591	D: 25.0±7.83 C: 24.5±8.98	N/I	100 mg	before ERCP
Geraci et al., 2019 (21) Italy	Total=40 D=20 C=20	D: 59.8±5.25 C: 58.6±4.25	D: 12/8 C: 9/11	N/I	D: 0 C: 0	100 mg	30-60 min before ERCP
Del-Olmo-Martínez et al., 2018 (22) Spain	Total = 1512 D=794 C=718	D: 72.9 ± 13.7 C: 73.1±14.23	D: 347/447 C: 348/370	N/I	D: 31 C: 40	100 mg	before ERCP
Okuno et al., 2018 (23) Japan	Total=147 D=74 C=73	D: 78±11.75 C: 83±13.25	D: 39/35 C: 37/36	D: 21.5±5.1 C: 20.0±4.6	N/I	25 mg	30 min before ERCP
Rainio et al., 2017 (24) Finland	Total=2000 D=1000 C=1000	D: 64±19.75 C: 63±20.5	D: 430/570 C: 421/579	D: 24.8±10.9 C: 24.8±10.85	D: 142 C: 137	100 mg	before ERCP
Leerhøy et al., 2016 (25) Denmark	Total=772 D =378 C =394	D: 65±18 C: 66±19	D: 216/162 C: 249/145	D: 26±5 C: 26±6	N/I	100 mg	after ERCP
Patil et al., 2016 (26) India	Total=400 D =200 C =200	N/I	D: 128/72 C: 123/77	N/I	D: 40 C: 35	100 mg	before ERCP
Lua et al., 2015 (27) Malaysia	Total = 144 D=69 C=75	D: 50.3±17.6 C: 49.6±16.8	D: 35/34 C: 50/25	N/I	D: 1 C: 0	100 mg	after ERCP

BMI, body mass index; C, control group; D, rectal diclofenac group; F, female; M, male; N, number; N/I, no information; ERCP, endoscopic retrograde cholangiopancreatography

successfully retrieved for full-text. Of these studies, 23 were excluded: 3 were reviews, 8 were meeting abstracts, 4 were editorial commentaries, 5 used different study models, and 3 had insufficient outcome data. Thus, the remaining 13 studies that met the inclusion criteria were included in the meta-analysis.

Among the 13 studies included in the analysis, four were randomized controlled trials (RCTs). The quality assessment using ROB-2 should be mentioned in the statistical analysis indicating that all RCTs studies were regarded to have low risk of bias (Figure 2A). Other 9 non-randomized studies were evaluated for quality with ROBINS-I should be mentioned in the statistical analysis. Of those, three studies were regarded to have moderate risk of bias, while others were regarded to have low risk of bias (Figure 2B).

The total sample size of the 13 studies was 8602 patients. The included studies were conducted in different geographic locations, ensuring less bias and a general outcome throughout distinct regions. The average age of patients was 70, ranging from 60 to 80 years of age. The proportion of female and male patients was comparable, with both comprising approximately 50%. Most patients were considered overweight with the average body mass index (BMI) of 23 kg/m2 ranging between 21 to 25 kg/m2 (Table 1).

The patients included were undergoing ERCP procedure for the first time by experienced endoscopists. The exclusion criteria applied across the studies included patients with acute or chronic pancreatitis, those with allergies or contraindications to NSAIDs (e.g., severe renal insufficiency, peptic ulcer), pregnant or breastfeeding patients or those with abnormal gastrointestinal anatomy.

Each study provided detailed information on the interventions and comparisons. In the rectal diclofenac groups, participants received either low dose rectal diclofenac (25 mg for body weight <50 kg or 50 mg for body weight > 50 kg) or high dose rectal diclofenac (100mg), administered either 30 minutes before or immediately after ERCP. The variety of doses and tim-

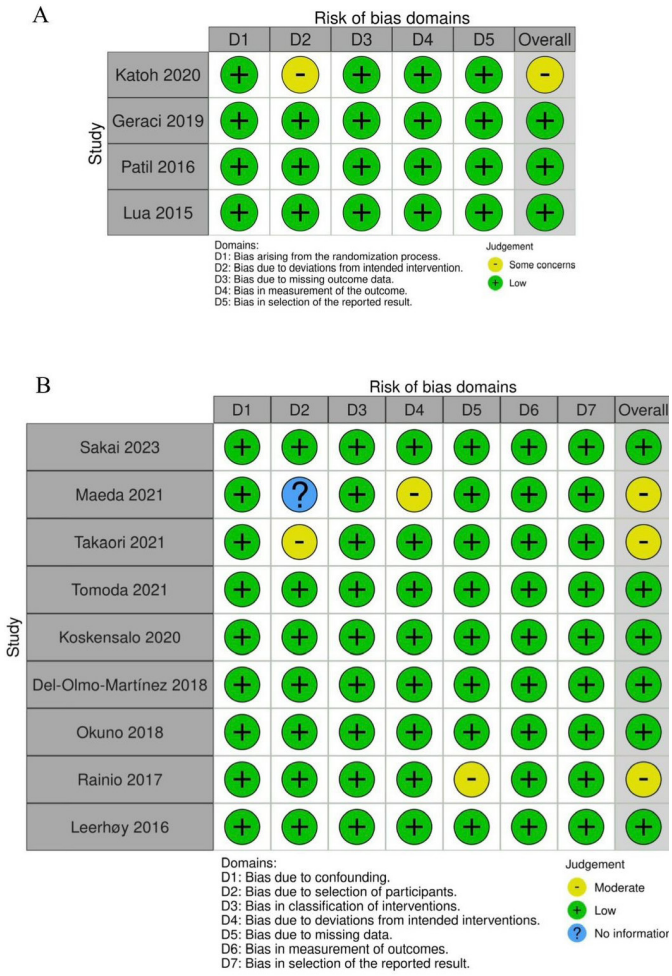

Figure 2. A) Risk of bias using Cochrane risk of bias tool for randomized trials (RoB2); B) Risk of bias using The Risk of Bias in Non-Randomized Studies – of Interventions (ROBINS-I)

ing applied in this study was intended to evaluate the optimal rectal diclofenac dose and timing of administration. In the control groups, participants received no treatment for comparison. The outcome data extracted were the prevalence and severity of PEP, impact of different doses and timing for the prevalence and severity of PEP, and adverse events.

In the control group, 132 out of 4338 patients developed mild PEP, while in the rectal diclofenac group, there were only 104 out of 4262 patients who developed mild PEP. The results indicated that rectal diclofenac marginally reduced the prevalence of mild PEP compared to the control, although this finding did not demonstrate statistical significance (RR 0.82; 95% CI: 0.64-1.06; p=0.13). The prevalence of mild PEP was significantly reduced by low-dose rectal diclofenac (RR 0.56; 95% CI: 0.32-1.00; p=0.05). The use of high-dose rectal diclofenac also reduced the prevalence of mild PEP, however no statistical significance was observed (RR 0.92; 95% CI: 0.69-.22; p=0.55). The timing of rectal diclofenac administration—whether given before or after the procedure—did not show a statistically significant difference in reducing the prevalence of mild PEP. In other words, both pre-procedure and post-procedure administration of rectal diclofenac appeared to provide comparable effectiveness for preventing mild PEP (Figure 3).

The prevalence of moderate to severe PEP was significantly lower in patients treated with rectal diclofenac compared to the control (RR 0.67; 95% CI: 0.51-0.89; p=0.006). High-dose rectal diclofenac significantly decreased the prevalence of moderate to severe PEP compared to the control (RR 0.63; 95% CI: 0.45-0.89; p=0.008). Meanwhile, the effect of low-dose rectal diclofenac in this regard did not demonstrate statistical significance (RR 0.78; 95% CI: 0.46-1.31; p=0.34). The timing of rectal diclofenac administration plays a key role in preventing moderate to severe PEP. Statistical analysis showed that administering rectal diclofenac before the procedure significantly lowered the prevalence of moderate-to-severe PEP compared to the control (RR 0.66; 95% CI: 0.47-0.92; p=0.01). Conversely, the administration of rectal diclofenac after the procedure did not show a significant reduction in the prevalence of moderate to severe PEP (RR 0.71; 95% CI: 0.43-1.18; p=0.19).

In terms of adverse events, the use of rectal diclofenac reported a comparable safety profile to the control group. The occurrence of adverse events related to the ERCP procedure was comparable in the rectal diclofenac and the control group, with no statistical difference (RR 0.84; 95% CI: 0.70-1.02; p=0.08). Additionally, no adverse event was observed in the administration of rectal diclofenac across all studies.

DISCUSSION

Several studies confirmed rectal nonsteroidal anti-inflammatory drugs (NSAIDs), such as rectal diclofenac or indomethacin, to effectively prevent PEP (24,25). A systematic review and meta-analysis further evaluated the efficacy of rectal diclofenac and indomethacin, yielding that a greater number of patients developed PEP in the indomethacin group than that in the diclofenac group (26). Recent trials have evaluated rectal diclofenac as prophylactic for PEP. Studies in Europe evaluated a high dose rectal diclofenac, while studies in Asia frequently evaluated low dose rectal diclofenac, thus the results were somehow contradictory (15-17).

In this study we have evaluated the efficacy and safety of rectal diclofenac in lowering the incidence and severity of PEP, as well as focusing on the optimal dose and timing of administration. The results indicate that rectal diclofenac provided a desirable efficacy in the prevention of overall PEP prevalence, highlighting the prevalence of moderate-to-severe PEP. This result is aligned with a study which indicated that rectal diclofenac significantly decreased the prevalence of PEP, with 4.1 % of patients in the rectal diclofenac group and 13.7% of patients in the control group developing PEP (19). A study evaluating several routes of diclofenac administration demonstrated that rectal diclofenac significantly reduced the prevalence of PEP and is superior to other routes of administration (17). Regarding this result, a study indicated that rectal diclofenac lowered the incidence of PEP significantly, with the most pronounced effect observed in moderate-to-severe PEP (22). Rectal diclofenac mitigated pancreatitis pathogenesis by suppressing the inflammatory responses through the inhibition of cyclooxygenase 2-prostaglandin A2 pathway (27). Rectal administration is more effective than other routes because it allows rapid absorption and bypasses first-pass metabolism, leading to higher local concentrations and enhanced anti-inflammatory effects (28). Rectal diclofenac significantly reduces the prevalence of moderate to severe PEP, but offers minimal benefit over no treatment in mild cases. This is because it effectively disrupts the intense inflammatory cascade responsible for more severe pancreatitis. In contrary, mild pancreatitis typically results from transient enzyme leakage and minor tissue damage that is often self-limiting, which explains the lack of a significant difference in outcomes (29).

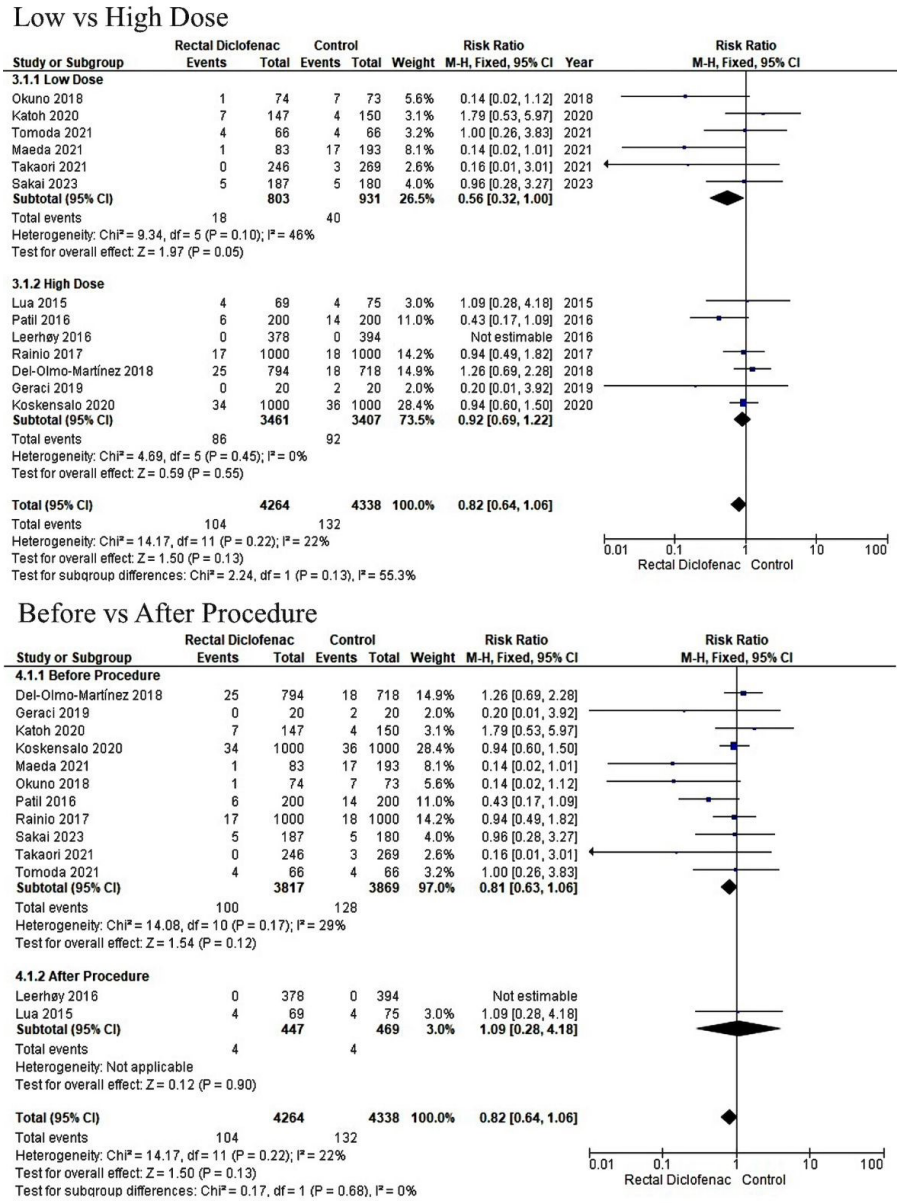

Figure 3. Forest plot of the incidence of mild post- endoscopic retrograde cholangiopancreatography (ERCP) pancreatitis (PEP) according to different doses and administration timing

This study suggested that administering a high-dose of 100 mg rectal diclofenac provided better efficacy in preventing moderate to severe PEP than low-dose rectal diclofenac and control groups. Dome studies demonstrated that prophylactic administration of 100 mg rectal diclofenac significantly reduces the prevalence of PEP up to 5% compared to the control (21). Comparing the efficacy of 25 mg versus 50 mg doses of rectal diclofenac it was found that the prevalence of PEP was significantly lower in the 50 mg group, revealing there was enhanced efficacy with a higher dose (30). A comprehensive review claimed that the efficacy of rectal diclofenac appears to be dose-dependent, with significantly reduced protection at doses lower than 50 mg. In contrast, a ceiling effect was suggested at doses above 100 mg, offering no additional benefit and potentially increasing the risk of adverse events (31).

Administering high-dose rectal diclofenac 30 minutes prior to the ERCP procedure was suggested to be more effective in preventing of moderate-to-severe PEP (17,22). This outcome was in accordance with a study by Wu et al. Reportedly, administering rectal diclofenac prior to ERCP halved the prevalence of PEP compared to administering post-ERCP, with 6.50% and 15.63% of patients developing PEP, respectively (32), additionally being associated with a shorter hospital stay (33). A study by Agahi et al. indicated that administration of rectal diclofenac both pre- and post-ERCP had no additional benefit in preventing PEP compared with pre-ERCP administration alone (34). It has been shown that pre-ERCP administration of NSAIDs ensures that therapeutic concentrations are present during the critical period of pancreatic injury and enzyme activation, thereby inhibiting the initiation and early propagation of the inflammatory cascade. Contrary, post-ERCP administration may be less effective, as the inflammatory cascade may have been already triggered, limiting its prophylactic potential (35).

Administration of rectal diclofenac showed no significantly increased risk of other adverse events related to the ERCP procedure, such as gastrointestinal (GI) bleeding, perforation, and cholangitis. No adverse event regarding the administration of rectal diclofenac was reported in this review. These findings indicate that rectal diclofenac was well tolerated, exhibiting a comparable safety profile to no treatment for patients undergoing ERCP

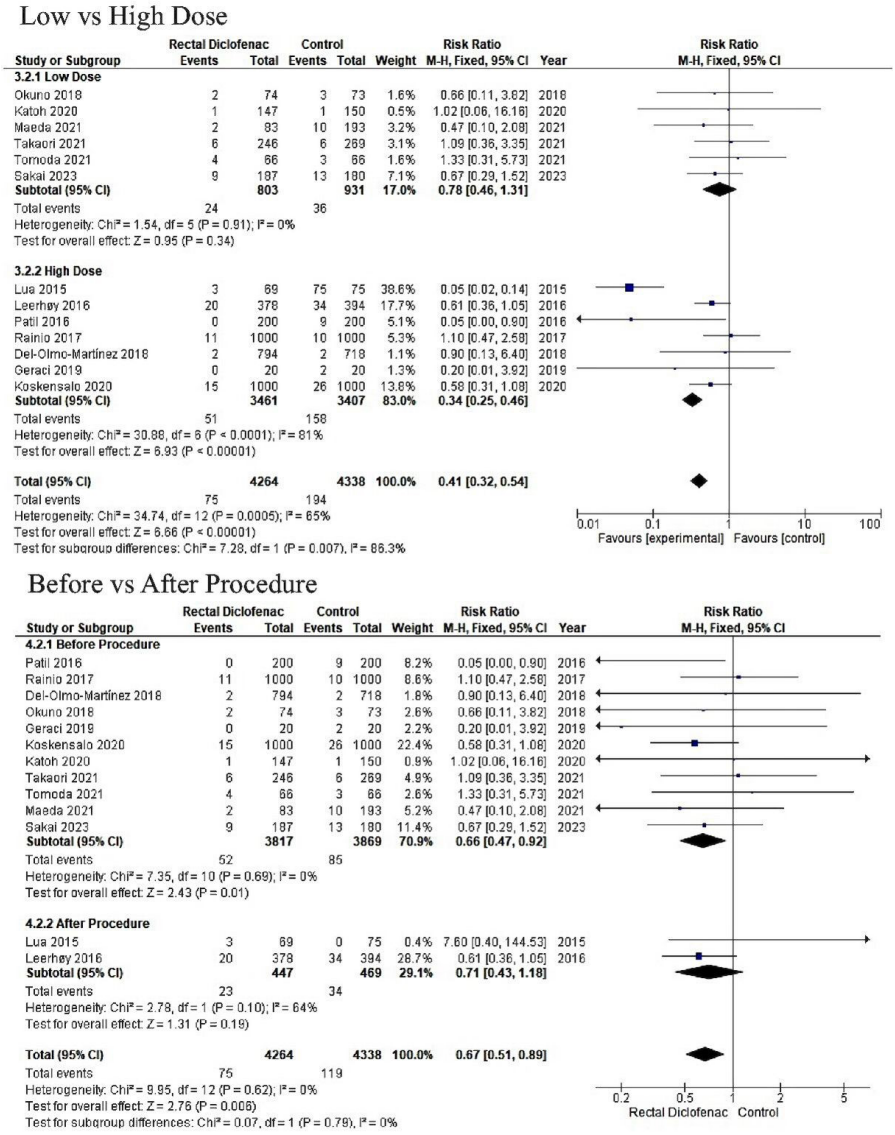

Figure 4. Forest plot of the incidence of moderate-severe post- endoscopic retrograde cholangiopancreatography (ERCP) pancreatitis (PEP) according to different doses and administration timing

procedure. Several meta-analyses concluded that rectal NSAIDs were safe and well-tolerated, with no significant side effects reported. Rectal administration enables effective local action while minimizing systemic exposure and associated side effects (29,36). Despite the encouraging outcomes reported, this study has several limitations. Firstly, the limited evaluation of confounding risk and long-term safety data may constrain the general applicability of the results reported. Secondly, the substantial heterogeneity observed across the included studies (I^2 = 65%)—particularly regarding the prevalence of moderate-to-severe PEP—indicates notable inconsistency in the reported outcomes. This variability is likely to be influenced by differences in the intervention protocols, especially the wide range of diclofenac doses (25 mg vs 100 mg) and the timing of administration (pre-ERCP vs post-ERCP). Such differences in dosing strategies may lead to variations in drug absorption and anti-inflammatory efficacy, ultimately affecting the pooled treatment effect. In addition, several potential confounders may further contribute to this heterogeneity. These include differences in baseline patient risk profiles for PEP, where known risk factors include female sex, younger age, and a history of pancreatitis or previous PEP, as well as variations in procedural complexity, such as difficult bile duct cannulation, repeated contrast injection into the pancreatic duct, and the performance of sphincterotomy. Furthermore, the use of concomitant prophylactic measures (e.g., pancreatic stents or other NSAIDs) may also influence both the incidence and severity of PEP. Collectively, these factors may partially explain the inconsistency observed across studies (37).

Future research should focus on conducting larger and multi-centred RCTs to further validate these findings and establish standardized rectal diclofenac protocols, including comparison with other preventive alternatives. We hope that by addressing these limitations, forthcoming studies may offer more reliable evidence on the use of rectal diclofenac as a prophylaxis for post-ERCP pancreatitis (PEP) in patients undergoing ERCP.

In conclusion, administering a high-dose 100 mg rectal diclofenac prior to the ERCP procedure provided a significant prophylactic efficacy against post-ERCP pancreatitis. Rectal diclofenac significantly lowered the incidence and severity of PEP. On top of that, rectal diclofenac was well-tolerated with no considerable adverse events reported. The efficacy of rectal diclofenac compared to other alternatives in preventing PEP requires further research.

AUTHOR CONTRIBUTIONS

Conceptualization, M.L. and T.S.; Methodology, M.L. and P.L; Supervision, T.S; Validation, T.S. and M.L.; Resources, P.L.; Investigation, M.L and P.L.; Formal Analysis, T.S.; Visualization, T.S. and P.L. All authors have read and agreed to the published version of the manuscript.

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TRANSPARENCY DECLARATION

Conflict of interests: None to declare.

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ORIGINAL ARTICLE

Effects of *Lactobacillus rhamnosus* probiotic supplementation on gut health in rabbits

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ABSTRACT

Aim: The research examined effects of prolonged oral *Lactobacillus rhamnosus* intake on rabbit intestinal tissue morphology.
Methods: The study included twelve male rabbits aged 10–12 months and weighing 1.5–2.0 kg who were randomly assigned to two groups of six animals each. The control group received distilled water but the experimental group received *L. rhamnosus* orally at 0.25 mL/kg/day for 45 days. The research team performed histological and morphometric analysis on duodenal samples to measure villus height and width and crypt depth and villus area and VH/CD ratio and muscularis thickness through ImageJ software.
Results: The extended probiotic treatment caused villus disorganization and epithelial necrosis and villus fusion and inflammatory infiltration and submucosal oedema. The quantitative analysis showed that all measured parameters including villus height and VH/CD ratio and villus area and muscularis thickness demonstrated significant decreases ($p<0.05$) when compared to the control group. The results show that absorptive capacity was impaired.
Conclusion: The duodenal mucosa developed structural and functional changes because of extended exposure to high *L. rhamnosus* concentrations, which indicates that long-term probiotic use could be harmful. Research needs to determine the correct dosage amounts and duration of treatment for non-rodent species.
Keywords: duodenum, histopathology, *Lactobacillus rhamnosus*, morphometry, probiotics, rabbits.

INTRODUCTION

The definition of probiotics includes live microorganisms which provide health advantages to hosts when taken in sufficient amounts to support intestinal microbial equilibrium and strengthen mucosal defence systems (1,2). The therapeutic potential of Lactobacillus Bifidobacterium and Saccharomyces genera bacteria has become increasingly important for treating digestive and metabolic and immune system disorders (3,4). Research studies show that probiotics enhance epithelial barrier strength and create immune tolerance through their production of short-chain fatty acids and bacteriocins which fight pathogenic bacteria (5–7). Research has focused on Lactobacillus rhamnosus because it shows excellent resistance to stomach acid and bile and strong ability to attach to intestinal cells and control inflammatory cytokines which help maintain both gut and body equilibrium (8,9). Research shows probiotics provide immediate health advantages yet studies show that taking high doses of probiotics for extended periods can harm gut bacteria and intestinal walls, which results in inflammation and reduced nutrient absorption (10–12). Most of the current literature is limited to rodent models (7), with scarce information on non-rodent species such as rabbits, which possess unique gastrointestinal physiology characterized by high microbial fermentation and susceptibility to dysbiosis (11). This gap limits the extrapolation of existing probiotic safety data to broader mammalian systems.
The aim of this study was to evaluate the histopathological and morphometric alterations in the duodenal mucosa of rabbits following 45-day oral supplementation with *Lactobacillus rhamnosus*, in order to assess potential risks associated with long-term probiotic use in non-rodent models.

MATERIALS AND METHODS

Materials and study design

The experiment was conducted at the Department of Basic Dental Sciences, University of Mosul, Iraq, from January to March 2025.
Twelve male rabbits that are healthy and domestic (*Oryctolagus cuniculus*) between 10 and 12 months old and weighing 1.5 to 2.0 kg each were kept in separate housing units with

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controlled environmental settings (temperature maintained at 22 - 25 °C and relative humidity at 50 to 60%).
Animals were provided standard rabbit chow and water ad libitum. Following acclimatization, rabbits were randomly assigned to two groups (N=6 per group): Group I (Control) obtained distilled water orally (vehicle) daily for 45 days, and Group II (Probiotic-treated) received oral drops of *Lactobacillus rhamnosus* (Vitron Farma, Turkey) at a dose of 0.25 mL/kg/day (equivalent to approximately 5 drops) for 45 consecutive days (10).
The experimental protocol was reviewed and approved by the Scientific and Ethical Committee of the Basic Dental Sciences Department at the College of Dentistry, in Mosul University, which granted approval under the reference number UOM. Dent25\/1076.

Methods

At the end of the experimental period, rabbits were euthanized humanely under appropriate anaesthesia. Duodenal segments (~0.5 cm^3) were excised, rinsed in phosphate-buffered saline, and fixed in 10% neutral buffered formalin for 24 hours. Standard histological procedures, dehydration in graded alcohols were followed: the tissue samples were prepared by clearing them with xylene and then embedding into paraffin wax before being sliced into 5 µg sections for staining with haematoxylin and eosin (H&E) (9).
Histological examination was carried out using a microscope (Olympus CX43) which had a digital camera for capturing images of the samples from the duodenal tissue of each rabbit animal under study. Five distinctive sections were prepared and stained with H&E. A total of three high power fields (HPFs) from each stained slide per rabbit were selected for detailed morphometric analysis; this resulted in 15 measurements being taken for each individual animal to provide a comprehensive overview of the mucosal architecture. The selection of these fields was made to ensure a representation of the mucosal structure, within the samples studied. Various measurements, such as the height of the villi and crypts well, as the ratio of villus height to crypt depth were calculated by utilizing Image J software (10) from the National Institutes of Health (NIH) in the United States.
Prepared slides were examined under a light microscope by a qualified histopathologist blinded to the group allocation. The histopathological–examination revealed multiple important findings, which included villus structure assessment and evaluation of epithelial health and presence of inflammation and submucosal swelling and gland tissue damage.
Morphometric measurements were conducted using Image-Pro Plus software (version 6.0, Media Cybernetics, Rockville, MD, USA). For each animal, ten well-oriented villi and ten crypts were analysed. The assessment included measurements of villus height (VH) and width (VW), crypt depth (CD), VH/CD ratio, villus area, and muscular thickness.

Statistical analysis

All data were expressed as mean ± standard deviation (SD). Statistical differences between the control and probiotic-treated groups were evaluated using an independent samples t-test. The research established $p \leq 0.05$ as its statistical significance threshold.

RESULTS

The duodenal sections from both experimental groups showed different structural patterns after 45 days of treatment through histological examination. The duodenal tissue in the control group maintained its typical structure because it showed well-organized villi which contained columnar epithelial cells and visible goblet cells. The submucosa and muscularis layers showed no signs of inflammation or disruption while maintaining their compact structure. The serosa displayed its typical four-layered pattern which makes up the intestinal wall structure.
The probiotic-treated group showed distinct pathological changes. The microscopic examination showed that villus structure became disorganized while their tips became flattened and adjacent villi merged together. The tissue showed two main patterns of damage which included dead epithelial cells and inflammatory cells that entered the lamina propria and submucosa. The tissue showed two main findings that included pronounced submucosal oedema and focal necrosis of enteric glands suggesting mucosal damage from extended contact with *Lactobacillus rhamnosus* (Figure 1 A–G).
The quantitative morphometric analysis verified all findings which were observed through histological examination. The probiotic-treated rabbits showed a substantial decrease ($p<0.05$) in villus height and area and VH/CD ratio and muscularis layer thickness but villus width and crypt depth remained unchanged ($p>0.05$). The research shows that probiotic treatment over time results in smaller absorptive areas and damaged mucosal tissue (Figure 2).

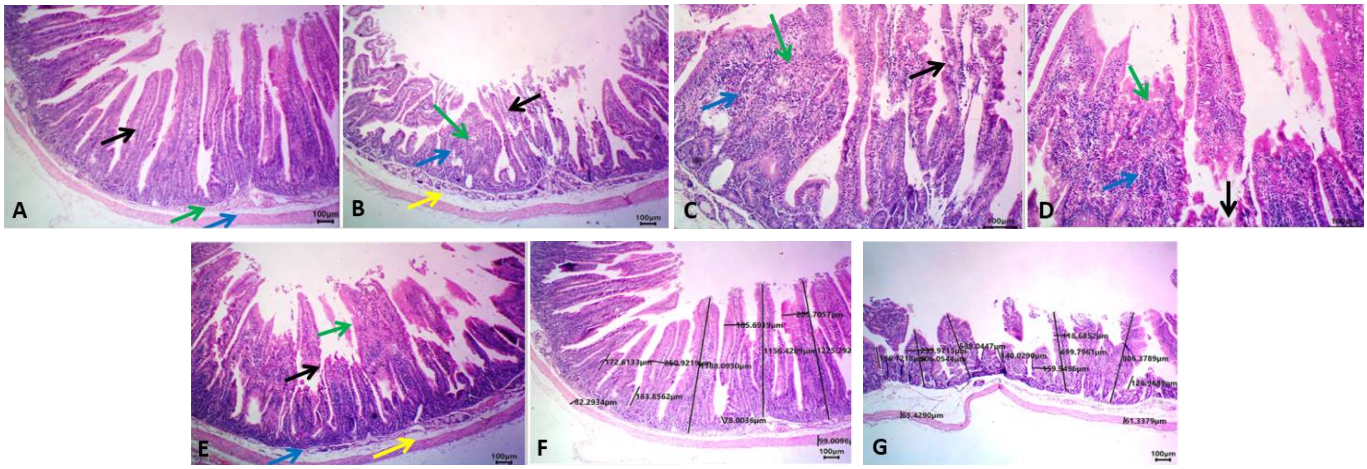

Figure 1. Photomicrographs of rabbit duodenal mucosa showing histological differences between control and probiotic-treated groups. A-B) Control group demonstrating intact villi and organized mucosa; C-G) Probiotic groups (T1–T2) showing villus disorganization, epithelial necrosis (black arrows), villus fusion (green arrows), inflammatory infiltration (blue arrows), and submucosal oedema (yellow arrows) (H&E stain; 40×–400×; scale bar = 100 µm)

Table 1. Histomorphometric parameters of the duodenum in control and probiotic-treated rabbits

Parameter	Mean±SD		p
	Control Group (N=6)	Probiotic Group (N=6)	
Villus height (VH) (μm)	1191.2±53.2	741.2±109.9	< 0.001
Villus width (VW) (μm)	197.8±31.8	163.4±55.1	0.262
Villus area (μm²)	209411±27323.9	131312.6±11974.6	< 0.001
Crypt depth (CD) (μm)	129±46.6	157.2±39.6	0.333
VH/CD ratio	8.1±1.2	5.2±1.1	0.007
Muscularis thickness (μm)	113.2±22.3	61±4.3	< 0.001

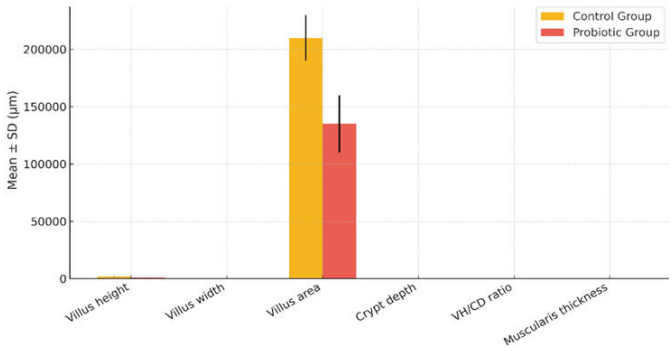

Figure 2. Quantitative comparison of duodenal histomorphometry parameters in control and probiotic-treated rabbit groups

DISCUSSION

The research demonstrates through experimental methods that rabbits who take *Lactobacillus rhamnosus* orally for long periods develop particular changes in their duodenal tissue structure and tissue appearance. The research shows that probiotics create beneficial effects on intestinal homeostasis but scientists have discovered new potential negative effects of extended and unsupervised probiotic intake. The probiotic-treated rabbits exhibited villus disorganization, epithelial necrosis, fusion of adjacent villi, inflammatory cell infiltration, and submucosal oedema—features indicating compromised mucosal integrity and the initiation of localized inflammation.

The structural changes at the mechanistic level result from disrupted tight-junction protein expression of occludin and claudins which control intestinal permeability. The disruption causes paracellular leakage and bacterial translocation resulting in immune activation through the process known as "leaky gut" (13). The tissue damage becomes more severe because inflammatory cells move into the area while TNF-α and IL-6 cytokines production increases (5,14). The study demonstrated that high probiotic doses, together with microbial imbalances, result in increased ROS production thus leading to oxidative stress and cell death of epithelial cells that prevents mucosal tissue repair (15).

The three mechanisms which disrupt tight-junction proteins occludin and claudin and cause oxidative stress and cytokine-driven inflammation work together to damage epithelial homeostasis and disrupt intestinal immune system function. The connected biological pathways show that patients with dysbiosis or intestinal barrier damage will experience adverse effects when taking high doses of probiotics for extended periods (16–18). The development of small intestine bacterial overgrowth (SIBO) occurs through dysbiosis causing bacterial growth that produces fermentation acids that damage epithelial tissue and lead to mucosal erosion and inflammation (19). The research data showed degenerative villus patterns and coalescence, which match the histological changes that occur in chronic enteropathies and experimental inflammatory gut models (20,21).

The morphometric analysis supported histological findings because it showed that probiotic treatment resulted in shorter villus height and smaller villus area and reduced villus-to-crypt ratio and thinner muscularis thickness. The intestinal absorptive capacity and nutrient uptake efficiency depend on these specific parameters. Their reduction shows that the mucosal absorption system and barrier function have experienced damage. The combination of villus atrophy and muscularis thinning occurs because of prolonged oxidative stress which damages epithelial cells and disrupts their normal renewal process according to previous studies on chronic intestinal stress (22,23).

The research provides new knowledge about non-rodent models because rabbits have a sophisticated hindgut fermentation system which responds strongly to changes in diet and microbial populations. Thus, this study is among the few to demonstrate deleterious duodenal remodelling associated with long-term *L. rhamnosus* administration in a rabbit model, extending the current understanding of probiotic safety beyond rodents (24,25).

The research findings show that translational medicine needs particular probiotic dosing protocols that should be tailored according to both the probiotic strain and the host species. The use of antibiotics for extended periods creates an imbalance in gut bacteria while damaging the intestinal tissue structure. Future research needs to use molecular techniques to study probiotic effects on intestinal tissue by measuring tight-junction proteins and cytokines and oxidative stress indicators. The research needs recovery-phase studies to verify that the documented histological and morphometric changes will fade away after participants stop taking the supplement.

The research study contains one essential limitation which needs to be acknowledged. The study depended on *Lactobacillus rhamnosus* as its probiotic strain at a set dose which restricted its applicability to different probiotic strains and treatment protocols. The research used rabbits as test subjects but this approach might not accurately show how human beings would react to treatment interventions. The study has two main limitations because it only examines histological and morphometric changes while ignoring molecular and biochemical assessments. Lastly the absence of a recovery group leaves uncertainty about whether the observed changes are reversible once the treatment ceases.

In conclusion, ultimately although probiotics show potential in maintaining gut health it is essential to monitor their prolonged use carefully. The negative impact on the lining of the intestine in rabbits as seen in this study highlights the importance of adopting specific and well-founded strategies when contemplating extended probiotic treatment, in both animal and human settings.

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TRANSPARENCY DECLARATION

Conflicts of interest: None to declare.

AUTHORS' CONTRIBUTION

Taghreed Al-Fakje designed and supervised the study, performed the experimental work, and contributed to data interpretation. Faeha Al-Mashhadane assisted in histological processing, statistical analysis, and manuscript preparation. Asmaa Al-Nuaimy participated in data collection, literature review, and manuscript editing. All authors read and approved the final version of the manuscript.

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Elevated neutrophil-to-lymphocyte ratio predicts poorer histopathological differentiation in colorectal cancer: a study from a Southeast Asian Tertiary Hospital

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ABSTRACT

Aim To evaluate the correlation between pre-treatment NLR and histopathological differentiation in colorectal cancer, and to assess the diagnostic accuracy of NLR as a predictive biomarker.
Methods A retrospective study was conducted at Adam Malik Haji Centre General Hospital Medan, North Sumatera. Medical records of 45 colorectal cancer patients treated between January and December 2024 were reviewed. Pre-treatment NLR values were calculated from peripheral blood counts. Receiver operating characteristic (ROC) curve analysis was performed to determine diagnostic performance, and correlation analysis was used to assess the relationship between NLR and tumour grade.
Results The mean NLR was 6.33±4.92. ROC analysis yielded an area under the curve (AUC) of 0.874, indicating excellent diagnostic ability. An NLR cutoff value of 6.25 provided a sensitivity of 87.5% and specificity of 72.5% for predicting poor histopathological differentiation. A moderate positive correlation ($r=0.612$; $p<0.001$) was found between higher NLR values and poorer differentiation.
Conclusion Pre-treatment NLR correlated with histopathological grade and showed promise as a simple, non-invasive biomarker for assessing tumour aggressiveness in colorectal cancer.
Keywords: biomarkers, tumour, inflammation, prognosis, survival analysis

INTRODUCTION

Colorectal cancer (CRC) is one of the most prevalent malignancies worldwide and remains a major cause of cancer-related mortality. Its incidence continues to rise in many low- and middle-income countries, including Indonesia, partly due to lifestyle transitions and delayed diagnosis (1,2). Despite advances in treatment, prognosis in many regions remains poor, largely because of late presentation and limited access to molecular prognostic tools (3).

In recent years, systemic inflammation has gained attention as a driver of cancer progression, influencing tumour initiation, invasion, and metastasis (4). Inflammation-based biomarkers derived from routine blood tests, such as the neutrophil-to-lymphocyte ratio (NLR), have shown promise as prognostic indicators in solid tumours. Multiple studies and recent meta-analyses have consistently reported that elevated pretreatment NLR is associated with tumour aggressiveness, recurrence, and reduced survival in patients with colorectal cancer (5–7). Importantly, the prognostic role of NLR remains in focus of current research, with clinical studies from the past five years reinforcing its relevance in modern oncology practice, particularly due to its low cost and accessibility (8,9).

Although the relationship between NLR and colorectal cancer outcomes has been explored previously, significant knowledge gaps remain. First, the association between NLR and histopathological differentiation, a key determinant of tumour biology and survival, has not been well established across diverse populations. A 2017 study from China reported a correlation between high NLR and poor differentiation in colorectal cancer (10). However, most available evidence originates from East Asian and Western cohorts, and data from Southeast Asia, particularly Indonesia, are scarce. Second, population-specific variations in immune response, tumour biology, and healthcare access may influence the prognostic value of NLR (11,12). Therefore, it is important to validate NLR as a biomarker in different ethnic and clinical settings before recommending its routine application in clinical practice.

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The aim of this study was to evaluate the association between preoperative NLR and the degree of histopathological differentiation among colorectal cancer patients treated at a tertiary hospital in Southeast Asia. Given that NLR is inexpensive, minimally invasive, and routinely available, confirming its prognostic value may support its integration into risk stratification strategies, particularly in resource-limited healthcare settings.

PATIENTS AND METHODS

Patient and study design

This observational analytic study employed a cross-sectional design to examine the association between the neutrophil-to-lymphocyte ratio (NLR) and histopathological differentiation in patients with colorectal cancer. Secondary data were retrospectively collected from medical records at Haji Adam Malik General Hospital, a tertiary referral centre in North Sumatra, Indonesia.

The study population consisted of all patients diagnosed with colorectal cancer and treated at the hospital between January and December 2024. A consecutive sampling technique was used to include all eligible patients who met the inclusion criteria until the required sample size was achieved.

Inclusion criteria were as follows: histopathologically confirmed colorectal cancer, age >18 years, no history of prior chemotherapy or radiotherapy, and absence of metastatic disease. Exclusion criteria included: a history of inflammatory bowel disease (e.g., ulcerative colitis or Crohn's disease), concurrent malignancies, medical conditions or medications known to affect leukocyte counts (e.g., bacterial infections, systemic inflammation, metabolic disorders, or use of corticosteroids or lithium), myeloproliferative disorders, and incomplete clinical or laboratory data.

Ethical approval for this study was obtained from the Research Ethics Committee of the Faculty of Medicine, Universitas Sumatera Utara.

Methods

Demographic, clinical, and laboratory data were extracted from electronic medical records. Laboratory results were obtained from routine preoperative blood tests, including total white blood cell count, absolute neutrophil count, and absolute lymphocyte count.

The neutrophil-to-lymphocyte ratio (NLR) was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count from preoperative complete blood count results. A predefined NLR cut-off value of 3.0 was applied to categorize patients into low NLR (<3.0) and high NLR (≥3.0) groups. This cut-off has been widely reported in previous colorectal cancer studies as clinically relevant for prognostic stratification (13,14).

Histopathological differentiation was determined from postoperative pathology reports and classified according to the World Health Organization (WHO) criteria. Tumours were categorized as well-differentiated adenocarcinoma (>95% gland formation, minimal cellular atypia, and preserved glandular architecture), moderately differentiated adenocarcinoma (50–95% gland formation, increased nuclear atypia, and irregular glandular structure), and poorly differentiated adenocarcinoma (<50% gland formation, marked pleomorphism, high mitotic activity, and a solid growth pattern indicative of aggressive behaviour) (15).

Disease staging was defined using the American Joint Committee on Cancer (AJCC) Tumor–Node–Metastasis (TNM) 8th edition system. Tumour invasion depth (T), regional lymph node involvement (N), and presence of distant metastasis (M) were extracted from surgical and imaging records to classify patients into stages I–IV (16).

Statistical analysis

Continuous variables were expressed as mean±standard deviation (SD) or median (interquartile range, IQR), depending on data normality. The association between NLR and histopathological differentiation was assessed using the ANOVA test. Diagnostic accuracy was evaluated using receiver operating characteristic (ROC) curve analysis to determine the optimal NLR cut-off value, sensitivity, and specificity. A $p<0.05$ was considered statistically significant. Results were presented in tables with descriptive summaries to support interpretation. A bivariate analysis was used to evaluate the relationship between the Neutrophil-to-lymphocyte ratio (NLR) and the degree of histopathological differentiation in colorectal cancer. Prior to the analysis, a normality test was performed to assess the distribution of the data. Given that the sample size was below 50, the Shapiro-Wilk test was utilized.

RESULTS

A total of 75 patients were initially identified during the study period. However, 30 patients were excluded for not meeting the inclusion criteria: 12 had undergone chemotherapy, radiotherapy, or presented with metastatic disease; three had diabetes mellitus; one had an autoimmune disorder; one had chronic kidney disease; one had heart failure; two had concurrent malignancies (endometrial and ovarian cancer); and 10 presented with acute infections. After applying the eligibility criteria, 45 patients remained in the final analysis, meeting the minimum sample size requirements determined by prior calculations.

Regarding sex distribution, 24 (53.3%) patients were male and 21 (46.7%) were female. The mean age of the cohort was 56.67 ± 14.6 years, with a median age of 57 years; the youngest patient was 26 years old and the oldest was 87 years.

Analysis of tumour localization revealed that the rectum was the most common site of malignancy, accounting for 28 (62.2%) patients, followed by the sigmoid colon in 11 (24.4%) patients. Histopathological examination showed that adenocarcinoma was the predominant tumour type, observed in 41 (91.1%) patients. Regarding disease staging, stage IIb was most frequently diagnosed, affecting 21 (46.7%) patients (Table 1).

The mean NLR across the study population was 6.33 ± 4.92, reflecting substantial variability in systemic inflammatory response among patients. The median NLR was 4.01, with values ranging from 1.03 to 18.3 (Table 2). Based on histopathological classification, 26 (57.8%) patients had well-differentiated tumours, 11 (24.4%) had moderately differentiated tumours, and 8 (17.8%) had poorly differentiated tumours (Table 2).

The diagnostic performance of NLR for distinguishing histopathological differentiation was assessed using receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC) was 0.874, indicating excellent discriminatory ability (AUC > 0.5), which was statistically significant ($p < 0.001$). The optimal NLR cut-off value was 6.25, yielding a

Table 1. Baseline clinicopathological characteristics of the study population

Variable	Values
Age (years)	
Mean±SD	56.67 ± 14.6
Median (min.-max.)	57 (26-87)
Sex (No; %)	
Man	24 (53.3)
Woman	21 (46.7)
Tumor size (cm)	
Mean+SD	5.2 ± 2.1
Median (min.-max.)	5.0 (2.0–11.5)
	No (%) of patients
Location	
Rectum	28 (62.2)
Sigmoid	11 (24.4)
Cecum	1 (2.2)
Ascending Colon	3 (6.7)
Transverse Colon	1 (2.2)
Descending Colon	1 (2.2)
Histopathological overview	
Adenocarcinoma	41 (91.1)
Mucinous adenocarcinoma	3 (6.7)
Signet ring carcinoma	1 (2.2)
Stage	
I	1 (2.2)
IIa	9 (17.8)
IIb	21 (46.7)
IIc	6 (13.3)
IIIa	1 (2.2)
IIIb	4 (8.9)
IIIc	4 (8.9)
Histopathological degree differentiation	
Well-differentiated	26 (57.8)
Moderately-differentiated	11 (24.4)
Poorly-differentiated	8 (17.8)
NLR	
Mean ± Standard Deviation	6.33±4.92
Median (min-max)	4.01 (1.03–18.3)

sensitivity of 87.5% and a specificity of 72.5%. The positive likelihood ratio (LR+) was 4.5, and the negative likelihood ratio (LR−) was 0.125, further supporting the robustness of NLR as a predictive biomarker (Table 2, Figure 1).

Due to non-normal data distribution, the Kruskal-Wallis test was performed, revealing mean NLR values of 3.93 ± 4.03 in the well-differentiated group, 7.98 ± 4.77 in the moderately differentiated group, and 11.09 ± 2.9 in the poorly differentiated group ($p < 0.001$). NLR values were highest in poorly

Table 2. Neutrophil-lymphocyte ratio (NLR) bivariate analysis of histopathological degrees

Histopathological degree	NLR average±SD	p*	r†
Well-differentiated	4.28 ± 4.37		0.612
Moderately-differentiated	7.91 ± 4.63	< 0.001	
Poorly-differentiated	10.83 ± 3.21		

*Kruskal Wallis test; †Spearman's Rho test

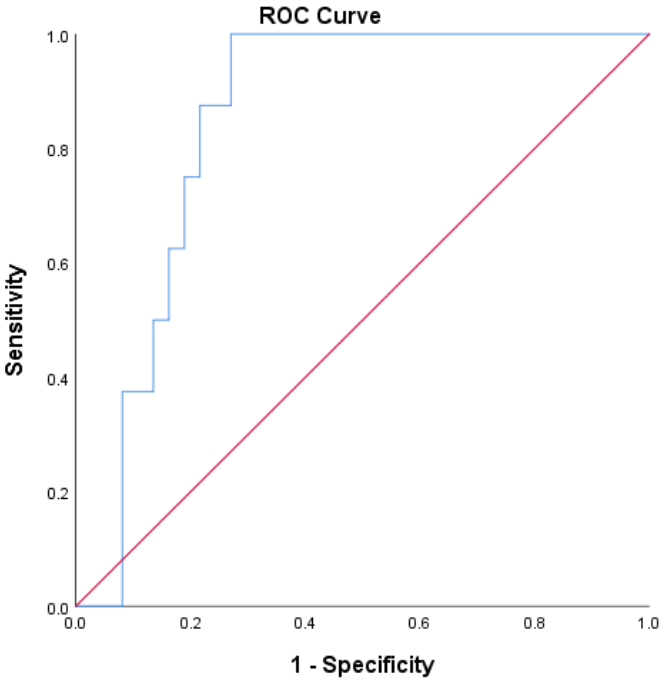

Figure 1. Neutrophil-lymphocyte ratio (NLR) and receiver operating characteristics (ROC) curve to histopathological degree

differentiated tumours, followed by moderately and well-differentiated tumours. Spearman's rho correlation analysis indicated a moderate positive correlation between NLR and histopathological differentiation ($r = 0.612$), suggesting that higher NLR values are associated with poorer tumour differentiation.

DISCUSSION

This study observed a higher prevalence of colorectal cancer among males, consistent with global data suggesting greater male susceptibility (9). This sex disparity may be attributed to both biological and lifestyle factors, including higher visceral fat accumulation, increased consumption of red and processed meats, higher rates of smoking and alcohol use, and pro-inflammatory metabolic profiles (9).

Age remains an important factor in colorectal cancer risk. The study population reflects the typical middle-aged to older adult demographic, aligning with previous reports highlighting regional variability in age at diagnosis (10). Given the rising incidence of colorectal cancer in younger populations, earlier screening protocols for individuals under 50 years have been suggested (11).

A central finding of this study is the significant association between systemic inflammation, as measured by the neutrophil-to-lymphocyte ratio (NLR), and histopathological differentiation. Elevated NLR was associated with poorer tumour differentiation, supporting previous evidence linking systemic inflammation with aggressive tumour biology (19,20). Mechanistically, neutrophils promote tumour angiogenesis and facilitate dissemination via secretion of factors such as vascular endothelial growth factor (VEGF), while lymphocytes mediate anti-tumour immunity through activation of CD8+ cytotoxic and CD4+ helper T cells, inducing tumour apoptosis and suppressing proliferation and metastasis (21,22). A high NLR thus reflects an imbalance between pro-tumour inflammatory activity and impaired anti-tumour immune response.

The clinical implications of this finding are significant. NLR is a simple, cost-effective biomarker that could be integrated into routine preoperative evaluation to aid in risk stratification, guide treatment planning, and identify patients who may require more intensive management or closer follow-up. Unlike some previous studies that assessed NLR only as a prognostic marker, this study additionally evaluated its diagnostic performance in relation to histopathological differentiation, highlighting its potential utility in clinical decision-making (23).

Histopathological differentiation itself remains a key determinant of treatment response and prognosis. Poorly differentiated tumours often show reduced responsiveness to neoadjuvant chemotherapy and radiotherapy, emphasizing the need for reliable preoperative markers of tumour aggressiveness (24). By linking NLR to differentiation, this study provides further evidence for its potential role in guiding individualized therapeutic strategies.

Several limitations should be acknowledged. The study was conducted at a single tertiary referral centre, which may bias the sample toward more advanced or complex cases. The relatively small sample size and the one-year study period may limit the generalizability of the findings and the robustness of statistical analyses. Additionally, the retrospective design relying on medical record data introduces the possibility of information and observer bias.

In conclusion, systemic inflammation, as reflected by NLR, is closely linked to tumour aggressiveness in colorectal cancer. Its incorporation into routine clinical assessment may facilitate early risk stratification and individualized treatment planning. Future large-scale, prospective, multicentre studies are warranted to validate these findings and to explore the integration of NLR with other inflammatory and molecular biomarkers to optimize prognostic and therapeutic decision-making.

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TRANSPARENCY DECLARATION

Conflicts of interest: None to declare.

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Bayesian model prediction for lung cancer survival based on demographic and laboratory results: a retrospective analysis

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ABSTRACT

Aim To examine the likelihood of predicting lung cancer survival versus death using Bayesian model based on demographic and laboratory data.

Methods A predictive design using electronic health records from 2012 to 2023 was implemented. IBM SPSS Statistics version 29.0 was used for data descriptive analysis and prediction models were built using SPSS Modeler version 18.0. Among the eight generated models, the Bayesian model demonstrated the highest accuracy (71.9%) and the best area under the curve (AUC) at 80.304, showcasing its superior predictive performance for lung cancer outcome.

Results A total of 1,843 patients without missing values were used. Males constituted 64.2 % of total sample. About 70 % of the patients were aged between 46 and 99 years. The Bayesian network identified seven key predictors for determining patient outcome (survival versus death). Among these, age was found as the most significant predictor of survival outcome.

Conclusion The Bayesian network outperformed other models in predicting lung cancer survival versus death probability. The integration of routine laboratory testing and demographic data in the machine learning model can help in the prediction of lung cancer survival versus death.

Keywords: death, machine learning, prediction, survival

INTRODUCTION

Lung cancer has become the most frequent disease globally and continues to be a major public health concern (1). In 2022, lung cancer has accounted for about 2.5 million new cases globally (2). Furthermore, lung cancer is a leading cause of illness worldwide (1). In the United States of America (USA), there is an increase in the incidence of lung cancer, which is accounted for 33 cases per 100,000 persons in 2022 (2). Lung cancer is considered to be a leading cause of death in the US with a mortality rate of 18.9 cases per 100,000 persons in 2022 (2). In Jordan, lung cancer is the fourth most frequent type of cancer and the average age at lung cancer diagnosis is 63.8 years (3).

Lung cancer development is significantly affected by demographic factors such as age and gender (4). Age is considered an important factor that affects the risk of developing lung cancer because this cancer becomes more common in older patients (5). Although lung cancer is uncommon in patients under 40 years of age, health care providers should not rule it out in younger patients (4). Lung cancer ranks first in men and second in women for both incidence and mortality (2). Regardless of the age at which it is recognized, men are more likely to have this malignancy than women (6). For both genders, lung cancer is a common cause of cancer-related death (7). In 2022, mortality rate among men was 18% higher than in women with lung cancer (2).

Laboratory tests are considered to be an important predictor of lung cancer such as white blood count (WBC), as higher WBC values were associated with lung cancer (8,9). Furthermore, low red blood cells (RBC) and haemoglobin (Hb) values were associated with lung cancer (10). Higher serum creatinine variation during hospitalization was associated with an increased risk of one-year mortality among patients with lung cancer (11). Manual diagnosis techniques for lung cancer are imaging tests, needle biopsy, sputum cytology, bronchoscopy, and lab tests (1).

In a recent study conducted in 2024, researchers used regression approaches, including the Cox proportional hazards (CPH) model to predict lung cancer survival (12). However, missing covariate data in clinical datasets is a significant barrier for

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regression-based models (12). Incomplete predictors cannot be included in regression-based predictive models. Machine learning (ML) algorithms imply that missing values occur randomly. However, if there is a specific missing pattern, such as a non-random process, ML algorithms may result in incorrect conclusions. Due to restrictions, commonly used imputation methods may not be accurate enough for datasets with missing values. To address the challenge of unavailable data in clinical risk assessment, we applied Bayesian network, which successfully manages data by creating a complex network structure with multiple components (12). The Bayesian network uses evidence to determine the posterior probability distribution of query variables (13). Furthermore, Bayesian Network is commonly utilized for predicting illness onset and progression, as well as evaluates treatment effectiveness. In cancer treatment, Bayesian network was used to estimate patient survival and evaluate treatment options, guiding the development of optimal strategies (12).

The aim of this study was to examine the likelihood of predicting lung cancer survival versus death using Bayesian network based on demographic and laboratory data.

PATIENTS AND METHODS

Patients and study design

Predictive and retrospective designs were used in this study. This study was conducted at Jordan University Hospital over the course of six months, from January 2024 to July 2024. Parients' information was managed with confidence. Each record was assigned an ID, allowing for anonymous processing of patient records. The extracted data were kept in a password-protected file at a secured computer in the researcher's office. The study data were only accessible to the researchers.

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki, Good Clinical Practices, and the Indian Council of Medical Research (ICMR). We included the electronic health records of patients who were diagnosed with lung cancer. The inclusion criteria specified patients with lung cancer regardless of the age. We excluded patients with any cancer other than lung cancer. The Jordan University Hospital Ethics Committee approved the study protocol prior to its initiation.

Methods

During the study period from January 2024 to July 2024, data on patients with lung cancer between 2012 and 2023 were obtained from Jordan University Hospital using a part of their electronic health records system (EHS). Extracting the data took four months due to the complexity of the organizational processes and the electronic health system. This dataset contains 1,843 records and eight variables. The selection of these variables was based on previous literature indicating that the choice of variables included in a study or model was guided by findings from previous publications. This approach is common in research to ensure that relevant and important factors are considered for predicting the outcome of lung cancer. Each record represents a particular patient within the database. These variables provide socio-demographic and lung cancer specific information. The variables that were requested included the stage of lung cancer, age, gender, RBC, Hb, WBC, and creatinine values, governorate, smoking, family history of cancer and patient outcome (survival versus death). The values such as the stage of lung cancer, smoking, and family history of cancer were excluded because they were not available in the electronic health records.

Statistical analysis

To build the Bayesian network, the following steps were performed as data preparation: checking for missing data cleaning by eliminating noisy data from the dataset and removing the duplicate and inconsistent data. All laboratory data were standardized using one international unit for analysis. All data were originally on five Excel sheets exported to SPSS files. Then, the files were merged into one data sheet by matching the cases with the ID numbers. Descriptive statistics were conducted using Statistical Package for Social Sciences, version 29.1 (14). IBM SPSS Statistics (Version 29.0) and IBM SPSS Modeler (Version 18.0) were employed for data manipulation, statistical analysis, and visualization in this study. These software applications enable effective data presentation for statistical and predictive analysis, as well as data management for descriptive and predictive modelling (15). Descriptive modelling found the major predictive variables for predicting lung cancer survival versus death. Predictive modelling was chosen based on accuracy and Area Under Curve (AUC) factors to create an appropriate model.

For applying data mining step, SPSS Modeler version 18.0 was used (15). Seventy and 30 percent of database records were selected as training and testing data, respectively. Training data were utilized to develop a predictive model, whereas testing data were used to evaluate the model's performance (16). The primary criteria for selecting the most effective AI model were the overall accuracy and AUC (17). The accuracy is the percentage of all the used datasets that are properly predicted out of all the instances (18). The AUC represents the performance metrics that determine the predictive ability of machine learning (ML) models and it measures the overall performance of the model (19).

The Bayesian network is a probabilistic graphical model that describes variables and their relationships using an acyclic graph with a directed structure (13). They are particularly useful in medical applications such as predicting lung cancer because they can model complex relationships between risk factors and symptoms, incorporating uncertainty and prior knowledge effectively. A probabilistic graphical model for information prediction about an uncertain area is called the Bayesian networks. According to Kim and Lee (13) this model has nodes that represent random variables and edges that reflect the conditional probability for the related random variables. Furthermore, the Bayesian network was selected due to several reasons other than achieving the highest performance, such as the likelihood of survival versus death calculated using the existing variables and their conditional likelihood correlations, effectively addressing the difficulty of risk assessment with partial knowledge (13). The Bayesian network supports expert knowledge to determine the conditional independence of risk variables. Additionally, they provide an intuitive visual representation of the relationships between survival and death parameters (20). Because the model is based on easily accessible covariates from daily clinical practice, it can be utilized as a predictive tool for particular lung cancer patients, assisting

doctors in decision-making process (13). Bayesian networks use a probabilistic framework to generate predictions, enabling outcomes to be interpreted in terms of probabilities.

RESULTS

The study included 1,843 patients between 2012 and 2023 of which 1,8184 (64%) were males. Age groups were categorized as children (2-18 years, 3.4%), young adults (19-45 years, 26.2%), and older adults (46-99 years, 70.4%). Laboratory assessments included haemoglobin (Hb), red blood cell (RBC) count, white blood cell (WBC) count, and creatinine serum levels. Below-normal Hb and WBC levels were observed in 628 (34.0%) and 569 (31.0%) of patients, respectively. Geographically, 1,084 (59%) were from Middle Governorates, followed by Northeast 423 (23.0%), South 175 (9.5%), and North Governorates 161 (8.7%). Notably, 1,479 (80%) of the patients belonged to the survived class (Table 1).

Table 1. Characteristics of the study sample (N=1,843).

Variable	No of patients (%)
Patient outcome	
Dead	364 (19.8)
Survived	1,479 (80.2)
Gender	
Male	1,184 (64.2)
Female	659 (35.8)
Age group	
Children (2-18)	63 (3.4)
Young adult (19-45)	482 (26.2)
Old adult (46-99)	1,298 (70.4)
WBC	
Normal	1,274 (69.1)
Above normal	569 (30.9)
Hb	
Normal	1,215 (65.9)
Below normal	628 (34.1)
Creatinine	
Normal	1,093 (59.3)
Abnormal	750 (40.7)
RBC	
Normal	1,133 (61.5)
Below normal	710 (38.5)
Governorates	
Middle	1,084 (58.8)
Northeast	423 (23.0)
South	175 (9.5)
North	161 (8.7)

Hb, haemoglobin; RBC, red blood cell; WBC, white blood cell

Among the eight generated models, the Bayesian network was the most effective one, achieving the highest overall accuracy score (80.304%) and the highest AUC score (0.719) (Table 2) followed by logistic regression (80.304%, AUC = 0.704), CHAID model (80.250, AUC = 0.692), neural network model (80.250, AUC = 0.685). Then, C5 model, Quest model, and C&R Tree model had the same performance (80.250, AUC = 0.5). Decision list model (70.912, AUC= 0.584) had the lowest performance.

Table 2. Characteristics of the study's models

Model	Overall accuracy (%)	Area under the curve
Bayesian network 1	80.304	0.719
Logistic regression 1	80.304	0.704
CHAID 1	80.250	0.692
Neural net 1	80.250	0.685
C5 1	80.250	0.5
Quest 1	80.250	0.5
C&R tree 1	80.250	0.5
Decision list 1	40.912	0.584

This study used the Bayesian network for binary classification to distinguish between deceased and surviving patients. The patient outcome node (survival versus death) in the graph represented a random variable, with death encoded as 0 and survival as 1. This encoding enabled the identification of probabilistic dependencies among discrete variables, facilitating the analysis of relationships within the dataset. The Bayesian network model contains eight nodes, including the parent node. It has 13 edges, representing the parameters that define the relationships between these nodes (Figure 1). Each node in the network represents a random variable of interest. For outcome (survival versus death) prediction of lung cancer, the predictor variables were age, gender, governorate, RBC, Hb, WBC, and creatinine values. The parent node is a node that has direct edges that lead to one or more child nodes (13). Directed edges between the nodes reflect the probability link among the variables in the network (20).

The Bayesian network found seven important predictors to predict the outcome of lung cancer (survival versus death). The

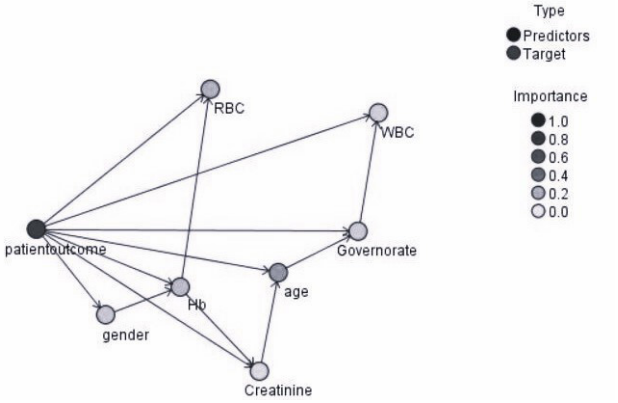

Figure 1. The structure of Bayesian network model
Hb, haemoglobin; RBC, red blood cell; WBC, white blood cell

age predictor's importance was 0.26, which is the most important predictor followed by RBC predictor's importance of 0.19, Hb predictor's importance was 0.16, gender predictor's importance 0.13, governorate predictor's importance 0.11, WBC predictor's importance 0.10, and creatinine predictor's importance was 0.06, which is the least important predictor (Figure 2).

For patients who had below normal RBC and Hb values, the conditional probability of death was 85%, while for patients who had normal RBC and Hb values, it was 80%. Survival probabilities were greatest among individuals with normal RBC and Hb values (0.81%). Survival probabilities among patients with normal creatinine value (0.69%) were higher

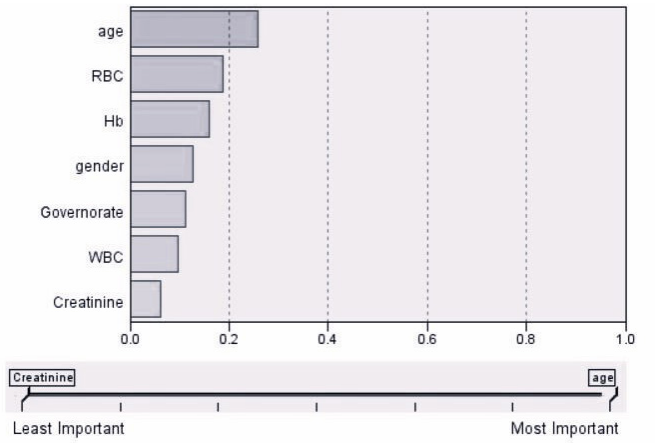

Figure 2. Predictors' importance for the outcome of lung cancer
Hb, haemoglobin; RBC, red blood cell; WBC, white blood cell

than in patients with abnormal creatinine value (0.31%). Furthermore, survival probabilities among patients who live in the North Governorates and had above normal WBC (0.14%) were lower than that in patients with normal WBC (0.86%). The older adults who live in the Middle Governorates had the highest probability of death (0.75%), while older adults residing in the North Governorates demonstrated the lowest recorded probability of death at just 0.04%. However, when WBC values were normal, the highest death probability was in the North Governorates (0.83%) and the Northeast Governorates (0.70%). Males with normal Hb levels had a higher survival probability (0.78%) compared to those with below-normal Hb levels (0.22%). Additionally, the probability of death among males was 0.42% higher than that of females (Table 3).

DISCUSSION

Machine learning is currently one of the most popular approaches to create a prediction model. It has been widely employed in medical science to assist healthcare professionals with prognosis, diagnosis, and other factor analysis. Machine learning algorithms are capable of managing massive amount of data and create prediction models for illness development, risk factors, and management, making them very useful. Using ML algorithms to analyse data can improve patient outcomes, identify needs, and enhance quality of life (21). In this paper, Bayesian network was used to predict survival versus death of lung cancer based on risk factors. Furthermore, Bayesian network is an extremely advanced modelling technique with numerous parameters (22). It is commonly used to forecast illness onset and progression, as well as evaluate treatment effectiveness, and for risk prediction of lung cancer (12).

Many researches have assessed the usefulness of machine learning algorithms in predicting the risk of cancer, but few of them used Bayesian network to predict the survival of lung cancer (12,20,23). However, the findings of this study contribute to clarifying the effectiveness of ML algorithms for the prediction of survival versus death likelihood among patients with lung cancer. The study model shows correlations between the outcome variable (whether the individual survived or died) and the seven predictors. This demonstrates how changes in the parent nodes' statuses might alter the probabilities of outcomes in the child nodes.

The Bayesian network provides a thorough description of the interactions and effects of a number of variables on patient outcomes (12). A recent study using a Bayesian network model to

Table 3. The Bayesian network model's determination of the probabilities of survival versus death and predictors

Parents Nodes	**Conditional probabilities of RBC**		**Conditional probabilities of creatinine**	
Dead or Survived/ Hb	**Normal**	**Below normal**	**Normal**	**Abnormal**
Survival/normal	0.81	0.19	0.69	0.31
Death/normal	0.80	0.20	0.57	0.43
Survived/below normal	0.28	0.72	0.43	0.57
Death/below normal	0.15	0.85	0.46	0.54
	Conditional probabilities of age (years)			**Conditional probabilities of WBC**
Dead or Survive/ Governorate*	**Children** (2-18)	**Young adult** (19-45)	**Old adult** (46-99)	**Normal**	**Above normal**
Survived /1	0.46	0.44	0.61	0.68	0.32
Dead/1	0.57	0.57	0.75	0.50	0.50
Survived/2	0.14	0.30	0.23	0.67	0.24
Dead/2	0.14	0.20	0.15	0.70	0.30
Survived/3	0.16	0.15	0.07	0.86	0.14
Dead/3	0.14	0.09	0.04	0.83	0.17
Survived/4	0.23	0.12	0.09	0.77	0.32
Dead/4	0.14	0.15	0.06	0.56	0.44
	Conditional probabilities of Hb			**Conditional probabilities of gender**	
Dead or survived/ Gender	**Normal**	**Below normal**		**Male**	**Female**
Survived/male	0.78	0.22		0.62	
Death/male	0.60	0.40		0.71	
Survived/female	0.53	0.47			0.38
Death/female	0.40	0.60			0.29

*1, middle, 2, Northeast, 3, North, 4, South; Hb, haemoglobin; RBC, red blood cell; WBC, white blood cell

evaluate lung cancer survival prognosis showed that internal validation demonstrated robust predictive performance, with the model achieving strong discrimination and calibration metrics, reflected by an AUC of 0.896 (12). The Bayesian network model had the highest overall accuracy score (80.304%) and the highest AUC score (0.719) in our study, whereas Bayesian networks had a low AUC score (0.614), which is utilized to predict survival among individuals diagnosed with lung cancer (23). The AUC decreases as the percentage of missing values in the validated dataset increases (12). This could mean that Bayesian network requires sufficient and accurate data for learning reliable conditional probabilities. Furthermore, the predictive study aimed to assess the potential value of blood inflammation in the diagnosis of lung cancer using a machine learning algorithm. It showed that the naive Bayes algorithm outperformed the other five ML systems in predicting adult lung cancer, with an AUC of 0.84 and an accuracy of 0.87 (10).

Age was the most important predictor for lung cancer, with incidence and mortality probability rising dramatically with age. Older patients (46-99 years) have the highest chance of mortality, which is consistent with evidence suggesting that increasing age is associated with significantly higher death outcomes in lung cancer (2). Researchers found that age was an important predictive factor for predicting lung cancer survival and prognosis using Bayesian network, as the median age of the patients with lung cancer was 63 years and it ranged from 22 to 92 years (12).

Laboratory tests can determine the survival and death probability of lung cancer. In our study, below normal red blood cell (RBC) and haemoglobin (Hb) were considered as important predictors for low survival probability among patients with lung cancer. Furthermore, a cross-sectional study found that Hb and RBC were significantly lower in lung cancer compared with healthy individuals (10).

Gender is the fourth important predictor. The study's findings are consistent with other trends found in the literature on lung cancer. According to global data, males are somewhat more likely to develop lung cancer than females, which is consistent with our study's higher male prevalence (2). The study's probability of survival and death was higher among males than females, which is consistent with global trends, particularly in high-income nations where early identification and advanced therapies are more widely available (2). A study of survival rates by gender found that treatment, healthcare systems, and comorbidities are likely to play significant effects (24).

In our study, the death probability was high in the Northeast Governorates (0.70), which is consistent with a Jordanian study revealing that Al- Mafraq city (northeast Jordan) has a high probability of lung cancer, which is possibly related to gaseous highly radioactive Radon element in Al- Mafraq city (25).

Other laboratory test that have been frequently obtained from patients was WBC, which is the sixth important predictor in this study. Elevated WBC is an alarming risk associated with lung cancer (8). In our study, above normal WBC was associated with poor survival rates and high death rate. Similarly, a predictive study aimed to create a prediction model that predicts future lung cancer diagnoses based on the available laboratory and clinical such as WBC, found that

WBC is an essential factor for predicting lung cancer death and has the ability to help prevent deaths from lung cancer through early identification utilizing a machine learning algorithm (9).

Creatinine serum value is considered as the least important predictor. Furthermore, abnormal serum creatinine is a predictor for low survival probability in our study. Creatinine could be a good biomarker for detecting early lung cancer (26). Researchers found that serum creatinine fluctuation increased the chance of death in patients with lung cancer (11).

The use of Bayesian network in lung cancer prediction incorporates previous knowledge and beliefs about causal links and conditional probabilities, as well as handling incomplete or uncertain data. The graphical structure of Bayesian network makes it easier to understand and convey variable interactions than more sophisticated ML models.

One of our study's strengths was that the Bayesian network efficiently overcomes the issues of missing data in forecasting patients' outcomes, while retaining high accuracy in prediction. Furthermore, the ML method was used in the study to analyse a large dataset. This study was especially unique in that it examined all patients with lung cancer, regardless of age, rather than merely the elderly.

Our research has a few limitations. While the aim was to include key variables relevant to predicting patient outcomes, such as lung cancer stage and medical imaging, these were excluded due to unavailability in the electronic health system. Additionally, the dataset's class imbalance, with most patients classified as survivors, may influence the results.

Prospective research is required to verify the use of the Bayesian network in future investigations. Further research is needed to compare the effectiveness of our ML model to the most used models for predicting lung cancer outcomes. Researchers often utilize Bayesian network in conjunction with medical data to investigate the links between risk variables and disease outcomes. Studies may focus on network structure refinement, inference algorithm improvement, and prediction validation against clinical data.

Healthcare providers can use the Bayesian network to evaluate survival versus death probabilities and guide hospital-based lung cancer treatment decisions, fostering personalized treatment approaches using routine demographic and laboratory data. In lung cancer survival prediction, the Bayesian network identifies the most influential predictors, such as age, gender, governorate, RBC, Hb, WBC, and creatinine values. This enhances model interpretability and aligns with clinician expertise, validating its practical utility.

Nursing practice implications involve utilizing predictive models to improve information systems, support clinical decisions, streamline documentation, and estimate death probabilities. By incorporating routinely available data into health records, the Bayesian network can deliver precise risk predictions. This AI approach elucidates complex relationships between lung cancer predictors, including demographics and laboratory results. Given its simplicity and interpretability compared to other ML methods, the Bayesian network is increasingly adopted in healthcare and can be seamlessly integrated into clinical workflows.

The Bayesian network is a robust tool for predicting lung cancer survival versus mortality. Its ability to manage uncertainty and integrate prior knowledge makes it highly beneficial for medical decision support systems. This study has examined the likelihood of predicting lung cancer survival versus death using Bayesian network based on demographic and laboratory data. Abnormal laboratory tests and older age were considered as important predictors for low survival probability. Incorporating demographic information and routine laboratory tests such as RBC, WBC counts, Hb, and creatinine levels enhance

the model's capacity to predict survival outcomes, supporting improved clinical decision-making for lung cancer care.

AUTHOR CONTRIBUTIONS STATEMENT

Conceptualization, IM, MA; Methodology, IM, MA; data collection: IM; analysis and interpretation, MA; manuscript writing, IM, MA; approval of the final article, IM, MA; accountability for all aspects of the work, IM, MA.

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TRANSPARENCY DECLARATION

Competing interests: None to declare.

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Med Glas (Zenica)

ORIGINAL ARTICLE

The correlation between interleukin-6 levels and quality of life in late-stage breast cancer patients with anxiety syndrome at Haji Adam Malik General Hospital, Medan

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ABSTRACT

Aim Breast cancer is the most common cancer among women worldwide, including Indonesia. Breast cancer patients are prone to have anxiety and it affects their quality of life. Interleukin-6 (IL-6) is one of the pro-tumorigenic cytokines that increases in breast cancer, and is associated with worse prognosis and cancer metastasis. Several studies demonstrated the correlation between inflammatory cytokines and anxiety disorders. Therefore, this study aimed to determine the correlation between IL-6 and quality of life with anxiety syndrome in patients with late-stage breast cancer.

Methods This cross-sectional study involved women with late-stage breast cancer (Stage III and IV). IL-6 level was assessed using Sandwich enzyme-linked immunosorbent assay (Sandwich-ELISA). The quality of life and anxiety syndromes were assessed using the European Organization for Research and Treatment of Cancer 30-Item Quality of Life Questionnaire (EORTC QLQ-C30) and Hospital Anxiety and Depression Scale (HADS)-A questionnaire, respectively. Kruskal-Wallis test was used to determine the correlation between variables. A $p<0.05$ was considered statistically significant.

Results Fifty-nine women met the inclusion criteria. Higher IL-6 level ($p<0.001$) and poor QoL ($p<0.001$) were related to severe anxiety disorders in late-stage breast cancer patients.

Conclusion IL-6 level and quality of life can be potentially used for the risk stratification and early detection of anxiety syndrome in late-stage breast cancer patients.

Keywords: anxiety, breast neoplasm, cytokine, quality of life

INTRODUCTION

Breast cancer is the most common cancer in women globally, surpassing lung cancer. Global Cancer Incidence, Mortality, and Prevalence (GLOBOCAN) 2020 reported an estimated 2.3 million new cases of breast cancer worldwide in 2020, with 684,996 deaths. Breast cancer accounts for 1 in 4 of all cancer cases and for 1 in 6 of all cancer deaths among women, ranking first in incidence in the vast majority of countries (1). The estimated breast cancer prevalence in Indonesia was 0.5%, and it ranked as the second most common cancer type following cervical cancer (2). The mortality rate of breast cancer has decreased in most Western countries due to modern therapy and early detection strategies, but developing countries have higher mortality rates of breast cancer than developed countries (15.0 per 100,000 vs 12.8 per 100,000) (1-3).

Various therapeutic methods have been developed based on the molecular characteristics of breast cancer. The¬treatment methods determined by¬the breast cancer stage, ranging from loco-regional therapies (surgery and radiotherapy), systemic, neoadjuvant therapy, and targeted drugs or immune checkpoint inhibitors (3).

Breast cancer is a multifactorial disease that involves genetic and environmental factors that play a role in its development, including inflammatory diseases (4). Interleukin-6 (IL-6) is one of the pro-tumorigenic cytokines. IL-6 dysregulation will trigger the pathogenesis of various diseases, such as cancer. There were increased IL-6 levels in breast cancer, and it was associated with a bad prognosis and metastasis (4). The survival rate of breast cancer reaches 85% and sequelae are common in breast cancer survivors, such as body image, psychological, and family problems (5). Breast cancer has negative psychological effects such as anxiety, stress, fear, and depression that are associated with quality of life in which these symptoms do not only affect breast cancer patients but also affect breast cancer survivors (5-7).

Anxiety is one of the most common psychological problems among breast cancer patients (8). The potent role of inflam-

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mation in mental disorders pathogenesis has attracted much attention for several past years, and some studies have proved the correlation between those variables (9,10). Breast cancer symptoms may lead to lower quality of life (5).

The aim of this study was to determine the correlation between IL-6 level and quality of life with anxiety syndrome in late-stage breast cancer patients in Haji Adam Malik General Hospital, Medan, Indonesia.

PATIENTS AND METHODS

Patients and study design

This cross-sectional study was conducted in the Oncology Surgery polyclinic of Haji Adam Malik General Hospital, Medan, Indonesia, from February 2024 to May 2024.

It used consecutive sampling method. The inclusion criteria were: women diagnosed with late-stage breast cancer (stage IIIA, IIB, IIIC, and IV) based on histopathological examination, aged 30 – 65 years, on chemotherapy and having completed at least three cycles of chemotherapy, and signed informed consent voluntarily. The exclusion criteria were: the participants had communication difficulties, comorbidities such as diabetes, cardiac disease, epilepsy, infection, and autoimmune disorders, a history of psychiatric illness, pregnant women, lactating mothers, patients who were consuming autoimmune medication or hydroxychloroquine, had surgeries or infection for the last one week before data collection. The association between IL-6 levels and quality of life with the severity of anxiety syndrome was analysed.

Ethical clearance and permission were obtained prior to data collection. We gave a brief explanation about this research, and the patients were asked to give informed consent before the data collection process was started. All data were kept confidential.

Methods

After the informed consent was signed voluntarily, participants were asked to fill in demographic data and a Hospital Anxiety and Depression Scale-A questionnaire (HADS-A) to assess their anxiety levels (5). The hospital Anxiety and Depression Scale consists of 7 anxiety questions that have scores of 0 – 3, respectively, and a total score of 0 - 21. The results were categorized into no anxiety (score of 0), mild (score of 1 – 10), moderate (score of 11 – 15), and severe (score of 16 – 21) anxiety.

Participants had venous blood samples (±5 mL) collected for IL-6 level measurement using a sandwich enzyme-linked immunosorbent assay (Sandwich ELISA) in the hospital laboratory. After the venous blood sampling, participants were asked to fill out the European Organization for Research and Treatment of Cancer 30-Item Quality of Life Questionnaire (EORTC QLQ-C30) for quality of life assessment (11). This questionnaire consists of five domains, including physical, emotional, social, role, and cognitive. The results were grouped into good, moderate, and poor quality of life. We provided an explanation about each questionnaire beforehand to avoid misunderstanding.

Statistical analysis

Descriptive analysis was conducted for all data. The numeric data were presented in mean± standard deviation (mean±SD) or median (min-max) for normally and abnormally distributed data, respectively. The data normality was assessed using the Kolmogorov-Smirnov test. The categoric data were presented in frequency (percentage). The correlations were determined using one-way ANOVA for normally distributed data or the Kruskal-Wallis test for abnormally distributed data. A $p<0.05$ was considered statistically significant.

RESULTS

This study included 59 selected women based on inclusion and exclusion criteria. The mean age was 51.14±7.26 years. Most women were unemployed (86.4%) and high school graduates, 54 (91.5%). They had been diagnosed with breast cancer for 1 to 6 years. Based on the disease stage, most women had stage IV breast cancer, 29 (49.2%). The mean of IL-6 level was 3.37±2.72 µg/dL. Based on the HADS-A and EORTC QLQ-30 scores, most women had mild anxiety, 29 (49.2%) and good quality of life, 37 (62.7%) (Table 1).

Table 1. Characteristics of the women with breast cancer

Characteristics	
Mean±SD age (years)	51.14±7.26
	No (%) of women
Occupation	
Employed	8 (13.6)
Unemployed	51 (86.4)
Education	
High school	54 (91.5)
Diploma or bachelor	5 (8.5)
Stage	
IIIA	5 (8.5)
IIIB	10 (16.9)
IIIC	15 (25.4)
IV	29 (49.2)
HADS-A	
Mild anxiety	29 (49.2)
Moderate anxiety	20 (33.9)
Severe anxiety	10 (16.9)
EORTC QLQ C-30	
Good	37 (62.7)
Moderate	6 (10.2)
Bad	15 (27.1)
Median duration of breast cancer (min – max) (years)	3 (1 – 6)
Mean±SD IL-6 (µg/dL)	3.37±2.72

HADS-A, Hospital Anxiety and Depression Scale-A questionnaire; EORTC QLQ C 30, European Organization for Research and Treatment of Cancer 30-Item Quality of Life Questionnaire

The correlations between variables were determined using the Kruskal-Wallis test. The analysis revealed a significant difference in the severity of anxiety syndrome based on the QoL level, which implies that there was a correlation between IL-6 and anxiety, with poor QoL associated with severe anxiety syn-

Table 2. The association between IL-6 level and quality of life with the severity of anxiety syndrome

Variable	**N (%) of participants**	**p**
IL-6 with the severity of anxiety syndrome	59 (100)	<0.001
Quality of life with the severity of anxiety syndrome	59 (100)	<0.001

drome ($p<0.001$). Furthermore, there was also a significant difference in the severity of anxiety syndrome based on the QoL level which implies that there was a correlation between QoL and anxiety, with poor QoL associated with severe anxiety syndrome ($p<0.001$) (Table 2).

DISCUSSION

The mean age of women in this study was 51.14 years, similarly to a study where the mean age of breast cancer patients was around 50 years of age (58.6 ± 9.4). The breast cancer risk increases with age starting from the 40s (12). Age is one of the breast cancer risk factors. The most common age of breast cancer patients is between 50 and 69 years (13). Most women were unemployed and high school graduates. Poverty, low educational status, and the absence of health insurance were associated with low breast cancer survival rates (14).

Based on the mean quality of life score, patients who had been diagnosed with breast cancer for more than one year have a better quality of life, particularly in the general health status and functional status, compared to those with less than one year of diagnosis. Anxiety increases at the time of cancer diagnosis but tends to diminish over time as patients adapt to their illness. (15). Most women in this study were stage IV breast cancer patients, which is in accordance with a study in which 55% of participants had stage IV breast cancer (16). The advanced stage of breast cancer was associated with the lower education levels, living in rural areas, limited access to advanced screening technology, and inequitable healthcare (17,18).

The mean of IL-6 level of our patients was 3.37. IL-6 plays an important role in the process of the normal mammary epithelial cells transformation through NF-kB activation. It is also associated with TNM staging, breast cancer metastasis and recurrence since high IL-6 levels are strongly associated with aggressive tumours (19). Based on HADS-A and EORTC QLQ-30 scores, most women had mild anxiety and good quality of life, respectively. Similarly, Hajj et al. demonstrated that most breast cancer patients who received chemotherapy experienced anxiety. It is estimated that 42.4% of breast cancer patients had at least mild anxiety (8,20). Social support had a positive role in reducing anxiety symptoms including support from family members, hospitals and nursing professionals (8).

Our study demonstrated a correlation between IL-6 levels and anxiety syndrome, consistent with other studies, suggesting that the immune-inflammatory system may play a role in anxiety (21,22). However, another study stated that IL-6 levels had less consistent association with anxiety (23). Age is another factor associated with anxiety syndrome. Older age is associated with lower anxiety, including in cancer patients. On the other hand, younger cancer patients are more prone to anxiety syndrome. It may be due to the higher impact of cancer on their life plans, and older patients had more life experience (24). Addionally, we found that quality of life and anxiety syndrome were also correlated; higher quality of life was associated with milder anxiety syndrome, similar to the other studies (20,24). Studies demonstrated that anxiety and depression during cancer diagnosis and therapy would increase physical and psychological symptoms that adversely impact the quality of life and decrease adaptation during therapy. Longer duration of breast cancer is associated with higher anxiety and depression which leads to decreased quality of life (25).

This was the first study in Indonesia to determine the correlation between IL-6 levels and quality of life with anxiety syndrome in late-stage breast cancer patients. Therefore, this study can be a guide for future studies.

Our study has several limitations: we only examined one pro-inflammatory cytokine level, and IL-6 without any adjusted or multivariate analysis. Furthermore, this was a single-centre study and was time-consuming due to the need for precise and timely blood sampling and sample storage.

In conclusion, the correlation between IL-6 levels and breast cancer stage and anxiety syndrome in late-stage breast cancer has been demonstrated in this study. Quality of life was correlated with anxiety syndrome in late-stage breast cancer. It implies that IL-6 level and quality of life have the potential to be applied for anxiety syndrome risk stratification or early detection in late-stage breast cancer patients, although further studies are required. Longitudinal, multicentre studies are needed to explore the causal relationship between these variables and to increase the generalizability of the study findings.

AUTHORS CONTRIBUTION

Conceptualization, E.Y.D, M.M.A, E.E, V.C, and K.B.S; Methodology, E.Y.D, M.M.A, and E.E; Software, E.Y.D; Validation E.Y.D; Formal Analysis, E.Y.D and M.M.A; Investigation E.Y.D; Resources, E.Y.D and M.M.A; Data Curation, E.Y.D, M.M.A, and E.E; Writing (original draft), E.Y.D and M.M.A, Writing (Review & Editing), E.Y.D, M.M.A, E.E, V.C, and K.B.S; Visualization, E.Y.D, M.M.A, and E.E; Supervision E.Y.D, M.M.A, E.E, V.C, and K.B.S; Project Administration, E.Y.D..

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Conflicts of interest: None to declare.

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ORIGINAL ARTICLE

Continuous positive airway pressure failure in the management of neonatal respiratory distress in a resource-limited setting: a prospective observational study

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ABSTRACT

Aim To determine the frequency of, and identify predictors for, continuous positive airway pressure (CPAP) failure in neonates of varying gestational ages in a resource-limited setting, where CPAP is often used beyond the preterm population.

Methods A prospective observational study included 119 neonates initiated on CPAP within 24 hours of birth for respiratory distress. We collected demographic, clinical, and perinatal data. CPAP failure was defined by persistent hypoxia or severe respiratory distress despite maximal settings, necessitating mechanical ventilation. Univariate and multivariable binary logistic regression analyses identify predictors of CPAP failure.

Results The CPAP failure rate was 33.6%. Univariate analysis identified lower gestational age (≤ 30 weeks), lower birth weight (≤1200 g), female sex, vaginal delivery, and a 5-minute Apgar score < 7 as significant predictors. However, in the multivariable model, only a 5-minute Apgar score **< 7** remained an independent predictor (Adjusted Odds Ratio AOR 5.315; p=0.006). Antenatal corticosteroid, age at CPAP initiation, and initial fraction of inspired oxygen $(FiO)_2$ were not significant.

Neonates with birth weight <1200g had shorter duration of successful CPAP use as revealed by Kaplan-Meier survival analysis (p<0.001)

Conclusion This study confirms that CPAP failure is a frequent and serious problem in our resource-limited NICU. A low 5-minute Apgar score is a significant predictor of failure. To save more newborns, we must focus on improving neonatal resuscitation and establish clear guidelines for respiratory care in our specific context.

Keywords: Apgar score, continuous positive airway pressure, developing countries, respiratory distress syndrome, newborn.

INTRODUCTION

Continuous positive airway pressure (CPAP) is the main non-invasive respiratory support for neonates with respiratory distress (1). Its primary mechanism is to maintain functional residual capacity by keeping alveoli open and reducing the work of breathing, which will improve oxygenation (2). CPAP effectively supports neonates across a range of respiratory conditions, including transient tachypnoea of the newborn (TTN), respiratory distress syndrome (RDS), congenital pneumonia, meconium aspiration, and apnoea of prematurity (3). TTN typically presents with tachypnoea and signs of respiratory distress within the first two hours of life in term and late-preterm neonates (4). It results from the delayed clearance of foetal lung fluid, leading to increased work of breathing. CPAP is often useful in these cases by providing respiratory support (5). Prematurity-related complications are considered a major factor in global under-5-year mortality. A significant number of these preterm births occurs in low- and middle-income countries (LMICs), where limited healthcare resources often lead to higher risks and poor outcomes (6).

In preterm neonates, RDS presents one of the most immediate and serious challenges caused by insufficient surfactant (7), which leads to the formation of hyaline membranes in the lungs and impairs gas exchange. During the first few days of life, the condition intensifies respiratory effort, leads to hypoxia, respiratory failure, and even death if not managed properly (8). The management strategy for RDS includes early CPAP use, surfactant replacement, and mechanical ventilation if needed (9). However, access to these therapies remains limited

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in many LMICs. The 2022 European Consensus Guidelines on managing respiratory distress syndrome advocate for starting CPAP as early as possible to improve outcomes in all neonates at risk of RDS (10), as it can reduce the need for surfactant replacement and mechanical ventilation (11). Even though implementing CPAP therapy in LMICs is feasible, a concerning 20–40% of neonates fail to respond to CPAP therapy (12). It is noted that CPAP failure has been associated with an increased risk of death and morbidities such as pneumothorax and bronchopulmonary dysplasia (13). Known predictors of CPAP therapy failure include lower gestational age, severe respiratory distress syndrome, high fraction of inspired oxygen (FiO_2), and delay in initiation of CPAP therapy (14).

Given the region's constrained resources, equipment, and healthcare infrastructure, this study aimed to assess the frequency of CPAP failure in neonates with respiratory distress, identify associated risk factors, and improve neonatal care in similar healthcare settings.

PATIENTS AND METHODS

Patients and study design

This prospective observational cohort study was done at Al-Batool Teaching Hospital (ATH), Mosul, Iraq, to assess CPAP failure rates and associated risk factors in neonates with respiratory distress admitted to the Neonatal Intensive Care Unit (NICU). Every year, about 1,500 neonates are admitted to the ATH neonatal unit. Care was provided by a consultant paediatrician and a general paediatrics resident, supported by a nurse-to-patient ratio of 1:5 to 40 neonates simultaneously.

The NICU is equipped to deliver nasal CPAP to up to 14 neonates concurrently, using SLE1000 devices with nasal prongs. Mechanical ventilation is limited to three neonates, and surfactant therapy was not administered due to limited availability.

Eligible newborns included those who received CPAP support in the first 24 hours of life due to clinical signs of respiratory distress, as one or more of the following: tachypnoea (>60 breaths/min), grunting, subcostal or intercostal retractions, central cyanosis, or apnoea.

A total of 119 neonates meeting the inclusion criteria were enrolled in this study from May to December 2024.

Enrolled neonates received routine care, including monitoring of oxygen saturation, heart rate, random blood sugar, and temperature, with respiratory distress assessment.

Every patient received empirical intravenous antibiotics and fluid therapy upon admission. Caffeine citrate was given for apnoea of prematurity.

Exclusion criteria were neonates with multiple congenital anomalies, sepsis, or those with CPAP discontinued for two hours or more. The study was approved by the Ethics Committee of the College of Medicine, University of Mosul.

Methods

Paediatric residents collected data using the New Ballard Score for gestational age (15), the Silverman Andersen Score (SAS) for respiratory distress assessment (16), and completed standardized forms. The measured parameters were gestational age, birth weight, sex, mode of delivery, use of antenatal steroids, 5-minute Apgar score, time of CPAP initiation, initial fraction of inspired oxygen (FiO_2), and type of respiratory distress. CPAP success or failure was the primary outcome.

Chest x-rays were done if symptoms persisted more than six hours, or earlier if pneumothorax or congenital anomalies were suspected. The CPAP began with a Peak End Expiratory Pressure (PEEP) of 5 cmH_2O, with an increase of 1 cmH_2O every 2-4 hours to a maximum limit of 8 cmH_2O. The initial FiO_2 was obtained from the digital oxygen blender integrated into the SLE1000 device (SLE Ltd., United Kingdom). Fraction of inspired oxygen (FiO_2) was then titrated in 5% increments every 30 minutes, up to 60%, and guided by continuous pulse oximetry to maintain Peripheral Oxygen Saturation (SpO_2) ≥ 90%.

The CPAP failure was defined by persistent hypoxia (SpO_2 <90%) despite maximal oxygen on CPAP, or ongoing severe respiratory distress (Silverman Anderson Score; SAS>6), or recurrent prolonged apnoeic episodes despite maximal PEEP. These neonates were put on mechanical ventilation, according to ventilator availability and clinical assessment. The CPAP was weaned when neonates were stable for 12-24 hours on current PEEP, showing no or mild respiratory distress (SAS <3), FiO_2 <27%, and SpO_2 consistently >95%. Nasal oxygen at 0.5 –2 L/min is then used to maintain adequate oxygenation after successful weaning from CPAP.

Statistical analysis

The Shapiro-Wilk test was used to assess the distribution of the continuous variable. The study population's features were described by median and interquartile range (IQR) for continuous variables, frequencies and percentages for categorical variables, presented in a table. The $\chi 2$ test and Fisher's exact test were used to evaluate associations between categorical variables, while the Mann-Whitney U test was used to assess associations for continuous variables.

Univariate binary logistic regression identified factors associated with CPAP failure, and then multivariable logistic regression was done to identify independent predictors of CPAP failure. Kaplan-Meier survival analysis was finally used to compare survival time to CPAP failure. P-value <0.05 is considered statistically significant.

RESULTS

This prospective observational study included 119 neonates. Shapiro-Wilk tests confirmed non-normal distribution for gestational age, birth weight, and CPAP duration ($p<0.05$).

Baseline characteristics stratified by CPAP success and failure reveal that male infants comprised 77 (64.7%) of the cohort. The median gestational age was 33 weeks, with 31 (26.1%) babies ≤30 weeks. Median birth weight was 1800g, and 25 (21%) were <1200g. Seventy (58.8%) neonates were delivered by Cesarean Section (CS) and 49 (41.2%) by normal vaginal delivery (NVD). Respiratory distress etiologies included 85 (71.4%) with RDS and 28 (23.5%) with TTN. Thirty-one (26.1%) neonates had an Apgar score <7. The median CPAP duration was three days. Ninety-six (80.7%) mothers had not received antenatal steroids. Maternal comorbidities included 10 with premature rupture of membranes, seven with hypertension, and two with diabetes. The prevalence of CPAP failure was 33.6% (Table 1).

Factors associated with CPAP failure demonstrate no statistically significant association between sex and CPAP outcome ($p=0.067$). However, a significant association was found with the mode of delivery ($p=0.003$): among CPAP failures, 60% were by NVD and 40% by CS, whereas in the no-failure group,

Table 1. Baseline statistics for the study population

Characteristic	Total 119 (100%)	CPAP success (N=79)	CPAP failure (N=40)	p
Sex (No; %)				
Male	77 (64.7)	56 (70.9)	21 (52.5%)	0.067
Female	42 (35.3)	23 (29.1)	19 (47.5%)	
Median gestational age (IQR)	33 (30-36)	34 (32-36)	29 (27-32)	< 0.001m*
Gestational age group (No; %)				
≤ 30 weeks	31 (26.1)	5 (6.3)	26 (65)	< 0.001*
> 30 weeks	88 (73.9)	74 (93.7)	14 (35)	
Median birth weight (IQR)	1800 (1250-2500)	2300 (1700-2600)	1100 (907.5-1475)	< 0.001m*
		No (%) of patients		
Birth weight group				
< 1200	25 (21)	3 (3.8)	22 (55)	<0.001*
≥ 1200	94 (79)	76 (96.2)	18 (45)	
Mode of delivery				
NVD	49 (41.2)	25 (31.6)	24 (60)	0.003*
CS	70 (58.8)	54 (68.4)	16 (40)	
APGAR score at 5 minutes				
<7	31 (26.1)	12 (15.2)	19 (47.5)	<0.001*
≥7	88 (73.9)	67 (84.8)	21 (52.5)	
Type of respiratory distress				
RDS	85 (71.4)	47 (59.5)	38 (95)	<0.001*
TTN	28 (23.5)	28 (35.4)	0	
MAS	5 (4.2)	4 (5.1)	1 (2.5)	
Pneumonia	1 (0.8)	0	1 (2.5)	
Maternal use of antenatal steroids				
Receive	23 (19.3)	18 (22.8)	5 (12.5)	0.180
Not receive	96 (80.7)	61 (77.2)	35 (87.5)	
PROM				
YES	10 (8.4)	8 (10.1)	2 (5)	0.341
NO	109 (91.6)	71 (89.9)	38 (95)	
Age at initiation of CPAP				
≤ 6 h	104 (87.4)	69 (87.3)	35 (87.5)	0.614
> 6 h	15 (12.6)	10 (12.7)	5 (12.5)	
Initial FiO_2				
≤35%	40 (33.6)	31 (39.2)	9 (22.5)	0.051
>35%	79 (66.4)	48 (60.8)	31 (77.5)	
Median duration of CPAP (IQR)	3 (2-4)	3 (2-4)	3 (2-4.75)	0.337m

* statistically significant; m Mann Whitney test

CS, Cesarean Section; FiO2, Fraction of Inspired Oxygen; IQR, Interquartile Range; MAS, Meconium Aspiration Syndrome; NVD, Normal Vaginal Delivery; PROM, Premature Rupture of Membrane; RDS, Respiratory Distress Syndrome; TTN, Transient Tachypnoea of Newborn;

31.6% were NVD and 68.4% CS. The type of respiratory distress was also significantly associated with CPAP outcome (p<0.001); RDS was common in both groups, but constituted 95% of the failure group (Table 2).

Mann-Whitney U tests revealed significant differences in both gestational age and birth weight between the CPAP failure and success groups (both p<0.001). Failure group neonates had a median gestational age of 29 weeks versus 34 weeks for the success group. Also, median birth weight in the failure group was 1100 grams compared to 2300 grams in the success group. CPAP duration showed no significant difference between the two groups (p =0.337).

Initial univariate analysis identified several factors significantly associated with CPAP failure. These included female sex (OR=2.203; p=0.049), gestational age of ≤30 weeks (OR=27.486; p<0.001), birth weight ≤1200 grams (OR=30.663; p<0.001), NVD (OR=3.240, p=0.004), and 5-minute Apgar score <7 (OR=5.052; p<0.001) (Table 2).

Following multivariate adjustment, only the 5-minute Apgar score remained an independent predictor of CPAP failure; neonates with a score <7 had significantly increased odds (adjusted odds ratio AOR=5.315, p=0.006).

There is an adequate model fit (p=0.892) in the Hosmer-Lemeshow test. Nagelkerke's R-squared was 0.599, with a significant overall association (p<0.001) with 84.9% classification accuracy.

Kaplan-Meier survival analysis demonstrated a statistically significant difference in successful CPAP support duration between neonates with birth weights <1200g and those ≥ 1200g (p<0.001). The survival curves showed a significantly higher CPAP failure rate in lower birth weight neonates, indicating a poorer prognosis for sustained non-invasive respiratory support in this vulnerable subgroup (Figure 1).

The forest plot based on multivariate analysis showed that a 5-minute Apgar score below 7 was the only factor independently linked to CPAP failure, as its confidence interval (CI) remained entirely to the right of the line of no effect, while the CI for other risk factors (lower gestational age, lower birth weight, female sex, and mode of delivery) crossed the line of no effect (AOR=1). This indicates that in our final adjusted model, their association with CPAP failure was not statistically significant (Figure 2).

Table 2. Factors associated with Continuous Positive Airway Pressure (CPAP) failure

Baseline characteristic	CPAP failure	CPAP no failure	Univariate regression		Multivariate regression	
			OR (95%CI)	p	AOR (95%CI)	p
	No (%) of patients					
Sex						
Male	21 (27.3)	56 (72.7)	2.203 (1.002-4.843)	0.049*	1.685 (0.521-5.447)	0.383
Female	19 (45.2)	23 (54.8)				
Gestational age						
≤30 weeks	26 (83.9)	5 (16.1)	27.486 (9.17-83.786)	<0.001*	8.340 (0.968-71.822)	0.054
> 0 weeks	14 (15.9)	74 (84.1)				
Birth weight						
≤ 1200 g	22 (88.0)	3 (12.0)	30.663 (8.345-114.884)	<0.001*	7.451 (0.645-86.091)	0.108
> 1200 g	18 (19.1)	76 (80.9)				
Mode of delivery						
NVD	24 (49.0)	25 (51.0)	3.240 (1.469-7.144)	0.004*	3.141 (0.993-9.933)	0.051
CS	16 (22.9)	54 (77.1)				
5-minute APGAR score						
<7	19 (61.3)	12 (38.7)	5.052 (2.110-12.97)	<0.001*	5.315 (1.606-17.594)	0.006*
≥7	21 (23.9)	67 (76.1)				
Antenatal corticosteroids						
receive	5 (21.7)	18 (78.3)	2.066 (0.705-6.049)	0.186	4.223 (0.760-23.458)	0.100
not receive	35 (41.0)	61 (59.0)				
Age at initiation of CPAP						
≤6 h	35 (33.7)	69 (66.3)	1.014 (0.322-3.198)	0.980		
>6 h	5 (33.3)	10 (66.7)				
Initial FiO_2						
≤35%	9 (22.5)	31 (77.5)	0.450 (0.189-1.072)	0.710		
>35%	31 (39.2)	48 (60.8)				

*statistically significant;
AOR, Adjusted Odds Ratio; CI, Confidence Interval; CS, Cesarean Section; FiO2, Fraction of inspired oxygen; NVD, Normal Vaginal Delivery; OR, Odds Ratio;
For statistical purposes, all values presented in the table are calculated and compared against the 'Second Row' (The Reference Category).

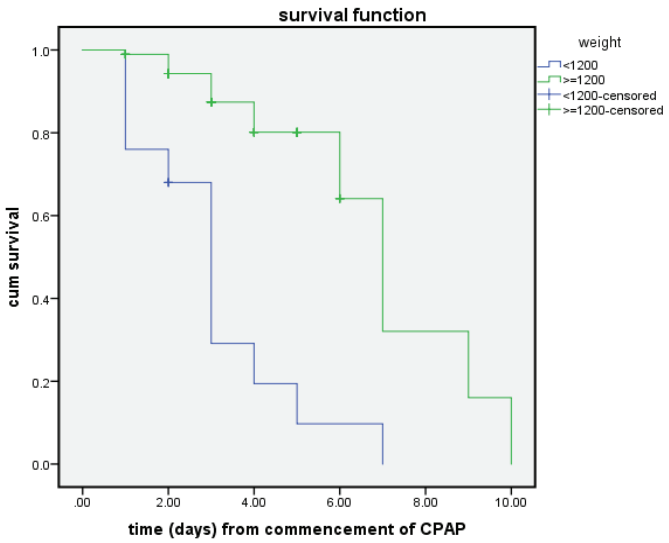

Figure 1. Kaplan-Meier survival curve of Continuous Positive Airway Pressure (CPAP) success in neonates with respiratory distress stratified by birth weight

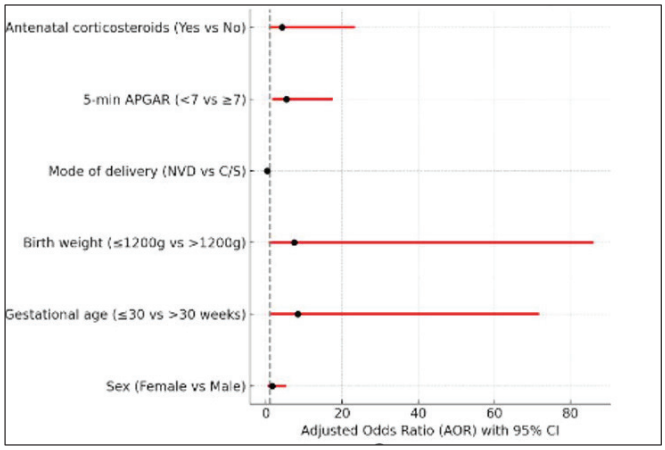

Figure 2. Forest plot of Adjusted Odds Ratio (AORs) for Continuous Positive Airway Pressure (CPAP) failure based on the multivariate analysis
The vertical dashed line at AOR = 1 indicates no effect; points to the right of the line suggest increased odds of CPAP failure; points to the left suggest protective factors (lower odds); only a 5-min APGAR score <7 showed a statistically significant association (CI does not cross 1)

DISCUSSION

This prospective observational study conducted in Mosul, Iraq, revealed a 33.6% CPAP failure rate, aligning with reports from other LMICs like Indonesia and Iran (17,18). The failure rate in our study is more than that in high-income countries, such as 21% in Australia and New Zealand (19), and 28% in Poland (20), due to available resources, including antenatal steroids, prophylactic CPAP use, and surfactant therapy which highlights a big gap in low-resource settings and the urgent need for improving the neonatal care.

In the univariate model, there is a higher risk of CPAP failure with lower gestational age (≤30 weeks), which agrees with studies that identified gestational age (≤30 weeks) as a significant early predictor of CPAP failure in neonates with respiratory distress (8,18). This finding highlights the susceptibility of premature lungs and the challenges in providing adequate respiratory support with CPAP alone.

Another strong predictor of CPAP failure was a very low birth weight of ≤1200 g. This is consistent with some other studies (8,17,20). Our Kaplan-Meier analysis provided further evidence for this, showing significantly shorter successful CPAP support duration in neonates <1200g. This reinforces the clinical reality that infants with very low birth weight often need more aggressive respiratory support and face a higher CPAP failure risk.

Our univariate analysis also indicated that female sex was associated with a higher incidence of CPAP failure. In contrast, some authors did not find sex as a significant risk factor (8,13). The reason for this discrepancy in our population is unclear, and future studies are needed to explore whether specific biological or physiological mechanisms might explain this association.

NVD was a significant risk factor for CPAP failure in univariate analysis, consistent with the higher observed failure prevalence in this group. However, this association lost statistical significance in the multivariate model. This suggests that the increased risk associated with NVD was not independent, but was largely explained by its correlation with other, more strong predictors of CPAP failure. This borderline result needs further investigation in larger, multicentre studies to clarify the independent role of delivery mode.

The lack of a significant association between antenatal corticosteroid administration on CPAP failure is concerning, given the well-established role of steroids in reducing the severity of RDS. However, the low antenatal steroid use rate in our cohort (19.3%) and Abdallah et al.'s cohort (25.5%) (8), likely limited the power to detect a significant effect and probably contributed to the higher overall CPAP failure rate and poorer outcomes.

In the multivariate model, only a 5-minute Apgar score <7 remained an independent predictor of CPAP failure. This suggests that the immediate postnatal condition, reflected by the Apgar score, is a critical determinant of CPAP success in our resource-limited context, potentially mediating prematurity and low birth weight effects. Gulczyńska et al. (20) also identified a low 5-minute Apgar score as a risk factor for CPAP failure. However, Abdallah et al. (8) found no such link, possibly due to differences in neonatal resuscitation practices or the availability of early respiratory supportive care. This finding underscores the importance of early resuscitation and stabilization to increase the likelihood of successful non-invasive ventilation.

On the other hand, the age at CPAP initiation and the initial FiO_2 were not significant predictors of failure, consistent with some other studies (8). This finding is in contrast with reports, which found initial FiO_2 as a significant CPAP failure risk factor (14,21). This discrepancy may be due to differences in CPAP protocols, the timing of the 'initial' FiO_2 assessment, and the resource availability, which could influence the thresholds for defining CPAP failure and escalating care between our resource-limited setting and those of the compared studies.

Limitations of this study include a single-centre study, which limits its generalizability to other settings; the relatively small sample size may have been underpowered to detect weaker independent associations for other significant factors, in addition to a lack of surfactant and limited access to mechanical ventilation, which affect the observed CPAP failure rate and the factors associated with it.

In conclusion, CPAP failure in LMICs is still a serious problem, with a low 5-minute Apgar score as a significant independent predictor, highlighting the importance of immediate postnatal stabilization and care. Further effort is needed to improve delivery room management and provide healthcare equipment to enhance neonatal outcomes.

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ORIGINAL ARTICLE

Parental trust and the role of misinformation in childhood immunization decisions

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ABSTRACT

Aim Vaccine hesitancy challenges global public health, with parental attitudes significantly impacting childhood immunization. This study examined parental perceptions of vaccine safety, effectiveness, and decision-making factors in Bosnia and Herzegovina.

Methods A cross-sectional survey was conducted in March 2025 with 233 parents at the Primary Healthcare Centre in Gračanica. A structured questionnaire based on the Parent Attitude about Childhood Vaccines (PACV) assessed sociodemographic data, vaccination experiences, information sources, and attitudes toward vaccines using a Likert scale.

Results Among 233 participants, 195 (83.7%) fully vaccinated their children, 30 (12.9%) practiced selective vaccination, and eight (3.4%) refused all vaccines. Vaccine hesitancy was significantly associated with lower education, (26.3% vs. 5.1%; p<0.001), rural residence (76.3% vs. 48.2%; p=0.002), and having three or more children (34.2% vs. 12.3%; p=0.01). Trust in healthcare professionals strongly influenced behaviour, with 178 (91.3%) of parents who fully trusted doctors adhering to the immunization schedule. Concerns about autism were reported by 14 (36.8%) hesitant parents and were significantly associated with delayed or refused vaccination (p<0.001).

Conclusion Although overall confidence was high, vaccine hesitancy persisted due to perceived risks. Strengthening healthcare communication and addressing misinformation, particularly autism concerns, may help improve vaccine uptake.

Keywords: health knowledge, immunization program, public health, vaccination coverage

INTRODUCTION

Vaccination is one of the most outstanding and cost-effective achievements in modern medicine and public health, significantly reducing the burden of infectious diseases by preventing illness, disability, childhood morbidity, and mortality worldwide (1). The World Health Organization (WHO) estimates that vaccines prevent 3.5 to 5 million deaths annually, predominantly among children under five (2). However, despite decades of success, immunization coverage has plateaued or declined in various regions over the last decade, raising concerns about the resurgence of vaccine-preventable diseases (3). In 2019, the WHO declared vaccine hesitancy, a delay in accepting or refusing vaccines despite availability, among the top ten global health threats (4). Recent data underscore the growing urgency: the European Region reported over 127,000 measles cases in 2024, the highest in more than 25 years, primarily driven by declining immunization rates and misinformation (5). This trend is particularly concerning in the Balkan region. In Bosnia and Herzegovina (B&H), the measles vaccination rate remains critically low, at approximately 55%, significantly below the 95% threshold required to prevent outbreaks (6). Insufficient vaccination coverage, exacerbated by COVID-19, has contributed to a third major measles outbreak since 2014, with 141 confirmed cases reported by February 2024, originating in Tuzla Canton, where this study was conducted (7).

Although vaccination programs are universally implemented, their success depends on public trust (8). Previous studies have demonstrated that parental attitudes trust in healthcare providers, and exposure to misinformation play key roles in vaccination decisions (9). Understanding the local factors influencing vaccine acceptance in low-coverage countries, such as B&H, is crucial for designing effective interventions. Existing research has broadly addressed vaccine hesitancy; however, a limited number of recent studies have focused on the parental population in B&H. Moreover, the few available studies were conducted before the COVID-19 pandemic (10,11), making this post-pandemic investigation particularly valuable for understanding how recent global health events have shaped parental attitudes toward childhood immunization. The resurgence of

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measles in this region highlights the urgent need for updated, context-specific data, especially with the rise of vaccine-related misinformation.

The main goal of this study was to investigate parents' attitudes toward childhood vaccination, identify the primary drivers of vaccine acceptance or hesitancy, and examine how demographic variables influence immunization decisions. Understanding these factors can guide evidence-based strategies to improve vaccination coverage in vulnerable populations.

PATIENTS AND METHODS

Patients and study design

This cross-sectional study was conducted in March 2025 at the Paediatric Department, Primary Healthcare Center Gračanica, Bosnia and Herzegovina. The inclusion criteria comprised parents or legal guardians of children aged 0–6 years attending the clinic for routine check-ups or vaccinations, which were randomly approached by staff and voluntarily agreed to participate. Exclusion criteria included refusal to participate or submission of incomplete questionnaires.

Informed consent was obtained from all participants in the study. The Ethics Committee of the Primary Healthcare Centre Gračanica approved the study.

Methods

Anonymous, paper-based questionnaires were handed out in the clinic's waiting room by healthcare workers. A total of 250 surveys were sent out, and 233 (93.2%) were fully completed and included in the final analysis. The 20-item questionnaire was developed by incorporating existing literature and modifying validated instruments, such as the Parent Attitudes about Childhood Vaccines (PACV) (12). The administration was done in the official languages of Bosnia and Herzegovina (Bosnian, Croatian, and Serbian). An English version was made for dissemination purposes. The questionnaire consisted of two sections: demographic and socioeconomic information (e.g., gender, age, education, number of children, residence), and parental attitudes toward vaccination, prior behaviours, information sources, trust in healthcare professionals, and views on mandatory immunization. Responses were measured on a five-point Likert scale (1 = Strongly Disagree to 5 = Strongly Agree) (13).

Statistical analysis

Descriptive statistics were used to summarize the sample characteristics. Categorical variables were presented as frequencies and percentages, and differences between groups (fully vaccinated vs. vaccine-hesitant) were assessed using the test or Fisher's exact test, as appropriate. A $p<0.05$ was considered statistically significant. For attitudinal variables measured on a five-point Likert scale, responses were dichotomized into "agree" (combining "agree" and "strongly agree") and "not agree" (including "undecided," "disagree," and "strongly disagree") for comparative analyses between the groups. To identify independent predictors of vaccine hesitancy, variables that were statistically significant in univariate analyses were entered into a multivariable logistic regression model. Adjusted odds ratios (aORs) with 95% confidence intervals (CIs) were reported. The model included sociodemographic factors (education level, place of residence, number of children), trust in healthcare providers, and belief-related variables (e.g., vaccine safety and perceived harm).

RESULTS

A total of 233 parents were included in the final analysis. Based on their self-reported vaccination practices, they were categorized into two groups: those who fully vaccinated their children, 195 (83.7%) and those who were vaccine-hesitant, 38 (16.3%). The vaccine-hesitant group encompassed both parents who partially vaccinated their childern and those who did not vaccinate them at all. Vaccine hesitancy showed a significant association with several sociodemographic characteristics. Parents with lower level of education, those living in rural areas, and those with three or more children were more likely to be hesitant (Table 1). A total of 10 hesitant parents (26.3%) had only completed primary education, compared to 10 (5.1%) parents in the fully vaccinated group ($p<0.001$). Rural residence was reported by 29 (76.3%) of hesitant parents versus 92 (48.2%) in the fully vaccinated group ($p=0.002$). Furthermore, having three or more children was more frequent among hesitant parents compared to those who fully vaccinated their children (34.2% vs. 12.3%; $p=0.01$). Gender was not significantly associated with the vaccination status ($p=0.62$), while age showed a modest but statistically significant association ($p=0.03$).

Table 1. Demographic characteristics of parents according to vaccination status

Variable	No (%) of participants		p
	Fully Vaccinated	Hesitant	
Gender			
Male	47 (24.1)	8 (21.1)	0.62
Female	148 (75.9)	30 (78.9)	0.62
Age (years)			
<20	1 (0.5)	4 (10.5)	0.03
20–29	83 (42.6)	13 (34.2)	0.03
30–39	92 (47.2)	22 (57.9)	0.03
40–49	19 (9.7)	2 (5.3)	0.03
Education			
Primary	10 (5.1)	10 (26.3)	<0.001
High school	116 (59.5)	22 (57.9)	<0.001
College	51 (26.2)	5 (13.2)	<0.001
Postgraduate	18 (9.2)	1 (2.6)	<0.001
No of children			
1	84 (43.1)	7 (18.4)	0.01
2	87 (44.6)	18 (47.4)	0.01
≥3	24 (12.3)	13 (34.2)	0.01
Residence			
Urban	82 (42.1)	8 (21.1)	0.002
Suburban	19 (9.7)	1 (2.6)	0.002
Rural	94 (48.2)	29 (76.3)	0.002

The source of vaccine information emerged as a major factor influencing parental behaviour (Table 2). Among parents who fully vaccinated their children, the majority cited healthcare professionals as their main source of information, 155 (79.5%).

Table 2. Sources of vaccine information by vaccination status

Source	No (%) of participants		p
	Fully vaccinated	Hesitant	
Doctor/healthcare worker	155 (79.5)	20 (52.6)	<0.001
Internet/social media	13 (6.7)	10 (26.3)	0.001
Family/friends	10 (5.1)	5 (13.2)	0.048
Government institutions	17 (8.7)	3 (7.9)	0.87

In contrast, only 20 (52.6%) hesitant parents reported relying on doctors or healthcare workers (p<0.001). Hesitant parents more frequently relied on less formal sources, including social media or friends and family.

Parental attitudes toward vaccination also differed sharply between the groups (Table 3). Parents of children who were fully vaccinated were significantly more likely to believe that vaccines are essential for health (91.3% vs. 57.9%; p<0.001) and that vaccination protects the wider community (93.3% vs. 55.3%; p<0.001). They were also more likely to trust the safety of vaccines and believe that side effects are rare, that vaccines do not weaken the immune system, and that their effectiveness is scientifically proven (all p<0.001). In contrast, vaccine-hesitant parents more often held beliefs linking vaccines to serious health conditions (36.8% vs. 10.3%) and showed greater distrust in medical professionals (42.1% v. 19.0%; p=0.002).

Table 3. Vaccine perception by vaccination status

Statement	No (%) of participants		p
	Agree (vaccinated)	Agree (hesitant)	
Vaccines are essential	178 (91.3)	22 (57.9)	<0.001
Vaccination protects the community	182 (93.3)	21 (55.3)	<0.001
Healthcare providers provide biased info	37 (19.0)	16 (42.1)	0.002
Vaccines cause autism/multiple sclerosis/cancer	20 (10.3)	14 (36.8)	<0.001
Side effects are rare	124 (63.6)	11 (28.9)	<0.001
Vaccine effectiveness proven	176 (90.3)	20 (52.6)	<0.001
Vaccination is safe	168 (86.2)	17 (44.7)	<0.001
Vaccines do not weaken immunity	162 (83.1)	16 (42.1)	<0.001

To determine which factors were independently associated with vaccine hesitancy, a multivariable logistic regression model was constructed, including all variables that reached statistical significance in the univariate analyses (Table 4). Four variables remained significant in the final model. Parents with lower educational attainment had nearly threefold higher odds of hesitancy. Attitudinal factors were also strong predictors: parental distrust in healthcare providers and the belief that vaccines are harmful both remained highly significant, with the latter showing the strongest association (aOR = 4.55; p<0.001).

Table 4. Multivariable logistic regression analysis of factors associated with vaccine hesitancy

Predictor	Adjusted OR (aOR)	95% CI	p
Lower education	2.85	1.30–6.28	0.007
Rural residence	2.67	1.40–5.12	0.003
Distrust in healthcare providers	3.21	1.58–6.52	<0.001
Belief that vaccines are harmful	4.55	2.15–9.62	<0.001

aOR, adjusted odds ratio; CI, confidence interval

DISCUSSION

This study explored parental attitudes toward childhood vaccination in Bosnia and Herzegovina, revealing that while a significant majority (83.7%) of parents reported full adherence to the national immunization schedule, vaccine hesitancy remains a concern.

Unlike earlier assumptions that widespread vaccine refusal is the main challenge (14), our data showed that full vaccine refusal remains low, while selective vaccination and vaccine delays driven by safety concerns are far more common. These findings support existing evidence that vaccine hesitancy lies on a behavioural spectrum and is primarily driven by uncertainty, misinformation, and fears related to vaccine safety, rather than by ideological opposition (15,16). Selective vaccination among some parents in our study points to partial hesitancy with less than 4% outright vaccine refusal, which is similar to data from Turkey (17) and Romania (18) and slightly exceeds the prevalence reported in China, where vaccine refusal was observed in 2.7% of the population (19).

The findings of this study identified four independent predictors of vaccine hesitancy: lower education level, rural residence, distrust in healthcare providers, and the belief that vaccines are harmful. These findings are consistent with global trends but also highlight unique contextual factors relevant to B&H (20). In our study parents with primary education had nearly threefold higher odds of hesitancy, while those living in rural areas were more than twice as likely to be hesitant.

A crucial factor in vaccine uptake is trust in healthcare professionals. The majority of parents who fully vaccinated their children identified healthcare workers as their main source of information, whereas hesitant parents relied more heavily on the internet and social media. Consistent with our findings, a study from the United States reported that lower trust in paediatricians was significantly associated with greater concerns about vaccines (21). Trust in healthcare providers plays a central role in shaping vaccine decisions, as transparent and evidence-based communication has been shown to reduce hesitancy and improve vaccine acceptance (22,23). Although parents seek balanced information, traditional campaigns often emphasize benefits while neglecting safety concerns. Social media and apps serve as key vaccine info sources, especially for new mothers who commonly consult online sources for health information (24).

The alleged link between vaccines and autism has been ever-present in public discourse since the 1990s; despite overwhelming scientific evidence disproving this link, the misconception remains widespread (25). In our study, this concern was more prevalent among hesitant parents and contributed to delayed or refused vaccinations, with similar findings reported in other studies, where fear of autism continues to be one of the most persistent drivers of vaccine hesitancy (25,26). The persistence of such misconceptions underscores the urgent need to address autism-related vaccine misinformation as a key step toward improving vaccine acceptance.

Despite the overall high trust in vaccines, concerns regarding safety and side effects were evident in our study.

To contextualize the findings, it is important to consider actual vaccination coverage in the local population. Unpublished data

from the Primary Healthcare Centre Gračanica indicate that the percentage of fully immunized children under six has fluctuated between 78% and 90% in recent years (Primary Healthcare Centre Gračanica, unpublished data). While these rates reflect relatively high vaccine uptake, they still fall short of the levels typically required to ensure herd immunity, especially for highly contagious diseases such as measles.

Bosnia and Herzegovina has seen a steady decline in routine immunization coverage over the past decade. According to WHO/UNICEF data, MMR1 coverage dropped from 89% in 2014 to below 70% by 2016-2019, with further declines during the COVID-19 pandemic, reaching a low of 58% by 2022 (27,28). This trend coincided with three major measles outbreaks: in 2014-2015 (over 5100 cases), 2019 (1332 cases), and most recently in 2023-2024, when 7445 cases were reported, primarily in Tuzla Canton (29-31). In all outbreaks, measles mainly affected unvaccinated or undocumented children, with a significant share among the youngest age groups (32-34).

With three measles outbreaks occurring within less than a decade, the Institute for Public Health of Federation B&H conducted a series of studies under the WHO Tailoring Immunization Programmes (TIP) that identified low confidence, complacency, and practical access barriers as key contributors to under-vaccination, particularly among parents with partially or non-vaccinated children (35). These findings are in line with our study results and confirm that the problem is not limited to global trends but has specific local determinants that require targeted public health strategies.

To strengthen vaccine confidence and accessibility in B&H, several awareness campaigns have been implemented to improve vaccination confidence, including awareness campaigns, healthcare worker communication training, and the introduction of a mobile app "My Calendar of Immunization" that tracks immunization status and sends reminders. Additional measures, like extended hours and walk-in sessions, have been recommended to improve service accessibility for working parents (36).

This study has several limitations. First, the sample was limited to parents from the municipality of Gračanica, which may restrict the generalizability of findings to other regions in Bosnia and Herzegovina with different demographic or socioeconomic profiles. Second, due to the cross-sectional design, the study cannot establish causal relationships between measured variables. Finally, data were based on self-reported vaccination behaviour and attitudes, which may be subject to recall bias or social desirability bias, particularly given the sensitive nature of the topic.

In conclusion, this study highlights the complex factors that shape parental vaccine decisions. While most parents recognize the benefits of vaccination, concerns about side effects and misinformation persist. Trust in healthcare professionals proved crucial for vaccine acceptance, underscoring the importance of clear and targeted communication. Strengthening confidence through transparent dialogue and addressing fears directly can improve vaccine uptake.

AUTHOR CONTRIBUTIONS

Conceptualization, E.M., and A.T.; Methodology, E.M; A.T.; Writing – review & editing, A.T., E.M.; Supervision, A.Ć., A.T.; E.B. ; Data curation A.T.; Writing – original draft preparation, A.T. E.M. M.B.; Software, E.M., E.B, M.B., Visualization, E.M., A.Ć.; E.B and M.B; Investigation, A.T. All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

Inflammatory myofibroblastic tumor in pediatric patients: a Bosnian cohort

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ABSTRACT

Aim Inflammatory myofibroblastic tumor (IMT) is a rare mesenchymal neoplasm of intermediate biological potential, characterized by spindle cell proliferation and significant inflammatory component. This study aimed to determine the clinicopathologic characteristics, clinical outcomes of IMT patients in the low-volume pediatric surgery service in Bosnia and Herzegovina.

Methods The study included data from three pediatric patients with IMT (two females, one male) diagnosed and operated from 2010 to 2024 at the Clinic of Pediatric Surgery, Clinical Centre, University of Sarajevo. Demographic, clinical, histopathological, immunohistochemical, and outcome parameters were analysed.

Results All tumors were located in the abdominal or abdominopelvic region; a median age of 4 years. Clinical manifestations included non-specific gastrointestinal symptoms (N=2) and systemic signs such as fever (N=2), weight loss and weakness (N=1). Complete surgical resection was conducted in all patients, and all experienced complete remission without recurrence. Histopathological analysis revealed consistent presence of spindle cells within a prominent inflammatory milieu, rich in plasma cells and lymphocytes. Immunohistochemically, all tumours were positive for vimentin, anaplastic lymphoma kinase (ALK), and smooth muscle actine (SMA), while ALK-FISH (fluorescence in situ hybridization) analysis (performed in one case) was negative.

Conclusion The constant of heterogeneous morphology, and significance of IMTs immunophenotype, particularly with the inflammatory component more pronounced, was found. ALK gene alterations were commonly associated with IMT, as well as with other types of pediatric neoplasms, however, favourable outcomes in our patients raise a question regarding further need to clarify the prognostic significance of molecular findings and their potential therapeutic implications.

Keywords: anaplastic lymphoma kinase, child, myofibroblasts, soft tissue neoplasms

INTRODUCTION

Inflammatory myofibroblastic tumor (IMT) is defined as a rare soft tissue neoplasm primarily composed of myofibroblasts and fibroblasts, infiltrated by inflammatory cells such as lymphocytes and eosinophils (1). Due to its described origin, the localization of this neoplasm varies significantly. It can occur in any region rich in soft tissue, but it is most commonly found in the lungs and abdominal cavity (2,3). Studies have shown that IMT is most frequently located in the liver and biliary tract (31.8% of cases), followed by the head and neck (20.6%), lungs (18.2%), abdomen (15.5%), and the urogenital tract (7.4%) (4).

Although initially considered a benign neoplasm, IMT is now classified as a subtype of sarcoma due to cases in which the tumor exhibited highly aggressive behaviour and poor prognosis (1,3). It is characterized by intermediate biological potential, as the likelihood of recurrence and metastatic potential is low (5). Specific data on the incidence in children remain unclear (6).

The aim of this study was to investigate the clinicopathologic, immunohistochemistry and molecular cytogenetics characteristics, and outcome of IMT patients in the low-volume pediatric surgery service in Bosnia and Herzegovina (B&H).

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PATIENTS AND METHODS

Patients and study design

This clinical, retrospective study was conducted at the Clinic of Pediatric Surgery of the Clinical Centre, University of Sarajevo, covering the period from 1 January 2010 to 31 December 2024. Three patients (two girls and one boy), who were diagnosed with inflammatory miofibroblastic tumor, were included.

The Ethics Committee of the Clinical Centre, University of Sarajevo, approved this study (No: 51-45-1-9463/25).

Methods

The data were collected by reviewing the available medical documentation through the electronic database of the Clinical Centre and the archives of the Clinic of Pediatric Surgery of the Clinical Centre.

The following features were reviewed: gender, age at the time of diagnosis, history, clinical presentation, tumor localization and characteristics, diagnostic workup, histopathological findings, molecular cytogenetics treatment and surgical intervention performed, disease outcome, complications, and recurrence.

Clinical diagnosis. The diagnostic workup for all patients included a detailed clinical history, physical examination, and comprehensive laboratory analyses: complete blood count, C-reactive protein, coagulation profile, and tumor markers including Alpha-fetoprotein (AFP), Cancer antigen 125 (Ca-125), and Carbohydrate antigen 19-9 (Ca 19-9). Imaging diagnostic techniques were used as well, including abdominal and pelvic ultrasonography (US) as the initial imaging modality, followed by computed tomography (CT) and/or magnetic resonance imaging (MRI) to characterize tumor's site, relation to adjacent structures and other characteristics.

Macroscopic descriptions. Surgical specimens (tumor masses with involved bowel segments, appendix, mesocolic lymph nodes, depending on the case) were fixed in 10% neutral buffered formalin. Upon examination, the size, weight, external appearance, cut surface characteristics (colour, consistency, presence of necrosis, cystic changes, or haemorrhage) and surgical margins.

Histopathological methods. Tumor tissue samples were fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned at 4-5μm. These sections were stained with Hematoxylin and Eosin (H&E) for light microscopic evaluation.

Immunohistochemistry and molecular cytogenetics. Immunohistochemical (IHC) analysis was performed on prepared histopathological samples, using standard technique. The antibody panel that was applied included: anti smooth muscle antibody (ASMA), smooth muscle actine (SMA), anaplastic lymphoma kinase (ALK), leucocyte common antigen (LCA); actine, muscle specific monoclonal antibody (HHF35), cytokeratin (CK), CD34, CD117, S100, vimentin and/or desmin.

Statistical analysis

The data were processed, and the results presented in numerical and tabular form. Descriptive statistical methods were used.

RESULTS

In total of three patients IMTs were identified. The median age was 4 years. Female to male ratio was 2:1. All of the patients (N=3) had tumor localized in the abdomen; in two patients the tumor was extensive, it was simultaneously presented in the abdomen and pelvis (Figure1). No recurrences were evident. The presence of elevated body temperature (N=2), occasional abdominal pain (N =1), nausea with vomiting (N =1), poor appetite and weight loss (N =1), diarrhoea (N =1), fatigue (N =1), and visible swelling in the abdomen (N =1) were noted. All of the patients showed complete remission (Table 1).

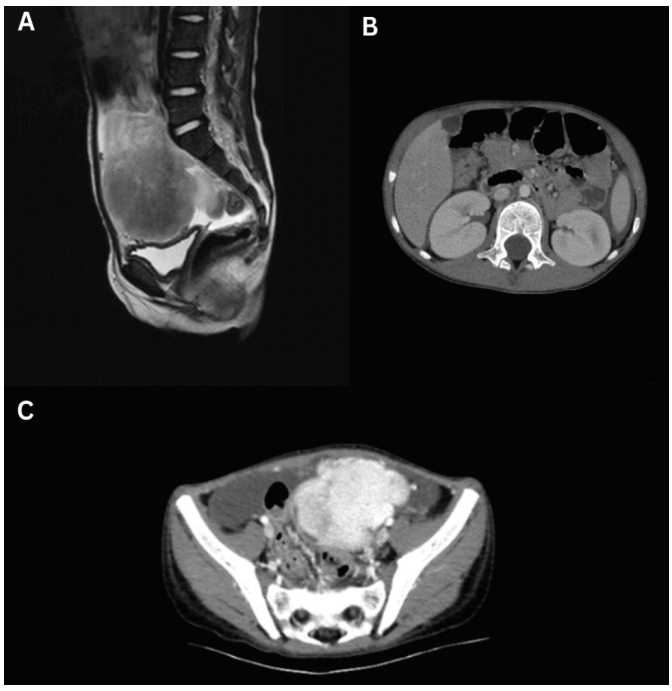

Figure 1. A) Magnetic resonance imaging (MRI) of inflmmatory myofibroblastic tumor (IMT) in patient ID2: a well-defined soft tissue mass (103x78.5x75 mm) with a pseudorenal shape and internal cystic components (12 mm). The tumor was compressing the bladder, uterus, ovaries, and iliac vessels without direct invasion of the sacrum or spinal canal. B) CT imaging of IMT in Patient ID2: a heterogeneous tumor mass (102x75x78 mm) in the midline of the pelvis, cranial to the bladder, compressing surrounding structures including iliac vessels and intestinal loops; C) CT imaging of IMT in patient ID3: an expansive tumorous lesion, parasagittal and on the left in location, that mildly protrudes into the anterior abdominal wall. It appears lobular and it is clearly demarcated from the surrounding soft tissue and vascular structures. The lesion shows intense enhancement, and laterally on the right, shows zones of lower opacification.

Macroscopic examination of the tumor and lymph nodes showed unencapsulated (N=2), multinodular (N=2) tumor, with the necrotic surface, covered with purulent debris (N=1), and with internal cystic components (N=1). Resected lymph nodes showed no metastases, but displayed reactive changes (N=2). The tumor sizes varied from 4 to 10 cm, with the average size of 7 cm (Figure 2). There was no evidence of infiltration of the surrounding tissues.

A description of histologic pattern of sampled tissues was found in the clinical histories of all patients (N=3). Spindle-shaped cells were found in all three patients, within fascicular arrangement (N=2), with prominent vascular network (N=3). Each pattern had strong inflammatory cell infiltration, with plasma cells (N=3), lymphocytes (N=2), eosinophils (N=2), neutrophils (N=1), hystiocytes (N=1) and mast cells (N =1). In one patient, a cellular mesenchymal tumor was defined. In other patient, smaller nodules exhibiting blood vessels resembling granulation tissue were observed, while remaining parts of the sample showed dilated vessels, macroscopically

mimicking cystic spaces. There were no signs of significant nuclear pleomorphism or atypical mitotic figures (Table 2).

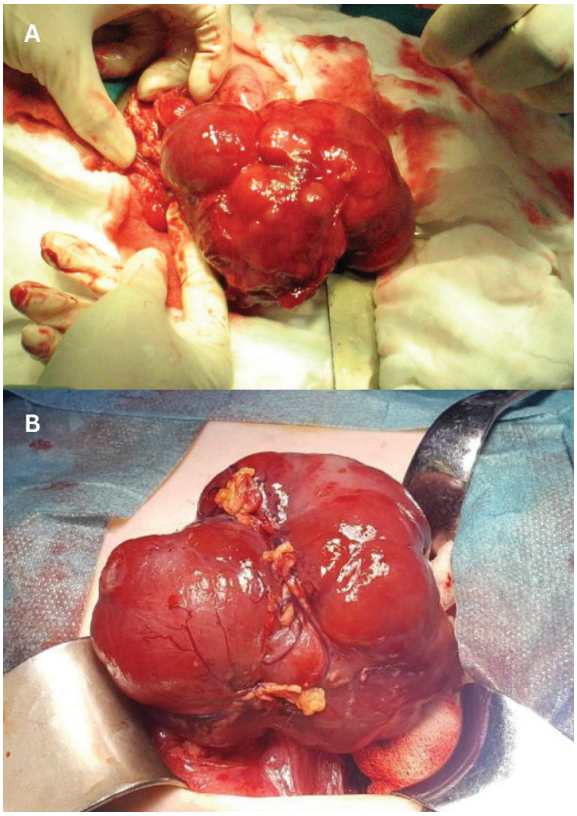

Figure 2. A) Intraoperative view of inflammatory myofibroblastic tumor (IMT) in patient ID3; B) intraoperative view of IMT in patient ID2 (E.M K.D. Images courtesy of the Clinic of Paediatric Surgery, Clinical Centre, University of Sarajevo (KCUS) (2020: A; 2013: B)

Immunohistochemical analysis demonstrated positivity for the intermediate filament protein type III, vimentin, as well as for ALK, and SMA, in all three patients. Protein S100 was variably negative, except in one patient, where it was focally positive. Desmin was positive, negative and variably negative. Additionally, analysed immunohistochemistry markers were mostly negative, with one exception showing positive results for CK and one showing variably negative results for HHF35 (Table 2).
ALK-FISH analysis was conducted in one patient and it was negative. Remaining two patients had no medical documentation available, regarding molecular cytogenetic studies.

DISCUSSION

Although IMT is a rare entity, several studies have described its clinical features and management; however, its low incidence usually makes it difficult for pediatric surgeons to accumulate experience in this disease (1-2). This is especially true for low-volume pediatric surgery centres in developing countries with low birth rates, such as B&H. The clinical information on IMT from B&H is very limited, and the present analysis is the first study with a long follow-up.
Histologic architecture and cellular features are highly variable, and while IMT is mostly locally aggressive with low metastatic potential, rare cases may demonstrate highly invasive behaviour and metastases. Tumor can appear in a wide range of localizations; any site rich in soft tissue. (1-5). Collectively, these factors accentuate the need for continued research to better understand IMT.
The heterogenic localization is one of the characteristics of this tumor, with mostly abdominopelvic site of primary tumor, but with a significant number in lungs and retroperitoneum, as well as other sited of soft tissues (3,4,7). Our patients showed 100% homogeneity regarding the site of development of IMT, orig-

Table 1. Clinical presentation, tumor site, and outcome of three patients with inflammatory myofibroblastic tumor

Patient ID	Age (years)	Sex	Location	Clinical presentation	Follow up (months)	Outcome
1	9	F	Abdomen	Occasional abdominal pain started 14 days ago, nausea, occasional vomiting, occasional diarrhoea with calm periods lasting 1-2 days, poor appetite, weight loss of 4 kg	110	CR
2	4	F	Abdomen, pelvis	Fatigue, elevated body temperature, visible swelling from the umbilicus to the pubis	6	CR
3	3	M	Abdomen, pelvis	Elevated body temperature	14	CR

F, female; M, male; CR, complete remission

Table 2. Morphologic, immunophenotypic, and molecular findings of three patients with inflammatory myofibroblastic tumor

ID	Tumor size (cm)	Immunohistochemical marker expression											ALK - FISH
		Vimentin	ASMA	SMA	S100	ALK	Desmin	LCA	CD34	CD117	HHF 35	CK	
1*	4	3+	N/A	2+	+	+	-	-	-	N/A	N/A	-	N/A
2†	10	+	N/A	+	-	+	-/+	-	-	-	-/+	-	N/A
3‡	7	3+	3+	2+	-	2+	+	N/A	-	-	N/A	+	Negative

N/A, not available/performed; Vimentin; fibroblast intermediate filament; ASMA, anti-smooth muscle antibody; SMA, smooth muscle actine; S100, calcium binding protein; ALK, anaplastic lymphoma kinase; Desmin, muscle-specific member of the intermediate filament, LCA, leucocyte common antigen; CD34, transmembrane glycoprotein, cell surface marker; CD117, cell-surface receptor; HHF-35, actine monoclonal antibody; CK, cytokeratin; ALK FISH – ALK gene fluorescence in situ hybridization test
Histologic pattern: *Spindle-shaped cells arranged in interwoven fascicles, intermixed with inflammatory infiltrates predominantly composed of lymphocytes and plasma cells; †Spindle-shaped myofibroblasts and fibroblasts with vesicular nuclei, arranged in fascicular and whorled patterns, with the stroma infiltrated by a prominent inflammatory component composed of plasma cells, lymphocytes, histiocytes, neutrophils, eosinophils, and occasional mast cells; marked vascular proliferation; ‡ Spindle cells, stroma rich in blood vessels and inflammatory infiltrates, consisting of plasma cells and eosinophils.

inating in the abdominal cavity. In our patients, clinical presentation included abdominal or gastrointestinal complaints, with unspecific symptoms such as elevated body temperature. A combination of symptoms such as fever, weight loss and overall weakness was found in one patient. Usual prevalence of 15-30% was noticed in other studies (8). More dominant gastrointestinal symptoms were found in our oldest patient (9-year old girl), in comparison with the younger ones (3 and 4 years of age).

Mean size of 6 cm found in other studies is similar to ours of 7 cm (7). The morphological abnormalities, such as necrosis, haemorrhage or calcification, are not often spotted within these tumors (8). We confirmed that finding, with traces of necrosis found in only one case. Even though it was found that abdominopelvic IMTs showed greater prevalence of recurrence in the comparison to other primary location sites, our patients went into complete remission after surgical treatment (7).

Three histological patterns with various combinations, were defined within the IMTs: a myxoid/vascular pattern, a compact spindle cell pattern, and a hypocellular fibrous pattern (8). There were no dominant morphologic subtypes of the tumor defined in our study. Plasma cells and lymphocytes are the most often inflammatory cells in IMTs (7). That is consistent with our findings. Myxoid hypocellular pattern, without dominant inflammatory component, can be found in infants, due to their immature inflammatory cell development (9). However, all our patients were older than one year. While no histopathological pattern was dominant, the tumor samples in these older children consistently demonstrated a significant inflammatory component. Observation might suggest that a more mature system can have influence on the tumor microenvironment (10).

Considering their myofibroblastic component, IMTs are positive for SMA in 85% of cases, and desmin in 65% (8). All of our patients showed positivity for these markers, underlining its connection with myofibroblasts.

ALK, anaplastic lymphoma kinase gene rearrangements were consistently found in IMT patients (7,8). ALK is a gene commonly associated with malignancies, such as anaplastic large cell lymphoma, rhabdomyosarcoma, leiomyosarcoma, Ewing sarcoma, and other "small blue cell" tumors (11-13). In IMTs, around 50% of tumors are associated with different kinds of ALK gene anomalies, including ALK rearrangements and discoveries of ALK fusion partners (14,15). Additionally, ALK expression is more common in younger patients (13).

In our three-patient series, no ALK gene abnormalities were detected (in the single case tested by FISH). Today, there is a rising of new hypotheses regarding whether ALK gene mutation can be connected to overall behaviour of tumor and prognosis of the disease, as in other kinds of pediatric tumors, such as neuroblastoma (16). Some studies raise questions of the correlation between potential *atypical* forms of IMTs and ALK rearrangements (15). However, it is yet very indefinite in what extent ALK can be connected to IMTs as well as how ALK rearrangements would be formed (14,17).

Our study is limited by its retrospective design and a small number of recruited patients. The study was conducted in a single institution, limiting its generalizability.

IMT is a very rare entity among children. We confirm the rarity and relatively good clinical outcomes of the patients with IMT in the low-volume pediatric surgery service from the developing country. Importance of surgery in its treatment is undeniable. Future efforts must be made on clarifying the molecular driver and immune interaction within IMT to potentially guide development of risk stratification strategies and less invasive therapies.

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TRANSPARENCY DECLARATION

Conflicts of interest: None to declare.

CrediT Author Contributions

Conceptualization: Asmir Jonuzi, Ilhana Tinjak and Zlatan Zvizdic. Methodology: Asmir Jonuzi, Ilhana Tinjak, Benjamin Kulovac, Nusret Popović, Emir Milisic, Predrag Ilić, Zlatan Zvizdic. Formal analysis and investigation: Asmir Jonuzi, Ilhana Tinjak, Melika Bukvić, Zlatan Zvizdic. Writing - original draft preparation: Asmir Jonuzi, Ilhana Tinjak, Benjamin Kulovac, Nusret Popović, Emir Milisic Predrag Ilić and Zlatan Zvizdic. Writing - review and editing: Asmir Jonuzi, Ilhana Tinjak and Zlatan Zvizdic. Supervision: Benjamin Kulovac and Zlatan Zvizdic.

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ORIGINAL ARTICLE

Effect of vitamin D supplementation on metabolic syndrome and atherosclerosis biomarkers in epilepsy: a randomized controlled trial

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ABSTRACT

Aim To analyze the effect of vitamin D administration on serum 25-hydroxyvitamin D (25[OH]D) level and biomarkers of metabolic syndrome and atherosclerosis: homeostatic model assessment of insulin resistance (HOMA-IR), adiponectin, homocysteine, and high sensitivity C-reactive protein (hs-CRP) in epilepsy patients receiving enzymatic antiseizure medications (ASMs).

Methods This double-blind, randomized, placebo-controlled trial included 40 adult epilepsy patients treated with enzymatic ASMs to receive vitamin D3 (2,000 IU/day) or placebo for 12 weeks. The primary outcome was a change in serum 25(OH)D level. Secondary outcome included changes in HOMA-IR, adiponectin, homocysteine, and hs-CRP. Data were analysed using a per-protocol approach.

Results Thirty-four patients completed the study. Vitamin D supplementation yielded a significantly greater increase in serum 25(OH)D (10.67±8.16 vs. –1.29±3.96 ng/mL; p<0.001) and adiponectin (1.38±3.05 vs. 0.34±1.89 µg/mL; p=0.045), as well as a significantly greater reduction in hs-CRP (–6.74±14.65 vs. 1.81±7.75 mg/L; p=0.041) compared with placebo. Conversely, no significant differences were observed between groups regarding the changes in HOMA-IR (–0.24±3.71 vs. –0.14±3.38; p=0.940) or homocysteine (7.90±1.79 vs. 8.15±2.70 µmol/L; p=0.290).

Conclusion Vitamin D supplementation (2,000 IU/day) effectively restores 25(OH)D level and improves adiponectin and hs-CRP in epilepsy patients on enzymatic ASMs, suggesting a potential benefit for cardiovascular risk reduction. However, vitamin D alone did not prevent the rise in homocysteine, likely due to the concurrent cessation of B-vitamin supplementation.

Keywords: anticonvulsants, cholecalciferol, C-reactive protein, insulin resistance

INTRODUCTION

Epilepsy is one of the oldest neurological diseases associated with a certain stigma in society, psychiatric comorbidity, and high economic costs (1) with prevalence and treatment availability varying across countries. Stigma associated with epilepsy significantly impacts the quality of life (QoL). In 2021, there were approximately 51.7 million people with active epilepsy, with the bulk of the burden (83.7%%) residing in low-income to middle-income countries (2). In addition, idiopathic epilepsy and epilepsy due to other causes, altogether, resulted in 16.2 million, (95% uncertainty interval [UI] 10.5–22.7) global years lived with disability (YLDs) in 2021 and were responsible for 1.8% (1.3–2.4) of total global YLDs (3). Approximately 30% of these patients progress to drug-resistant epilepsy, necessitating lifelong treatment with antiseizure medications (ASMs) (4). However, the long-term use of enzymatic ASMs (such as phenytoin, carbamazepine, and phenobarbital) induces hepatic cytochrome P450 (CYP-450) enzymes, leading to the accelerated catabolism of vitamin D and subsequent deficiency (5–7). This deficiency is critical because vitamin D plays a vital role in immune and metabolic regulation, not just bone health. Consequently, patients on enzymatic ASMs face a dual pathology. The medication-induced depletion of vitamin D exacerbates their susceptibility

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to metabolic syndrome, the prevalence of which ranges from 23.5% to 52.6% in this population (8,9). This unique metabolic vulnerability significantly increases the risk of coronary heart disease and cerebrovascular disease, leading to accelerated atherosclerosis (10). Given this heightened cardiovascular risk, early detection using specific biomarkers is essential to improve patient prognosis (11). Several biomarkers are established indicators of metabolic syndrome and atherosclerosis, including homeostasis model assessment of insulin resistance (HOMA-IR) (12), adiponectin (13), homocysteine (14), and high sensitivity C-reactive protein (hs-CRP) (15). While these biomarkers are widely used in general internal medicine, their specific utility in monitoring ASM-induced metabolic changes remains underutilized.

To date, no studies have specifically explored whether correcting vitamin D deficiency can reversibly improve these specific biomarkers of metabolic syndrome and atherosclerosis in the epilepsy population. Existing literature discusses the relationship between vitamin D and metabolic health in the general population, but fails to account for the unique metabolic disruption caused by enzymatic ASMs in epilepsy patients.

The aim of this study was to analyse the effect of vitamin D administration on biomarkers of metabolic syndrome and atherosclerosis in epilepsy patients receiving enzymatic ASMs.

PATIENTS AND METHODS

Patients and study design

This study encompassed a randomized, double-blinded, parallel controlled trial design on adult patients with epilepsy. The patients were enrolled either in the group that received vitamin D supplementation at a dose of 2,000 IU or the placebo group. This study was conducted from October 2024 to May 2025 at the Department of Neurology, Mohammad Hoesin Hospital, Palembang, Indonesia.

The study was registered in the Indonesia Clinical Research Registry (INA-CRR) on 17 February 2025 (registration number: INA-13022025BB3713B).

This clinical trial also has met the ethical eligibility of the Ethics Institute of Mohammad Hoesin Hospital Palembang (Ethics approval number: DP.04.03/D.XVIII.06.08/ETIK/280/2024).

Methods

Patients diagnosed with epilepsy who were ≥18 years old, received enzymatic ASMs therapy for at least 6 months, and were willing to participate in the study and signed an informed consent form were included in this study. Exclusion criteria were as follows: patients who had a history of chronic kidney disease, chronic liver disease; patients who routinely received vitamin D supplementation every day for the past 2 weeks; hypercalcemia (serum calcium level >10.5 mg/dL); vitamin D level above normal range (>100 ng/mL); patients suffering from acute infections in the past 2 weeks; serious physical injury or trauma in the past month; history of active non-metabolic autoimmune or inflammatory disease; active cancer patients in the last 6 months; being on immunosuppressant treatment; history of acute heart disease in the last 3 months; and history of motor seizures in the last week. Patients were dropped from the study if they did not adhere to the intervention for two consecutive weeks, decided to quit the study, or experienced moderate or severe side effects from the intervention.

The sample size calculation was based on the study's primary outcome, serum 25(OH)D level. We determined the minimum sample size required to detect a large standardized effect size (Cohen's d=1.0) between the Vitamin D and placebo groups. The calculation utilized the formula for the difference between two independent means, assuming a two-tailed significance level (α) of 0.05 ($Z_{\alpha/2}$=1.96) and a power (1−β) of 80% (Z_{β} =0.84). Based on a standardized variance (S^2) of 1.0 and a detectable mean difference (Δ) of 1.0, the calculation indicated a minimum of 16 patients per group. To account for a potential dropout rate of 20%, a total of 20 patients were enrolled in each group.

After recruitment of study participants, randomization was conducted to determine whether the study participants belonged to the group that received vitamin D supplementation or a placebo. Research participants were numbered 1 to 40 based on the order of arrival (*consecutive*) and were asked to choose one envelope to determine whether they were in group A (vitamin D) or in group B (placebo). Previously, 20 envelopes containing the letter A and 20 envelopes containing the letter B were prepared and placed in one selection box. The study participants were chosen manually in front of a third party (outpatient pharmacists at Mohammad Hoesin Hospital, Palembang). The pharmacists provided intervention according to the selected envelope. The intervention was in the form of vitamin D3 administration at a dose of 2,000 IU or placebo containing sucrose per day for 12 weeks. The researchers and the study participants were blinded.

Monitoring of the intervention was carried out every month by means of routine control of research subjects at the Neurology Clinic of Mohammad Hoesin Hospital. During the visit, the patients brought all the intervention drugs given, and the researchers would count the remaining amount of drugs available to assess the patients' compliance. During the intervention, other vitamins and supplements were discontinued. The primary outcome was the change in serum 25-hydroxyvitamin D (25[OH]D) level from baseline to 12 weeks. Secondary outcome included changes in biomarkers of metabolic syndrome and atherosclerosis, including HOMA-IR (calculated by multiplying fasting glucose [in mg/dL] by fasting insulin [in μU/mL], divided by 22.5), adiponectin, homocysteine, and hs-CRP.

Blood samples were collected in the morning after a 10-12 hour fasting period to measure the levels of 25(OH)D, fasting glucose, insulin, adiponectin, homocysteine, and hs-CRP, both before and 12 weeks after the intervention.

Blood samples were then analyzed in the laboratory of Mohammad Hoesin Hospital, Palembang. Especially for adiponectin and homocysteine, blood samples were centrifuged, and the plasma was collected, centrifuged, and preserved at -20°C. Serum adiponectin and homocysteine levels were quantified using commercial ELISA kits according to the manufacturers' protocols. Adiponectin was measured using a sandwich ELISA (EUROIMMUN EQ 6446-9601, Germany; LOD 0.064 ng/mL; intra/inter-assay CV: 4.6–7.5%/6.6–8.4%; 1:1000 dilution). Homocysteine was quantified by a competitive inhibition ELISA (CED984Ge, Cloud-Clone Corp., China; range 98.77–8000 ng/mL; sensitivity <40.22 ng/mL; intra/inter-assay CV <10%/<12%).

Statistical analysis

Normality of numerical variables was assessed using the Shapiro–Wilk test. Normally distributed data were analysed using parametric tests, including the independent samples t-test for comparisons between the groups and the paired t-test for within-group changes. For non-normally distributed data, non-parametric tests (Mann–Whitney U test for between-group comparisons and Wilcoxon signed-rank test for within-group changes) were applied.

The analysis followed a per-protocol approach, and participants who did not complete the intervention were excluded from the final analysis. Missing data due to drop-out or non-adherence were not imputed. Results are presented as mean ± standard deviation (SD) or median (minimum–maximum), and 95% confidence intervals were reported where applicable. A $p<0.05$ was used to determine statistical significance for all analyses, and no adjustment for multiple comparisons was applied.

RESULTS

At the beginning of the study, a total of 40 patients were included and put into two main groups, the group that received vitamin D and placebo with equal distribution (n=20). A total of 34 patients completed the study, with similar distribution between the groups (n=17), thus meeting the minimum sample size (Figure 1).

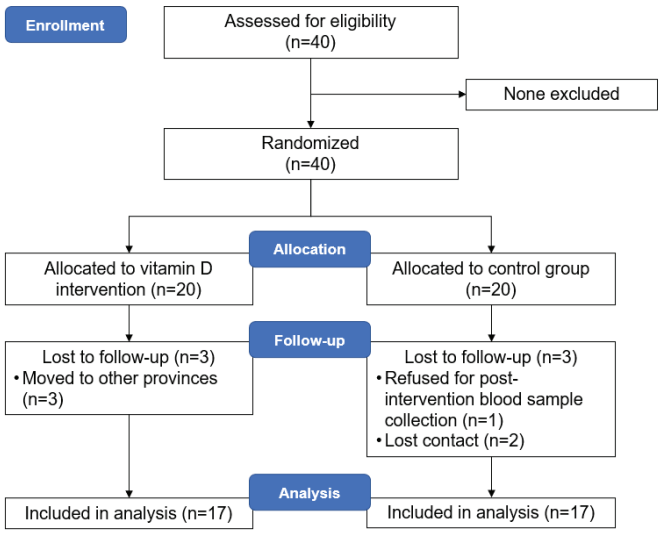

Figure 1. Flowchart of patient selection

The study population was predominantly young adults (18–45 years; 88.2%) with a balanced sex distribution, where slightly more than half were male (52.9%). Baseline age showed a normal distribution, with the vitamin D and placebo groups having mean ages of 28.53 ±5.94 and 36.59±14.12 years, respectively. Most patients had received ASMs for more than two years (73.5%), with therapy duration showing a non-normal distribution and median values of 6 years (range: 1–30) in the vitamin D group and 5 years (0.66–20) in the placebo group. Enzyme-inducing ASMs were most frequently used (55.9%), followed by enzyme inhibitor (17.6%) and combined regimens (26.5%), with comparable distributions across the two groups (Table 1).

Only a small proportion of participants had adequate vitamin D status at baseline, with most exhibiting deficient levels (50.0%). Following supplementation, the vitamin D group showed a marked shift toward normal 25(OH)D levels, with no remaining cases of deficiency. In contrast, the placebo group continued to show persistent insufficiency and deficiency. The distribution of HOMA-IR, hs-CRP, and adiponectin categories remained comparable between the groups, both before and after the intervention, suggesting no major baseline or placebo-driven changes in insulin resistance, inflammatory status, or adiponectin levels. Homocysteine showed a different pattern, where a majority of participants initially had abnormally low levels (47.1% in vitamin D; 41.2% in placebo), but post-intervention most patients in both groups shifted into the normal range (5–15 µmol/L) (Table 2).

Table 1. Baseline characteristics of antiseizure medication (ASM)-treated patients receiving vitamin D or placebo

Characteristic	Vitamin D	Placebo	Total
Age (mean±SD)	28.53±5.94*	36.59±14.12*	
	No (%) of patients		
Age group (years)			
18–45	17 (50.0)	13 (38.2)	30 (88.2)
46–65	0 (0)	4 (11.8)	4 (11.8)
Sex			
Male	9 (26.5)	9 (26.5)	18 (52.9)
Female	8 (23.5)	8 (23.5)	16 (47.1)
Type of ASMs			
Enzyme induction	8 (23.5)	11 (32.4)	19 (55.9)
Enzyme inhibition	3 (8.8)	3 (8.8)	6 (17.6)
Combination	6 (17.6)	3 (8.8)	9 (26.5)
Duration of therapy			
6 months–2 years	4 (11.8)	5 (14.7)	9 (26.5)
>2 years	13 (38.2)	12 (35.3)	25 (73.5)
Duration of therapy (median, min-max)	6 (1–30) †	5 (0.66–20)†	

Shapiro-Wilk normality test: *normally distributed data, †non-normally distributed data
ASMs, antiseizure medications;

Serum 25(OH)D increased significantly in the vitamin D group after 12 weeks (19.14±7.62 to 29.81±6.44 ng/mL; $p<0.001$), with no significant change in the placebo group (22.60±9.18 to 21.31±7.96 ng/mL; $p=0.198$). HOMA-IR slightly decreased in both groups but without statistical significance. Adiponectin increased modestly following vitamin D supplementation (6.98±7.33 to 7.45±5.52 µg/mL; $p=0.039$), whereas no meaningful change was observed in the placebo group (8.18±9.05 to 8.51±9.99 µg/mL; $p=0.449$). Homocysteine levels rose markedly in both groups. hs-CRP decreased significantly after vitamin D supplementation (8.54±15.37 to 1.75±1.10 mg/L; $p=0.012$), while no significant change occurred in the placebo group (2.76±2.70 to 4.56±7.98 mg/L; $p = 0.638$) (Table 3).

Vitamin D supplementation yielded a significantly greater increase in serum 25(OH)D (10.67±8.16 vs. –1.29±3.96 ng/mL; $p<0.001$), and adiponectin (1.38±3.05 vs. 0.34±1.89 µg/mL; $p=0.045$), as well as a significantly greater reduction in hs-CRP (–6.74±14.65 vs. 1.81±7.75 mg/L; $p=0.041$), compared with placebo. Conversely, no significant differences were observed between the groups regarding the changes in HOMA-IR (–0.24±3.71 vs. –0.14±3.38; $p=0.940$), or homocysteine (7.90±1.79 vs. 8.15±2.70 µmol/L; $p=0.290$), (Table 4).

Table 2. Characteristics of 25(OH)D, homeostatic model assessment of insulin resistance (HOMA-IR), adiponectin, homocysteine, and high sensitivity C-reactive protein (hs-CRP) levels before and after the intervention

Biomarkers (reference value)	No (%) of patients					
	Before intervention		Total	After intervention		Total
	Vitamin D	Placebo		Vitamin D	Placebo	
25(OH)D (ng/mL)						
Normal (≥30)	1 (2.9)	1 (2.9)	2 (5.9)	9 (26.5)	2 (5.9)	11 (32.4)
Insufficient (21–29)	7 (20.6)	8 (23.5)	15 (44.1)	8 (23.5)	8 (23.5)	16 (47.1)
Deficient (≤20)	9 (26.5)	8 (23.5)	17 (50.0)	0 (0)	7 (20.6)	7 (20.6)
HOMA-IR						
Normal (<2.5)	8 (23.5)	10 (29.4)	18 (52.9)	7 (20.6)	10 (29.4)	17 (50.0)
Insulin resistance (≥2.5)	9 (26.5)	7 (20.6)	16 (47.1)	10 (29.4)	7 (20.6)	17 (50.0)
Hs-CRP (mg/L)						
Low-moderate risk (≤3)	12 (35.3)	10 (29.4)	22 (64.7)	12 (35.3)	13 (38.2)	25 (73.5)
High risk (>3)	5 (14.7)	7 (20.6)	12 (35.3)	5 (14.7)	4 (11.8)	9 (26.5)
Adiponectin (μg/mL)						
Normal (≥5)	9 (26.5)	11 (32.4)	20 (58.8)	10 (29.4)	10 (29.4)	20 (58.8)
Low (<5)	8 (23.5)	6 (17.6)	14 (41.2)	7 (20.6)	7 (20.6)	14 (41.2)
Homocysteine (μmol/L)						
Normal (5–15)	1 (2.9)	3 (8.8)	4 (11.8)	16 (47.1)	13 (38.2)	29 (85.3)
Low (<5)	16 (47.1)	14 (41.2)	30 (88.2)	0 (0)	1 (2.9)	1 (2.9)
High (>15)	0 (0)	0 (0)	0 (0)	1 (2.9)	3 (8.8)	4 (11.8)

Table 3. Comparison of 25(OH)D levels and biomarkers of metabolic syndrome and atherosclerosis in the vitamin D supplementation and placebo groups before (Pre) and after (Post) the intervention

Biomarker	Treatment group					
	Vitamin D			Placebo		
	Pre'	Post	p	Pre	Post	p
25(OH)D (ng/mL)	19.14±7.62*	29.81±6.44*	0.000†	22.60±9.18*	21.31±7.96*	0.198†
HOMA-IR	3.79±3.32	3.56±2.73	0.758c‡	3.11±3.04	2.97±2.89	0.795‡
Adiponectin (μg/mL)	6.98±7.33	7.45±5.52	0.039c	8.18±9.05	8.51±9.99	0.449‡
Homocysteine (μmol/L)	3.55±1.10	11.45±2.13	0.000c‡	4.17±2.58	12.32±3.33	0.000‡
Hs-CRP (mg/L)	8.54±15.37	1.75±1.10	0.012c‡	2.76±2.70	4.56 ± 7.98	0..638‡

Normality test of data distribution with Shapiro-Wilk normality test: *normally distributed data, †Paired t-test, ‡Wilcoxon signed Ranks Test, α=0.05, significant if p<0.05
HOMA-IR, homeostatic model assessment of insulin resistance; Hs-CRP, high sensitivity C-reactive protein;

DISCUSSION

This study demonstrated that high-dose vitamin D supplementation (2,000 IU/day) significantly improved serum 25(OH)D levels and positively modulated key biomarkers of metabolic syndrome and atherosclerosis, specifically adiponectin and hs-CRP, in patients with epilepsy receiving enzymatic ASMs.

Our baseline data confirm that vitamin D deficiency is a prevalent comorbidity in this population. Half of the enrolled patients exhibited Vitamin D deficiency prior to intervention, a finding consistent with previous meta-analyses regarding ASM-treated patients (16). This susceptibility is largely attributed to the use of enzymatic ASMs, which induce cytochrome P450 enzymes, thereby accelerating the catabolism of vitamin D and other micronutrients (17) often requiring long-term treatment. Following the 12-week intervention, 100% of the supplementation group achieved non-deficient levels, suggesting that 2,000 IU/day is an effective dosage for correcting deficiency in patients on polytherapy, contrasting with previous studies where lower doses (400–1,000 IU) were insufficient (18).

A key finding of this study was the significant increase in adiponectin level in the vitamin D group compared to placebo. Adiponectin is a cardio-protective adipokine often suppressed in epilepsy patients due to mitochondrial dysfunction and medication side effects (19,20). Biologically, vitamin D likely upregulates adiponectin through the activation of the vitamin D receptor (VDR) on adipocytes, which directly stimulates adiponectin gene transcription. Concurrent with this rise in adiponectin, we observed a significant reduction in hs-CRP levels. vitamin D exerts anti-inflammatory effects by suppressing NF-κB activation and downregulating pro-inflammatory cytokines (21,22). The reduction of hs-CRP combined with elevated adiponectin suggests that vitamin D supplementation may offer a dual protective mechanism against the chronic inflammatory state associated with atherosclerosis in epilepsy.

Despite improvements in inflammatory markers, we did not

Table 4. Comparison of vitamin D levels and biomarkers of metabolic syndrome and atherosclerosis between vitamin D supplementation and placebo groups

Biomarker	Group	Mean±SD	Median	Min.	Max.	95%CI	p
25(OH)D (ng/mL)							
Pre	Vitamin D	19.14±7.62*	30.4	7.9	33.8	–9.36	0.240[§]
	Placebo	22.60±9.18*	20.4	10.4	47.5	–2.43	
Post	Vitamin D	29.81±6.44*	30.4	20.8	45.6	3.46	0.002[§]
	Placebo	21.31±7.96*	20.4	9.5	35.5	–13.54	
Δ	Vitamin D	10.67±8.16*	12.1	–3.7	26.5	7.41	0.000[§]
	Placebo	–1.29±3.96*	-0.6	–12.0	4.0	–16.51	
HOMA-IR							
Pre	Vitamin D	3.79±3.32	2.77[†]	0.4	10.75	0.68	0.683[¶]
	Placebo	3.11±3.04	1.60[†]	0.50	11.30	–0.70[‡]	
Post	Vitamin D	3.56±2.73	3.27[†]	0.58	10.07	0.41	0.413[¶]
	Placebo	2.97±2.89	2.23[†]	0.41	10.47	–0.43[‡]	
Δ	Vitamin D	–0.24±3.71*	–0.67	–6.10	6.59	–2.57	0.940[¶]
	Placebo	–0.14±3.38*	–0.16	–5.64	7.84	–2.39	
Adiponectin (µg/mL)							
Pre	Vitamin D	6.98±7.33	5.12[†]	1.81	29.71	0.44	0.433[¶]
	Placebo	8.18±9.05	5.34[†]	2.44	40.0	–0.46[‡]	
Post	Vitamin D	7.45±5.52	5.89[†]	2.35	20.66	0.87	0.865[¶]
	Placebo	8.51±9.99	5.63[†]	2.62	45.0	0.88[‡]	
Δ	Vitamin D	1.38±3.05	1.91[†]	-8.03	6.22	0.041–	0.045[¶]
	Placebo	0.34±1.89	0.23[†]	-3.79	5.0	0.049[‡]	
Homocysteine (µmol/L)							
Pre	Vitamin D	3.55±1.10	3.10[†]	1.96	5.91	0.83	0.865[¶]
	Placebo	4.17±2.58	3.48[†]	2.24	13.21	–0.85[‡]	
Post	Vitamin D	11.45±2.13	11.46[b]	8.7	17.65	0.13	0.131[¶]
	Placebo	12.32±3.33	12.8[†]	3.37	17.64	–0.15[‡]	
Δ	Vitamin D	7.90±1.79	7.69[†]	5.04	11.74	0.28	0.290[¶]
	Placebo	8.15±2.70	8.90[†]	0.65	11.93	0.30[‡]	
Hs-CRP (mg/L)							
Pre	Vitamin D	8.54±15.37	2.0[†]	1.0	58.0	0.86	0.865[¶]
	Placebo	2.76±2.70	1.6[†]	1.0	12.0	–0.88[‡]	
Post	Vitamin D	1.75±1.10	1.2[†]	1.0	4.0	0.23	0.245[¶]
	Placebo	4.56±7.98	2.8[†]	1.0	34.5	–0.24[‡]	
Δ	Vitamin D	–6.74±14.65	–0.7	–54.9	1.0	0.038–	0.041[¶]
	Placebo	1.81±7.75	0[†]	–5.0	31.2	0.046[‡]	

Shapiro-Wilk normality test, *normally distributed data: †non-normally distributed data; ‡Monte Carlo simulation, number of samples 10.000; §Independent sample t-test, ¶Mann-Whitney U test; α=0.05, significant p<0.05
HOMA-IR, homeostatic model assessment of insulin resistance; Hs-CRP, high sensitivity C-reactive protein;

observe a significant change in insulin resistance (HOMA-IR). The relationship between vitamin D and insulin sensitivity remains complex; while some studies suggest a benefit, studies which include a meta-analysis have found no significant effect of supplementation on HOMA-IR, particularly in short-term interventions (23,24). It is possible that the 12-week duration was sufficient to alter inflammatory signalling (hs-CRP) but insufficient to effect structural changes in glucose homeostasis, or that HOMA-IR in this population is driven more by ASM-induced hepatic changes than by vitamin D deficiency.

Unexpectedly, homocysteine levels increased significantly in both the vitamin D and placebo groups. This finding contradicts the hypothesis that vitamin D would lower homocysteine via cystathionine beta-synthase (CBS) enzyme upregulation (25). The universal rise in homocysteine in our cohort is most likely explained by the study protocol, which required the cessation of vitamin B supplementation (B6, B12, folate) to isolate the effects of vitamin D. Homocysteine metabolism is fundamentally dependent on these B-vitamin cofactors for remethylation and transsulfuration (26). The withdrawal of these supplements in patients taking enzyme-inducing ASMs who are already prone to folate depletion likely unmasked an underlying deficiency, leading to the observed rise in homocysteine regardless of vitamin D status. This underscores the critical importance of maintaining vitamin B supplementation alongside vitamin D in this patient group.

This study has several limitations. First, variables such as dietary intake, sun exposure, and physical activity were not strictly controlled, potentially introducing confounding factors. Second, baseline levels of vitamin B6, B12, and folate were not measured, preventing a definitive correlation between micronutrient status and the observed rise in homocysteine. Finally, the sample size, while sufficient for the primary outcome, may have been underpowered to detect smaller changes in secondary metabolic parameters like HOMA-IR. Future studies with larger cohorts and comprehensive micronutrient profiling are warranted to validate these findings.

In conclusion, vitamin D administration in epilepsy patients receiving enzymatic ASMs effectively restores vitamin D levels and improves specific biomarkers of metabolic syndrome and atherosclerosis, namely adiponectin and hs-CRP. However, vitamin D alone appears insufficient to control homocysteine levels in the absence of B-vitamin supplementation. These results support the integration of vitamin D into the standard of care for epilepsy to mitigate long-term cardiovascular risk.

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TRANSPARENCY DECLARATION

Conflict of interest: None to declare.

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ORIGINAL ARTICLE

The correlation between polymorphism of interleukin 3 receptor alpha Rs6603272 G/T gene with negative symptoms in Bataknese schizophrenia patients

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ABSTRACT

Aim There are many studies about the correlation between Bataknese and schizophrenia patients, one of the most common ethnic groups in Indonesia. Cytokine and its receptor disorder is one of the scopes of immunology research in schizophrenia. Interleukin 3 (IL-3) is an important cytokine with various biologic roles in immune response. We would like to determine the association between the polymorphism of IL3RA gene with negative symptoms in Bataknese schizophrenia patients.

Methods This study used comparative case control approach. The negative symptoms were assessed using Positive and Negative Syndrome Scale (PANSS). Blood sampling and deoxyribonucleic acid (DNA) isolation used salting out method followed by IL3RA RS6603272 G/T genotype identification using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The data were analysed using $\chi 2$ and Kruskal Wallis test. A $p<0.05$ was considered statistically significant.

Results Two hundred participants were divided into schizophrenia and healthy control groups evenly. G alleles (49.5% and 50.5%) and GT genotypes (49% and 53%) were the most common alleles and phenotypes in both groups with no significant difference in both groups ($p>0.05$). The analysis also did not reveal significant correlation between the polymorphism of IL3RA RS6603272 G/T with the negative symptoms of schizophrenia ($p > 0.05$).

Conclusion There was neither significant difference of the presence of G and T alleles, also GG, GT, and TT genotypes between two groups nor the correlation between polymorphism of IL3RA RS6603272 G/T with schizophrenia negative symptoms.

Keywords: Bataknese, IL3RA RS6603272 G/T gene, polymorphism, schizophrenia negative symptoms

INTRODUCTION

Schizophrenia is a chronic and complex neuropsychiatric disorder with a significant impact on the quality of life of patients and their families. Schizophrenia spectrum disorders affect more than 21 million people globally. Seven out of 1,000 people have schizophrenia spectrum disorder during their lifetime, and the symptoms typically appear in the second or third decade of life. The most common onset of schizophrenia is late adolescence and 20s, but males usually have an earlier onset than females (1,2).

The prevalence of schizophrenia had been increasing from 13.1 million in 1990 to 20.9 million cases in 2016, and most cases (70.8% or 14.8 million) were found in the age group of 25 – 54 years old. East Asia and South Asia had the highest schizophrenia cases, about 7.2 million and 4.0 million, respectively, in 2016. National Basic Health Survey in 2018 revealed that Bali and North Sumatra are provinces with the highest schizophrenia prevalence in Indonesia (11% and 5%, respectively). Medan city has a household prevalence of schizophrenia, in which 6.77% of household members had schizophrenia (2-6). Bataknese is one of the most common ethnic groups in Indonesia, particularly in North Sumatra province, and it is between mountains and a lake that has an island in the middle. Bataknese is the oldest Proto-Melayu ethnicity that has retained its language and tradition for a thousand years, and the use of the native language can still be found. Bataknese was divided into six categories: Mandailing, Angkola, Toba, Dairi or Pakpak Dairi, and Simalungun (7,8).

There are various studies about the correlation between genes and schizophrenia (9). A study in the Psychiatry Department Dr. Soetomo Regional General Hospital Surabaya revealed the high prevalence of COMTVal158Met polymorphism (6.7%) with heterozygote alleles of 21,946 (40%), nucleotide variance

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of T substitute to A of 21,971 (3.3%), and G nucleotide variance in the polymorphism of DISC1 gene in schizophrenia patients (10,11). The investigation of the correlation of 174G/C interleukin-6 polymorphisms with schizophrenia in Batak population showed 174G/C polymorphism as a risk factor (12). It was also demonstrated that there was a strong correlation between interferon gamma +874 A/T in schizophrenia. A significant correlation between the polymorphism of the interleukin-10 1082 G/A gene in Bataknese was found (13,14). Various studies have demonstrated significant correlations between genetic variants in schizophrenia patients (13-16) .

One of the complexities of schizophrenia is the lack of a central pathophysiology mechanism and a main biological marker. The most common hypothesis is the combination of genetic and environmental factors during early life, which is consistent with the neurodevelopmental hypothesis. Although the schizophrenia gene component has a huge impact, most genetic architectures remain unknown. Schizophrenia is a multifactorial disorder that includes interactions of various susceptible genes, epigenetic processes, and environmental factors (17-19). The dysfunction of the immune system and inflammation contribute to the development of schizophrenia. The specific mechanism regarding the correlation of the neuroimmune system and schizophrenia is being investigated, but it is believed that immune dysregulation may impact brain development and function, potentially contributing to the onset of schizophrenia and its clinical manifestations (20,21).

The disorder of cytokines and their receptors is one of the research focuses regarding immunology in schizophrenia (22). Interleukin-3 (IL-3) is an important cytokine that has various biologic roles in immune response. It affects inflammation response in the adult brain development through the regulation of activated microglia, altering the susceptibility of nerve inflammation, and schizophrenia patients have abnormal serum IL-3 levels. A study in China demonstrated that the polymorphism of the Interleukin-3 Receptor Alpha (IL-3RA) gene was correlated with schizophrenia. Furthermore, the IL-3RA rs6603272 gene also plays a role in schizophrenia (20,22).

The correlation between schizophrenia and nerve degeneration in schizophrenia is a complex study. The exact mechanism is still unknown, but there was evidence that nerve inflammation and degeneration contribute to the negative symptoms in schizophrenia (23). However, the specific cause-and-effect relationship between nerve inflammation, degeneration, and negative symptoms in schizophrenia requires further research for better comprehension (24).

The aim of this study was to investigate the correlation of polymorphism of the IL-3RA gene to negative symptoms in the Bataknese schizophrenia and healthy control groups.

PATIENTS AND METHOD

Patients and study design

The study was conducted in the Prof. Dr. M. ldrem Psychiatry Hospital for case group sampling, Blood Transfusion Unit of Medan City for control group sampling, and Integrated Laboratory of Faculty of Medicine, Universitas Sumatera Utara for gene polymorphism examination between April and July 2024. We wanted to determine the association between the polymorphism of interleukin 3 receptor alpha RS6603272 G/T gene with negative symptoms in Bataknese schizophrenia patients.

Consecutive sampling was applied in this study. The inclusion criteria were as follows: Bataknese patients with schizophrenia whose diagnoses were established according to the third edition of the Guidelines for the Classification and Diagnosis of Mental Health Disorders (25); individuals with Bataknese ancestry up to two generations; aged 18–60 years; presenting predominantly negative symptoms (N score < 21); and who were cooperative and agreed to participate in the study. The exclusion criteria included a history of neurological, endocrine, or autoimmune diseases; a history of alcohol or substance use other than nicotine and caffeine; and current or previous use of immunosuppressive drugs (including corticosteroids or cancer treatment regimens).

The participants were divided into schizophrenia and healthy control groups.

We gave a brief explanation to participants before data collection, and they were asked to sign informed consent. The data were kept confidential.

This study had been approved by the Ethics Committee of Faculty of Medicine of (Number: 362/KEPK/USU/2024).

Methods

After informed consent was obtained, the schizophrenia group was asked to fill out the Bahasa version of the Positive and Negative Syndrome Scale (PANSS) (26). The healthy control group was asked to fill out the Mini International Neuropsychiatric Interview (Mini ICD-10) (27) first to exclude other psychiatric disorders. After that, 7 mL of blood was withdrawn from the median cubital vein in stable schizophrenia patients. For the control group, blood was withdrawn just before the donor process. Blood was examined in the Integrated Laboratory of Medicine Faculty at Universitas Sumatra Utara.

After the blood was withdrawn, it was placed in a vacutainer containing ethylenediamine tetraacetic acid (EDTA) and stored at a temperature of 4–80 °C until deoxyribonucleic acid (DNA) isolation was performed. DNA was isolated using the salting-out method (28). After the DNA isolation, IL3RA RS6603272 G/T genotypes were identified using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method (29). Fragments in 133 base pairs (bp), 25 bp, and 158 bp from IL3RA RS6603272 G/T were amplified using forward primer 5 ACGACCTGTACTTGAACGTTGCCAAGTAGGTGGGC-3, reverse primer: 5-AGCATCCGTTTTGTAGTGAAGAC-3. The PCR products were stored at 37 °C overnight with amplified and digested DNA fragments using the EcoR01091 enzyme and then separated in a 3% agarose gel that was stained with ethidium bromide (24). The examination of DNA concentration, check, and purity were conducted using a nanophotometer n-50 with a mean of 1.8.

Statistical analysis

The data were collected using software statistical analysis with the following steps: variable analysis and presented in the frequency tables. The analysis comprises frequency and percentage. The comparative test ($\chi 2$ test) was conducted to assess the difference in allele and IL-3RA RS6603272 G/T genotype between Bataknese schizophrenia and healthy control groups. The odds ratios were calculated (OR>1 indicates that independent variables were not risk or protective factors; OR=1 indicates that independent variables were not risk or protective factors; and OR<1 indicates that independent variables were

the protective factor of dependent variables). The comparative tests were conducted to determine the correlation between the polymorphism of IL3RA RS6603272 G/T and schizophrenia negative symptoms using the Kruskal-Wallis test.

RESULTS

Two hundred band agarose electrophoresis gels were analysed on the IL3RA RS6603272 G/T gene, and the reading was done using the exact method with Sun et al. (20). No deviation from the Hardy–Weinberg Equilibrium was detected for the three polymorphisms (p>0.05). The analysed demographic characteristics in this study (schizophrenia group) were sex, age, onset of the disease, duration of the disease, and the total PANSS negative symptoms score, while in the healthy control group, the characteristics analysed were sex and age. The categorical data were presented in N (%). In contrast, the numeric data were presented in mean and minimum–maximum value.

Most of our participants in both groups were males, comprising 56 (56%) in the healthy control group and 79 (79%) in the schizophrenia group, respectively. The mean age in the healthy control and schizophrenia groups was 28.89 and 32.38 years, respectively. The median onset age of schizophrenia onset was 27 years, and the median duration was 5 years. Most of the schizophrenia participants in the schizophrenia group were rehospitalization patients who received polytherapy, in which clozapine was the most common antipsychotic drug (98%). Most MoCA-Ina scores indicated moderate cognitive impairment. The median of the PANSS negative symptoms score was 25 (Table 1).

Table 1. Demographic characteristics of two patient groups

Variable	Healthy control (N=100)	Bataknese schizophrenia (N=100)
Sex (No; %)		
Male	56 (57)	79 (79)
Female	44 (44)	21 (21)
Age (Mean±SD)	28.89±6.075	32.38±4.4269
	Median (interquartile range)	
Onset (age)	-	27 (14.0-41.00)
Duration (years)	-	5.00 (2.00-21.00)
The total of PANSS negative symptoms score	-	26.93 (21.00-42.00)

PANSS, Positive and Negative Syndrome Scale

The polymorphism of the IL3RA RS6603272 G/T gene consisted of two alleles: G and T. The allele variable was a categorical (nominal) variable and presented in a frequency distribution. The G allele was the most common in both groups. There was no significant difference in G and T allele presence between the two groups (p>0.05), with an odds ratio (OR) of approximately 0.961 (close to 1), indicating that the independent variable was not a risk factor for schizophrenia. The genotype variant of the IL3RA RS6603272 G/T gene consisted of a combination of the G and T alleles, represented by the GT, GG, and TT genotypes. The GT genotype was the most common in the schizophrenia and control groups (49% and 53%, respectively) (Table 2).

The result of binary binomial logistic regression analysis demonstrated no significant difference regarding the presence of the GT genotype in healthy controls and schizophrenia groups, with a p=0.479 and OR of 1.288 (GT versus TT). However, based on the OR value, the distribution of GT and GG genotypes in healthy controls was a risk factor for schizophrenia. The p=0.667 (OR 1.190)-for the GT and GG genotypes was found. This result indicates that there were no significant differences in the presence of the GG genotype between the two groups, and the OR value revealed that the difference in the distribution of GG and TT genotypes was not considered a risk factor for schizophrenia (Table 2).

Table 2. Differences of IL3RA RS6603272 G/T gene alleles and genotypes in healthy control and schizophrenia groups

Variable	No (%) of patients		p	OR (95% CI)
	Schizophrenia	Healthy control		
IL-3RA RS6603272 Allele				
G	102 (49.5)	104 (50.5)	0.920	0.961 (0.649-1.422)
T	98 (50.5)	96 (49.5)		
Total	200	200		
The Polymorphism IL-3RA RS6603272 Genotypes				
G/T	49 (49)	53 (53)	0.478	1.288 (0.641-2.588)
G/G	26 (26)	26 (26)	0.667	1.190 (0.538-2.636)
T/T	25 (25)	21 (21)		
Total	100	100		

Bataknese schizophrenia patients with mean scores of 27.57, 24.85, and 25.84 for the GG, GT, and TT genotypes, respectively, with p = 0.762 (> 0.05), were found. Furthermore, the mean score of the Bataknese schizophrenia group with G and T alleles was 27.41 and 26.95, respectively, with a p=0.308 (> 0.05). It can be concluded that there is no significant correlation between the polymorphism of IL3RA RS6603272 G/T with negative symptoms in the Bataknese schizophrenia group (Table 3).

Table 3. Correlation between T genotype and polymorphism of IL-3 RS 6603272 G/T gene with negative symptoms in schizophrenia patients

Variable	Genes	N	Mean	p
Genotypes (N=100)	GG	49	27.57	0.763
	GT	26	24.58	
	TT	25	25.84	
Allele (N=200)	G	102	27.41	0.308
	T	98	26.95	

PANSS, Positive and Negative Syndrome Scale

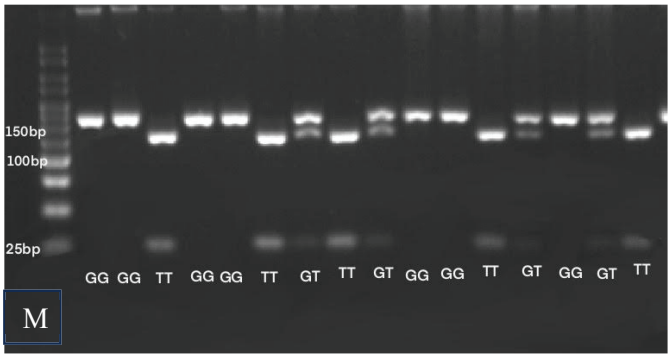

Figure 1. Agarose electrophoresis gel of the polymorphism of IL3RA RS6603272 G/T Gene

1st row = marker (M), base pair 25 = base pair ladder, base pair 158= allele G, base pair 23 and 133 = T allele; 2nd row = GG genotype; 3rd row = GT genotype, 6th row = TT genotype

DISCUSSION

Most participants in this study were males with an age range of 23 – 50 years in the schizophrenia group. It is similar with previous studies (1,19,20) although there are opposite results with no significant prevalence of schizophrenia between males and females (30). The reason for higher schizophrenia prevalence in males may be due to the lower medication adherence and higher relapse rate in males, as well as a relative difference in help-seeking between males and females, and a higher substance misuse rate in males (31,32). The analysis of age of onset in this study was in line with other studies which found that the most common onset of schizophrenia was in the age group of 19 to 51 years (33). An epidemiology study from Spain also found that the highest prevalence of schizophrenia was found in males in the age group of 35- 54 years (1,33).

There was no significant difference in the presence of the allele between the two groups. However, a study in China found a significant difference in alleles between schizophrenia and healthy control groups, in which the T allele is more common in schizophrenia patients compared to healthy controls, with a significant correlation (22). The inconsistency between those studies may be due to different inclusion criteria and ethnicity. Furthermore, environmental and lifestyle factors may play an important role in schizophrenia development, leading to the variance in results between studies. Interleukin-3 may contribute to various immunological disorders, consistent with the hypothesis that cytokine disorders in schizophrenia have a strong genetic basis. However, the functional aspect of the rs6604272 polymorphism in IL-3RA is still unknown. Further studies are required to determine if this gene polymorphism is associated with increased peripheral IL-3 value in schizophrenia patients, particularly chronic patients (33-35).

We found no significant difference in the genotype polymorphism of the IL3RA RS6603272 G/T gene between the two groups. It was not consistent with previous studies that demonstrated a correlation between the polymorphism of the IL3RA RS6603272 G/T gene and schizophrenia prevalence. The inconsistency between these studies may be due to differences in inclusion criteria, sample size, and ethnicity. Additionally, the increased serum IL-3RA level was confirmed in the studies prior to the polymorphism study. The different results of similar polymorphism may appear due to several factors, such as different effects from similar polymorphism genetic factors, which depend on the individual's whole genetic arrangement, also environmental factors, such as dietary habits, lifestyle, toxic exposure, and stress (22,36). The epigenetic modification role, such as DNA methylation and histone modification, can alter gene expression without altering the DNA sequence (37). The polymorphism regarding the disease risk may only express those risks in certain conditions. These single or combination factors may cause similar polymorphism with different results in different individuals or conditions. The different findings in this study may be influenced by factors such as varying standards for antipsychotic drugs, sample size, and differing inclusion and exclusion criteria across studies (38).

The analysis revealed no significant correlation between IL-3RA RS6603272 G/T gene alleles and genotypes with schizophrenia negative symptoms. This study is the first to determine the correlation between the polymorphism of the IL3RA RS6603272 G/T gene and negative symptoms. However, numerous studies have investigated the correlation between gene polymorphism and negative symptoms, particularly those involving immune-related genes. Several studies have reported correlated results, finding a significant positive correlation between immune-related genes and schizophrenia negative symptoms, specifically the polymorphisms of IL-1β, IL-2, IL-6, and TNF-α genes (39,40).

Several studies also demonstrated the correlation between peripheral inflammation biomarkers and negative symptoms. An increased inflammation cascade, characterized by heightened immune activity, pro-inflammatory cytokine production, and oxidative stress, was found in schizophrenia patients. The interaction between these factors contributes to serotonergic, dopaminergic, and glutamatergic neurotransmission disorders, leading to negative symptoms (21,40,41). Peripheral immune response reflects central neuroimmune status; therefore, systemic cytokine concentration is more relevant to reflect how inflammation leads to a specific symptom profile in schizophrenia. The balance between peripheral Th1 and Th2 inflammatory responses and cytokine production is crucial for efficient immune response; therefore, their imbalance may affect Th2 differentiation and secretion. Reportedly, increased IL-3 serum levels in schizophrenia patients, particularly in chronic patients, and their correlation analysis demonstrated the correlation between increased serum IL-3 levels and negative symptoms score. A genetic study also demonstrated that IL-3 and IL-3RA genes are located near the schizophrenia-associated genetic marker in pseudo-autosomal region (PAR1) from X and Y chromosomes, in which the abnormality of IL-3 and its receptor contributes to increased granulocyte-macrophage colony stimulating factor (GM-CSF), which encourages inflammation, and may play a role in the manifestation of schizophrenia symptoms (21,38,42).

Previous studies supported the hypothesis that pro-inflammatory cytokines in the peripheral body system increased in schizophrenia. However, the manifestation of schizophrenia symptoms varied and was affected by the complex interaction between genetic, epigenetic, environmental, and neuronal developmental factors. These interactions may lead to similar polymorphism in patients with different symptoms. Each person has a unique genetic arrangement, and other genetic variants may interact with polymorphisms, altering their effects. This interaction may alter how certain polymorphisms contribute to the development of symptoms. Schizophrenia-related polymorphism can lead to different symptom profiles, depending on certain life events in the environmental context (22,38,43).

This study was the first in Indonesia and Medan to investigate one ethnic group, the Bataknese, specifically reporting the demography of genotypes and alleles of the polymorphism of the IL3RA RS6603272 G/T gene in North Sumatra. This study may be the foundation of future studies regarding the correlation polymorphism of gene for another ethnic group in Indonesia or other countries with schizophrenia to extend the knowledge between gene and schizophrenia.

Our study has several limitations such as the confounding factors of cytokines that cannot be fully controlled, and the small sample size.

In conclusion, there was neither significant difference regarding the presence of G and T alleles also GG, GT, and TT genotypes between both groups nor significant correlation between the polymorphism of IL3RA RS6603272 G/T allele with negative symptoms of schizophrenia.

AUTHORS CONTRIBUTION

Conceptualization D.A.A, E.E, and M.M.A; methodology, D.A.A, E.E, and M.M.A; software, D.A.A; validation D.A.A; formal analysis, D.A.A and E.E; investigation. D.A.A; resources D.A.A and E.E; data curation D.A.A and E.E; writing (first draft) D.A.A and E.E; writing (review and editing), D.A.A, E.E, M.M.A, V.C, Z.Y; visualization D.A.A, E.E, M.M.A, V.C, Z.Y; study registration and funding, D.A.A.

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Conflicts of interest: None to declare.

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ORIGINAL ARTICLE

The impact of cognitive impairments on the quality of life of patients with multiple sclerosis

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ABSTRACT

Aim Multiple sclerosis (MS) is a chronic, inflammatory, (auto)immune disease of the central nervous system. Cognitive impairments are common in MS patients and significantly affect their quality of life. This study aimed to assess the impact of cognitive disorders on the quality of life in MS patients.
Methods A prospective study included 135 MS patients and 50 healthy individuals. Participants were divided into three groups: 85 patients with MS lasting over one year, 50 newly diagnosed MS patients, and 50 healthy individuals. Clinical assessment included the Mini-Mental Status Examination and cognitive function tests (Wechsler Intelligence Scale, Revised Beta Test, Raven's Coloured Progressive Matrices, Wechsler Memory Scale, Audio-Verbal Learning Test, Rey-Osterrieth Complex Figure Test, Verbal Fluency Test, and the SF-36 quality of life scale).
Results Cognitive impairments were found in 40-60% of MS patients, with mnestic functions most affected (30-60%). Patients with longer disease duration had poorer visuospatial, visuoconstructive, and visuoperceptual abilities. Immediate working memory, attention, and logical memory were also more impaired. Quality of life was significantly reduced in both MS groups, with lower MMSE score correlating with greater impairment.
Conclusion Cognitive impairments in MS patients affect memory, executive function, and attention, with global intellectual decline occurring later. A strong link between cognitive dysfunction and reduced quality of life highlights the importance of cognitive assessment for evaluating disease severity and therapy effects.
Key words: cognition, executive function, neuropsychological tests

INTRODUCTION

Multiple sclerosis (MS) is a chronic, inflammatory, autoimmune disease of the central nervous system (CNS), characterized by inflammation, demyelination, gliosis, and neuronal loss (1). Although the exact etiology remains unknown, it is believed that a combination of genetic predisposition and environmental factors such as vitamin D deficiency, birth season, tobacco exposure, and Epstein-Barr virus, play a role in the disease development (2,3).
The prevalence of MS worldwide ranges from 5 to 300 per 100 000 people and increases at higher latitudes (4).
Although the overall prevalence of MS generally decreases with increasing proximity to the equator, recent epidemiological data indicate a rising incidence in certain regions, which may reflect changes in environmental exposure, genetic susceptibility, or improved diagnostic practices (5-7).

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The clinical course varies, with the relapsing-remitting form being most common, often progressing to a secondary-progressive form. Less common types include primary progressive and progressive-relapsing MS. Symptoms may present in episodes or as gradual progression (8,9).
Cognitive dysfunction is a common, yet often under recognized manifestation of MS, affecting up to 65% of patients (10). It typically involves deficits in working memory, attention, and executive functions, while global intellectual capacity often remains preserved in the early stages of the disease (11). These higher cognitive functions are supported by intricate neural networks encompassing the associative cortex and limbic structures particularly the hippocampus and anterior cingulate gyrus (12,13).
Disruption of these systems is closely associated with cognitive decline in MS and contributes significantly to reduced quality of life. Cognitive impairment interferes with daily functioning, including interpersonal relationships, work performance, and emotional well-being, and is frequently accompanied by other "invisible" symptoms such as fatigue and depression (14).
The World Health Organization defines quality of life (QoL) as an individual's perception of their position in life, within their

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cultural context and value system, in relation to personal goals and expectations (15). QoL encompasses physical, psychological, social, and functional dimensions.

In routine clinical practice, an increasing number of patients with MS present with cognitive difficulties, including impairments in memory, attention, verbal expression, and concentration, despite being physically relatively stable (16).

These so-called "invisible symptoms" are frequently underdiagnosed and inadequately addressed, although they substantially compromise quality of life, occupational performance, and interpersonal relationships (17).

A deeper understanding of cognitive dysfunction in MS, along with its quantification and correlation with clinical manifestations, contributes to the advancement of individualized treatment, patient education, and rehabilitation strategies. Furthermore, cognitive decline may serve as an early predictor of MS progression (18).

The majority of available literature addresses cognitive impairments in patients with longer disease duration, often without comparative analysis involving control groups or longitudinal follow-up (19).

In the region of Southeast Europe such studies remain scarce. Notably, investigations that explore the association between objective neuropsychological findings and quality of life parameters are especially underrepresented.

The aim of this study was to examine the impact of cognitive impairments on the quality of life in patients with MS, emphasizing their effect on physical, psychological, and social functioning.

PARTICIPANTS AND METHODS

Participants and study design

This prospective study was conducted at the Clinic of Neurology, University Clinical Centre Tuzla, Bosnia and Herzegovina. The duration of the study was 2.5 years during the period June 2021 – December 2023.

A total of 185 patients were included in the study, comprising 135 patients diagnosed with multiple sclerosis (MS) and 50 healthy patients as controls. Patients were divided into three groups: Group I consisted of 85 patients with MS duration longer than one year, Group II included 50 patients newly diagnosed with MS with disease duration less than one year, and Group III consisted of 50 healthy patients, matched by age, gender, and education level to the MS groups. Patients were selected consecutively during the study period.

Inclusion criteria for Group I and Group II were a confirmed MS diagnosis based on the current McDonald criteria and in group I patients had to have a brain magnetic resonance scan no older than one year prior to the initial testing (20, 21). Group III included patients without a history or clinical signs of neurological or cognitive disorders. Exclusion criteria for MS patients included comorbid diseases and central nervous system (CNS) injuries, such as trauma, metabolic disorders, epilepsy, and vascular lesions. Demographic data including age, gender, and education level were collected for all patients. The approval from the Ethics Committee from University Clinical Centre Tuzla was obtained.

Methods

All MS patients underwent brain MRI at baseline. Neurological examinations were conducted by a neurologist, while neuropsychological testing was performed by both a psychologist and a neuropsychiatrist. Clinical evaluation included the Expanded Disability Status Scale (EDSS) (22), the Mini-Mental State Examination (MMSE) (23), and the SF-36 Quality of Life Scale (24). To assess cognitive functions, a comprehensive neuropsychological battery was used, consisting of the Wechsler Intelligence Scale WB-II (25), Revised Beta Test (26), Raven's Coloured Progressive Matrices (27), Wechsler Memory Scale (28), Audio Verbal Learning Test (AVLT) (29), Rey-Osterrieth Complex Figure Test (RCFT) (30), and the Verbal Fluency Test (FAS) (31). All participants underwent initial testing, with follow-up testing performed one year later.

Statistical analysis

The Mann–Whitney U test, Wilcoxon test, and $\chi 2$ test were used to examine differences between the groups. Associations among variables test scores, and scale domains were evaluated using regression analysis and Spearman's correlation coefficient. A $p< 0.05$ was considered statistically significant.

RESULTS

Group I included 85 MS patients (70.5% female; mean age 42.0±9.3 years), Group II included 50 newly diagnosed MS patients (82% female; mean age 37.5±10.8 years), and Group III included 50 healthy patients matched by age, gender, and education. Among MS patients, 121 (82%) had relapsing-remitting and 14 (18%) had secondary-progressive MS (Table 1).

Table 1. Demographic and clinical characteristics of the patients

Variable	Testing I			Testing II		
	Control	Group I	Group II	Control	Group I	Group II
Number of patients	50	85	50	50	80	45
RRMS	0	71	50	0	66	45
SPMS	0	14	0	0	14	0
Age (mean±SD) (years)	38±5.8	42±9.3	37.5±10.8	39±5.8	43±9.3	38.5±10.8
Gender (M/F) (N)	35 / 15	60 / 25	41 / 9	35 / 15	59 / 21	39 / 6
Education (mean±SD) (years)	16±1.8	12±2.5	12±2.4	16±1.8	12±2.5	12±2.4
EDSS (mean±SD)	0	2.5±2.3	1.9±1.8	0	3±2.6	1.6±1.5
Duration of disease (mean±SD) (years)	0	6±4.0	< 1	0	7±4.0	1

*Testing I, initial testing; Testing II, testing after one year; Control, patients without multiple sclerosis; Group I, patients with multiple sclerosis which lasted more than one year; Group II, patients with newly diagnosed multiple sclerosis; RRMS, relapsing remitting form of multiple sclerosis; SPMS, secondary progressive form of multiple sclerosis; EDSS, extended scale of disability status in multiple sclerosis

Table 2. Distribution of cognitive disorders, initial testing

Variable	No (%) of patients					
	Group I (N=85)			Group II (N=50)		
	Normal	Below average	Pathological	Normal	Below average	Pathological
WBac	50 (58.82)	12 (14.12)	23 (27.06)	40 (80.00)	4 (8.00)	6 (12.00)
WBc	33 (38.82)	46 (54.12)	6(7.06)	22 (44.00)	23 (46.00)	5 (10.00)
CPM	82 (96.47)	2 (2.35)	1 (1.18)	47 (94.00)	2 (4.00)	1 (2.00)
β	56 (65.88)	11 (12.94)	18 (21.18)	40 (80.00)	3 (6.00)	7 (14.00)
WB mc	33 (38.82)	28 (32.94)	24(28.24)	25 (50.00)	18 (36.00)	7 (14.00)
WB lm	35 (41.18)	9 (10.59)	41 (48.24)	23 (46.00)	8(16.00)	19 (38.00)
AVLT 1-5	42 (49.41)	18 (21.18)	25 (29.41)	27 (54.00)	11 (22.00)	12 (24.00)
AVLT 6	19 (22.35)	17 (20.00)	49 (57.65)	13 (26.00)	13 (26.00)	24 (48.00)
AVLT 7	18 (21.18)	15 (17.65)	52 (61.18)	14 (28.00)	16 (32.00)	20 (40.00)
RCFT rc	48 (56.47)	7 (8.24)	30 (35.29)	37 (74.00)	6 (12.00)	7 (14.00)
RCFT vm	31 (36.47)	8 (9.41)	46 (54.12)	24(48.00)	5 (10.00)	21 (42.00)
FAS	56 (65.88)	5 (5.88)	24 (28.24)	38 (76.00)	4 (8.00)	8 (16.00)

Wbac, Wechsler Bellevue scale form II -subtest: Assembling cubes; WBc, Wechsler Bellevue scale form II - subtest: Common terms; CPM, Raven's Coloured Progressive Matrices; β, Revised Beta test maze; WB mc, Wechsler Bellevue scale form II - subtest: mental control; WB lm, Wechsler Bellevue scale form II – subtest: logical memory; AVLT , Auditory-verbal learning test; RCFT rc, Rey Complex Figure Test - recognition trial; RCFT vm, Rey Complex Figure Test - visual memory; FAS, FAS verbal fluency tests

Significant differences in test performance were found between MS groups and healthy controls ($p<0.05$). Visuospatial and visuoconstructive abilities were more impaired in Group I than in Group II: cube subtest (WB ac) in 23 (27%) versus 6 (12%), crossing out subtest (RCFT re) in 30 (35.3%) versus 7 (14%) cases. Executive functions, as measured by the maze subtest, were preserved in 67 (79%) patients of Group I and 43 (86%) of Group II. Verbal fluency (FAS) was within normal range in 24 (28%) patients of Group I and 8 (16%) of Group II. Working memory, attention, and short-term memory were impaired in 24 (28.2%) patients of Group I and 13 (16%) of Group II, while logical memory was impaired in 41 (48.2%) and 19 (38%), respectively. Short-term verbal memory (AVLT A6) was impaired in 49 (57%) of Group I and 24 (48%) of Group II, while long-term verbal memory (AVLT A7) showed deficits in 52 (61%) of Group I and 20 (40%) of Group II patients. Non-verbal recall (RCFTvm) was impaired in 46 (54%) of Group I and 21 (42%) of Group II patients. Verbal fluency impairment due to demyelination was present in 24 (28.2%) patients of Group I and 8 (16%) of Group II (Tables 2, 3).

In Group I, all psychological tests correlated significantly with physical QoL ($p<0.05$); the mental and total QoL score correlated significantly with all tests except numerical memory. In Group II, SF-36 dimensions correlated significantly with all tests except the beta maze and numerical memory (Table 4).

At follow-up, Group I showed the strongest correlation between quality of life and the RCFT test. In Group II, the strongest correlation was with MMSE, WB mc, and RCFT. Physical and mental QoL dimensions, as well as total SF-36 score, correlated positively and significantly with MMSE in both MS groups. During the second testing, correlations in Group II remained significant for most psychological tests,

Table 3. Distribution of cognitive disorders, testing after one year

Variable	No (%) of patients					
	Group I (N=80)			Group II (N=45)		
	Normal	Below average	Pathological	Normal	Below average	Pathological
WBac	50 (62.50)	12 (15.00)	18 (22.50)	35 (77.78)	6 (13.33)	4 (8.89)
WBc	26 (32.50)	49 (61.25)	5 (6.25)	13 (28.89)	29 (64.44)	3 (6.67)
CPM	74 (92.50)	0 (0.00)	6 (7.50)	43 (95.56)	2 (4.44)	0 (0.00)
β	57 (71.25)	8 (10.00)	15 (18.75)	37 (82.22)	4 (8.89)	4 (8.89)
WB mc	23 (28.75)	31 (38.75)	26 (32.50)	20 (44.44)	14 (31.11)	11 (24.44)
WB lm	33 (41.25)	12 (15.00)	35 (43.75)	19 (42.22)	9 (20.00)	17 (37.78)
AVLT 1-5	34 (42.50)	17 (21.25)	29 (36.25)	19 (42.22)	15 (33.33)	11 (24.44)
AVLT 6	17 (21.25)	19 (23.75)	44 (55.00)	11 (24.44)	13 (28.89)	21 (46.67)
AVLT 7	17 (21.25)	16 (20.00)	47 (58.75)	8 (17.78)	13 (28.89)	24 (53.33)
RCFT rc	47 (58.75)	4 (5.00)	29 (36.25)	34 (75.56)	2 (4.44)	9 (20.00)
RCFT vm	29 (36.25)	13 (16.25)	38 (47.50)	24 (53.33)	6 (13.33)	15 (33.33)
FAS	57 (71.25)	6 (7.50)	17 (21.25)	32 (71.11)	7 (15.56)	6 (13.33)

Wbac, Wechsler Bellevue scale form II -subtest: Assembling cubes; WBc, Wechsler Bellevue scale form II - subtest: Common terms; CPM, Raven's Coloured Progressive Matrices; β, Revised Beta test maze; WB mc, Wechsler Bellevue scale form II - subtest: mental control; WB lm, Wechsler Bellevue scale form II – subtest: logical memory; AVLT , Auditory-verbal learning test; RCFT rc, Rey Complex Figure Test - recognition trial; RCFT vm, Rey Complex Figure Test - visual memory; FAS, FAS verbal fluency tests

Table 4. Correlation of psychological testing results with SF - 36 quality of life scale of two study groups, initial testing

Tests	Group I (N = 85)		Group II (N = 50)	
	b	p	b	p
MMSE	1.8715	0.0308*	1.7793	0.2432
Wbac	0.1448	0.9032	-0.7157	0.7833
WBc	1.2413	0.3767	0.2402	0.8874
CPM	-0.6093	0.5278	0.2281	0.8831
β	0.3079	0.8088	0.0432	0.9846
WB mc	-4.4289	0.0016*	-1.6309	0.4819
WB lm	1.3079	0.228	1.7972	0.3536
AVLT 1-5	0.9852	0.4016	-3.2439	0.2642
AVLT 6	2.2738	0.2714	2.6429	0.3723
AVLT 7	-1.2207	0.5215	-0.1473	0.9652
RCFT rc	0.1747	0.5549	0.9593	0.2157
RCFT vm	0.5844	0.0899	0.0259	0.9727
FAS	0.0195	0.9602	0.409	0.5703
D - W test	(R) = 0.6113	0.0005*	(R) = 0.5834	0.1915
	AC 2.1109		AC 2.0517	

b, Partial correlation coefficient of multiple regression correlation analysis; p, Possibility of random difference two-sided testing of the hypothesis; MMSE, Mini mental status examination; Wbac, Wechsler Bellevue scale form II -subtest: Assembling cubes; WBc, Wechsler Bellevue scale form II - subtest: Common terms; CPM, Raven's Coloured Progressive Matrices; β, Revised Beta test maze; WB mc, Wechsler Bellevue scale form II - subtest: mental control; WB lm, Wechsler Bellevue scale form II – subtest: logical memory; AVLT, Auditory-verbal learning test; RCFT rc, Rey Complex Figure Test - recognition trial; RCFT vm, Rey Complex Figure Test - visual memory; FAS, FAS verbal fluency tests; (R), multiple correlation coefficient; D - W test, Durbin Watson test; AC, autocorrelation

Table 5. Correlation of psychological testing results with SF - 36 quality of life scale of two study groups, testing after one year

Tests	Group I (N = 80)		Group II (N = 45)	
	b	p	b	p
MMSE	0.6482	0.4613	5.759	0.0018*
Wbac	-2.4887	0.1886	-9.1236	0.0038*
WBc	0.7909	0.6231	0.358	0.874
CPM	0.3813	0.4621	1.4019	0.3466
β	-0.0546	0.9706	0.6627	0.7973
WB mc	-0.2788	0.8991	-0.653	0.7925
WB lm	0.6237	0.7189	0.2282	0.9215
AVLT 1-5	-2.9256	0.1095	0.0268	0.9922
AVLT 6	1.9576	0.4079	-1.8345	0.6923
AVLT 7	-0.7322	0.683	2.0087	0.637
RCFT rc	1.3474	0.0188*	1.5841	0.0420*
RCFT vm	0.8836	0.061	0.0789	0.9163
FAS	0.1797	0.708	0.2756	0.7155
D - W test	(R) = 0.5986	0.0023*	(R) = 0.7285	0.0110*
	AC 2.0315		AC 1.4302	

b, partial correlation coefficient of multiple regression correlation analysis; p, possibility of random difference two-sided testing of the hypothesis; MMSE, Mini mental status examination; Wbac, Wechsler Bellevue scale form II -subtest: Assembling cubes; WBc, Wechsler Bellevue scale form II - subtest: Common terms; CPM, Raven's Coloured Progressive Matrices; β, Revised Beta test maze; WB mc, Wechsler Bellevue scale form II - subtest: mental control; WB lm, Wechsler Bellevue scale form II – subtest: logical memory; AVLT, Auditory-verbal learning test; RCFT rc, Rey Complex Figure Test - recognition trial; RCFT vm, Rey Complex Figure Test - visual memory; FAS, FAS verbal fluency tests; (R), multiple correlation coefficient; D - W test, Durbin Watson test; AC, autocorrelation

with fewer associations seen for the cube subtest, Raven's Coloured Progressive Matrices (CPM), beta maze, and logical memory (Table 5).

DISCUSSION

Multiple sclerosis (MS) is a neurodegenerative disease that most often affects women of reproductive and working age. In this study, women were more represented in both MS groups (70.5% and 82%, respectively), which is consistent with previous findings (4, 32). The majority of patients had the relapsing-remitting form of MS (82%), in line with the distribution reported in other studies (32, 33).

Cognitive impairments were identified in 50% of patients in Group I and 42% in Group II, which corresponds to the prevalence range of 40–70% reported in the literature (10, 11, 13, 34). These impairments mainly affected memory, attention, and executive functions, especially in patients with longer disease duration. Intellectual abilities and executive functions remained relatively preserved in the early stage, which supports previous findings (35) that executive deficits are less frequent in newly diagnosed patients.

Visuospatial and visuoperceptual functions were more impaired in Group I, while attention and short-term memory showed slightly worse outcomes in Group II. These results align with the study (36), which noted early difficulties in attention, concentration, and processing speed among MS patients. As multiple sclerosis progresses, memory difficulties tend to worsen. In the early stages, individuals often experience problems with retrieving information, while later stages are more commonly associated with challenges in acquiring new information (37). It was found that longer disease duration is linked to more severe impairments in verbal episodic memory and logical thinking (33, 34).

These findings support the observation in our study that verbal learning and memory are more significantly affected in patients with longer disease duration.

Our results showed the quality of life (QoL) of MS patients was significantly reduced across all measured dimensions and strongly correlated with both MMSE and cognitive test results; physical and mental dimensions of QoL showed statistically significant associations with most neuropsychological scores, especially in patients with more advanced disease. This relationship has been confirmed in previous studies emphasizing the link between cognitive status and social, emotional, and functional outcomes (34). Cognitive difficulties reported by patients with multiple sclerosis are strongly linked to poorer physical and mental quality of life, as well as a decreased likelihood of being employed, even when factors like age, fatigue, depression, and disability level are taken into account (38).

It is important to note that executive functions and non-verbal intelligence did not show a significant correlation with quality of life in the early stages of our study, suggesting that preserved higher-order functions may mask deeper cognitive decline. This finding aligns with longitudinal studies that have examined cognitive functions in MS patients and their association with quality of life, emphasizing that even mild cognitive deficits can substantially impact daily living, even among patients with minimal physical disability (35).

The limitations of this study include the absence of standardized neuropsychological protocols specifically designed for MS patients. The tests used were time-consuming and required

specialized examiner skills. Future studies should incorporate validated cognitive assessment tools tailored to MS populations and include larger samples to increase generalizability.

In conclusion, this study confirms that cognitive impairments are prevalent in MS and significantly reduce patients' quality of life. Our longitudinal comparison revealed that early cognitive decline primarily involves attention, working memory, and short-term verbal memory, whereas more advanced stages show greater impairments in verbal learning, logical memory, visuospatial abilities, and long-term memory.

The originality of this study lies in its longitudinal design, involving the assessment of cognitive functions at both early and advanced stages of the disease, in comparison with a control group of healthy individuals. Furthermore, the study introduces an innovative approach by integrating a broad range of validated neuropsychological tests along with the SF-36 questionnaire for quality of life assessment, enabling a multidimensional analysis of the impact of cognitive impairments on patients' functional status. Additionally, this research contributes to the existing body of literature by quantifying the relationship between cognitive status and subjectively perceived physical and mental health as an essential component in planning comprehensive care for patients with MS.

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TRANSPARENCY DECLARATION

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ORIGINAL ARTICLE

Ambient temperature and ruptured abdominal aortic aneurism: a retrospective study from Bosnia and Herzegovina

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ABSTRACT

Aim Ruptured abdominal aortic aneurysm (RAAA) is a life-threatening emergency with high mortality. While conventional risk factors are well recognized, emerging evidence suggests environmental temperature may also influence rupture risk. This relationship has not been studied in Bosnia and Herzegovina. The aim of this study is to investigate the association between ambient temperature and RAAA prevalence.
Methods A retrospective observational study was conducted at the Clinical Center of the University of Sarajevo between January 2021 and February 2025. Data from 105 RAAA patients were analyzed using demographic, clinical, and temperature data, with time series analysis assessing patterns around rupture events.
Results The mean patient age was 71.5±7.6 years; 91 (86.7%) were male. The average aneurysm diameter was 85.1±17.7 mm. Hypertension (68.6%), smoking (55.2%), and diabetes (37.7%) were the most prevalent comorbidities. The mortality rate was 38.7% (40 patients). Most ruptures occurred during colder months, with a peak in January (16.1%) and a low in August, March, and February (each 4.7%). The mean ambient temperature during the 10 days before rupture was 11.41± 6.16 °C, not significantly different from the temperature on the rupture day (p=0.991). However, minimum daily temperature was significantly lower than the mean daily temperature on rupture days (6.48±5.92 °C vs. 11.42±17.61 °C; $p < 0.001$).
Conclusion A seasonal RAAA pattern with winter clustering was observed, but no consistent short-term link to ambient temperature was found, warranting further study with advanced models.
Keywords: abdominal aortic aneurysm, aortic rupture, cardiovascular diseases, temperature, seasons

INTRODUCTION

Abdominal aortic aneurysm rupture (RAAA) represents a life-threatening vascular emergency with high mortality rates, often ranging between 80% and 90% (1). While established risk factors such as age, male gender, smoking, hypertension, and aneurysm diameter are well-documented, emerging evidence suggests that environmental factors, particularly ambient temperature, may also influence the risk of rupture (2). Studies from various geographic regions have indicated seasonal and temperature-related variations in the prevalence of AAA rupture, with peaks observed during colder months or periods of sudden temperature fluctuations (3-5).
The underlying mechanisms by which temperature may influence aneurysmal rupture remain poorly understood but are hypothesized to involve blood pressure variability, vasoconstriction, increased sympathetic activity, and inflammatory responses triggered by thermal stress (5-7). In colder temperatures, the combination of elevated systemic vascular resistance and increased arterial wall stress may contribute to a higher risk of aneurysm rupture, particularly in vulnerable patients (6,7).
Despite growing interest in the role of environmental factors in cardiovascular emergencies, the association between ambient temperature and RAAA remains insufficiently explored in many regions. No comprehensive studies to date have addressed this relationship within Bosnia and Herzegovina. Improved understanding of temperature-related influences on rupture risk may enhance clinical risk stratification and support more effective allocation of healthcare resources—particularly in settings with limited access to specialized vascular care and emergency surgical services.
The aim of this study was to investigate the relationship between ambient temperature and the prevalence of RAAA in Bosnia and Herzegovina, using retrospective clinical data and meteorological records. By identifying temperature-related

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patterns, we seek to contribute to the broader understanding of environmental determinants in cardiovascular emergencies and offer insights for improved patient care and seasonal preparedness.

PATIENTS AND METHODS

Patients and study design

This retrospective, observational study conducted to evaluate the association between ambient temperature and the prevalence of RAAA in Bosnia and Herzegovina at the Department for Cardiovascular surgery of the Clinical Center of the University of Sarajevo between January 2021 and February 2025. Inclusion criteria were: patients aged 18 years or older, and patients with a confirmed diagnosis of RAAA established through computed tomography. Exclusion criteria included traumatic aortic injury, thoracic aortic aneurysms, and incomplete clinical or admission data.

Methods

Patient data were retrospectively extracted from hospital medical records and included demographic information (age, gender), relevant cardiovascular risk factors and comorbidities such as arterial hypertension, coronary artery disease, diabetes mellitus, chronic kidney disease, and active or former smoking status. In addition, data on the clinical presentation, hemodynamic status on admission, date and time of hospital admission, CT imaging findings, surgical intervention (if applicable), and in-hospital outcome (including mortality) were collected.
The date of RAAA was defined as the documented date of acute symptom onset or the date of emergency medical presentation, depending on the available clinical documentation.
Meteorological data were obtained from the official records of the Federal Hydrometeorological Institute of Bosnia and Herzegovina. For the duration of the study period, daily meteorological variables including mean, minimum, and maximum ambient temperatures were recorded for each calendar day. For every included patient, the corresponding temperature values on the date of rupture were extracted. To assess potential delayed or cumulative effects of ambient temperature on aneurysm rupture risk, lagged temperature exposures from 1 to 10 days prior to the event were also analyzed. Temperature data were matched by geographic location of the hospital or patient residence, where available, to ensure regional accuracy.

Statistical analysis

Descriptive statistics were used to summarize patient demographics and clinical characteristics. Categorical variables were expressed as frequencies and percentages, and continuous variables as mean ±standard deviation (SD) or median with interquartile range, depending on distribution. Normality of the data was evaluated using Kolmogorov-Smirnov tests, and the t-tests were applied only when the assumption of normal distribution was satisfied. The association between ambient temperature and RAAA prevalence was evaluated using time-series analysis and Poisson regression models, adjusted for day of the week, seasonality, and long-term trends. Additional subgroup analyses were performed based on age, gender, and comorbidities. A $p<0.05$ was considered statistically significant.

RESULTS

A total of 105 patients were included in the study. The cohort was predominantly male, 91 (86.7%), and the majority were residents of Sarajevo, 62 (59.0%). Arterial hypertension was the most common comorbidity, present in 72 (68.6%), followed by diabetes mellitus in 40 (37.7%) and chronic kidney disease in 23 (21.9%) patients. The mean age of patients was 71.5±7.6 years, with an age range of 54 to 88 years. The mean maximum diameter of the abdominal aortic aneurysms was 85.1±17.7 mm, ranging from 50 mm to 137 mm (Table 1).

Table 1. Demographic, clinical characteristics, postoperative complications and surgery outcome of patients with ruptured abdominal aorta (RAAA) in the period 2022-2024

Variable		**Mean±SD**
Age (years)		71.5±7.6
		No (%) of patients
Gender	Male	91 (86.7)
	Female	14 (13.3)
Place of residence	Sarajevo	62 (59.0)
	Zenica	19 (18.1)
	Mostar	2 (1.9)
	Travnik	23 (21.0)
Comorbidities	Hypertension	72 (68.6)
	DM	40 (37.7)
	CKD	23 (21.9)
	Smoking	58 (55.2)
	Dyslipidemia	33 (31.4)
	CAD	38 (36.1)
Onset of symptoms of RAAA	< 6 hours	50 (47.6)
	>6 hours	55 (52.4)
Postoperative complications		47 (44.3)
Surgery outcome	Recovered	65 (61.3)
	Death	40 (38.7)
		Mean±SD
Abdominal aneursym diameter (mm)		85.1±17.7

DM, diabetes mellitus; CKD, chronic kidney disease; CAD, coronary artery disease;

At the time of presentation, 50 (47.6%) patients reported symptom onset within less than six hours, while 55 (52.4%) presented more than six hours after the onset of symptoms. Postoperative complications were documented in 47 (44.3%), whereas 58 (55.7%) patients had no reported complications. Regarding surgical outcome, 65 (61.3%) patients survived and were discharged in stable condition, while 40 (38.7%) experienced death.
The highest rate of ruptures was in January, 17 (16.1%) and the lowest in February, March and August, 5 (4.7%). The rate was highest in autumn and winter and (September-February), and lowest in summer (June-August) (Figure 1).
According to the data obtained from the national meteorological registry, the mean ambient temperature during the overall observation period was 11.42±8.08 °C, with recorded values ranging from –0.3 °C to 29.8 °C. The mean temperature during the 10 days preceding aneurysm rupture was 11.41±6.16 °C (range: 1.76–25.8 °C). No statistically significant difference

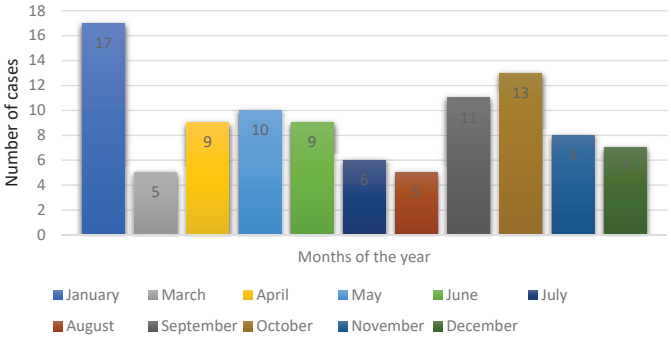

Figure 1. Monthly number of ruptured abdominal aorta (RAAA) (N=105)

between the mean temperature during the 10-day pre-rupture period (p=0.991) compared to the rupture day. However, when evaluating the temperature characteristics specifically on the day of rupture, a statistically significant difference was observed between the minimum daily temperature (6.48±5.92 °C) and the corresponding daily mean temperature (11.42±17.61 °C) (p<0.0019). In contrast, no significant difference was found between the mean monthly temperature (10.91±7.20 °C) and the daily mean temperature on the day of rupture (11.42±17.61 °C; p = 0.342). (Table 2)

Table 2. Ambient temperature parameters and comparative analysis related to aneurysm rupture timing

Temperature parameter	Mean±SD (°C)	Range (°C)	Comparison	p
Overall observation period	11.42±8.08	-0.3 - 29.8	.	-
10 days preeceding aneursym rupture	11.41±6.16	1.76 - 25.8	Day of rupture vs. 10-day pre-rupture mean	0.991
Day of rupture – minimum daily temperature on the day of rupture	6.48±5.92	–0.1 - 19.3	Minimum daily vs. daily mean temperature	<0.001
Day of rupture – daily mean temperature	11.42±8.08	0.5 - 27.6	Daily mean vs. mean monthly temperature	0.342

DISCUSSION

This study examined the clinical characteristics, seasonal distribution, and potential environmental influences on RAAA in a patient cohort from Bosnia and Herzegovina. The findings confirm well-established demographic and clinical patterns, while also contributing region-specific insights to the ongoing debate regarding the impact of ambient temperature on vascular emergencies.

Consistent with global data, the majority of patients in this study were older males, reflecting the recognized demographic profile for AAA development and rupture (8,9). The predominance of arterial hypertension and smoking as comorbidities aligns with international research, which consistently identifies these factors as key contributors to aneurysmal disease (10,11). Although diabetes mellitus was also present in a substantial proportion of patients, its role in aneurysm pathophysiology remains complex, with some studies suggesting a protective effect against rupture (12).

The mortality rate associated with RAAA in this cohort remained high, echoing outcomes reported in emergency vascular surgery worldwide (9,10). The challenge of timely diagnosis and surgical intervention, particularly in regions with limited vascular surgery infrastructure, may partly explain the observed outcome. Similar trends have been observed in other low- and middle-income countries, where delayed presentation and transfer times remain significant barriers to survival (13-15).

Seasonal variation in RAAA prevalence was evident, with clustering of cases in colder months, particularly during winter. This pattern has been reported in several international studies, including those from Europe and Asia, which have hypothesized that colder temperatures may act as a physiological stressor by increasing sympathetic tone and arterial pressure, thereby precipitating rupture in vulnerable aneurysms (16-19). While some researchers have suggested a clear winter peak, others have observed more variable patterns, possibly influenced by regional climate and healthcare access (19).

With respect to ambient temperature, this study did not identify a statistically significant relationship between the mean temperature on the day of rupture and in the days preceding the event. This finding is in contrast to studies (3-5) from countries with more pronounced seasonal shifts, where associations between cold weather and increased RAAA prevalence have been reported. Our findings are in line with those from Germany (20), where no significant association between ambient temperature or atmospheric pressure and the incidence of RAAA in a Mid-European population was reported. Similarly, we found no statistically significant differences between the mean temperature on the day of rupture and the preceding days. This convergence of findings across different European settings suggests that, in certain temperate regions, short-term fluctuations in temperature may not exert a strong or direct effect on aneurysm rupture risk (18-20). Some studies may reflect shared methodological constraints, particularly the limited sample size and reliance on basic statistical comparisons, such as t-tests, which may lack the sensitivity to detect subtle or delayed environmental effects (19,20). Also it was found that environmental variables such as temperature and pressure do not significantly influence RAAA prevalence in Germany, supporting the idea that local climate characteristics or patient profiles might mediate environmental impacts (20). While other international studies (3-5) utilizing time-series and distributed lag non-linear models have demonstrated associations between cold weather and increased rupture risk, especially in regions with more extreme seasonal variation, our results and those from Mid-European Region (20) emphasize the need for region-specific analyses and advanced modeling techniques. Future research incorporating larger multicenter cohorts and sophisticated temporal models may help clarify whether the lack of association in our and a study from Mid-European Region (20) reflects true absence of effect or insufficient analytical power.

A limitation of the study was that it examined only temperature as a factor. Future research should also consider other environmental elements, such as noise level and access to green spaces, which may affect heart and blood vessel health.

In conclusion, while this study supports known demographic and clinical characteristics of RAAA and suggests a possible seasonal trend, it does not provide conclusive evidence of temperature as a short-term trigger. Nonetheless, it offers valuable baseline data for Bosnia and Herzegovina and underscores the need for larger, multi-center studies utilizing advanced epidemiological methods to better understand environmental influ-

ences on vascular emergencies. These findings could inform future risk prediction models and support seasonal planning in vascular surgery services.

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TRANSPARENCY DECLARATION

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ORIGINAL ARTICLE

Comparison of inflammatory parameter levels after sclerotherapy of varicose veins with polidocanol

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ABSTRACT

A**im** To determine changes in inflammatory parameter values after ultrasound-guided foam sclerotherapy (UGFS) of varicose veins. The values of inflammatory parameters taken immediately after treatment and seven days after treatment were compared.

M**ethods** A total of 41 patients with verified varicose vein disease were included. A total of 82 samples were taken from the cubital vein and-C-reactive protein, D-dimer, fibrinogen and leukocytes were analysed.

Results Significant differences were observed in D-dimer values. Significant changes were recorded in D-dimer levels, which showed significant increases 7 days after treatment. D-dimer levels increased from an average of 287.3 ng/mL to 350.9 ng/mL, which is a statistically significant change with a p-value of 0.04. The difference was not statistically significant for other parameters.

Conclusion D-dimers have been shown to be a specific inflammatory parameter in vein sclerotherapy. Increased values of D-dimers did not affect blood hypercoagulability. Sclerosing agents are effective for seven days after treatment, and recovery should be adjusted to this finding. Although an increase in D-dimer values seven days after treatment has been proven, vein sclerotherapy treatment remains a safe, extremely effective and reliable method of treating varicose veins, and will have an increasingly wide usage in the future.

Keywords: C- reactive protein, D-dimer, fibrinogen, UGFS

INTRODUCTION

Varicose veins are as old as Hippocrates (1). The veins of the legs become dilated and of blue colour due to excessive accumulation of blood. This disease and the effort to identify and treat it has a very long history (2).

In the late 1970s, the German pharmaceutical company BASF (Badische Anilin & Sodafabrik), which produced polidocanol, abandoned its use as an anaesthetic because it was found to cause venous thrombosis. And so, by chance, it turned out that this substance could be a good sclerosant. Otto Henschel, who was the scientific director of Kreussler Pharma in Germany at the time, became interested in this substance, and in 1963 Dr. Peter Lunkenheimer was the first to use polidocanol in his patients (3,4).

Polidocanol (Laureth-9 or Lauromacrogol 400) is a synthetic fatty alcohol (alkyl polyglycol ether lauryl alcohol). Chemically, it can be represented as a polymer belonging to the class of alcohols and glycols (5). After intralesional administration, polidocanol induces endothelial cell injury by interfering with calcium signalling and nitric oxide pathways. After endothelial damage, platelets aggregate at the site of injury and attach to the venous wall, resulting in a dense network of platelets, cellular debris, and fibrin that occludes the vessel (6). Polidocanol acts as a detergent, forming aggregations of molecules in the form of micelles. The micelles then interact with the endothelial cell membrane and disrupt the cells by solubilizing essential molecules from the membrane surface. Therefore, when injected intravenously, lauromacrogol 400 (polidocanol) destroys the wall of the blood vessels of the affected varicose veins and thus permanently clogs them (7). Polidocanol induces at first endothelial damage, which causes platelets to aggregate at the site of damage and attach to the vein wall. Then, a dense network of platelets, cellular debris, and fibrin occludes the vessel (8).

The mechanism of sclerotherapy involves the destruction of the venous endothelium of the blood vessel, exposure of the basal layer of collagen to the sclerosing agent, induction of vasospasm and, ultimately, complete fibrosis of the blood vessels. With ultrasound monitoring, sclerosants have been observed to enter the deep venous system via perforators or junctions; but, the clinical incidence of deep vein thrombosis or pulmonary embolism after sclerotherapy is very low (9).

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The use of sclerosing agents as foam has been shown to be more effective and requires lower concentration then the liquid sclerosing agent. The use of foam reduces the amount of agent, maximizes the sclerosing effect by increasing the surface area of contact with the varicose vein walls, and prevents intravascular bleeding (10). Foam sclerotherapy has been reported to have a success rate of 85–95% compared with 35% for liquid in eliminating reflux in large cutaneous veins. Similarly, histological studies have shown similar effects of sclerosing agents at concentrations of 0.5% as a foam and 3% as a liquid (11).
This procedure has been widely used in the last thirty years, while in Bosnia and Herzegovina the procedure expansion started 15 years ago. Our institution has been performing it for the last 15 years and has treated over 3,000 patients (12).
So far, a large number of studies have been conducted measuring laboratory parameters that affect coagulability and inflammation (13,14). However, there are no studies examining sclerotherapy and the selected parameters, such as D-dimers, fibrinogen and thrombocytes.
The aim of this study was to investigate specific inflammatory parameters that may be indicative of inflammatory conditions of the veins and could contribute to future research and clinical practice.

PATIENTS AND METHODS

Patients and study design

The study was designed as a prospective, observational, interventional clinically controlled study. It was conducted at the Cantonal Hospital Zenica and competent laboratories. The study included patients who underwent minimally invasive treatment of varicose veins by ultrasound guided foam sclerotherapy (UGFS) during the period January 2023 to April 2024. A total of 41 patients were included in the study (82 samples in total).
The inclusion criteria were: patients older than 18 and younger than 65 years of age, patients with clinically and ultrasound-verified varicose veins of the lower extremities, disease stage from Ido IV according to the Clinical, Etiological, Anatomical and Pathophysiological (CEAP) classification (4).
The exclusion criteria were patients younger than 18 and older than 65 years, disease stage according to CEAP classification 0, V and VI, previous deep vein thrombosis and pulmonary thromboembolism, chronic diseases: myocardial infarction, degenerative diseases of blood vessels, patients who did not consent to participate in the trial, patients who independently withdrew from research, patients who did not have all the planned parameters taken, peripheral arterial ischemia, pregnancy, disability. Patients with varicose veins stage V and VI were excluded, as the disease is developed and advanced with additional complications such as venous ulcers, which can affect the values of the measured parameters being tested. Also, chronic venous thrombosis and thromboembolic incidents often give false positive laboratory values, and such patients were excluded from the study.
Each patient gave written consent for the study and was informed in detail about all protocols and procedures of this study. Patients under the age of 18 could not give their consent, and according to the pathophysiology of venous diseases, they could not be part of this study. Patients older than 65 are often burdened with additional comorbidities, which could affect the results of the study.
The Ethics Committee of the Cantonal Hospital Zenica gave its approval for this study.

Methods

The study was conducted over a period of fourteen months. During the study, all patients had a minimum of two visits to the doctor, initial and control treatment, and two visits to the laboratory. The first visit was during the procedure and vein treatment itself, then they were referred to the laboratory, and the same procedure was repeated after 7-10 days.
After the physical examination, each patient underwent an ultrasound (Color Doppler) examination of the blood vessels of the lower extremities (Biosound esoate MyLab 25 Xvision probe 5-10Hz, Genoa/ Italy). The examination of the arterial and deep venous system was performed in the supine position, while the superficial venous system was performed in the standing position. Measurements were performed to assess the sufficiency of the saphenofemoral junction and the small saphenous vein.
Immediately before the treatment, patients were administered low-molecular-weight heparin at a dose determined according to body weight (15).
Sclerotherapy was performed with patients in the standing, sitting and lying positions.
After the preparation, the treatment protocol for each patient was standardized. The sclerosant Polidocanol (Aetoksisclerol®, Laboratoires Kreussler, Paris, France) was used.
Sclerosing foam was obtained using a sterile disposable syringe set (20 mL two-piece, three-way stopcock syringe connector). The mixture of sclerosing fluid and room air was 1+4 (one volume of sclerosing agent + four volumes of air; i.e., a ratio of 1/5). Foam preparation and venipuncture were performed according to the Tessari method (16).
Sclerotherapy was guided by ultrasound and performed by direct needle puncture (22 Gauge, length 40 mm, Terumo® Leuven; Belgium), with the patient in the supine and standing positions. The sclerosing agent was applied as a foam. Immediately after the treatment for 10-15 minutes, the patient had blood drawn from the cubital vein by venipuncture for laboratory analysis as follows: complete blood count (CBC) – leukocytes (reference value 4-10x10^9/L) (Sysmex xn-350, Kobe/ Japan), C reactive protein (CRP) (reference value<5 mg/dL) (Olympus AU400, Nagano/Japan), fibrinogen (reference value 1.9-4.3 g/L) (Stago Gennevilliers/France), D-dimer (reference value 50-500 ng/mL) (Afias 1, Gangneung/South Korea).
A follow-up examination and monitoring were performed 7-10 days after the treatment. At that time, a new re-evaluation and examination of the patient were performed. For a more detailed analysis, the patients were statistically processed and divided according to the concentration of the sclerosing agent with which they were treated. The patients were treated with five different concentrations of polidicanol: three patients with 1%, seven with 1.5%, 21 with 2%, six with 2.5% and four patients with 3% polidicanol.

Statistical analysis

Numerical variables were presented as arithmetic means and standard deviations (SD), and as medians, interquartile ranges (IR Q1–Q3), and ranges (min-max), if they did not follow normal distribution. Quantitative variables were tested for normality using the Shapiro-Wilk test and evaluated with QQ-plots and

histograms. Ordinal and categorical variables were described using medians and interquartile ranges, or, as discrete values, presented with frequency distributions. For dependent measurements, a paired t-test was applied if the assumptions for its use were met. The statistical significance level was set at <0.05.
We presented our results in tables and graphs, and stated which p values and which hypothesis test were used. The confidence interval of 95% was used in the results.

RESULTS

Among the 41 patients, 14 (34%) were male and 27 (66%) were female. The mean age of the patients in the total sample was 42±13 years. The median age was 42 years (IR= 32 and 49) years. Analysis of inflammatory markers in the patients treated with polidocanol between the time points immediately after the treatment and seven days after the treatment showed the difference. The most prominent findings were the significant changes in D-dimer level, which showed a significant increase 7 days after treatment. Mean value of D-dimer levels increased from 287.3 ng/mL to 350.9 ng/mL (p=0.04) (Table 1 and figure1). Also, for one patient D-dimer value of 1.278.0 ng/mL was recorded 7 days after the treatment.
We performed a more detailed statistical analysis of different concentrations of polidocanol in order to check whether a particular concentration of polidocanol had a significant effect on the results. The results showed no statistically significant difference in D-dimer values according to different concentrations of sclerosing agents (Figure 2).

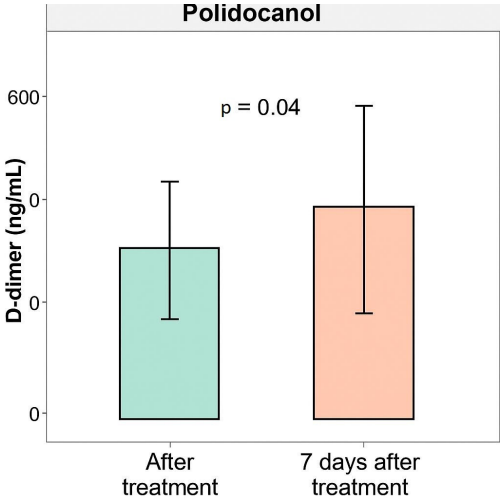

Figure 1. D-dimer values after treatment and 7 days after treatment

Concentration of other markers, including C-reactive protein (CRP), fibrinogen, and leukocytes, did not show statistically significant differences during this post-operative period. CRP level showed a slight decrease from an average of 2.5 mg/dL to 2.0 mg/dL. Similarly, fibrinogen level decreased slightly from an average of 3.7 g/L to 3.6 g/L, leukocytes decreased from 6.4 x 109/L to 6.2 x 109/L (Table 1).

Table 1. Values of laboratory measurements immediately after the treatment and 7 days after the treatment

Variable (reference value)	Sample timing		Difference*	95% CI*,†	P*
	After treatment	7 days after treatment			
C-reactive protein (<5) (mg/dL)			0.51	-0.23; 1.3	0.2
Mean (±SD)	2.5 (±2.7)	2.0 (±1.9)			
Median (Q 1–Q3)	1.5 (0.8–2.4)	1.3 (0.9–2.6)			
Range (Min–Max)	0.4–12.1	0.2–11.0			
D-dimer (50-500) (ng/mL)			-64	-124; -3.2	0.040
Mean (±SD)	287.3 (±129.3)	350.9 (±200.7)			
Median (Q1–Q3)	245.0 (188.0–345.0)	314.0 (243.0–439.0)			
Range (Min–Max)	151.2–722.0	127.0–1.278.0			
Fibrinogen (1.9-4.3) (g/L)			0.12	-0.12; 0.35	0.3
Mean (±SD)	3.7 (±0.7)	3.6 (±0.7)			
Median (Q1–Q3)	3.7 (3.3–4.2)	3.6 (3.2–4.0)			
Range (Min–Max)	2.0–4.8	2.1–5.6			
Leukocytes (4-10) (10^9/L)			0.23	-0.26; 0.71	0.4
Mean (±SD)	6.4 (±1.3)	6.2 (±1.5)			
Median (Q1–Q3)	6.2 (5.6–7.0)	6.3 (5.0–7.0)			
Range (Min–Max)	4.0–9.2	3.6–10.1			

*refers to the Difference, p and 95% CI (Confidence interval) columns;
† indicates the 95% CI that refers to where the two are in the table

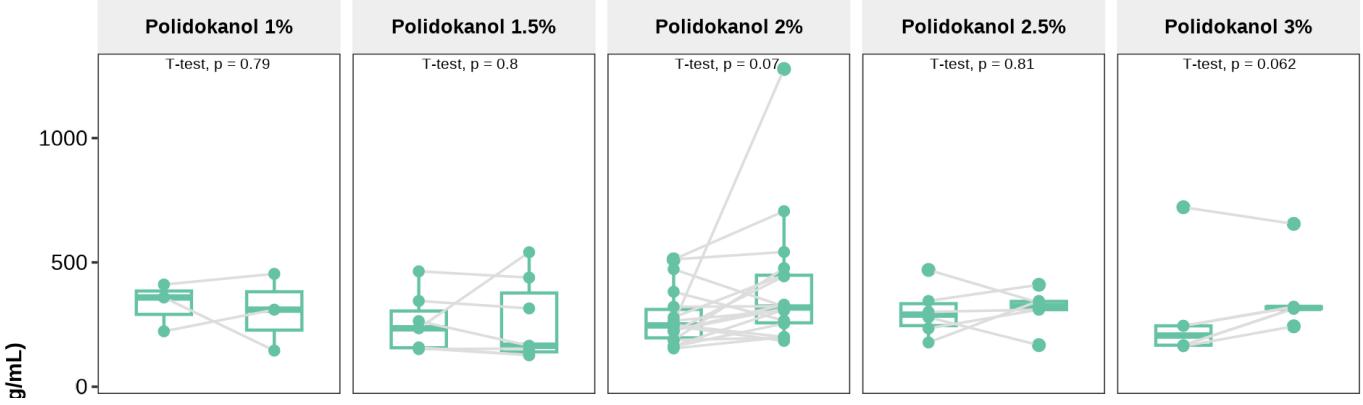

Figure 2. D-dimer values immediately after and 7 days after treatment, according to different concentrations of sclerosing agents

There was no statistically significant difference in CRP values right after the treatment and seven days after the treatment. The mean value decreased by 0.51 (±2.4) mg/dL (Table 1).
No statistically significant difference was found in fibrinogen values after the treatment and 7 days after the treatment. The average value decreased by 0.12 (±0.7) g/L. No statistically significant difference was found in leukocytes values after the treatment and 7 days after the treatment. The average value decreased by 0.23 (±1.5) × 10^9/L (Table 1).

DISCUSSION

Nowadays, there is a large number of non-invasive methods of the varicose vein treatment that have proven to be very effective and reliable (17). Today, detergent-based sclerosing agents have gained the greatest popularity. Polidocanol (lauromacragol) and sodium tetradecyl sulfate are the two sclerosing agents that are most common and most widely used due to their effectiveness, non-toxicity, and affordable price (18).
In our results, among patients treated with polidocanol, biohumoral response showed difference, both immediately after the treatment and at the second measurement seven days after the treatment. The most significant changes were in D-dimer, where measurements after seven days were significantly increased. D-dimer level in patients treated with polidocanol was also increased. Our results are in line with a large number of other studies that have confirmed the elevation of D-dimer levels after sclerotherapy with polidocanol or sodium tetradecyl sulfate (STS) (19,20).
D-dimer values showed a significant difference in measurements immediately after the treatment and seven days after the treatment in patients treated with polidocanol. Previous studies on D-dimers have consistently confirmed that differences between measurements exist, primarily due to procoagulant activity; however, these differences are insufficient to cause harmful effects or disrupt the overall function of the coagulation system during or after venous system treatment (21).
C-reactive protein CRP and fibrinogen are acute phase proteins that increase as nonspecific inflammatory markers. In the literature, elevated values of D-dimer and CRP parameters can be found in patients with varicose veins and some other comorbidities, such as hypertension (22). Since these parameters are nonspecific, it is very difficult to analyse and isolate their response to an exact and specific change occurring in the body. The reason for the increase in values may be a damaged wall and destroyed endothelium, which enhance procoagulant activity in the blood, and thus activate processes that increase the value of the aforementioned parameters (17,23). Recent research shows that CRP values are slightly elevated in patients with varicose veins and in patients who have undergone minimally invasive treatments. Changes are minimal and negligible in medical practice (23).
C-reactive protein did not show a statistically significant difference between measurements taken immediately after treatment and seven days later. It is interesting that the average value after 7 days was lower. Reportedly, no significant difference in CRP levels immediately and 7 days after polidocanol treatment was found (24,25).
Fibrinogen levels were slightly changed but without statistically significant difference after treatment and seven days later. Fibrinogen is one of the main parameters in the procoagulant activity of blood and its values can be changed under the influence of various parameters. Even varicose veins themselves can cause an elevation of blood fibrinogen level (27). Changes in fibrinogen level in our study were minimal. A large number of studies also showed minimal non-significant differences in fibrinogen levels (26,27).
The results of the analysis of formed blood elements were without significant difference in values. Most similar studies state that sclerosing agents do not have a significant effect on formed blood elements (28,29).
A limitation of this study is the relatively small number of patients and samples, although it was sufficient to achieve moderate statistical power; additionally, the study was conducted at a single centre. Also, data obtained after the first follow up were insufficient for some patients. The majority of patients had one-month and three-month follow-up. We did not show all clinical outcomes and stratification of patients because they did not have an impact on the results.
Polidocanol is a safe sclerosant and does not cause severe complications such as thromboembolic effects nor does it provide a biohumoral response related to the onset of thrombotic incidents. It is important to choose an adequate sclerosant depending on the diameter of the vein. For total endothelial dysfunction, a minimum concentration of sclerosant of 0.5% is required. Sclerosant agents are effective for 7 days after treatment, so recovery should be adjusted to this knowledge (compression therapy, repeated treatment, etc.). For optimal outcomes, it is important that the patient is adequately prepared, the appropriate vein is selected, the correct concentration is used, and the patient is properly monitored with diagnostic and laboratory tests.
Sclerotherapy is an extremely effective and reliable method for treating varicose veins and is expected to see increasingly widespread use.

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TRANSPARENCY DECLARATION

Competing interests: None to declare.

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ORIGINAL ARTICLE

Impact of 3D printing on vascular surgery training and preoperative planning

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ABSTRACT

Aim To investigate the impact of three-dimensional (3D) printing technology on vascular surgery, focusing on its role in preoperative planning and surgical training.

Methods A systematic review was conducted of studies published between 2017 and 2024 that evaluated 3D printing in vascular surgery. Databases searched included MEDLINE and CENTRAL. Eligible studies reported applications of 3D printing in preoperative planning, simulation, or surgical education.

Results Analysis of relevant studies revealed that 3D-printed vascular models improved surgical precision, reduced fluoroscopy time, enhanced technical skill acquisition, and increased trainees' confidence. Models based on computed-tomography angiography data provided accurate, patient-specific anatomy for classic or endovascular aortic aneurysm repair and other vascular procedures.

Conclusion 3D printing significantly enhances vascular-surgery training and preoperative preparation by providing realistic, patient-specific simulations that improve both technical competence and surgical outcomes.

Keywords: education, medical, models, simulation, three-dimensional, training

INTRODUCTION

Three-dimensional (3D) printing has transformed modern medical practice, particularly in vascular surgery where precision is critical. Patient-specific anatomical models generated from imaging data enable surgeons to plan procedures with greater accuracy and provide trainees with realistic simulation tools. Recent evidence confirms that 3D printing improves procedural safety, efficiency, and educational outcomes.

Endovascular procedures have greatly improved vascular disease care, but maintaining surgeon proficiency is difficult due to frequent updates and costly simulators. 3D printing technology, which allows for affordable, customized, and complex model creation, is emerging as a valuable tool in medical training and surgical planning. By integrating 3D printing with imaging data, medical professionals can visualize complex anatomies and develop patient-specific models that enhance education and clinical outcomes (1).

Mixed reality improves vascular surgery training by providing immersive visualization of complex aortic anatomy, especially useful during COVID-19. A Chinese study compared its effectiveness to traditional methods in teaching residents about aortic diseases (2). Similarly, cardiac catheterization with central vein cannulation can pose risks such as thrombosis and infections due to multiple attempts or improper placements (3). A cost-effective cardiovascular simulator was developed to improve training and device testing for cardiac procedures, replicating realistic heart anatomy and blood flow. It serves as a valuable tool for practicing interventions like catheterization and TAVR, enhancing preclinical evaluation. The system addresses limitations of commercial simulators by being more affordable and comprehensive for educational and development purposes (4).

A 3D-printed model was successfully developed and used for endovascular simulation, with development stages and applications (5). Patient-specific 3D models aid in planning intracranial aneurysm treatment and enhance residents' understanding of vascular anatomy and procedures (6).

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Transparent 3D models of aortic disease, created from anonymized CT-angiography images using Vat-photopolymerization, showed that these models are feasible as teaching tools. Preliminary data indicate they help improve understanding of aortic disease among surgical trainees (7). 3D-printed tissue constructs, incorporating stem and endothelial cells with appropriate bioinks and printing methods, offer a less invasive treatment option. Proper selection of bioink and printing techniques is essential for developing functional bioprinted tissues and promoting vascularization (8).

The aim of this study was to analyse current literature to evaluate how 3D printing influences vascular-surgery training and preoperative planning.

MATERIALS AND METHODS

Materials and study design

Systematic review followed the PRISMA guidelines. Publications from 2017 to 2024 were screened. Eligibility criteria focused on studies that reported the application of 3D printing in vascular surgery and interventions. Articles exploring the use of 3D printing in vascular surgery, medical simulation, and training were included. Only studies published in English were considered. Conference abstracts, editorial commentaries, and review articles were excluded (Figure 1).

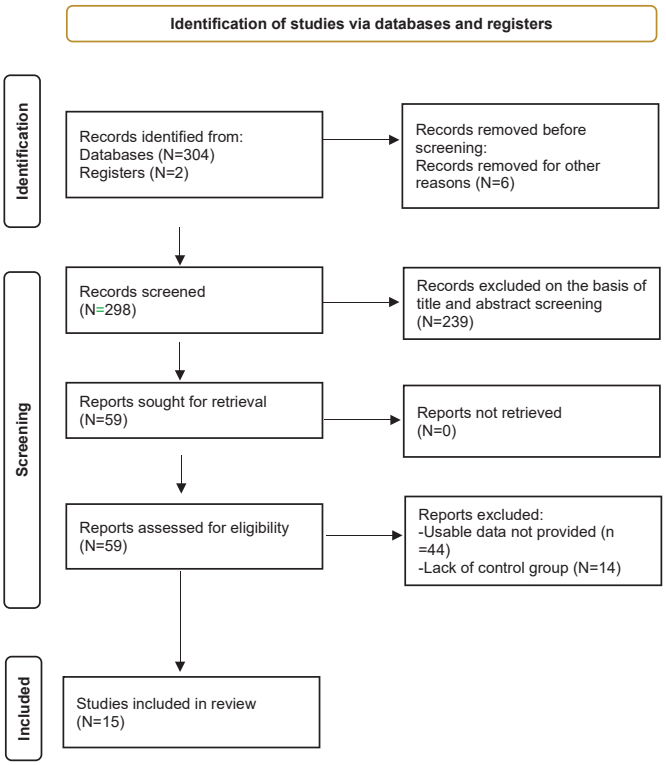

Figure 1. Preferred Reporting Items for Systematic Review (PRISMA)

Information sources and search strategy of literature published between 2017 and 2024 was conducted using various online databases, including MEDLINE (via PubMed) and the Cochrane Central Register of Controlled Trials (CENTRAL). Furthermore, reference lists from systematic reviews and other relevant articles were screened to identify additional information and research results. Additional sources, such as international clinical trial registries (9), were also reviewed. The search strategy incorporated terms such as "Vascular Surgery," "3D Printing," "Surgical Education," "Training Simulation," and "Anatomical Models." Data extraction was performed using a structured Excel spreadsheet, capturing essential information, including study characteristics (e.g., first author, publication year), primary research focus, and key findings.

Study selection and data extraction were systematically organized using Zotero 6.0.30 for efficient management (10). Titles and abstracts were screened to identify potentially relevant studies, followed by a comprehensive full-text review to confirm eligibility based on established inclusion criteria. Any disagreements between reviewers were resolved through discussion. Data extraction was performed using a structured Excel spreadsheet, capturing essential information, including study characteristics (e.g., first author, publication year), primary research focus, and key findings (Table 1).

Methods

Outcome measures. Two reviewers (article authors) independently evaluated each study, resolving disagreements through discussion, to ensure a thorough and unbiased assessment of evidence on 3D printing in vascular surgery training and preoperative planning. The aim of this study was to review surgical skill and competency, as measured by the Objective Structured Assessment of Technical Skills (OSATS) score (11).

Primary outcomes. a) Improvement in surgical skill competency. The primary aim of this study was to evaluate changes in surgical skill competency, measured using the Objective Structured Assessment of Technical Skills (OSATS), before and after training with 3D-printed models, in order to quantify enhancement in trainees' surgical skills. b) Improvement in preoperative planning accuracy. This was assessed by comparing the alignment of preoperative plans developed using 3D-printed models with the actual surgical outcomes, providing evidence for the practical value of these models in refining surgical precision.

Secondary outcomes. Composite technical metrics encompasses several technical improvements, including reduced operative time, fewer intraoperative errors, and enhanced accuracy during procedural steps. Together, these metrics provide a comprehensive evaluation of technical advancements facilitated by 3D printing in vascular surgery.

Eligibility for performing complex procedures were determined if a training with 3D-printed models enables trainees to qualify for more advanced vascular surgeries:

a) monitoring serious adverse events –it compared the prevalence of serious adverse events in surgeries planned with and without 3D -printed models;

b) prevalence of treatment-emergent adverse events arising specifically from the introduction of 3D-printed models into surgical planning and training was recorded and evaluated;

c) boost in trainee confidence levels among trainees was measured before and after training sessions involving 3D models using a Likert scale (12), highlighting the models' impact on psychological preparedness and self-assurance;

d) reduction in surgical completion time and effect of 3D preoperative planning on efficiency was examined by comparing the time required to complete surgeries before and after using 3D-printed models;

e) fluoroscopy time during endovascular procedures was evaluated before and after training with 3D models to determine

Table 1. Overview of studies regarding 3D printing in vascular surgery

Author	Year	Focus of study	Key findings
Foresti R., et al.	2024	3D Vascular modelling/ practice	A new 3D-printed modular system for endovascular training was developed using real patient data. In a small study, trainees using a stepwise difficulty approach completed sessions faster and reported high satisfaction, demonstrating that the system is an effective, customizable, and accessible simulation tool to improve training efficiency.
Raffaele A., et al.	2024	3D Vascular modelling/ practice	A 3D-printed arm model was created using CT scans, including bones and vessels, with latex dipping for veins and Material Jetting for arteries. The model also included a silicone skin mould. Experts rated it highly for realism and functionality, demonstrating its potential as a training tool for paediatric ultrasound-guided vascular procedures, despite some limitations in ultrasound appearance.
Li W., et al.	2023	Teaching tool for trainees	The study found that MR-assisted training combined with 3D-printed models improved residents' understanding of aortic anatomy and pathophysiology, boosted their confidence, and increased enthusiasm for learning about aortic diseases. Most residents rated the models as realistic and effective for training.
Mir A., et al.	2023	3D Vascular modelling/ practice	The study reviews methods for 3D bioprinting vascularized tissues, highlighting the importance of using appropriate bioinks, incorporating stem and endothelial cells, and selecting suitable printing techniques to improve tissue development and address current technical challenges.
Nguyen P., et al.	2023	3D Vascular modelling/ practice	The study found that 3D-printed patient-specific vascular models made with FDM, SLA, SLS, and PolyJet techniques are highly accurate, with minimal errors, making them suitable for clinical planning and training.
Rynio P., et al.	2022	3D Vascular modelling/ practice	This accuracy study evaluated 3D-printed templates of the visceral aorta and aortic arch against CT angiography images. The results showed high reliability and excellent agreement between measurements of aortic and branch diameters, with minimal bias. The models demonstrated very close geometric accuracy, indicating they are reliable tools. These findings support their use in endovascular and interventional radiology practices for guiding the fabrication of customized stent-grafts.
Magagna P., et al	2022	3D Vascular modelling/ practice	This pilot study demonstrated that 3D printing is a feasible and useful tool for preoperative planning, simulation, and patient communication in complex aortic and vascular surgeries. Further research is recommended.
Göçer H., et al.	2021	3D Vascular modelling/ practice	The study found that 3D-printed vascular models closely matched actual measurements and influenced procedural planning, leading to more accurate access site and device choices. Practicing with these models can improve success rates, reduce errors, prevent unnecessary procedures, and serve as valuable educational tools in vascular interventions.
Matyjas M., et al	2021	3D Vascular modelling/ practice	The 3D-printed embolization simulator was highly effective for training, improving beginners' confidence and reducing procedure times, making it a valuable educational tool.
Kiraly L., et al	2021	3D Cardio-Vascular modelling/ practice	This study demonstrates that 3D-printed models of the heart and vessels improve diagnosis, surgical planning, and safety in complex congenital cardiac surgeries. Preoperative rehearsals on these models led to modified operative plans and no increased complications, highlighting their value despite resource requirements. The partnership also suggests a potential for future bioprinted patient-specific implants.
Kärkkäinen JM., et al	2019	3D Vascular modelling/ practice	A 3D-printed AAA model was used to simulate EVAR procedures, demonstrating that experienced operators completed the procedure faster and more independently than less experienced trainees. The model effectively replicates all steps, making it useful for training and assessing endovascular skills in vascular surgery.
Spinelli D., et al	2019	3D Vascular modelling/ practice	3D-printed, disassemblable aortic models based on patient CT images were used to enhance training for 37 surgical trainees. Evaluation showed that demonstrating these models significantly improved trainees' understanding of aortic disease, indicating that 3D models are a feasible and effective teaching tool in surgical education.
Torres I., et al	2018	3D Vascular modelling/ practice	3D printing of vascular models enhances endovascular training by improving surgical planning and skill development, especially for complex procedures. Different methods are reviewed, and the process takes about a week, showing that these models are effective tools for surgical education and confidence.
Rotman OM., et al	2018	3D Vascular modelling/ practice	A new, cost-effective cardiovascular flow simulator was developed to train medical professionals and test devices in central vein catheterization. It features realistic anatomy, pulsatile blood flow, and variable arm positions, enabling catheter insertion and navigation. The simulator aims to improve training, reduce risks, and support device development before animal testing.
Mafeld S., et al	2017	3D Vascular modelling/ practice	The study demonstrated that creating and using a 3D-printed anatomically accurate human aorta for endovascular training is feasible. Feedback from 96 physicians indicates the models are valuable, but further validation is needed.
Park CK.	2017	3D Neuro-Vascular modelling/ practice	This review discusses how advancements in 3D printing technology have enabled the creation of precise, realistic neurosurgical disease models. These models improve spatial understanding, aiding in surgical planning, simulation, and training, and are now ready for routine clinical use.

the models' role in minimizing radiation exposure during endovascular procedures;
f) understanding of anatomical models change in trainees' comprehension of vascular anatomy was assessed through pre- and post- training tests, demonstrating the educational impact of 3D-printed models;
g) measuring the consistency and agreement among surgeons.

RESULTS

Clinical applications and benefits analysis reported significant enhancements in surgical performance among trainees who utilized 3D-printed aneurysm models, noting marked reductions in fluoroscopy time and contrast volume, which contributed to improved safety and efficiency (3,5).
The study involved participants aged 19-75 (median age 54), including 43 women and 33 men. The most common aneurysms were in the internal carotid artery (30%), anterior communicating artery (26%), middle cerebral artery (23%), and posterior circulation (21%). Without 3D printed models, the inter-observer agreement among Medical Doctors (MD1, MD2, MD3) was good, with kappa values of 0.703 (MD1 vs. MD2) and 0.64 (MD1 vs. MD3). After using 3D models, the agreement improved to very good levels: kappa values (k) increased to k=0.85 (MD1 vs. MD2), k=0.70 (MD1 vs. MD3), and k=0.72 (MD2 vs. MD3). The k measures the consistency among surgeons, with higher values indicating better agreement (6).
Six anonymized CT angiography cases of aortic disease were converted into reassemblable 3D models using vat photopolymerization. These models were shown to 37 surgical trainees after a seminar, and a validated questionnaire assessed their understanding before (T0) and after (T1) viewing the models. Participants (average age 28, mostly male) demonstrated a significant increase in understanding, from a median score of 7.25 at T0 to 8.00 at T1 (p=0.002). It emphasized the educational value of 3D-printed aortic models, which helped trainees better comprehend the intricate anatomy of aortic diseases, a critical aspect for precise surgical planning and execution (7).
Successful vascularization depended on integrating stem and endothelial cells, choosing appropriate bioinks, and selecting suitable printing methods (such as extrusion, inkjet, stereolithography, and laser-based techniques) for both scaffold and scaffold-free constructs. Scaffold materials include hydrogels and polymers, while scaffold-free methods use decellularized matrices, spheroids, and protein-based inks (8).
Additionally, it was found that 3D-printed models for arteriovenous malformation surgery not only facilitated more effective preoperative planning but also boosted surgeons' confidence, a vital factor for successful outcomes. Over 18 months, 3D printed models of arteriovenous malformations from CT angiography scans of six patients were created. These vascular models showed precise details of the nidus, vessels, and skull relationship, aiding in surgical planning. Despite clear visualization of vessels, differentiating arteries from veins by colour was a limitation. The models, placed beside surgeons during procedures, improved identification and surgical preparation (13).
Fluid pumps have been integrated to simulate a pulsatile circulatory system, creating a realistic environment for practicing techniques such as percutaneous transluminal angioplasty and ultrasound-guided femoral artery access. All trainees completed the task, with an average final simulation time of 16.24±8.05 minutes. The total training time, including non-evaluated sessions, was shorter for the SS group (23.13±9.2 minutes) compared to the random simulation (RS) group (44.6±12.8 minutes), with a significant difference (p=0.0075). In the standard simulation (SS) group, training times increased progressively over three sessions, while times in the RS group remained steady, indicating that the SS group's difficulty levels increased gradually. The second training session for the SS group used a stepwise difficulty 3D model, whereas the RS group's second session involved a random difficulty 3D model (14).
In several cases, simulations were conducted under fluoroscopy, providing realistic radiological guidance for the trainees. The simulator, designed with computer-aided tools and 3D printed, was used for embolization training. The trainees completed pre- and post- training questionnaires assessing their expertise and confidence levels. Participants watched an instructional video and performed four embolizations on the simulator. Five experts and twelve novices participated, with experts being experienced radiology residents and fellows, and novices being medical students and less experienced residents. Experts rated the simulator highly for embolization training. Performance times differed significantly between experts (189±42s) and novices (235±66s) with a p-value of .001. The simulation effectively replicated the embolization process, including complications, and enhanced educational outcomes. Notably, novices reported a significant boost in confidence (p=0.001) (15).
Simulating endovascular aortic replacement (EVAR) procedures using a 3D-printed aneurysm of aorta abdominalis (AAA) model connected to a fluid pump, two expert implanters and 20 vascular surgical trainees with different level of experience performed the procedures. Results showed that experienced trainees had significantly shorter total procedure times (32±9 minutes) and fluoroscopy times (13±5 minutes) compared to less experienced trainees (44±6 minutes and 23±8 minutes, respectively). All experts completed the procedure within 45 minutes and did so independently, whereas only 46% of the less experienced trainees achieved this, with only 15% completing the entire procedure independently. Benchmark implanters consistently outperformed trainees in nearly all EVAR metrics, highlighting their higher proficiency and the simulator's potential to differentiate skill levels effectively (16).
In the study that evaluated the dimensional accuracy of patient-specific vascular models created using various 3D printing technologies Fused-Deposition Modelling (FDM), Stereolithography (SLA), Selective Laser Sintering (SLS) and PolyJet 3D printing, models demonstrated a high level of dimensional accuracy, indicating they are suitable for clinical use in surgical planning and training. All methods produced high-quality models with overall errors ranging from approximately -0.72% to 0.53% compared to original CT angiography data. The digital segmentation process introduced a small average error of about -0.83%. Errors due to the printing process themselves were minimal, with models slightly underestimated on average (17).
The accuracy of 3D-printed models of the visceral aorta and aortic arch, compared to CT angiography images, showed measurements at key landmarks with excellent reliability, with intraclass correlation coefficients above 0.9. Bland-Altman analysis revealed minimal measurement biases, ranging from 0.05 to 0.47 for arch templates and 0.06 to 0.38 for abdominal templates. Hausdorff mean distances averaged 0.47 mm for the

aortic arch and 0.24 mm for the abdominal models, indicating high geometric accuracy suitable for clinical applications. Additionally, the ability to rapidly design and print patient-specific stents addresses delays associated with traditional manufacturing, which often takes 12–15 weeks (18).

In a study describing the reconstruction of an 8-year-old girl's left arm from CT scans—including bones, arteries, and veins—using semi-automatic segmentation, different 3D printing methods were tested. Latex dipping produced vessels that closely resembled real paediatric veins in echogenicity and flexibility, while arteries were printed using material jetting technology. An external mould was also printed to create soft tissues with silicone. Twenty experts evaluated the model, rating it highly realistic in morphology and function for simulation purposes, particularly for vessel and soft tissue response to puncture. However, its ultrasound appearance received lower scores (19).

The Vicenza "San Bortolo" Hospital and the University of Padova created 20 patients 3D-printed aortic models for education, aiding in planning, training, and patient communication. These models improved device customization, surgical accuracy, and surgeon confidence, especially in complex surgeries. They also provide realistic practice for young surgeons to develop open surgical skills outside the operating room. Although cadaveric and virtual simulations are useful, 3D models offer a more accessible and precise way to understand patient-specific anatomy, potentially reducing operative times and radiation exposure (20).

A retrospective analysis of 16 patients (avg. age 72) who underwent superficial femoral artery balloon angioplasty compared pre-procedural CT images with 3D-printed models. Measurements from manual and software segmentation methods were similar and both smaller than the actual balloon sizes used. Stenosis severity assessments were consistent between methods but lower than angiographic reports. The use of 3D models led to changes in vascular access site selection in 31.2% of patients and altered wire and catheter choices in eight cases, demonstrating the models' influence on procedural planning (21).

DISCUSSION

This systematic review can even underscore the transformative role of 3D printing in vascular surgery, highlighting its potential to revolutionize both training and preoperative prospects and illustrate the feasibility and utility of 3D-printed models in endovascular simulations, demonstrating their capacity to replace costlier and less accessible traditional approaches. 3D printing provides vascular surgeons with tangible, patient-specific representations that facilitate personalized planning and rehearsal of complex interventions (). The technology improves comprehension of vascular anatomy, optimizes stent-graft selection, and enhances communication within surgical teams.

Simulation-based education using 3D models standardizes skill acquisition and bridges the gap between theory and clinical practice. Specific applications include preoperative rehearsal for congenital heart surgery (1,14), TAVR valve testing and procedural simulation (2,3). Computational modelling has improved process optimization. Despite advancements, challenges remain in cell maintenance, printing thick tissues, replicating tissue complexity, and scaling. Exploring newer technologies like 4D printing may help overcome these limitations and improve vascularized tissue fabrication (12).

3D strengths lie in its focus on innovative and promising cell encapsulation techniques using hydrogels, covering a range of versatile technologies and providing a broad overview (). It addresses a critical unmet medical need by exploring potential therapies for limb ischemia and emphasizes the ability to tailor hydrogel properties for specific applications (). However, it has weaknesses in that it lacks detailed discussion of clinical trial results and real-world efficacy, providing limited insight into biological challenges such as immune responses and long-term cell survival ().

Additionally, the heavy focus on technical aspects may overlook important translational and regulatory hurdles, and there is little exploration of future directions, scalability, and practical implementation of these therapies (22).

However, challenges remain - high equipment costs, material limitations, and the need for trained technical staff restrict widespread adoption (2,23).

The lack of standardized workflows across institutions also contributes to inconsistent results. Continued innovation in biocompatible materials and cost-efficient production is essential for routine clinical integration. Time and cost constraints remain significant barriers, especially in emergency contexts requiring rapid production. Moreover, the lack of standardized workflows and methodologies across institutions contributes to variability in procedural durations and outcomes, complicating widespread implementation ().

In conclusion, 3D printing significantly improves both vascular-surgery training and preoperative planning. Its adoption leads to higher procedural accuracy, greater trainee confidence, and more efficient operations. Future research should focus on validating these outcomes in larger, multicentre trials and extending data collection beyond 2026 to ensure contemporary relevance.

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TRANSPARENCY DECLARATION

Conflicts of interest: None to declare.

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ORIGINAL ARTICLE

Evaluation of predictive factors for conception after saline infusion sonosalpingography

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ABSTRACT

Aim To evaluate predictive factors of spontaneous conception after saline infused sonosalpingography (SIS).
Methods A prospective study was conducted from August 2023 to December 2024 in Mosul/Iraq. A total of 100 women were categorized into two groups: pregnant after SIS and non-pregnant after SIS. For the achievement of conception, the two groups were followed for 6 months and their clinical characteristics were compared. Seventy women were polycystic ovary syndrome (PCOS) negative, and 30 were positive.
Results Among 100 women (both with and without PCOS) 58% showed the duration of infertility for positive pregnancy test ≤2 years versus 42% women >2 years. The time ≤2 months to get pregnant after SIS showed 79% women versus 21% >2 months. The pregnancy prevalence of 24% was markedly higher in the first 2 months post SIS equal to 79% (100 women with and without PCOS). The sub analysis of 70 women without PCOS showed the duration of infertility for pregnant women of ≤2 years in 66.7% versus 33.3% >2 years. The time needed to get pregnant after SIS was 60% ≤2 months versus 40% >2 months. Pregnancy rate was 21.1% and higher in the first 2 months after hydrosalpingography equal to 60% in 71 women without PCOS (PCOS positive women were excluded). Anti mullerian hormone showed a significant difference in pregnant women.
Conclusion The SIS had positive effect on spontaneous conception in the first two months after the procedure.
Keywords: female infertility, pregnancy outcome, salpingography

INTRODUCTION

It is well known that chromopertubation enhanced laparoscopy (LC) is gold standard diagnostic technique for tubal patency assessment but its drawbacks are hospital staying, invasiveness and necessity for general anaesthetic equipment (1). X-ray executed Hysterosalpingogram (HSG) is considered a classical technique for estimating patency of fallopian tubes but exposure to radiation and allergy from iodine contrast agents are the main restrictions (2,3). For child bearing aging women, there are multifactorial etiologies influencing pregnancy incidence like uterine, partner, ovarian and tubal factors, nearly 30 to 35% account for tubal factors (4-6) therefore assessing tubal factors is crucial and fundamental point for pregnancy workout (7).
In the last few years saline infused sonosalpingography (SIS) has become the first imaging diagnostic route for evaluating patency of fallopian tubes substituting hysterosalpingography (8,9). No iodine allergy or radiation risk limitations are present (as in HSG) as its dynamic ultrasound imaging tool with saline solution purely or mixed with air pushed to uterus and fallopian tubes (10). On the contrary, SIS is an easier and cheaper procedure than HSG or LC especially when an experienced radiologist could discriminate between fallopian tubes temporal spasm and true occlusion.
Many studies have been suggested that the pregnancy rate is increased following SIS as minor obstructions can be opened by flushed fluid to fallopian tubes (11,12) or have enhancing effect on endometrium perfusion (13) also ovulation induction could be promoted (14).
Having a look on the follow-up facts and different theories, there is a good percentage of patients who have achieved natural pregnancy after SIS interference, subsequently, it has been proposed that expectant management could have a role in some couples without the need for overtreatment and medical resources wasting (11). Yet, couple's clinical characteristics and tubal evaluations results are not being studied widely to help make treatment choices i.e. non- or interventional ways, and also if the expectant management is preferred, the time interval or for how long is not yet well cleared.
The aim of our research was to evaluate whether certain patient clinical features (age, body mass index (BMI), type and period of infertility and anti-mullerian hormone (AMH) serum level have significant and notable function in track down treatment options whether expectant or interventional routes post SIS procedure.

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PATIENTS AND METHODS:

Patients and study design

The study prospectively involved 140 women who were subjected to SIS from August 2023 to December 2024 at an outpatient private clinic for diagnostic imaging at Mosul city/Iraq, of which 100 women where eligible for the study; 40 women were excluded due to loss of follow-up or matched exclusion criteria. Inclusion criteria included female at reproductive age with history of primary or secondary infertility. Exclusion criteria were invasive assisted conception technique (intrauterine insemination exam and *in vitro* fertilization exam) carried out between SIS and conception, pelvic inflammatory disease, inability to have lithotomy position.

The outcome of pregnancy was followed up for 6 months after SIS with patients arranged in two groups according to pregnancy outcome: pregnant women after SIS and non-pregnant women after SIS.

Selected clinical conditions were taken and interpreted as possible influencing factors on pregnancy outcome such as body mass index (BMI), type and duration of infertility, age, AMH for ovarian reserve assessment, polycystic ovary syndrome (PCOS), obstetric and pelvic previous operation, SIS results of bilateral tubal occlusion, patency of at least one tube, patent or closed known side tube due to history of contralateral salpingectomy.

An acknowledged consent was signed by all the participants.

The study was conducted following approval from the Medical Ethics Committee of Mosul Medical College, University of Mosul.

Methods

Candidates were asked to attend no later than the 12th day of their menstrual cycle. The ultrasound exam was done by experienced medical imaging professional with more than 5 years' experience in gynaecological and obstetrical checking.

After bladder voiding, a lithotomy position was adapted by the patient, Cusco vaginal speculum was inserted in a place with cervical disinfection (povidone iodine solution); it was routinely done for all patients. A two-dimensional transvaginal ultrasound probe (GE Versana Premiere, China) was inserted first to assess the uterus and adnexa before saline infusion through the catheter system. The pre-warmed saline solution of about 20 to 35 mL was infused to the uterine cavity to evaluate tubal patency by noticing the fluid spillage in the peritoneal cavity (anteriorly or posteriorly to the uterus) by the transvaginal probe.

The results were classified as: bilateral tubal occlusion, patency of at least one tube, patency of one side tube due to contralateral tube removal (could be due to ectopic pregnancy as a cause). A Versana premiere (GE Health Care, China) with transvaginal transducer frequency range of 4.0-10.0MHz was handled.

Statistical analysis

Shapiro-Wilk test to check the normality of distributions was used, continuous variables presented as mean ± standard deviations (SD) when they fell within the normal distribution, and median and range (maximum, minimum) when they did not. Group based variables were presented as a number of cases and percentages (%). Based on the normality of distribution of the variables, distinction between two independent variables were assessed using the Student-T test for independent variables or the Mann-Whitney U test. The comparative review among group based variables was done using $\chi 2$. Logistic regression analysis was performed to identify variables correlated with spontaneous conception. A p=0.05 was determined as statistically significant.

RESULTS

A total of 24 (out of 100; 24%) women with the diagnosis of infertility conceived within 6 months after doing the hydrosalpingography. The median age for both PCO positive (N=29) and PCO negative (N =71) non-pregnant women was 27.0 years, while for the pregnant group it was 26.0 years (p=0.653). BMI did not show significant difference in the obese women who did not become pregnant comparing to the women who became pregnant, 30 (39.5 %) and 15 (62.5 %), respectively (p=0. 922).

The prevalence of normal SIS in the group of women who got pregnant was 79.2%, as opposed to 93.4% in those who did not get pregnant, while close SIS of the group of women who conceived was 20.8%, as opposed to 6.6% in those who did not conceive (p=0.042). The prevalence of pelvic operation without CS of the group of women who got pregnant was 54%, as opposed to 80.2% in those who did not get pregnant, while pelvic operation with CS, of the group of women who got pregnant was 46%, compared to 20% of those who did not get pregnant (p=0.011). The percentage of women without PCOS was 54.16% of the group who got pregnant compared to 76.31% in those who did not get pregnant, while in the women with PCOS 45.84% women conceived in contrast to 23.68% in those who did not conceive (p=0.037). No significant difference was observed in infertility type, -AMH, AFC. On the other hand, for comparison of pregnant groups, the median of occurrence of pregnancy time after SIS ≤2 months was 1.5 versus 4.5 in those with occurrence of pregnancy time after SIS >2 months (p= 0.000).

The median of duration of infertility in the group ≤2 years was 18 months versus 48 months in those who had the duration of infertility >2 years (p= 0.000).

The cumulative rate of spontaneous pregnancy following SIS within six months was 24% and the rate of pregnancy was markedly higher in the first 2 months after doing the SIS at 79% and in the first month at 37.5 % (Table 1).

Logistic regression analysis showed that no variable capable of influencing the prevalence of pregnancy between the two groups, with an exception of PCO positive women.

A total of 15 (out of 71; 21.1%) infertile women conceived within 6 months after executing SIS.

The median age for PCO negative non-pregnant women was 28.9 years, while for the pregnant group it was 29.06 years (p=0.95). BMI did not show significant difference even in obese women who did not get pregnant comparing to the women who got pregnant, 18 (32.7%) versus six (40.0%) (p=0.59). The AMH showed significant difference in pregnant women comparing to non-pregnant women, 1.5 versus 1.2, respectively (p=0.027) (Table2).

The prevalence of normal SIS in the group of women who got pregnant was 66.7%, in contrast to 91% in those who did not

Table 1 Clinical characteristics of 100 women with and without polycystic ovary syndrome (PCOS)

Variable	Non – pregnant (N=76)	Pregnant (N=24)	p
Median age (min. – max.)	27.0 (15-45)	26.0 (17-45)	0.653
	No (%) of women		
BMI (kg/m^2)			
Underweight (<18.5)	2 (2.6)	0 (0.0)	No pregnant women no p value
Normal (18.5 - 24.9)	29 (38.2)	3 (12.5)	0.607
Overweight (25 - 29.9)	15 (19.7)	6 (25)	0.624
Obesity (≥30)	30 (39.5)	15 (62.5)	0.922
Type of infertility			
Primary	37 (48.7)	10 (41.7)	0.54
Secondary	39 (51.3)	14 (58.3)	
SIS			
Normal	71 (93.4)	19 (79.2)	0.042
Closed	5 (6.6)	5 (20.8)	
Pelvic operation			
Without CS	61 (80)	13 (54)	0.011
With CS	15 (20)	11(46)	
PCO			
NO	58 (76.32)	13 (54.16)	0.037
YES	18 (23.68)	11 (45.84)	
Occurrence of pregnancy time after SIS (months)			
<2		19 (79)	0.0001
>2		5 (21)	
Duration of infertility (years)			
≤2		14 (58)	0.000
>2		10 (42)	
	Median (min. – max.)		
Occurrence of pregnancy time after SIS (months)			
≤2		1.5 (0.5-2.)	0.000
> 2		4.5 (3-6)	
Duration of infertility (years)			
≤2		18 (24-15)	0.000
>2		48 (96-36)	
Anti-Mullerian hormone (ng/mL)	2.3 (0.03-17)	2.5 (0.5-11.8)	0.840
Antral follicle count	7.0 (0.0 -30)	10.5 (0.0-40)	0.061

BMI, body mass index; SIS, saline infusion sonosalpingography; CS, caesarean section

get pregnant, while close SIS, of the group of women who got pregnant was 33.3% compared to 9.0% in those who did not get pregnant, (p=0.004). The prevalence of pelvic operation without CS of the group of women who conceived was 66.7%, in contrast to 89.1% in those who did not conceive, while pelvic operation with CS of the group of women who got pregnant was 33.3%, versus 10.9% in those who did not, (p=0.034). No significant difference was noted in the infertility type and AFC.

Table 2. Clinical characteristics of 71 women without polycystic ovary syndrome (PCOS)

Variable	Non – pregnant (N=55)	Pregnant (N=15)	p
Mean age (±SD) (years)	28.96 ±6.7	29.06 ±7.2	0.950
	No (%) of women		
BMI (kg/m^2)			
Underweight (< 18.5)	2 (3.6)	0 (0.0)	No pregnant women no p value
Normal (18.5 - 24.9)	25 (45.5)	4 (26.7)	0.19
Overweight (25 - 29.9)	10 (18.2)	5(33.3)	0.205
Obesity (≥30)	18 (32.7)	6 (40.0)	0.59
Type of infertility			
Primary	24 (43.6)	5 (33.3)	0.47
Secondary	31 (56.4)	10 (66.7)	
SIS			
Normal	50 (91.0)	10 (66.7)	0.004
Closed	5 (9.0)	5 (33.3)	
Pelvic operation			
Without CS	49 (89.1)	10 (66.7)	0.034
With CS	6 (10.9)	5 (33.3)	
Occurrence of pregnancy time after SIS (months)			
≤2		9 (60.0)	0.02
>2		6 (40.0)	
Duration of infertility (years)			
≤2		10 (66.7)	0.001
>2		5 (33.3)	
	Median (min. – max.)		
Anti-Mullerian hormone (ng/mL)	1.2 (0.03-14)	1.5 (0.5-4.5)	0.027
Antral follicle count	7.0 (0.0 -30)	10.0 (0.0 -26)	0.106

BMI, body mass index; SIS, saline infusion sonosalpingography; CS, caesarean section

On the other hand, for comparison of the pregnant groups, the median of occurrence of pregnancy time after SIS ≤2 months was 1.0 versus 4.0 in those with occurrence of pregnancy time after SIS >2months (p= 0.02).

The median of duration of infertility ≤2 years was 24 versus 66 in those whose with the duration of infertility >2 years (p= 0.001). The cumulative rate of spontaneous pregnancy following SIS within six months was 21.1% and the rate of pregnancy of 60% was notably higher in the first 2 months after SIS (Table 2).

Logistic regression analysis showed that AMH was the only independent variable capable of influencing the prevalence of pregnancy between the two groups: OR (odds ratio)=1.58; 95% CI: 1.11, 1.67 (p=0.03).

DISCUSSION

The advantage of SIS was very clear in women with short duration of infertility, low prevalence of CS operation, and higher AMH level; this fact encourages expectant management rather than overtreatment and wasting medical resources. The results support the fact that infertility period is positively allied with

spontaneous pregnancy after executing SIS, (14-16); it can be explained by the presence of continual obstruction of the fallopian tubes resulting in long term inflammatory process affecting the internal tubal structures (15,17). The AMH showed no significant difference when 100 patients were included (with and without PCOS factor), which could be explained by the high number of small peripheral follicles in the ovaries causing false high serum AMH level. In sub-analysis of 71 women (not having PCO factor), AMH was the sole factor in declaring dissimilarity in the prevalence of spontaneous conception among the two groups. This emphasizes AMH value as a potent predictive variable (14,16,18). Pregnancy rate was 24% in the group with 100 women included (with and without PCO), which is comparable to 26.59% in other studies (11); it may be attributed to the small percentage of bilateral tubal occlusion of 10% in our study comparing to 9.5% in other studies (11). However, pregnancy rate of 16% was found in some studies (18) with bilateral tubal occlusion of 13.4%. BMI showed no statistical difference between the two groups, which is in disagreement with other reports (18,19) showing that obesity may cause irregular menstrual cycle, anovulation, higher miscarriage rate, poor fertility treatment response and reduce the rate of pregnancy; this may be attributed to the relatively small sample of the study.

Conception in first month after SIS showed a higher percentage of 37.5%, which is compatible with prior studies (11,14,20) encouraging the suggestion that spontaneous pregnancy is favoured during the same cycle of menstruation following SIS.

The mean time to pregnancy within 6 months passing SIS in our study was 1.7 months, which was lower comparing to other studies, 3.4±2.9 (18) and 5.3 (11). This supports the expectant management for a certain period of time for infertile couples following SIS for the achievement of spontaneous pregnancy and decreased overtreatment and medical risks.

Unexpectantly, five patients with bilateral obstruction of the fallopian tubes conceived spontaneously following SIS, which was reported in other studies (15,18). It might be due to the removal of the debris and secretion inside tubes by the mechanic process of liquid passage, and spasm of tubes, which also contributes to the false positive tubal occlusion.

A coherence review for randomized trials achieved that tubal intervention may give significant raise in the conception odd and live birth as in other studies (21, 22). This favourable and effective impact could be clarified by a breakdown of minor adhesions by contrast agent passage, which also clears debris and plugs of mucus. The passage of agent may also expand tubal stenosis (20,21).

The restraint of this study is a relatively small sample and limited period of follow-up to months. A larger sample size is recommended to validate the study results.

In conclusion: our study supports the favourable and positive effect of SIS on the achievement of spontaneous pregnancy. This is true within the first month and encourages choosing expectant management overtreatment and assisted reproductive technique especially in women with short duration of infertility, lower CS interference and good AMH serum level.

Author Contributions

Conceptualization, D.R. and L.A.; Data curation, D.R. and L.A.; Formal Analysis, M.A. .; Investigation, D.R., M.A. and L.A.; Methodology, D.R., and M.A.; Project administration, M.A. and L.A.; Software, M.A. and D.R.; Visualization, D.R. and L.A.; Writing – original draft, D.R and M.A; Resources, L.A. and M.A.; Supervision, D.R. and L.A.; Validation, D.R, AND; Writing – review & editing, D.R, M.A. and L.A. All authors have seen and approved the final manuscript.

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Conflicts of interest: None to declare.

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ORIGINAL ARTICLE

Pregnancy outcome after in vitro fertilization: a retrospective cohort study

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ABSTRACT

Aim To determine the outcome of pregnancies in women whose pregnancies resulted from in vitro fertilisation (IVF) procedure.

Methods This retrospective cohort study was conducted at the Clinic for Gynaecology and Obstetrics, University Clinical Centre Tuzla, Bosnia and Herzegovina. Data from the delivery protocol were analysed over a five-year period between 1 January 2019 and 31 December 2023. A total of 257 deliveries from IVF pregnancies were analysed, constituting the study group, while the control group consisted of an equal number of women with spontaneously conceived pregnancies. The following data were analysed: baseline obstetrics and neonatal characteristics, and the presence of complications during pregnancy and delivery.

Results The average age of pregnant women was higher in the study compared to the control group (p=0.001). The average body weight and body length at birth were lower in the study group (p=0.001). The twin pregnancies were more frequent in the study group (p=0.0001). The largest number of primiparous women (p=0.0001) was recorded in the study group. A higher number of newborns with Apgar scores <7 at the first and fifth minutes was found in the study group (p=0.0001). There was higher prevalence of preterm births and cesarean section in the study group (p=0.0001). Fetal asphyxia and breech presentation were more prevalent in the study group (p=0.008 and p<0.0001).

Conclusion Pregnancies resulting from IVF were still riskier than those resulting spontaneously.

Key words: asphyxia neonatorum, cesarean section, premature birth, pregnancy, twin

INTRODUCTION

In modern times, due to the increased incidence of infertility (1), the number of couples requiring some form of assisted reproductive technology (ART) to achieve offspring has increased (2). ART includes *in vitro* fertilization (IVF), intrauterine insemination (IUI), egg donation and sperm donation, embryo donation, intracytoplasmic sperm injection (ICSI), cryopreservation (egg, sperm, and embryo freezing, surrogacy, preimplantation genetic testing (PGT -A) (2).

The standard IVF procedure involves the extracorporeal fertilization of sperm and egg through spontaneous interaction, embryo culture for 2 to 5 days, and embryo transfer into the uterus (3). It was reported that 10 to 13 million or more infants have been born from ART in the 40 years since the first ART-conceived infant was born in 1978 (4). A "good perinatal outcome" for newborns from pregnancies resulting from IVF was considered as a single pregnancy newborn born at term (>37 weeks of gestation) with a body weight >2500 grams (5). The success of the IVF procedure was related to the number of embryos transferred, with poorer outcome considered due to the increased prevalence of multiple pregnancies (6). Data showed that these pregnancies have an increased risk of poor perinatal outcome compared to pregnancies resulting from spontaneous conception (7).

In addition to the significantly higher risk of multiple pregnancies, IVF pregnancies also have an increased number of preterm births and newborns with low birth weight (8). Numerous studies have proven an increased risk for the occurrence of preeclampsia, gestational hypertension, placenta previa, and gestational diabetes (9).

There are numerous studies, systematic reviews and meta-analyses relating to this very interesting and researched topic (10,11). However, in our country, Bosnia and Herzegovina (B&H), research on infertility in general was scarce (only about the causes of infertility) (12); but no research on pregnancy outcomes after IVF procedures was found. Bosnian researcher investigated progesterone induced blocking factor taken in early pregnancy predicting the pregnancy

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outcome in women undergoing in vitro fertilization procedure (13). Also, reproductive characteristics and success rate of intracytoplasmic sperm microinjection in spinal cord injury infertile men were investigated (3). In addition, in B&H the difference in *Lactobacillus* abundance between age groups during ovarian stimulation in fresh *in vitro* fertilization (IVF) cycles (14) was investigated including an experience after the first 105 IVF cycles and how to make ART affordable (15).

The aim of this study was to determine the obstetrics outcome of pregnancies in women whose pregnancies resulted from IVF procedures. We want to investigate whether these are truly high-risk pregnancies, as obstetric scientific circles believe, or whether those are pregnancies with a normal favourable obstetric outcome and the usual complication prevalence, as in spontaneous pregnancies. The results of our study should be useful to a wider circle of obstetricians in B&H, and beyond, who encounter pregnancies resulting from IVF procedures in their daily practice.

PATIENTS AND METHODS

Patients and study design

This was a retrospective cohort study conducted at the Clinic for Gynaecology and Obstetrics, University Clinical Centre Tuzla, B&H. Data from the delivery protocol were analysed over a five-year period between 1 January 2019 and 31 December 2023. A total of 257 deliveries from IVF pregnancies were analysed, constituting the study group, while the control group consisted of an equal number of women with spontaneously conceived pregnancies. The control group was selected by taking the next pregnant woman in the delivery protocol following each IVF pregnancy case.

The Ethics Committee of the University Clinical Centre Tuzla approved the study.

Methods

The following data were analysed: average maternal age, parity (I, II, >III), number of newborns (singleton or multiple pregnancies), average birth weight of the newborns, average length of the newborns, Apgar score <7 after the first and fifth minutes, mode of delivery (vaginal or cesarean section), and the presence of complications during pregnancy and delivery. The complications analysed during pregnancy and delivery included: gestational hypertension, gestational diabetes, premature rupture of membranes, fetal asphyxia, breech presentation, placental abruption, and placenta previa, previous spontaneous abortion, preeclampsia).

Statistical analysis

For the statistical analysis of the data, descriptive statistics (mean value, standard deviation - SD), and percentage along with Student's t-test, Mann-Whitney U test, Chi-square test were employed; odds ratio was used in statistical data processing. A difference of $p<0.5$ was considered statistically significant.

RESULTS

The total number of deliveries from IVF pregnancies in five-year period were 257 (out of 14881 deliveries; 1.72%) (Table 1). A total number of newborns in the study group was 315.

Average age of pregnant women was higher in the study compared to the control group, 34.005.19 and 28.00±5.41, respectively ($p<0.001$) (Table 2).

The average body weight and body length at birth were in the study group statistically significantly lower compared to the control group, 2.990.00±774.41 and 3430±577.42, 53.00±4.69 and 55.00±3.30, respectively ($p<0.001$) (Table 2).

Twin pregnancies were more prevalent in the study group, in which one (0.38%) pregnant woman had triplets. Twin pregnancies were more frequent in the study compared to the control group, 57 (22.2%) and 3 (1.1%), respectively ($p<0.0001$) (Table 3).

The largest number of primiparous women, 226 (87.9%) ($p<0.0001$) was recorded in the study group, while in the control group there was the largest number of secondiparous women, 148 (57.6%) ($p<0.0001$) (Table 3).

It was found that a higher number of newborns with Apgar score <7 at the first and fifth minute was recorded in the study group, 76 (23.8%) and 37 (11.6%), respectively ($p<0.0001$). There was a higher prevalence of preterm births in the study group, and cesarean section deliveries were more frequent in the study group, 74 (28.8%) and 220 (85.6%), respectively ($p<0.0001$) (Table 3).

Table 1. Number of deliveries after in vitro fertilisation during the study period

Year	Total number of deliveries	Number (%) of deliveries (IVF)	Number of newborns
2019	3328	71 (2.1)	92
2020	2941	45 (1.5)	53
2021	2746	41 (1.5)	47
2022	2615	52 (1.9)	68
2023	2551	48 (1.8)	55
Total	14881	257 (1.72)	315

Table 2. Baseline maternal and neonatal characteristics of the study and control group

Characteristic	Study group		Control group		
	No of patients	Median±SD (min-max)	No of patients	Median±SD (min-max)	p
Maternal age (years)	257	34.00 ±5.19 (22.00-50.00)	257	28.00 ±5.41 (18.00-43.00)	<0.001
Birth weight (gr)	319	2.990.00 ±774.41 (300.00-2.990)	259	3430 ± 577.42 (1.000-3430)	<0.001
Birth length (cm)	319	53.00 ±4.69 (33.00-53.00)	259	55.00 ± 3.30 (34.00-55.00)	<0.001
Apgar score					
at first minute	319	8.00 ±1.49 (0.00-8.00)	259	9.00 ± 1.08 (4.00-9.00)	<0.001
at fifth minute	319	9.00 ±1.13 (0.00-9.00)	259	9.00 ± 0.61 (5.00-10.00)	<0.001

Table 3. Perinatal characteristics in the study and control group

Variable	No (%) of patients Study group	No (%) of patients Control group	p	Odds Ratio (95% CI)
Primiparous women	226 (87.9)	109 (42.4)	<0.0001	3.90 (6.31,15.52)
Gemels	57 (22.2)	3 (1.1)	<0.0001	24.13 (7.45,76.19)
Apgar score				
in first minute <7	76 (23.8)	30 (11.6)	<0.0001	3.18 (1.99,5.06)
in fifth minute <7	37 (11.6)	8 (3.1)	<0.0001	5.23 (2.39,11.48)
Preterm delivery	74 (28.8)	18 (7.0)	<0.0001	5.37 (3.10,9.31)
Cesarean Section	220 (85.6)	79 (30.7)	<0.0001	18.43 (11.43,29.60)

A higher number of pregnant women with hypertension was found in the study group, 41 (15.95%), without statistically significance difference between the groups (p=0.157). Diabetes and preeclampsia were found in equal numbers in both the study and control groups, three (1.16%) without statistical significance. Thrombophilia and previous spontaneous abortion were found in a higher number in the study group, without statistical significance (p=1.0). Premature rupture of membranes was present in a higher number in the study group compared to the control group, 50 (19.45%) and 43 (16.73%) without statistical significance (p=0.49) (Table 4).

Fetal asphyxia was found to be significantly more prevalent in the study group, 79 (30.73%), (p=0.008). Breech presentation was statistically more significant in the study group, 37 (14.39%), (p<0.0001). In the control group, there was a statistically significantly higher number of women who previously had cesarean section, 35 (13.61%), (p=0.02) (Table 4).

Table 4. Complications in pregnancy and delivery in the study and control group

Complication	No (%) of patients		p	Odds Ratio (95% CI)
	Study group	Control group		
Hypertension	41 (15.95)	29 (11.28)	0.157	-
Preeclampsia	3 (1.16)	3 (1.16)	1.0	-
Diabetes	3 (1.16)	3 (1.16)	1.0	-
Thrombophilia	17 (6.61)	7 (2.72)	0.05	-
Premature rupture of membranes	50 (19.45)	43 (16.73)	0.49	-
Previous spontaneous abortion	17 (6.61)	7 (2.72)	0.05	-
Previous cesarean section	18 (7)	35 (13.61)	0.02	0.48 (0.26, 0.87)
Fetal asphyxia	79 (30.73)	52 (29.23)	0.008	1.75 (1.17, 2.62)
Breech presentation	37 (14.39)	7 (2.72)	<0.0001	6.01 (2.62, 13.75)
Placental abruption	4 (1.55)	3 (1.16)	0.381	-
Placenta previa	1 (0.38)	3 (1.16)	0.617	-

DISCUSSION

The mechanisms involved in the association between IVF and poorer perinatal outcome were not entirely clear. One possible explanation was that perinatal complications may be due to the underlying cause of infertility itself, as numerous maternal factors associated with infertility also increase the risk of poorer perinatal outcome in women undergoing IVF (16). In our study 1.72% were pregnancies form IVF; in the study group the highest number were primiparous women. Our results were consistent with global studies that have found that women who give birth after IVF were mostly primiparous (17).

The age of the pregnant woman not only affects pregnancy outcome, but it is also closely related to infertility rates (18). Increasing age, the number of aneuploid oocytes also increases infertility. In women over 40 years of age, about 90% of oocytes are aneuploid (19). Older pregnant women were at higher risk of delivering low-birth-weight infants and experiencing preterm births (20). Our study showed that women undergoing IVF were significantly older, with average age of 34.0 years, compared to 28.0 years in the control group. This age was slightly higher compared to a study that reported average age of 31.87, even older women were found also in the control group, with an average age of 31.72 years (21). Numerous studies show that compared to fertile women, women who conceive through IVF are older, have more frequently preexisting chronic conditions, and have an increased risk of poorer perinatal outcome (22,23). We did not reduce the age rank for pregnant women (22-50) from the IVF group because we included all IVFs during the examined period; the reason is that one of the key characteristics and causes of infertility, especially in modern times, is the phenomenon of delayed motherhood, i.e. giving birth at a later age.

The average birth weight was significantly lower in women undergoing IVF, compared to women with spontaneously conceived pregnancies. Our data were similar to a study, where the average birth weight was higher in the fertile group, while in the IVF group, it was lower. The study also demonstrated an increased risk of delivering low-birth-weight infants and a higher prevalence of preterm births (24).

Women undergoing IVF have a higher percentage of preterm births, even when adjusting for maternal age and parity (25,26). The most common causes of preterm birth were cervical insufficiency and inflammatory processes, subsequently leading to premature rupture of membranes (26). A meta-analysis covering 25 studies found a twofold increased risk of preterm birth in singleton pregnancies following IVF compared to spontaneously conceived pregnancies (27). In our study, the percentage of preterm births among women with IVF was 28.79%, which was slightly lower compared to a study where it was 39.4% among primiparas and 42.2% among multiparas; low-birth-weight infants accounted for 36.8% of primiparas and 33.4% of multiparas. (21).

Apgar score <7 at the first minute was found in 24.12% of newborns, and Apgar <7 at the fifth minute was found in 11.74% of newborns in the study group. These values were higher compared to those from a study (24) where Apgar score <7 at the first minute was found in 4.9% of newborns and at the fifth minute in 1.3%. Fetal asphyxia was more common in the study group. The presence of Apgar score <7 in our study group can be attributed to the significantly lower average birth

weight of these newborns, and prematurity, which was also more frequent in the study group in our study.

Prematurity may also account for an increased number of breech presentations, which were more common in preterm births. Breech presentation was recorded in 14.39% pregnant women in our study. The IVF pregnancies held an increased risk of abnormal fetal head rotations, breech presentation and cesarean section (28,29). In our study a higher frequency of cesarean section of 89.10% was found in IVF pregnancies. This prevalence is significantly higher compared to a study, where the cesarean section rate was 58% (30).

The trend of increased cesarean sections rates was not only observed among spontaneous pregnancies but particularly among IVF pregnancies (31). Women who conceive through IVF are twice as likely to deliver by cesarean section. (27) The higher prevalence of cesarean section may be explained by the more frequent presence of placental insufficiency, preeclampsia, and other pregnancy complications. The increased prevalence of multiple pregnancies was one of the main reasons for the higher number of cesarean sections (26,32). Multiple pregnancies were associated with a higher risk of maternal complications (preeclampsia, gestational diabetes) and abnormal fetal presentation during delivery (26,32). An additional explanation for the increased number of cesarean sections could be the higher average maternal age, with many of these women experiencing their first pregnancy (32).

Twin pregnancies were a key factor for complications for both the mother and the newborn in pregnancies resulting from IVF. Twin pregnancies lead to numerous complications both during pregnancy and at delivery (26). There were more frequent abnormal positions of one or both fetuses, higher rates of prematurity, and, a greater likelihood of operative delivery (26). Many clinical studies recommend transferring only one embryo, which reduces the rate of twin pregnancies and thus improves perinatal outcome (33). In our study, there were 22.17% twin pregnancies and one (0.38%) pregnancy with triplets. Reportedly, a 20-fold increase in the prevalence of twin pregnancies in IVF pregnancies was found (21).

Among the recorded complications during pregnancy and delivery, hypertension was most frequently noted in our study. Since these were older pregnant women compared to the control group, they more frequently experience hypertension, whether it is chronic hypertension or hypertension that first appears during pregnancy. The risk of hypertensive disorders and preeclampsia in pregnancies following IVF is higher. In IVF pregnancies hypertension was present from 8.1% to 12.4% (34). Numerous studies have shown that preeclampsia results from improper placental vascularization, immune intolerance, endothelial cell activation, and excessive systemic inflammatory response (35).

IVF was an independent risk factor for developing gestational diabetes, with insulin resistance and hyperinsulinemia being implicated (1), as well as an increased prevalence of polycystic ovarian syndrome (PCOS) among patients undergoing IVF (36). In our study, diabetes was diagnosed in 1.16% of the pregnant women in the study group.

The risk of abnormal placentation involves several factors common to both fertile and infertile patients, such as advanced maternal age, endometrial damage, uterine scarring, and a short interval between a previous cesarean section and the current pregnancy (37). Many studies have shown increased frequency of abnormal placentation in women with ovulation stimulation and IVF procedures (38). An increased prevalence of placenta previa and placental abruption was reported (39).

Due to concerns about pathological conditions associated with thrombophilia, infertility patients undergoing IVF are now screened for thrombophilia prior to the procedure (40), and it can lead to thrombosis of uterine blood vessels, thereby causing placentation disorders (41). Thrombophilia was present in 6.61% of pregnant women in the study group.

Pregnant women undergoing IVF were at an increased risk of premature rupture of membranes. The cause was unknown, and it was one of the main causes of preterm birth (42,43). In our study, there was a slightly higher number of cases of premature rupture of membranes in the study group (no statistical significance).

The conducted study has certain limitations: it involves a five-year period and a small sample of IVF pregnancies, the data were obtained from the delivery protocols using available information, the study was conducted only in one institution. A study conducted in multiple institutions and over a longer time period would have greater significance.

Our study is the first of its kind in Bosnia and Herzegovina, it was conducted in one of the clinics that counts the largest number of births per year, so it is certainly a study with a fairly large sample. Through the study, we obtained results that may break the taboo and fear of obstetricians when it comes to pregnancies from IVF procedures.

In conclusion, pregnancies resulting from IVF are still riskier than those resulting spontaneously, which was confirmed by our study. We do not believe that the complications are a result of IVF, but rather that patients who undergo IVF and consequently their pregnancies, are more complex and have more risks and complications than pregnancies from natural conception, which we aimed to prove in our study.

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TRANSPARENCY DECLARATION

Conflict of interest: None to declare.

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ORIGINAL ARTICLE

Impact of daily administration of teriparatide on the histological pattern of testicular structure in adult male rats

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ABSTRACT

Aim Teriparatide, a synthetic analog of the active fragment of human parathyroid hormone (PTH 1–34), is a clinically approved anabolic treatment for osteoporosis. While its bone-forming capacity is well-documented, evidence regarding its systemic safety, particularly in relation to male reproductive health, remains limited. This study was designed to investigate the histological consequences of prolonged teriparatide exposure on testicular architecture in adult male rats, addressing a critical gap in its toxicological profile.

Methods Twenty adult male albino rats were randomly allocated into two groups. The control group received subcutaneous saline (1 mL/kg/day), and the treated group was administered teriparatide (10 µg/kg/day) daily for 60 days. Testes were collected post-euthanasia, fixed in formalin, and processed for histological examination. Spermatogenic activity was assessed using Johnsen's scoring system, and morphometric parameters, including seminiferous tubule diameter and epithelial height, were quantified. Statistical significance was determined using the Mann–Whitney U test ($p \leq 0.05$).

Results Histological findings revealed preserved cytoarchitecture with active spermatogenesis in control testes. The teriparatide-treated group exhibited significant degenerative changes, including epithelial detachment, interstitial edema, vascular congestion, and Leydig cell necrosis. Johnsen's scores were significantly reduced in the treated group (median = 4.5; IQR=1.5) compared to controls (median = 8.0; IQR=2.0; $p<0.001$). Seminiferous tubule diameter and epithelial height were markedly decreased in the treated animals.

Conclusion The findings suggest that prolonged teriparatide exposure may impair spermatogenesis and alter testicular structure. Re-evaluation of its reproductive safety is warranted, particularly for males of reproductive age.

Keywords: histopathology, reproductive toxicity, spermatogenesis, teriparatide, testis

INTRODUCTION

Osteoporosis represents a progressive metabolic skeletal disorder characterized by decreased bone mass and microarchitectural deterioration of bone tissue, ultimately predisposing individuals (particularly postmenopausal females) and elderly males to fragility fractures and associated morbidity (1,2). Among the Food and Drug Administration (FDA)-approved therapeutic agents for severe osteoporosis (2), teriparatide, synthetic recombinant fragment of human parathyroid hormone (PTH 1–34), has gained clinical prominence due to its potent anabolic effect on osteoblasts, in contrast to antiresorptive agents such as bisphosphonates (3,4). By stimulating bone formation and improving trabecular connectivity and mineral density, teriparatide has redefined treatment paradigms in osteometabolic care. However, beyond its osteogenic actions, teriparatide also exerts systemic effects by modulating calcium-phosphate homeostasis through enhanced intestinal calcium absorption and renal calcium reabsorption—mechanisms partially mediated via activation of vitamin D metabolism (5,6). These systemic influences raise concerns regarding their potential effects on other physiological systems, particularly those reliant on tightly regulated calcium dynamics, such as the male reproductive axis (7).

The testis, as the central organ in male fertility, exhibits high metabolic demand and functional dependence on both endocrine and paracrine signals. Sertoli cells within seminiferous tubules coordinate germ cell differentiation, while Leydig cells in the interstitial space maintain androgen production (8). Importantly, calcium ions regulate crucial reproductive functions, including spermatogonial maturation, steroidogenesis, and sperm motility (7,9). Hence, agents that alter calcium equilibrium may indirectly compromise testicular architecture and function (8).

Recent experimental evidence has increasingly implicated parathyroid hormone analogs in eliciting testicular pathology via multifactorial pathways. Among these, oxidative imbal-

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ance, compromised microvascular integrity, and interference with endocrine regulatory circuits have been highlighted as plausible contributors to gonadal dysfunction (10,11). Notably, extended exposure to teriparatide in preclinical models has been associated with pronounced histoarchitectural disruptions (most prominently), disorganization of the seminiferous epithelium and morphological degeneration of Leydig cells, thus raising concerns regarding its potential gonadotoxic effects (12,13).

Nonetheless, the reproductive safety landscape of teriparatide remains inadequately delineated, particularly in the context of therapeutic use among males with active fertility potential.

The aim of this study was a critical assessment of the impact of chronic teriparatide administration on testicular histology and spermatogenic outcomes within a controlled rodent model, thereby contributing mechanistic insights into the drug's broader endocrine footprint. By focusing on morphometric indices and degenerative patterns, this research seeks to elucidate whether systemic PTH modulation may pose an unrecognized risk to male reproductive health.

MATERIALS AND METHODS

Materials and study design

This controlled laboratory investigation was executed between March and May 2024 within the Department of Anatomy, College of Veterinary Medicine, University of Mosul, Iraq.

The study was structured to assess the histological implications of prolonged teriparatide exposure on testicular morphology and spermatogenic function in a standardized rodent model.

All procedures adhered strictly to the international guidelines for the ethical treatment of laboratory animals and received a formal approval from the Animal Research Ethics Committee of the College of Dentistry, University of Mosul (Approval No. UOM Dent 24/1029).

Twenty healthy adult male albino rats (*Rattus norvegicus*), aged 6–7 months and weighing between 300–350 grams, were housed under controlled environmental conditions (temperature: 22±2 °C, humidity: 55±10%, 12/12 h light/dark cycle) with unrestricted access to standard pellet feed and water. The animals received standard commercial rodent food while having unlimited access to filtered water. The researchers established these conditions to prevent outside factors from affecting testicular morphology and spermatogenesis while studying teriparatide effects.

The animals were randomly assigned into two equal groups (N=10 per group): Group I (Control) received subcutaneous injections of sterile normal saline (1 mL/kg/day) for 60 days; Group II (Teriparatide-treated) received daily subcutaneous injections of teriparatide at 10 µg/kg body weight for 60 days.

Methods

Teriparatide (recombinant human PTH 1–34) was prepared fresh under aseptic conditions prior to each administration, with dosage individually adjusted based on daily body weight measurements. At the end of the experimental period, animals were anesthetized using a ketamine–xylazine mixture (80 mg/kg and 10 mg/kg intraperitoneally, respectively, in both groups) and euthanized by cervical dislocation. Both testes were carefully dissected, trimmed of extraneous tissues, and fixed in 10% neutral-buffered formalin for no less than 48 hours.

Dose justification and human equivalence. A daily teriparatide dose of 10 µg/kg/day was used because this amount was established through preclinical research to assess the drug's safety outside bone tissue. The FDA-approved body-surface-area (BSA) conversion method (14) allows to calculate the human-equivalent dose (HED). The conversion is based on Km factors (rat Km = 6; human Km = 37), which are species-specific constants representing the ratio of body weight to surface area. Using this approach, the calculated HED was 0.00162 mg/kg, equivalent to a daily dose of about 1.62 µg/kg.

The calculated dose for a 70-kg adult amounts to approximately 113 µg per day. The rat dose was set above the therapeutic human dose of 20 µg/day administered subcutaneously, producing a 5.6-fold higher systemic exposure. This approach was intended to reveal subtle reproductive effects, yet remained within the accepted preclinical safety margins (15,16).

Histological processing and evaluation. Following fixation, samples were dehydrated using graded ethanol concentrations, embedded in paraffin blocks, and sectioned at 5 µm thickness using a rotary microtome (Leica RM2235, Leica Biosystems, Wetzlar, Germany). Sections were stained with haematoxylin and eosin (H&E) for general histopathological evaluation. The structural integrity of seminiferous tubules, germinal epithelium, interstitial tissue, vascular compartments, and Leydig cells was assessed via light microscopy (Olympus CX43, Olympus Corporation, Tokyo, Japan).

To semi-quantify spermatogenic activity, Johnsen's scoring system was employed. Ten seminiferous tubules per animal were randomly selected, and scores were assigned on a 1–10 scale based on the most advanced germ cell type present.

Morphometric parameters, including seminiferous tubule diameter and germinal epithelial height, were measured using ImageJ software (NIH, USA), with calibrated digital images captured from multiple fields.

Statistical analysis

Data were first examined for normality using the Shapiro–Wilk test. Depending on the distribution, results were expressed either as mean ± standard deviation (SD) or median with interquartile range (IQR). Comparative analysis between the two groups was conducted using the Mann–Whitney U test for nonparametric data and Student's t-test for parametric variables. A p-value ≤ 0.05 was considered statistically significant. Group size was set a priori following established guidance for adequately powered morphometric endpoints in rodent studies. A prospective power analysis did not perform, but the observed standardized effects for the main outcomes (seminiferous tubule diameter and epithelial height) resulted in post hoc power values greater than 0.99 at $\alpha=0.05$, which confirmed the adequacy of the selected sample size. Effect sizes and confidence intervals to complement p-value (17) were additionally reported.

RESULTS

Microscopic evaluation of testicular tissue in the Control group (Group I) revealed a well-preserved seminiferous epithelium with active spermatogenesis. Germ cells at various stages—spermatogonia, primary and secondary spermatocytes, spermatids, and spermatozoa—were arranged in an orderly, stratified manner. Sertoli cells were clearly visible at the

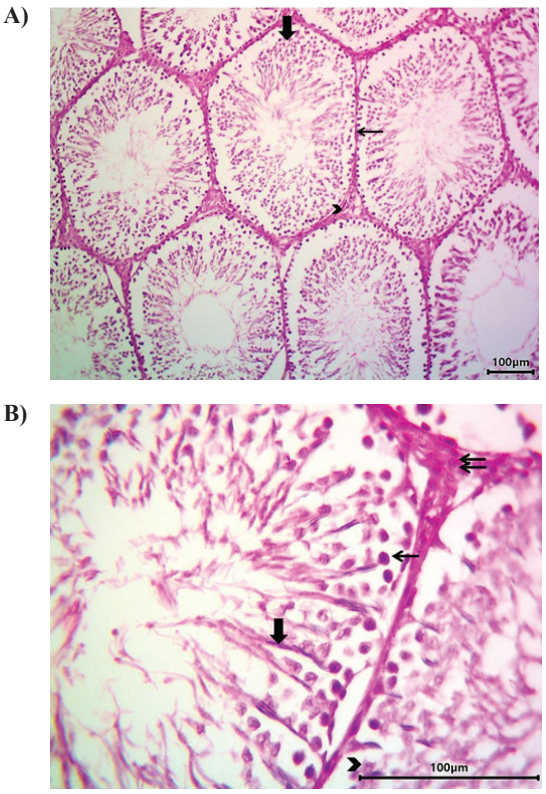

Figure 1. A) Histological section of the testis from a control rat showing preserved seminiferous tubule architecture with active spermatogenesis (arrow). The germinal epithelium is intact and displays an orderly arrangement of spermatogenic cells at various maturation stages (bold-arrow) (100×); B) intact seminiferous tubules with Spermatocytes (arrow), Spermatid (bold-arrow), Sertoli cells (arrowhead) and Leydig cell (double-arrow), (400×). H&E staining

basal compartment of the seminiferous tubules, while Leydig cells appeared structurally intact within the interstitial space. Vascular architecture was normal, and no signs of edema, congestion, or necrosis were observed (Figure 1A and 1B).

Conversely, rats treated with teriparatide (Group II) exhibited substantial histopathological alterations. Seminiferous tubules showed reduced cellular density and severe hypospermatogenesis. Key pathological features included germinal epithelial disorganization, vacuolization, interstitial edema, vascular congestion, and focal necrosis of Leydig cells. Spermatogenic arrest was evident in most tubules, with predominant degeneration of spermatocytes and detachment of the germinal epithelium (Figures 2A–D).

Semi-quantitative grading using Johnsen's scoring system revealed a statistically significant reduction in spermatogenic activity among the treated animals. The median Johnsen score in the control group was 8.0 (IQR=2.0), whereas the teriparatide-treated group exhibited a median score of 4.5 (IQR=1.5). Statistical comparison using the Mann–Whitney U test confirmed that the difference was highly significant ($p<0.001$), indicating disrupted spermatogenesis in response to prolonged teriparatide exposure.

Quantitative morphometric evaluation demonstrated a pronounced reduction in both seminiferous tubule diameter and germinal epithelial height in the teriparatide group compared to controls. The mean seminiferous tubule diameter in the control group was 224.6±8.2 µm, while in the treated group, it significantly decreased to 172.4±6.5 µm ($p<0.001$). The germinal

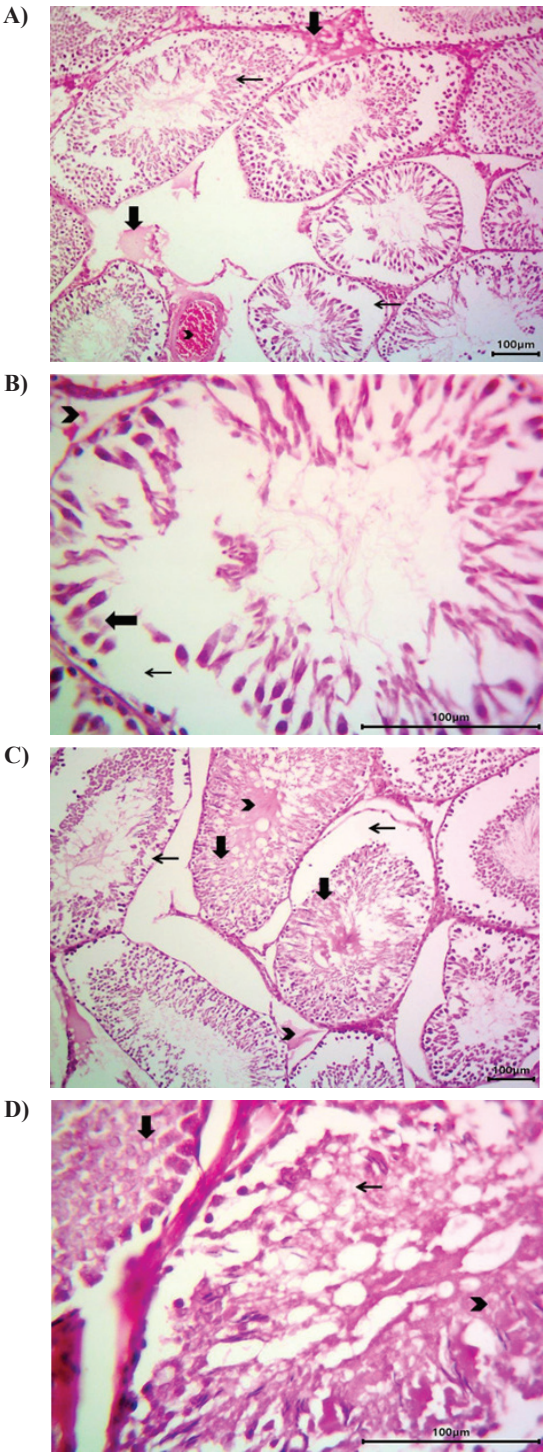

Figure 2. A) Testicular section from teriparatide-treated rats showing necrosis and degeneration of spermatogenesis cells with hypospermatogenesis (arrow), edema between seminiferous tubules (bold-arrow) and congested blood vessels (arrowhead), (100×); B) Higher magnification reveals hypospermatogenesis in the seminiferous tubules (arrow), necrosis and degeneration of the spermatocytes (bold-arrow) and necrosis of Leydig cells (arrowhead), (400×); C) hypospermatogenesis and detachment in the seminiferous tubules (arrow), necrosis and degeneration of the spermatocytes (bold-arrow) and edema (arrowhead), (100×); D) necrosis (arrow) and degeneration (bold-arrow) of the spermatocytes and edema (arrowhead), (400×). H&E staining.

epithelial height was also markedly reduced from 58.7±3.1 µm in controls to 39.2±2.4 µm in the treated rats ($p<0.001$).

These morphometric differences further substantiate the degenerative histological observations and suggest that teri-

Table 1. Morphometric parameters of seminiferous tubules in two groups

Parameter	Mean±SD		p
	Control group	Treated group	
Seminiferous tubule diameter (µm)	224.6±8.2	172.4±6.5	<0.001
Germinal epithelium height (µm)	58.7±3.1	39.2±2.4	<0.001

paratide may compromise testicular cytoarchitecture through direct or indirect mechanisms (Table 1). These histological and morphometric findings are suggestive of endocrine disruption, likely mediated through perturbations in the hypothalamic–pituitary–gonadal axis (Figure 3).

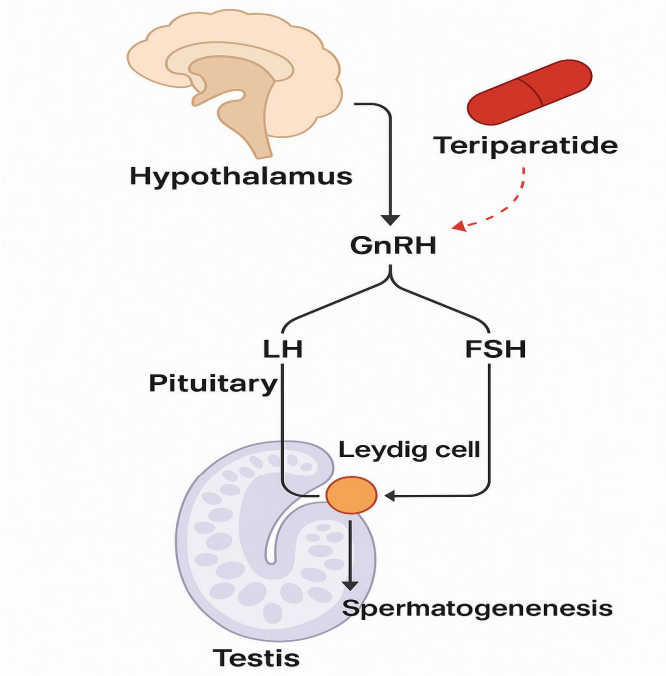

Figure 3. Schematic representation of the hypothalamic–pituitary–gonadal (HPG) axis showing the proposed inhibitory effect of teriparatide on GnRH signalling and downstream gonadotropin secretion, potentially impairing spermatogenesis (Created by the authors)

DISCUSSION

The current study presents compelling histopathological and morphometric evidence indicating that prolonged teriparatide administration induces marked disruptions in testicular architecture and spermatogenic function in adult male rats. These findings align with a growing body of preclinical data that suggest parathyroid hormone analogues may exert off-target effects beyond the skeletal system, particularly on hormonally regulated organs such as the testes (11,12). The 60-day exposure window was chosen to capture one complete spermatogenic cycle in rats. The seminiferous epithelium cycle lasts 12.8 days so a 58–60-day study period allows to measure chronic effects across the entire germ cell lineage which enhances the translational value for male fertility risk assessment (19). Previous research using rodents established that two months of experimental time in rodents equals several years of human exposure thus enabling the assessment of long-term teriparatide effects on testicular structure (20,21).

In the control group, the preservation of seminiferous tubule integrity, orderly germinal epithelium, and active spermatogenesis confirmed normal testicular function. Conversely, the teriparatide-treated group exhibited extensive degeneration, including epithelial detachment, interstitial edema, vascular congestion, and Leydig cell necrosis, features that collectively signal impaired spermatogenic progression and disrupted androgenic activity. The statistically significant reductions in both Johnsen scores and seminiferous tubule dimensions support these morphological observations and reinforce the hypothesis that teriparatide may interfere with the homeostatic regulation of male fertility. The underlying mechanisms through which teriparatide induces such testicular damage may be multifactorial. One plausible pathway involves its known effect on systemic calcium-phosphate metabolism, which may secondarily impact the hypothalamic–pituitary–gonadal (HPG) axis (22). Teriparatide stimulates calcium absorption via the gut and renal reabsorption, potentially altering neuroendocrine feedback loops involving gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) (5,6). It is plausible that chronic perturbations within the hypothalamic–pituitary–gonadal (HPG) axis, potentially induced by teriparatide, may disrupt upstream neuroendocrine signals emanating from the hypothalamus and pituitary gland. Such dysregulation may ultimately compromise testicular steroidogenesis and germinal cell maturation. This hypothesis gains support from our histological observations, which revealed marked Leydig cell degeneration—an outcome that directly implicates diminished intratesticular testosterone synthesis. In turn, impaired androgenic support could destabilize Sertoli cell function, thereby attenuating the microenvironment required for spermatogenic progression (23,24).

The observed morphometric problems match the effects of low androgen because research has shown that decreased testosterone production leads to smaller seminiferous tubules and reduced epithelial cell height (25,26). Research studies conducted with rodent models show that spermatogenesis problems and germ cell death are associated with oxidative stress biomarkers malondialdehyde (MDA) and superoxide dismutase (SOD) (27, 28).

Moreover, oxidative stress may constitute an auxiliary mechanism contributing to testicular damage. The metabolic shifts triggered by prolonged teriparatide exposure could elevate intracellular reactive oxygen species (ROS), undermining cellular viability and promoting germ cell apoptosis (11). These mechanistic inferences align with prior findings by Li et al. (13), who reported structural disruption and reduced spermatogenic indices following extended administration of PTH analogs. Likewise, it was demonstrated that calcium–phosphorus dyshomeostasis could impair Leydig and Sertoli cell physiology, leading to subfertility (29). It was further emphasized that exogenous anabolic hormones may induce interstitial congestion and inflammatory infiltration within the testis—hallmarks mirrored in our treated cohort (30).

The convergence of these findings raises the possibility that teriparatide, when administered chronically, may function as a pharmacological endocrine-disrupting compound (EDC). This potential classification is particularly salient in clinical contexts involving younger male patients, where long-term reproductive implications must not be overlooked.

It is the first experimental study which evaluated how daily teriparatide treatment affects testicular tissue structure in adult

rats. Our research introduces new findings about teriparatide reproductive safety through complete morphometric and histological assessments which differ from previous studies that concentrated on skeletal results. The study's original findings demonstrate why healthcare providers should track parathyroid hormone analogue side effects that occur outside the skeleton when patients use these medications for extended periods. There are some limitations of the study. Despite the robustness of histological and morphometric data, the study is constrained by the absence of serum hormonal profiling (e.g., testosterone, LH, FSH) and oxidative stress biomarkers (e. g., MDA, SOD), which limits mechanistic inference. Furthermore, the use of a single dose and fixed exposure duration precludes evaluation of dose-dependent or reversible effects. Finally, extrapolation to human physiology remains tentative, necessitating further translational research to validate these findings.

In conclusion, the present investigation established a histomorphological link between prolonged teriparatide administration and testicular degeneration in adult male rats. The observed alterations, including epithelial atrophy, Leydig cell necrosis, and reduced seminiferous tubule dimensions, strongly implicate HPG axis dysregulation and androgenic insufficiency. Accordingly, these findings urge clinicians to adopt a cautious approach when prescribing teriparatide to individuals of reproductive potential and emphasize the necessity for longitudinal evaluation of its gonadotoxicity. Further research should include hormonal profiling and molecular markers to clarify the mechanisms underlying testicular damage. Evaluating dose dependency, treatment reversibility, and long-term reproductive safety, especially in clinical settings, will be crucial for guiding therapeutic use of teriparatide in reproductive-age individuals.

Author Contributions: OMA, conceptualization, methodology, experimental work, data analysis, manuscript writing. GAT, histological evaluation, supervision, manuscript review and editing.

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TRANSPARENCY DECLARATION

Conflicts of interest: None to declare.

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ORIGINAL ARTICLE

Evaluation of brain injury biomarkers in mild traumatic brain injury with and without computed tomography findings

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ABSTRACT

Aim Mild traumatic brain injury (mTBI) presents diagnostic challenges, with head computed tomography (head CT) often overutilized in emergency settings. Blood biomarkers such as glial fibrillary acidic protein (GFAP) and ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) have shown promise in early injury detection. The aim of this study was to evaluate the diagnostic utility of GFAP and UCH-L1 in identifying intracranial injuries early and potential reduction in unnecessary head CT scans in mTBI patients.
Methods A prospective study was conducted on 102 adult patients with mTBI. Serum levels of GFAP and UCH-L1 were measured within 12 hours after injury and compared with head CT findings using appropriate statistical analyses.
Results Both biomarkers demonstrated 100% sensitivity and moderate specificity, with high negative predictive value (NPV), supporting their utility in ruling out injuries detectable on CT.
Conclusion GFAP and UCH-L1 are effective early biomarkers for excluding significant intracranial injuries and may help optimize head CT scan utilization in the acute management of mTBI.
Key words: GFAP, neurotrauma, UCH-L1

INTRODUCTION

Mild traumatic brain injury **(**mTBI) is a prevalent public health concern due to its high incidence, acute effects, and potential long-term cognitive and functional consequences (1). Head CT remains the standard imaging modality used for the initial assessment of patients with suspected TBI in emergency departments (2). However, growing evidence suggests that head CT is frequently overused in mild cases, often yielding negative findings, which leads to unnecessary radiation exposure and increased healthcare costs (3). Furthermore, the diagnostic and prognostic accuracy of standard clinical tools, such as the Glasgow Coma Scale (GCS), is limited by external factors including intoxication, comorbidities, advanced age, or the presence of other injuries (4).

While neuroimaging can visualize structural brain damage and guide surgical decisions, it provides limited information about a patient's long-term functional prognosis, particularly in cases of mild or moderate injury (5). Studies have shown that a substantial proportion of mTBI patients present with no head CT abnormalities despite exhibiting neurological symptoms, further highlighting the need for complementary diagnostic tools. To address these limitations, research has increasingly focused on fluid-based biomarkers that are released into the systemic circulation following injury to brain tissue (6). Among these, glial fibrillary acidic protein (GFAP) and ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) have emerged as promising neuron-specific biomarkers with demonstrated diagnostic utility in mTBI (7). GFAP is an intermediate filament protein expressed predominantly in astrocytes, where it plays a role in synaptic regulation, inflammatory responses, and axonal regeneration (8). Its serum concentration increases as a consequence of astrocytic damage, peaking around 20 hours after trauma, and remaining elevated for up to 7 days. The optimal time window for GFAP sampling is considered to be between 6 and 18 hours post-injury. UCH-L1 is a neuron-specific protease involved in the degradation of ubiquitinated proteins. It is highly abundant in neurons and constitutes approximately 5% of total brain protein (6). Upon injury, UCH-L1 level rises rapidly, peaking within 8 hours and returning to baseline shortly thereafter. The biomarker is most effective in the early stages of trauma, with an optimal sampling window between 2 and 8 hours (5). Both biomarkers are minimally expressed in non-neuronal tissues, contributing to their high specificity for central nervous system injury (6).

Numerous studies have evaluated the utility of GFAP and UCH-L1 in the detection of intracranial injury following

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mTBI. Some of them have demonstrated that the combined use of these biomarkers, interpreted against predefined thresholds, yields high negative predictive value (NPV) in ruling out CT-detectable lesions in mTBI patients (9,10). Additionally, these biomarkers are being investigated for their prognostic potential in predicting long-term neurological outcomes (11).
Although blood biomarkers including GFAP and UCH-L1 offer a promising adjunct to identify patients at risk of intracranial injury, validated clinical guidelines still rely mainly on head CT, and biomarker-guided strategies are not yet widely implemented in everyday practice (12) . Recent multicentre data using a rapid point of care assay suggest that GFAP and UCH-L1 testing can support decision-making on head CT indication and may offer a cost and time effective approach to the management of patients with mTBI in emergency settings (13). Further support for the use of GFAP and UCH-L1 as a rule out tool to safely reduce head CT utilization in mTBI comes from a recent European multicentre study, which showed that combined testing of these biomarkers can reliably exclude intracranial lesions while decreasing unnecessary CT scans (14).
This single-centre study evaluated GFAP and UCH-L1 as rapid blood biomarkers for the early exclusion of intracranial injury in patients with suspected mTBI within 12 hours of trauma, with a particular focus on their practical clinical use beyond multicentre or laboratory-based trials. The aim was to determine their diagnostic accuracy (sensitivity, specificity and negative predictive value) and to assess whether they can safely reduce reliance on head CT.

PATIENTS AND METHODS

Patients and study design

This prospective observational study was conducted at the University Clinical Centre (UCC) Tuzla over an eight-month period (August 2024-March 2025). A total of 102 adult patients (≥18 years) with mTBI were included. mTBI was defined based on a Glasgow Coma Scale (GCS) score of 13 -15 on admission (15) in accordance with internationally accepted criteria (16).
The study protocol was reviewed and approved by the Ethics Committee of UCC Tuzla (Approval No. 02-09/2-114/23) and a written informed consent was obtained from all participants prior to inclusion.
Inclusion criteria were: age ≥18 years, hospital admission within 12 hours post-injury, GCS 13–15, and availability of both serum biomarker tests (GFAP and UCH-L1) and head CT imaging. Exclusion criteria included GCS <13, penetrating head trauma, polytrauma, current treatment with anticoagulants, venous blood sampling conducted more than 12 hours after injury, preexisting neurological diseases and severe intoxication interfering with neurological assessment. The diagnosis of mTBI was based on accepted clinical criteria, including GCS 13–15, loss of consciousness <30 minutes, and post-traumatic amnesia <24 hours (16). A CT-positive finding was defined as the presence of intracranial haemorrhage (subarachnoid, subdural, epidural, or intracerebral), cerebral contusions, or skull fractures. Patients without acute intracranial findings were classified as CT-negative.

Methods

Venous blood samples were collected upon hospital admission and in all cases within 12 hours of the traumatic event. Blood was drawn into 6 mL serum tubes with clot activator (Vacusera CAT Serum, Disera A.Ş., İzmir, Turkey) and immediately transported to the Department of Biochemistry at the UCC Tuzla Polyclinic for Laboratory Diagnostics for processing. In the laboratory, samples were allowed to clot for approximately 30 minutes at room temperature (total time from venipuncture to centrifugation ≈30 minutes), followed by centrifugation at 3000 rpm for 7 minutes (≈21 000 g-minutes) in accordance with the manufacturer's instructions for the Alinity assay (Abbott, USA), to ensure complete separation of serum and removal of cellular components (17). Prior to analysis, all serum samples were visually inspected for haemolysis, lipemia, and icterus. Samples exhibiting visible discoloration or turbidity were excluded from testing to avoid analytical interference. The haemolysis, icterus and lipemia (HIL) index detection module was not enabled on the Alinity i analyser, therefore visual inspection served as the preanalytical quality-control step. All biomarker analyses were performed within two hours after centrifugation.
Serum concentrations GFAP and UCH-L1 were quantified using chemiluminescent microparticle immunoassay (CMIA) technology on the Alinity i analyser (Alinity i TBI assay, Abbott, USA). Diagnostic cut-off values were 35 pg/mL for GFAP and 400 pg/mL for UCH-L1, as recommended in the Alinity i TBI assay documentation. Samples exceeding either threshold were interpreted as positive. According to the manufacturer's algorithm, the overall TBI test result was considered positive if either biomarker exceeded its respective threshold (17).

Statistical analysis

Descriptive statistics were expressed as mean ± standard deviation (SD) for continuous variables with normal distribution, and as median with interquartile range (Q1–Q3) for non-normally distributed variables. Categorical data were presented as frequencies and percentages. Differences in the distribution of categorical variables were assessed using Pearson's $\chi 2$ test. Comparisons of continuous variables between two independent groups were performed using the Mann–Whitney U test. Correlations between continuous variables (age, GCS, GFAP, UCH-L1, and time from injury to blood draw) were evaluated using Spearman's rank correlation coefficient (rho). Diagnostic performance of GFAP and UCH-L1 was assessed using receiver operating characteristic (ROC) curve analysis. For predefined decision thresholds, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy were calculated from 2×2 contingency tables using head CT findings as the reference standard. A $p< 0.05$ was considered statistically significant.

RESULTS

A total of 102 patients were enrolled in the study, comprising 76 (75%) males and 26 (25%) females ($p<0.001$), yielding a male-to-female ratio of 2.92. The median age of participants was 49.5 years, with an interquartile range (IQR) of 32.0 to 63.0 years. The youngest patient was 18 years old, the oldest was 87. All patients presented with GCS between 13 and 15 (median 15; IQR: 14–15). Ninety-nine (of 102) patients had blood drawn within 6 hours of injury, and three were sampled after 6 hours. Overall median time from injury to blood draw was 3.0 hours (IQR 2.0–4.0) (Table 1). No statistically significant sex differences were found in any of the continuous variables analysed, including GCS scores, GFAP, and UCH-L1

Table 1. Characteristics of the continuous parameters of the age, GCS, GFAP, UCH-L1 and time from injury to sampling for the entire study population (N=102)

Parameters	Mean (SD)	Min. – Max.	Median	IQR (25%-75%)	
Age (years)	47.77 (18.845)	18-87	49.50	32.00	63.0
GCS	14.60 (0.601)	13-15	15.00	14.00	15.0
GFAP (pg/mL)	1160.14 (4010.33)	8.4-26014.4	114.45	26.27	515.85
UCH-L1 (pg/mL)	2112.90 (3610.94)	112.7-28830.3	797.75	361.0	2168.05
Time* (hours)	3.4412 (1.40419)	2.00-9.00	3.00	2.00	4.00

Time*, interval from injury to blood sampling (hours);
SD, standard deviation; Min.-Max, minimum-maximum; IQR, interquartile range; GSC, Glasgow Coma Scale; GFAP, Glial Fibrillary Acidic Protein; UCH-L1, Ubiquitin Carboxy-terminal Hydrolase L1.

concentrations (p>0.05 for all comparisons). The age showed a positive correlation with GFAP and UCH-L1, but a negative correlation with GSC, GSC was negatively correlated with GFAP, UCH-L1 and time from blood draw; GFAP exhibited a positive correlation with UCH-L1 (Table 2).

Among 102 patients, 58 (57%) had injuries caused by traffic accidents, 31 (30%) were caused by falling, and 13 (13%) had injuries from other causes (fights, occupational) (p<0.001).

Out of a total of 102 patients, 35 (34%) had CT-confirmed mTBI, 67 (66%) had no detectable abnormalities on CT imaging (p=0.002).

Table 2. Correlation between continuous parameters: age, GCS, GFAP, UCH-L1 and time from injury to sampling (N=102)

Parameters		Age (years)	GSC	GFAP (pg/mL)	UCH-L1 (pg/mL)
GSC	rho	-0.474			
	p	<.001			
GFAP (pg/mL)	rho	0.505	-0.679		
	p	<.001	<.001		
UCH-L1 (pg/mL)	rho	0.255	-0.362	0.501	
	p	<.001	<.001	<.001	
Time* (h)	rho	0.107	-0.233	0.159	0.047
	p	0.283	0.018	0.112	0.636

Time*, interval from injury to blood sampling (hours);
GSC, Glasgow Coma Scale; GFAP, Glial Fibrillary Acidic Protein; UCH-L1, Ubiquitin Carboxy-terminal Hydrolase L1.

Biomarker analysis revealed that 80 (78%) patients tested positive for mTBI based on GFAP and/or UCH-L1 results (TBI test), 22 (22%) were negative. A statistically significant association was found between positive biomarker profiles and CT-confirmed injuries (p<0.001). All patients with these CT-detected injuries also had positive TBI test.

The diagnostic performance of GFAP was assessed using ROC curve analysis. At a cutoff of 35.0 pg/mL, GFAP demonstrated 100% sensitivity, 52.24% specificity, and a negative predictive value of 100% for detecting CT-confirmed mTBI (Table 3, Figure1). Although GFAP showed perfect sensitivity, the moderate specificity led to a number of false positives. The positive predictive value was 52.24%, and diagnostic accuracy reached 68.63%. Similarly, UCH-L1 was evaluated using ROC analysis to determine diagnostic effectiveness. At a threshold of

Table 3. Contingency table and performance of serum Glial Fibrillary Acidic Protein (GFAP) Ubiquitin Carboxy-terminal Hydrolase L1 (UCH-L1) in detecting mild traumatic brain injury (mTBI) vs cranial CT

Performance metrics	UCH-L1 positive	UCH-L1 negative	GFAP positive	GFAP negative
CT positive	35	0	35	0
CT negative	40	27	32	35
Sensitivity	100 (90 - 100)		100 (90 - 100)	
Specificity	40.30 (28.49 - 53.0)		52.24 (39.67 - 64.60)	
NPV	100 (87.23 - 100)		100 (90 - 100)	
PPV	46.67 (41.82 - 51.58)		52.24 (45.99 - 58.42)	
Accuracy	60.78 (50.62 - 70.31)		68.63 (58.69 - 77.45)	

Values in the contingency table represent the number of patients (N). GFAP, Glial Fibrillary Acidic Protein; UCH-L1, Ubiquitin Carboxy-terminal Hydrolase L1; NPV, Negative Predictive Value; PPV, Positive Predictive Value

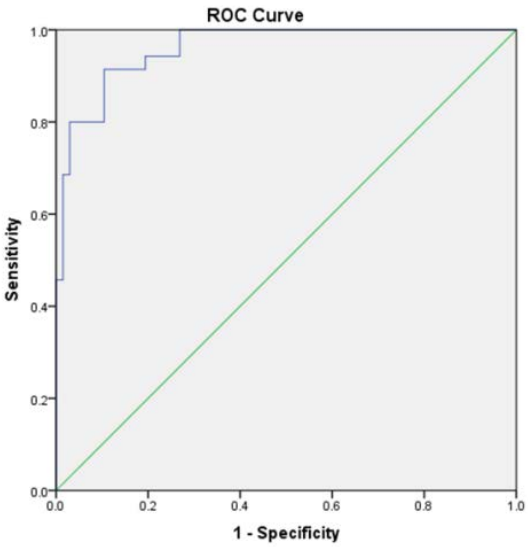

Figure 1. Receiver operating characteristic (ROC) curve showing performance of Glial Fibrillary Acidic Protein (GFAP) in detecting mild traumatic brain injury confirmed by cranial CT

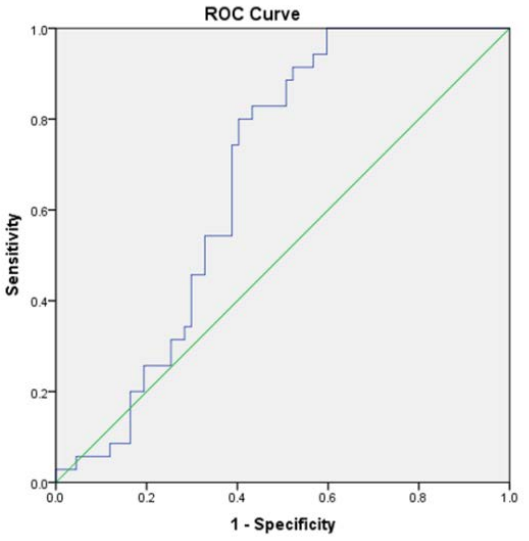

Figure 2. Receiver operating characteristic (ROC) curve showing performance of Ubiquitin Carboxy-terminal Hydrolase L1 (UCH-L1) in detecting traumatic brain injury confirmed by cranial CT

400.0 pg/mL, UCH-L1 achieved 100% sensitivity, 40.30% specificity, and NPV of 100% (Table 3, Figure 2)

DISCUSSION.

mTBI remains a diagnostic challenge because its clinical presentation is often subtle and non-specific, and conventional head CT is frequently normal even in patients with clinically relevant injury (1,5). Despite these limitations, CT has remained the primary imaging modality in routine practice, although its extensive use raises concerns about radiation exposure, overuse of healthcare resources and financial costs (18). These issues have intensified interest in non-invasive alternatives, particularly blood-based biomarkers, among which GFAP and UCH-L1 have emerged as leading candidates for mTBI evaluation (11,19).

In our cohort of 102 patients with mTBI, both GFAP and UCH-L1 were significantly inversely correlated with GCS, consistent with their role as markers of astrocytic and neuronal injury. GFAP showed a strong negative correlation (rho = −0.679, $p < 0.001$) and UCH-L1 a moderate negative correlation with GCS (rho = −0.362, $p < 0.001$), indicating higher biomarker levels in patients with lower consciousness scores. These results are consistent with prospective and multicentre studies reporting increasing GFAP and UCH-L1 concentrations with decreasing GCS and with CT-detectable intracranial lesions, even in clinically classified mTBI (6,11,19,20).

GFAP and UCH-L1 were positively correlated with each other (rho = 0.501, $p < 0.001$), supporting their complementary nature in capturing astroglial (GFAP) and neuronal (UCH-L1) injury, as highlighted in biomarker reviews and clinical validation studies (6,11,19). Correlations between biomarker levels and time from injury were weak, which is likely to reflect the narrow sampling window in our cohort (median 3.0 hours, IQR 2.0–4.0) within the 12-hour timeframe recommended by the manufacturer. This is consistent with kinetic studies showing that GFAP and UCH-L1 rise rapidly after trauma and remain diagnostically informative during the early post-injury phase, so variation with sampling time is limited when blood is drawn within the first hours after injury (6,19,20). Taken together, the strong correlations with GCS and minimal dependence on sampling time suggest that acute GFAP and UCH-L1 levels primarily reflect the burden of neuronal and astrocytic injury rather than the exact timing of blood sampling within this early window, supporting their potential use to identify patients with clinically relevant brain injury despite mild symptoms and to guide more selective use of head CT imaging (6,11,19,20).

In our single-centre study at UCC Tuzla, both GFAP and UCH-L1 demonstrated excellent diagnostic sensitivity (100%) and a negative predictive value of 100% for CT-positive intracranial lesions, supporting their use as rule-out tools. However, specificity was only moderate (52.24% for GFAP and 40.30% for UCH-L1), resulting in many biomarker-positive but CT-negative patients. A similar pattern was reported in the large multicentre ALERT-TBI trial, where combined GFAP/UCH-L1 testing with predefined, lower cut-off values (22 pg/mL and 327 pg/mL, respectively) in 1959 adults with TBI achieved high sensitivity (97.6%) and NPV (99.6%), but only modest specificity (11). Unlike ALERT-TBI, which included a broader spectrum of TBI severity, our study focused exclusively on adults with mTBI within 12 hours of trauma and applied higher decision limits (GFAP 35 pg/mL; UCH-L1 400 pg/mL). Across these methodological differences, the consistent pattern of very high sensitivity and NPV but limited specificity indicates that GFAP and UCH-L1 are particularly suited for ruling out clinically significant intracranial lesions, whereas positive results should be interpreted cautiously and always in conjunction with clinical assessment and neuroimaging.

Recent clinical and observational studies, together with meta-analyses, have further clarified the role of GFAP and UCH-L1 in mTBI, consistently showing higher biomarker concentrations in head-injured patients than in controls, with the greatest increases in those with CT- or MR-confirmed lesions and generally better discrimination for GFAP than for UCH-L1 (6,14,21–23). European cohorts of patients treated in hospital emergency departments, together with studies using rapid testing platforms, have additionally shown that combined measurement of these biomarkers provides high negative predictive value and supports early risk stratification in routine care (14,22–25). Contemporary reviews also note that including patients with orthopaedic or other extracranial injuries may reduce specificity, since mild biomarker elevations can occur without intracranial damage. However, such populations reflect real-world trauma practice, and available data indicate that the high NPV of GFAP and UCH-L1 for safely excluding clinically significant intracranial lesions is preserved (23–26).

Although the findings of this study are encouraging, several limitations should be acknowledged. It was a single-centre study with a relatively small sample, especially in the CT-positive group, which limits the precision and generalisability of the results. Major confounders such as polytrauma, anticoagulant therapy, known neurological disease and clinically significant intoxication were excluded, but patients with isolated orthopaedic or other extracranial injuries were included. These concomitant injuries may have modestly increased GFAP and UCH-L1 concentrations and contributed to the moderate specificity and the number of patients with elevated biomarker levels but no CT-detectable intracranial lesions. As brain MRI was not performed, we cannot determine whether such discordant results represent true false positives relative to CT or intracranial injuries that remain undetectable on CT. The study was not powered to compare biomarker performance between isolated head injury and additional extracranial trauma or to perform robust analyses by lesion type or prognostic outcome. Larger multicentre studies with integration of MRI findings and more detailed control of extracranial injuries and other confounders are needed to refine decision thresholds and to define the clinical scenarios in which these biomarkers provide the greatest benefit. Despite these limitations, this single-centre mTBI study showed that GFAP and UCH-L1 achieved excellent diagnostic sensitivity and a negative predictive value of 100% for excluding CT-positive intracranial lesions, supporting their use as rule-out triage tools to safely reduce unnecessary cranial CT in selected patients. However, their moderate specificity means that positive biomarker results should always be interpreted in conjunction with clinical assessment and neuroimaging, and further multicentre validation is required to better define the role of GFAP and UCH-L1 in mTBI management.

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TRANSPARENCY DECLARATION

Competing interest: None to declare.

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Med Glas (Zenica)

ORIGINAL ARTICLE

Minimal clinically important difference (MCID)-based analysis of single- versus two-level laminectomy for lumbar spinal stenosis: a prospective study

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ABSTRACT

Aim To compare outcomes and minimal clinically important differences (MCID) between single-level laminectomy (SLL) and two-level laminectomy (TLL) for lumbar spinal stenosis (LSS).
Methods This prospective study included 119 patients with confirmed LSS who underwent SLL or TLL at Cantonal Hospital Zenica from January 2018 to January 2025. Assessments were performed preoperatively and at 1 and 6 months postoperatively using the Oswestry Disability Index (ODI), Swiss Spinal Stenosis Questionnaire (SSSQ), and Visual Analogue Scale (VAS) for back and leg pain, along with evaluation of motor, sensory, and urinary function. MCID values were used to assess meaningful improvement.
Results At six months, SLL patients had lower ODI (15.0 vs. 18.0; $p = 0.006$), VAS-LB (3.0 vs. 5.0; $p < 0.001$), and SSSQ score (17.2 vs. 21.6; $p < 0.001$) comparing to TLL patients. Motor deficits (14.8% vs. 35.5%; $p = 0.019$) and urinary dysfunction at 1 month (5.7% vs. 22.6%; $p = 0.013$) were less frequent in SLL. More SLL patients achieved MCID for ODI (80.7% vs. 58.1%; $p = 0.024$) and SSSQ (73.9% vs. 48.4%; $p = 0.017$) at 6 months, with TLL patients 28% and 34% less likely to reach MCID for ODI and SSSQ, respectively. No significant differences were found preoperatively or at 1 month.
Conclusion The study suggests that SLL and TLL have comparable outcome, with a slight tendency toward better functional improvement and fewer deficits after SLL.
Keywords: degenerative spinal stenosis, disability evaluation, nerve root compression, outcome assessment

INTRODUCTION

Lumbar spinal stenosis (LSS) is the narrowing of the lumbar spinal canal, lateral recess, or neural foramina, either congenitally or due to degenerative spondylosis (1). Aging, chronic wear, and trauma contribute to intervertebral disc degeneration, leading to posterior protrusion, osteophyte formation, facet hypertrophy, synovial cysts, and *ligamentum flavum* thickening (2). According to Framingham study 19.4% of individuals aged 60–69 had a spinal canal diameter <10 mm (3). This condition significantly impacts patients' quality of life by often restricting mobility and everyday activities (4). The LSS most commonly presents with neurogenic claudication, typically involving a single spinal level. Multi-level involvement is considerably rare, resulting in a limited number of studies specifically addressing multi-level LSS (5).

The assessment of LSS relies on clinical, radiological, and self-reported evaluations, providing a foundation for long-term patient monitoring (1). To standardize the quantification of clinical status, Jaeschke originally defined the minimal clinically important difference (MCID) as the smallest score change perceived as beneficial by patients, warranting therapeutic modification in the absence of significant adverse effects or excessive costs (6). Over time, this concept has been refined to encompass the minimal clinically meaningful score change, the threshold risk reduction necessary to justify treatment, or the mean score difference between cohorts with optimal and suboptimal outcomes (7). Despite the established role of MCID in evaluating treatment efficacy, research comparing the outcome of single-level laminectomy (SLL) versus two-level laminectomy (TLL) for LSS remains limited. While MCID-based assessments have been conducted in LSS populations, no prior studies have specifically examined MCID-based outcomes in the context of SLL versus TLL.

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The aim of this study was to evaluate and compare clinical outcome following single-level laminectomy (SLL) versus two-level laminectomy (TLL) in patients with lumbar spinal stenosis (LSS). Additionally, the study seeks to conduct a minimal clinically important difference (MCID)-based analysis to determine whether the perceived benefits of surgical intervention differ between cases involving compression of a single nerve root versus compression of two nerve roots.

PATIENTS AND METHODS

Patients and study design

This prospective study was conducted at the Neurosurgery Department of Cantonal Hospital Zenica from January 2018 to January 2025. A total of 119 consecutive patients meeting the inclusion criteria were enrolled, all with radiologically and clinically confirmed degenerative LSS, treated surgically with SLL or TLL. Exclusion criteria encompassed congenital LSS, involvement of more than two levels, coexisting disc herniation, spondylolisthesis (spinal segmental instability), spinal trauma, spinal tumours, and a history of lumbar spine surgery. Patients were divided into two groups: those who underwent SLL and those who underwent TLL.

All included patients provided signed informed consent.

Ethical approval was obtained from the Ethics Committee of Cantonal Hospital Zenica, Bosnia and Herzegovina.

Methods

Demographic data on age and sex were extracted from medical records. All patients underwent lumbar spine MRI (1.5 T Magnetom Avanto, Siemens, Germany) to determine the affected vertebral level and grade LSS using the Schizas classification (8): A - no or minor stenosis with visible cerebrospinal fluid (CSF), B - moderate stenosis with identifiable rootlets filling the dural sac, C - severe stenosis without visible rootlets and a homogeneous gray dural sac signal, while posterior epidural fat remains present, D - complete absence of identifiable rootlets and posterior epidural fat. The MRI was used to verify the type of spinal stenosis, distinguishing central canal stenosis, lateral recess stenosis, and foraminal stenosis.

Preoperatively, the patients completed the Oswestry Disability Index (ODI), Swiss Spinal Stenosis Questionnaire (SSSQ), and Visual Analogue Scale (VAS) to assess functional impairment and pain severity. The ODI, consisting of ten sections, evaluated disability related to lower back pain, with scores ranging from 0 (no disability) to 100 (maximum disability) (9). The SSSQ included 12 items, with symptom severity and physical function subscales, yielding a total score between 12 and 55, where higher values indicate more severe symptoms (10). Pain intensity in the lower back (VAS-LB) and lower extremities (VAS-LE) was measured using the VAS, scored from 0 (no pain) to 10 (worst pain) (11).

Neurological assessment included an evaluation of motor deficit using the Medical Research Council (MRC) muscle power scale (12): 0 – no visible contraction, 1 – minimal visible contraction, 2 – movement without overcoming gravity, 3 – active movement overcoming gravity, 4 – movement against some resistance, and 5 – normal strength. Patients with an MRC score <5 were classified as having a motor deficit. Sensory function was assessed using the Sensory Assessment Scale (SAS) for L1-S3 dermatomes, scored as 0 – absent, 1 – reduced, and 2 – normal (12, 13). A SAS score <2 indicated sensory impairment. Urinary function was evaluated on a scale of 0 – complete dysfunction, 1 – partial dysfunction, and 2 – normal function (12), with score <2 indicating urinary dysfunction.

The indication for surgical intervention was the presence of neurogenic claudication accompanied by MRI-confirmed lumbar spinal stenosis classified as Schizas grade D (Figure 1A and 1B) or grade C (Figure 1D and 1E). The surgical procedure consisted of laminectomy with removal of the spinous process and flavectomy, aiming to achieve adequate decompression of the central dural sac.

Postoperative follow-up was conducted at one (±5 days) and six (±15 days) months, assessing ODI, SSSQ, VAS-LB, VAS-

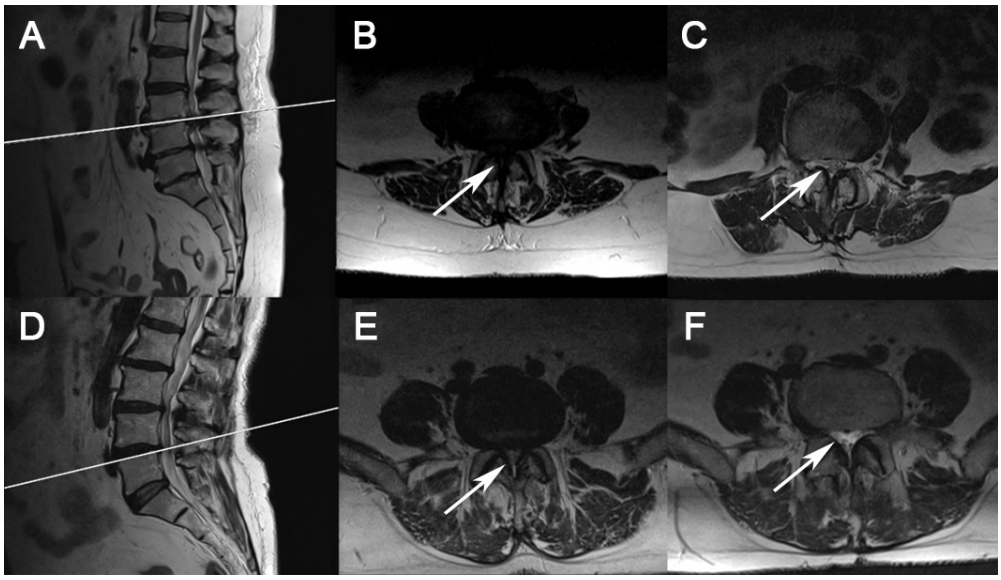

Figure 1. Preoperative and postoperative MRI of the lumbar spine. Patient 1. A) Sagittal T2-weighted MRI showing lumbar spinal stenosis (LSS) at L3/L4 and L4/L5, treated with two-level laminectomy; B) preoperative axial MRI demonstrating Schizas grade D stenosis (arrow); C) postoperative axial MRI at 6 months showing restored rootlets and cerebrospinal fluid space (arrow). Patient 2. D) sagittal MRI of a patient with LSS at L4/L5; E) axial MRI demonstrating Schizas grade C stenosis (arrow); F) postoperative axial MRI at 6 months showing successful decompression with visible rootlets and cerebrospinal fluid space (arrow).

LE, as well as motor, sensory, and urinary function. The average hospitalization duration was three days, during which wound status and signs of infection were monitored. Wound re-evaluation was performed on the 10th postoperative day. The MCID was defined as a change of -10.8 for ODI (14), -5.8 for SSSQ (14), and -3.0 for both VAS-LB and VAS-LE (15), calculated for the intervals from preoperative assessment to 1-month postoperative and preoperative to six-month postoperative. Postoperative evaluation included follow-up MRI of the lumbosacral spine at six months to assess the extent and adequacy of decompression (Figure 1C, F).

Statistical analysis

Continuous variables were presented as median with interquartile range (IQR), based on distribution normality assessed using the Kolmogorov-Smirnov test, while categorical variables were expressed as frequency (N) and percentage (%). Group differences were analysed using the Mann-Whitney U test for continuous variables and Pearson's χ^2 test for categorical variables. For the MCID-based analysis, relative risk (RR) with corresponding 95% confidence intervals (CI) was calculated in the context of TLL versus SLL. Statistical significance was set at $p<0.05$.

RESULTS

The median age was 54 years (IQR: 42.5–61.0) in the SLL group and 56 years (IQR: 44.0–62.0) in the TLL group (p=0.524). The SLL group included 39 males (44.3%) and 49 females (55.7%), while the TLL group had 15 males (48.4%) and 16 females (51.6%) (p=0.445). Central canal stenosis was present in 54 (61.4%) SLL and 18 (58.1%) TLL patients (p=0.741), lateral recess stenosis in 76 (86.4%) and 27 (87.1%) (p=0.913, and foraminal stenosis in 56 (63.6%) and 20 (64.5%) patients, respectively (p=0.921). Schizas grade C occurred in 54 (61.4%) SLL and 15 (48.4%) TLL patients, and grade D in 34 (38.6%) and 16 (51.6%) patients, respectively (p=0.307) (Table 1). Among 103 SLL patients (Figure 2A), the most commonly treated levels were L3 (N=31; 30.1%) and L4 (N=30; 29.1%). In 33 TLL patients (Figure 2B), the most frequently decompressed levels were L3–L4 (N=13; 41.9%) and L4–L5 (N=9; 29.0%).

The ODI scores were lower in the SLL comparing to TLL group at one month, 34.0 vs. 39.0 (p=0.010), and at six months, 15.0 vs. 18.0 (p=0.006). The VAS-LB was lower at six months in SLL patients, 3.0 vs. 5.0 (p<0.001). The SSSQ scores were lower in the SLL comparing to TLL group preoperatively, 37 vs. 44 (p=0.005), at one month, 24.5 vs 30.8 (p<0.001), and at six months, 17.2 vs. 21.6 (p<0.001). Motor deficits were more frequent in TLL comparing to SLL patients at six months, 35.5% vs. 14.8% (p=0.019), and urinary dysfunction was higher at one month, 22.6% vs. 5.7% (p=0.013) (Table 2).

A higher number of patients who underwent SLL achieved MCID for ODI at six months, 71 (80.7%), compared to those with TLL, 18 (58.1%) (p = 0.024). Similarly, MCID for SSSQ at six months was more frequently reached in the SLL group, 65 (73.9%), than in the TLL group, 15 (48.4%) (p=0.017). Patients who underwent TLL were 28% less likely to achieve MCID for ODI at six months (RR=0.72; 95% CI: 0.52–0.99) and 34% less likely to achieve MCID for SSSQ at six months (RR=0.66; 95% CI: 0.45–0.96) compared to those who underwent SLL (Table 3).

Table 1. Characteristics of the patients with single-level laminectomy (SLL) and two-level laminectomy (TLL)

Variable	SLL (N=88)	TLL (N=31)	p
Median age (IQR) (years)	54 (42.5-61.0)	56 (44.0-62.0)	0.524
	No (%) of patients		
Sex			0.445
Male	39 (44.3)	15 (48.4)	
Female	49 (55.7)	16 (51.6)	
Type of stenosis			
Central canal	54 (61.4)	18 (58.1)	0.741
Lateral recess	76 (86.4)	27 (87.1)	0.913
Foraminal	56 (63.6)	20 (64.5)	0.921
Schizas grade			0.307
C	54 (61.4)	15 (48.4)	
D	34 (38.6)	16 (51.6)	

IQR, interquartile range;

DISCUSSION

To our knowledge, this is the first study comparing MCID achievement between SLL and TLL, indicating better functional improvement and symptom relief in patients with single level LSS.

A significant finding indicating better six-month outcomes is that 80.7% of patients in the SLL group achieved ODI-based MCID, compared to 58.1% in the TLL group. These results

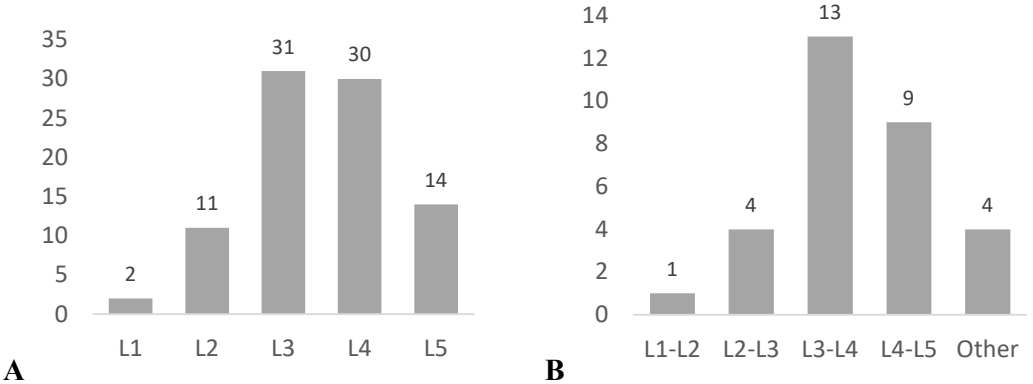

Figure 2. Distribution of A) single-level (SLL) and B) two-level laminectomies (TLL) by vertebral region

Table 2. Results of observed variables preoperatively, at 1-month and 6-month follow-up

Variable	Follow-up period	SLL (N=88)	TLL (N=31)	p
		Median (IQR)		
ODI	Preoperative	52.0 (41.0-62.0)	52.0 (41.0-65.0)	0.824
	1 month	34.0 (28.0-41.0)	39.0 (30.0-45.5)	0.010
	6 months	15.0 (11.0-17.0)	18.0 (12.0-22.0)	0.006
VAS-LB	Preoperative	7.0 (5.0-7.0)	8.0 (5.0-10.0)	0.039
	1 month	5.0 (3.0-7.0)	6.0 (4.0-8.0)	0.058
	6 months	3.0 (2.0-6.0)	5.0 (3.0-8.0)	<0.001
VAS-LE	Preoperative	8.0 (6.0-8.0)	8.0 (6.0-10.0)	0.481
	1 month	5.0 (3.5-8.0)	6.0 (4.0-9.0)	0.107
	6 months	3.0 (2.0-6.0)	4.0 (3.0-7.0)	0.058
SSSQ	Preoperative	37 (25-48)	44 (34-48)	0.005
	1 month	24.5 (17.5-31.2)	30.8 (23.8-33.6)	<0.001
	6 months	17.2 (12.3-21.8)	21.6 (16.7-23.5)	<0.001
		No (%) of patients		
Motor deficit	Preoperative	36 (40.9)	15 (48.4)	0.529
	1 month	21 (23.9)	13 (41.1)	0.067
	6 months	13 (14.8)	11 (35.5)	0.019
Sensitive deficit	Preoperative	74 (84.1)	25 (80.6)	0.780
	1 month	51 (59.0)	22 (70.1)	0.286
	6 months	28 (31.8)	11 (35.5)	0.826
Urinary dysfunction	Preoperative	9 (10.2)	8 (25.8)	0.069
	1 month	5 (5.7)	7 (22.6)	0.013
	6 months	4 (4.6)	4 (12.9)	0.203
Surgical wound infection	6 months	3 (3.4)	1 (3.2)	0.981
Revision	6 months	4 (4.5)	5 (16.1)	0.051

SLL, single-level laminectomy; TLL, two-level laminectomy; IQR, interquartile range; ODI, Oswestry disability index; VAS-LB, visual analogue scale for lower back; VAS-LE, visual analogue scale for lower extremities; SSSQ, Swiss spinal stenosis questionnaire

Table 3. Minimal clinically important difference (MCID) of perceived benefits of surgical intervention between patients with single-level laminectomy (SLL) versus two-level laminectomy (TLL)

Variable	Preoperative follow-up period	No (%) of patients MCID		p	Risk ratio (95% CI)
		SLL (N=88)	TLL (N=31)		
ODI	1 month	39 (44.3)	13 (41.9)	0.984	0.95 (0.59-1.52)
	6 months	71 (80.7)	18 (58.1)	0.024	0.72 (0.52-0.99)
VAS-LB	1 month	58 (65.9)	17 (54.8)	0.378	0.83 (0.58-1.18)
	6 months	74 (84.1)	26 (83.9)	0.999	1.00 (0.83-1.19)
VAS-LE	1 month	58 (65.9)	14 (45.2)	0.069	0.69 (0.45-1.04)
	6 months	69 (78.4)	27 (87.1)	0.430	1.11 (0.93-1.32)
SSSQ	1 month	43 (48.9)	11 (35.5)	0.281	0.73 (0.43-1.22)
	6 months	65 (73.9)	15 (48.4)	0.017	0.66 (0.45-0.96)

CI, confidence interval; ODI, Oswestry disability index; VAS-LB, visual analogue scale for lower back; VAS-LE, visual analogue scale for lower extremities; SSSQ, Swiss spinal stenosis questionnaire

are consistent with a previous study (16), which demonstrated superior recovery in terms of ODI and VAS scores, as well as walking distance, among LSS patients treated with SLL versus TLL. It was confirmed that single-level treatment of LSS compared to multilevel treatment yields significantly better favourable outcome for patients (17). It is crucial to differentiate between studies where treatment was performed at a different level once "moderate" stenosis is confirmed. The better outcome in our study likely stem from the indication for surgery being MRI-verified Schizas C or D grade with corresponding imaging, limiting comparability. This is supported by the fact that patients' clinical presentation preoperatively was significantly more severe, as indicated by VAS-LB and SSSQ score. Similarly, it was reported that patients undergoing TLL had significantly more severe symptoms before surgery (18).

Findings from previous studies remain inconsistent. Some reports suggest that the number of operated levels does not significantly influence surgical outcome (19), while others indicate that multilevel surgery in LSS patients results in similarly favourable outcome and complication rate as single-level procedures (20). A recent meta-analysis showed that decompression involving two or more levels produced postoperative results comparable to single-level decompression, suggesting it may be a non-inferior approach (21). In the present study, MCID analysis showed a trend toward more favourable postoperative outcome in patients who underwent single-level lumbar decompression, which may be related to compression involving only a single nerve root and a less severe clinical presentation. Previous research suggests that patients with a longer duration of LSS-related pain may experience poorer outcome, and that demographic, health, and clinical factors are more predictive of clinical results than surgery-related factors (22). In some cases, decompression at two levels has been associated with greater clinical improvement at six months, with significant benefit achieved in around 60% of cases and MCID reached in 77.6% of patients (23). However, excessive surgical intervention, particularly beyond two levels, may compromise spinal stability and negatively affect long-term recovery (24). Patient selection should therefore consider both the severity of stenosis and the risk of postoperative complications (23).

Future research should standardize MCID assessment in LSS, as variations in definition and reporting limit comparability. Differences may arise from baseline severity, patient expectations, and methodological inconsistencies (25,26). Establishing uniform criteria could improve outcome evaluation and surgical decision-making, especially when comparing single- and multilevel decompression (27).

This study has several limitations. The short follow-up period may not adequately assess long-term functional outcomes, the potential for symptom recurrence, or the need for reoperation. Additionally, the absence of standardized MCID thresholds for LSS complicates cross-study comparisons and may impact the interpretation of clinical improvement.

In conclusion, this study suggests that SLL may be associated with higher MCID achievement rates compared to TLL, potentially reflecting better functional improvement and symptom relief. These findings raise the possibility that two-level compression could influence surgical outcome, perhaps contributing to a more complex disease course and less favourable postoperative recovery. However, due to variability in MCID thresholds and the relatively short fol-

low-up period, further studies using standardized outcome measures and longer-term data are needed to better understand these associations and assess the durability of surgical benefits.

AUTHOR CONTRIBUTIONS

Conceptualization, E.B and H.B.; methodology, E.B., H.B. and G.L.; software, E.B.; validation, F.A. and A.M.; formal analysis, E.B.; investigation, E.B., H.B., F.A., A.M.; resources, E.B.; data curation, E.B.; writing—original draft preparation, E.B., F.A., A.M. and M.H.; writing—review and editing, H.B. and G.L.; visualization, E.B.; supervision, H.B., F.A. and G.L.; project administration, E.B.; All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

Von Mises stress analyses using finite element model in two patients with chronic low back pain

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ABSTRACT

Aim To investigate biomechanical properties of low back pain, which is a pervasive issue with a profound impact on an individual's quality of life due to pain and daily activity limitations.

Methods Two female patients suffering from chronic low back pain were presented. Patient 1 had severe compression fractures at L2 and L4, while Patient 2 had degenerative lumbar scoliosis due to L4-5 disc degeneration. We utilized Finite Element Analysis based on patient-specific CT scan data of lumbosacral 3D reconstruction to quantify the stress intensity as a measurement, known as Von Mises Stress. The reconstruction of the data was performed using specialized software, and simulations were conducted using Ansys 2020. To validate our results, we compared them to previous simulations.

Results Patient 1 exhibited the highest Von Mises stress in the annulus fibrosus at L2-L3 during axial rotation (84.168 MPa) and in the nucleus pulposus at L2-3 during anterior flexion (16.722 MPa). Patient 2 displayed the highest Von Mises stress both in the annulus fibrosus and nucleus pulposus at L1-L2 during axial rotation (0.515 MPa and 0.0594 MPa, respectively).

Conclusion Our findings can help identify the segments at the highest risk for developing lumbar spondylosis and disc degenerative disorders by quantifying Von Mises stress in the lumbosacral region.

Keywords: disc degeneration; finite element analysis; low back pain; lumbosacral spondylosis.

INTRODUCTION

Lumbosacral spine is referred to as the lower segment of the spinal column, consisting of the lumbar vertebrae (L1-L5) followed by the sacrum (S1). The intervertebral discs, which consist of the annulus fibrosus and the nucleus pulposus, are located between each adjacent vertebrae. Spine comprises complex structures such as vertebrae, intervertebral discs, ligaments, and muscles. Degeneration can occur in the intervertebral discs, which can cause pain in the lower back (1,2).

Low back pain (LBP) represents a prevalent issue that significantly impacts quality of life. It is primarily attributed to the considerable discomfort experienced in the lower back, especially the lumbosacral region, and the subsequent impairment in carrying out routine daily activities. Lumbar disc degeneration is a progressive disease that alters the geometric morphology and biomechanical properties, affecting the transmission and allocation of the lumbar spine (2,3). Lumbar disc degeneration can be caused by age, gender, body mass index (BMI), and lumbar load (3). The most common cause of LBP in older individuals is attributed to degenerative processes affecting various components of the spinal column, including each spinal segment, facet joints, intervertebral discs, and the supporting muscles and ligaments of the vertebral column. The degenerative process is caused by compressive forces, commonly known as axial loading, acting upon each segment of the spinal column and intervertebral disc (2,3,4). Understanding the underlying mechanism of lumbar disc degeneration that causes pain is essential when analysing the basic principles of spine biomechanics, developing new surgical methods, and identifying optimal treatment options (1,5).

Numerous analyses of in vitro experiments have been conducted to comprehend the impact of spine biomechanics in disc degeneration. These experiments include bony stress (6), lumbar spine range of motion (ROM) (7), intradiscal pressure

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(8), and lumbar spine strain rate (9). However, studies relying on experiments are not sufficient, as such tests depend on the availability of specimens and cannot adequately control for different disc degenerative changes. Identifying mechanical differences caused by intervertebral disc degeneration can be simplified and made more accurate using finite element modelling (10-12). By determining the magnitude of von Mises stress obtained in each lumbosacral segment, we can estimate which segments are at the highest risk and can develop into lumbar spondylosis or further disc degenerative disorders.

The aim of this study was to understand the biomechanical properties of lumbar spine in patients suffering from low back pain by determining the von Mises stress using finite element modelling.

PATIENTS AND METHODS

Patients and study design

Two patients who experienced low back pain (LBP) attended to Sultan Agung Hospital in 2022 were presented. Patient 1 is a 62-year-old female who suffered a severe old compression fracture of the second lumbar (L2) and a mild compression fracture of the fourth lumbar (L4) disc. Patient 2 is a 46-year-old female who suffered degenerative lumbar scoliosis due to intervertebral disc L4-5 degeneration.

Both patients underwent 3D reconstruction lumbosacral CT scan and the obtained output was saved as DICOM (Digital Imaging and Communications in Medicine) file. The measured anthropometric parameters were lumbar lordosis, ventral disc height, and dorsal disc height (13). Lumbar lordosis angle was measured by Cobb's method by forming two lines (Figure 1A), i.e. the line drawn perpendicular to lumbar vertebra one upper plate and lumbar vertebra five endplate perpendicular line (14, 15). Ventral disc height (VDH) and dorsal disc height (DDH) (Figure 1B) are the distance between the ventral and dorsal ends of the lumbar disc (13). Measurement was done using mimics 21.0 software with the measure features.

The geometry model was produced from the CT scan data in the form of a DICOM file which then entered the Mimics 21.0 software (Figure 1C and 1D) for the lumbar vertebral bone segmentation process and the smoothing model process, so that the finished model was entered into the Geomagic Studio 12.0 software for the surfacing process (Figure 1E and 1F). SolidWorks 2018 and Design Modeller software were used for the process of making intervertebral discs. For meshing and simulation (Figure 1G and 1H), ANSYS 2020 R1 software was used (2,10,14,16-17).

Methods

The simulation of loading on the first lumbar to the first sacral began by conducting a meshing convergence study. The model lumbar one to sacral one used in this convergence study contained 31 models consisting of six cortical bone, five cancellous bone, five upper endplate, five lower endplate, five annulus fibrosus, and five nucleus pulposus. The element size used in this simulation refers to literature studies that have been carried out and follow research conducted by Kang et al. (18). Material properties on each lumbar vertebral component were from Kang et al. previous research (18). Following that, each

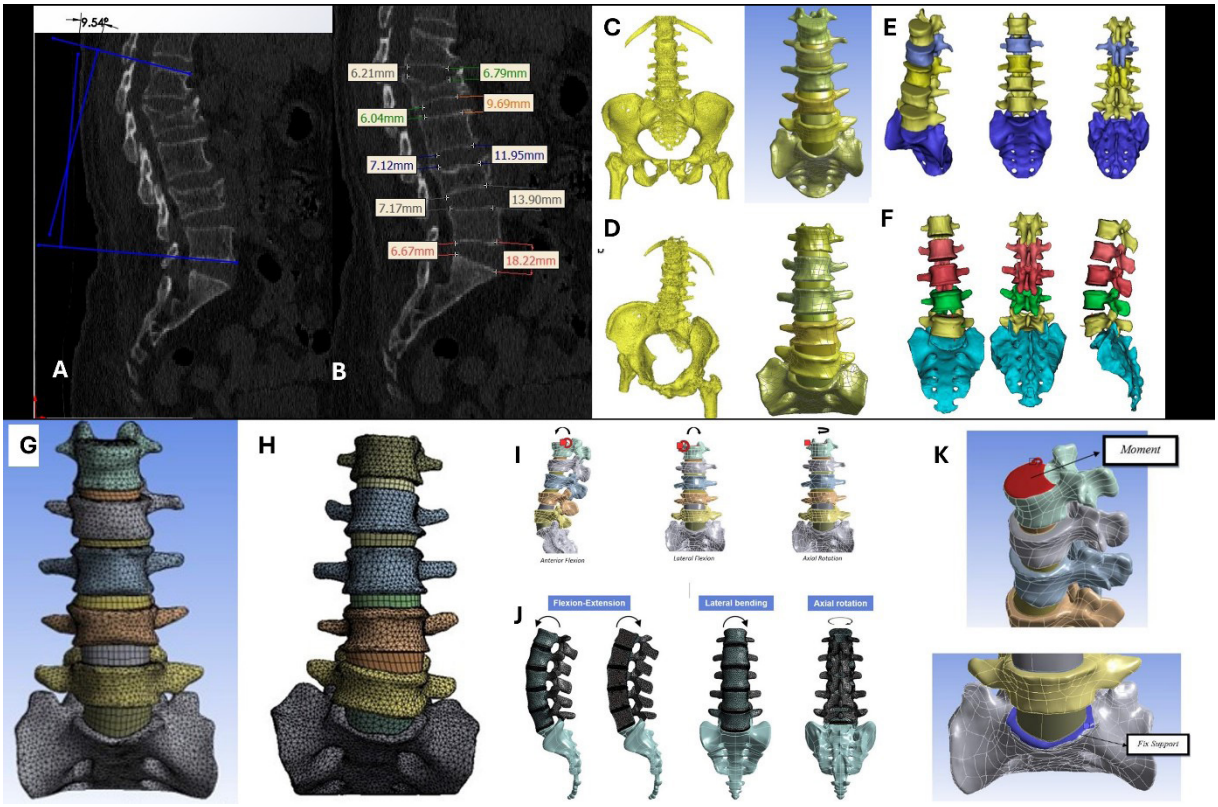

Figure 1. A) Measurement of lumbar lordosis angle; B) Ventral and dorsal disc height measurement; C) Convert geometry model and segmentation process using Mimics 21.0 software in patient 1; D) Convert geometry model and segmentation process using Mimics 21.0 software in patient 2; E) Smoothing and finalization using Geomagic Studio 12.0 software in patient 1; F) Smoothing and finalization using Geomagic Studio 12.0 software in patient 2; G) Meshing process using ANSYS 2020 R1 in patient 1; H) Meshing process using ANSYS 2020 R1 in patient 2; I) Simulation with several loading directions using ANSYS 2020 R1 in patient 1; J) Simulation with several loading directions using ANSYS 2020 R1 in patient 2; K) Moment and fix support location

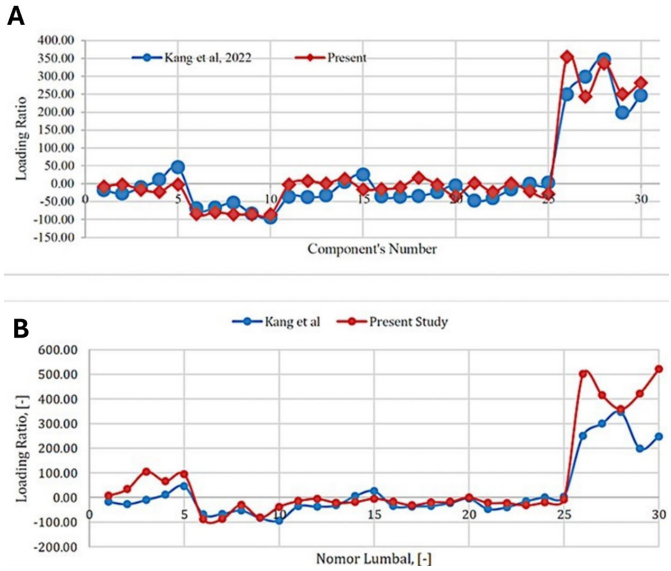

Figure 2. Validation by comparing the result of simulation with Kang et.al simulation. A) showing result for patient 1; B) showing result for patient 2

spinal component was modelled as an isotropic linear-elastic material defined by Young's modulus (representing stiffness) and Poisson's ratio (representing lateral deformation during axial loading) (18). For the validation process, material properties of both normal and osteoporotic bones were used; however, for the subsequent analyses in this study, only the material properties of normal bones were applied.

The loading ratio is a calculation of the ratio of changes in the von Mises stress value between normal bone conditions and bone conditions that experience osteoporosis. The loading ratio calculation was carried out with the formula (A-B)/B x 100%, where A and B stand for the condition of osteoporotic bones and normal bones, respectively (Figure 2).

The load given to the lumbar vertebrae was adjusted to the basic motion in the human lumbar vertebrae (Figure 1I and 1J), namely anterior flexion-extension, lateral bending, and axial rotation (18). The sacrum bone affords fixed support while the upper surface of the lumbar vertebral one cortical bone receives load in the form of motion (Figure 1K).

Statistical analysis

The validation process of the simulation results was determined by comparing the loading ratio findings of the author's simulation with the simulation findings carried out by Kang et al. (18). We used the material properties of each lumbar spine component based on the previous study by Kang et al. (18) (Table 1). Validation using geometric parameters could not be performed because the initial CT data used to reconstruct each lumbosacral segment were obtained from scans conducted at Sultan Agung Islamic Hospital, whereas Kang et al. used a different CT dataset. The original geometric data (e.g., exact vertebral and disc dimensions) from Kang et al. were not available for direct comparison; therefore, one-to-one geometric validation between the two models was not feasible.

Table 1. Material properties of lumbar vertebral component

Components of the lumbar vertebrae	Modulus young (MPA)		Rasio poisson (-)	
	Normal	Osteoporosis	Normal	Osteoporosis
Cortical Bone	12000	8040	0.3	0.3
Cancellous Bone	200	34	0.25	0.25
Endplate	1000	670	0.3	0.3
Nucleus Pulposus	1	9	0.49	0.4
Annulus Fibrosus	4.2	5	0.45	0.45

RESULTS

Based on the lumbar lordotic angle measured using Cobb's method, Patient 1 had a lumbar lordosis of 9.54°, whereas Patient 2 had a lumbar lordosis of 26.50° (Table 2). The loading ratio data obtained from the simulation results of the first lumbar bone to the first sacral bone showed the same trend as the loading ratio reported by Kang et al. (18) (Figure 2).

Computer simulations were performed on both patients from vertebra lumbar one to sacral one with three different loading conditions, with a moment of 400 Nm on the upper surface of lumbar one. Von Mises stress was calculated from the computer simulation result on vertebral body (Figure 3A and 3D), annulus fibrosus (Figure 3B and 3E) and nucleus pulposus (Figure 3C and 3F), respectively.

Table 2. Disc height measurement

Vertebra lumbal	Ventral Disc Height (VDH) (mm)		Dorsal Disc Height (DDH) (mm)	
	Patient 1	Patient 2	Patient 1	Patient 2
L1 – L2	6.79	7.53	6.21	3.22
L2 – L3	9.69	8.32	6.04	3.32
L3 – L4	11.95	8.46	7.12	3.84
L4 – L5	13.90	13.35	7.17	6.01
L5 – S1	18.22	12.06	6.67	5.03

Simulation of Patient 1

Through variations in loading conditions, different maximum points of von Mises stress occurred in the modelling of the lumbar one to sacral one. Under anterior flexion and lateral flexion loading conditions, the maximum von Mises stress point occurred in the upper endplate component of L3-L4 with the von Mises stress value of 391.16 MPa. Under axial rotation loading conditions, the cortical bone component had the highest value of von Mises stress in 172.52 MPa.

The minimum von Mises stress points in the various loading conditions of the modelling of lumbar one to sacral one all occurred in the nucleus pulposus component of L5-S1. Under anterior flexion loading variation, the von Mises stress value in the nucleus pulposus component of L5-S1 is 0.88315 MPa. Under axial rotation and lateral flexion loading variations, the von Mises stress values were 5.4451 MPa and 2.0629 MPa, respectively.

In this study, a three-dimensional simulation was performed to visualise the distribution of von Mises stress on the surfaces of the cortical bone, annulus fibrosus, and nucleus pulposus. The distribution of von Mises stress on the cortical bone surface was evenly spread without any region of stress concentrated in the central area of the cortical bone. The colour contours depicted the formations resulting from different loading conditions on the intervertebral disc, which consists of the annulus fibrosus and nucleus pulposus models. In the nucleus pulposus model with lateral flexion loading variations, it is evident

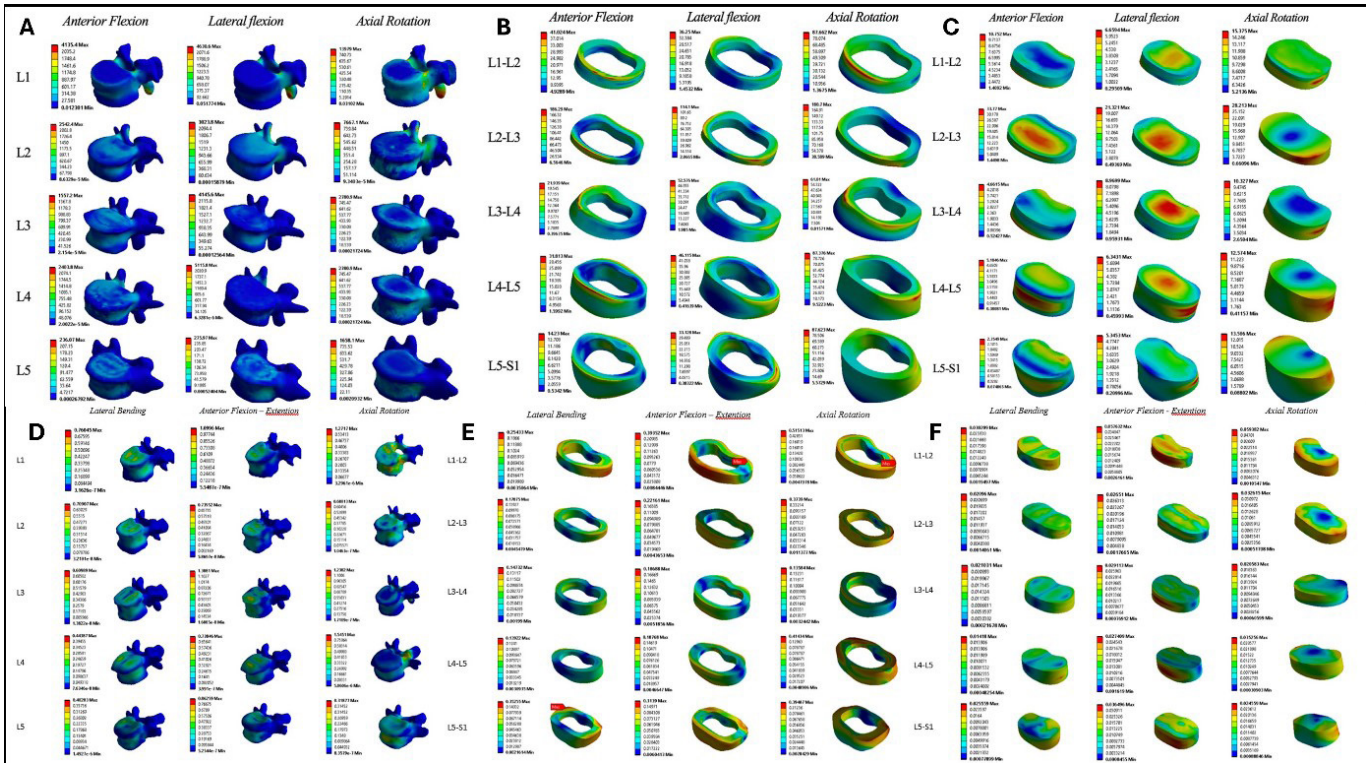

Figure 3. Von Mises stress in patient 1 and 2. A) Von Mises stress of vertebral body patient 1; B) Von Mises stress of annulus fibrosus patient 1; C) Von Mises stress of nucleus pulposus patient 1; D) Von Mises stress of vertebral body; E) Von Mises stress of annulus fibrosus patient 2; F) Von Mises stress of nucleus pulposus patient 2

that in the central region of the L2-3 to L4-5 nucleus pulposus models experienced high von Mises stress. This is indicated by the presence of red colour in the simulation results.

Based on the simulations performed on the modelling of the lumbar one to sacral one in patients, lower back pain attributable to lumbar spondylosis can be observed. The highest von Mises stress on the intervertebral disc for different loading conditions was seen in the annulus fibrosus and nucleus pulposus of L2-L3. The von Mises stress in the annulus fibrosus of L2-L3 is 64.967 MPa under anterior flexion loading, 84.168 MPa under axial rotation loading, and 44.141 MPa under lateral flexion loading. In the nucleus pulposus, the von Mises stress was 16.722 MPa under anterior flexion loading, 12.747 MPa under axial rotation loading, and 10.544 MPa under lateral flexion loading.

Simulation of Patient 2

It was observed that the average value of von Mises stress in the annulus fibrosus and nucleus pulposus components followed the same pattern, with the most significant von Mises stress values occuring in the discs of the lumbar vertebrae L1-L2 under each lateral bending, anterior flexion-extension, and axial rotation loading conditions respectively, were 0.09 MPa, 0.13 MPa, and 0.20 MPa. The von Mises stress values (MPa) for the transient annulus fibrosus for the nucleus pulposus are 0.01 MPa, 0.02 MPa, and 0.02 MPa. Then, the von Mises stress of the annulus fibrosus and nucleus pulposus components on the lumbar intervertebral disc L2-L3 decreases under each loading condition of lateral bending, anterior flexion-extension, and axial rotation loading respectively, were 0.06 MPa, 0.08 MPa, and 0.12 MPa for the annulus fibrosus, while for the nucleus pulposus are 0.009 MPa, 0.012 MPa, and 0.011 MPa. The values of the annulus fibrosus and nucleus pulposus components in the lumbar intervertebral disc L3-L4 also decreased under each condition of lateral bending, anterior flexion-extension, and axial rotation loading conditions are 0.05 MPa, 0.08 MPa, and 0.05 MPa for the annulus fibrosus, and for the nucleus pulposus they are 0.009 MPa, 0.012 MPa, and 0.008 MPa, respectively.

After obtaining the average von Mises stress value, we determined the maximum stress value and location, especially in the annulus fibrosus component, which support the load received by the lumbar vertebrae bone while surrounding and protecting the component in it, namely the nucleus pulposus, which is a gel liquid. Annulus fibrosus that has undergone degeneration (thinning) can cause pain, and worst of all, can suffer damage where it is no longer able to protect the nucleus pulposus, so that when the vertebral bone receives the load of the nucleus pulposus, it can come out through the gap of the fibrosus annulus that has been damaged, or what is commonly called the Hernia Nucleus Pulposus (HNP) (25). This often occurs mainly in the dorsal part of the intervertebral discs. Stress concentration of the annulus fibrosus component is highest in lumbar vertebrae 1-2 when experiencing axial rotation and anterior flexion-extension loads, namely 0.51 MPa and 0.39 MPa. However, for lateral bending stress concentration loads found in the annulus fibrosus vertebra lumbar 5 - sacral 1 is 0.35 MPa. This is due to the patient experiencing degenerative lumbar scoliosis in the lower lumbar vertebrae so that the bone structure becomes asymmetrical but more inclined to the right of the patient. The result of degenerative lumbar scoliosis is also seen when the lumbar vertebral bone experiences axial rotation load, the maximum stress value of the annulus fibrosus is found, which is also significant in the discs of the lumbar 4-5 and the lumbar 5 – sacral 1 are 0.41 MPa and 0.39 MPa. The contour plots of the lumbar vertebrae illustrate both the magnitude of the von Mises stress and the locations of stress concentration within the annulus fibrosus and nucleus pulposus.

DISCUSSION

Simulation analyses of Patient 1

Based on the loading data obtained from the simulations conducted, von Mises stress occurred while the modelling was exposed to variations of lateral flexion loading conditions. The highest von Mises stress value was noted in the upper endplate component of L3-L4 with a von Mises value of 451.46 MPa. The lowest von Mises stress value was noted in the nucleus pulposus component when subjected to anterior flexion loading conditions, with a value of 0.88315 MPa.

Patient 1 suffered an old compression fracture of the second and fourth lumbar, leading to loss of lordosis (9.54º), while the normal range of lumbar lordosis in the adult population measured by Cobb's angle technique is between 40-60º (19). The loss of lumbar lordosis due to an old compression fracture can indeed have an impact on von Mises stress distribution in the spine. Lumbar lordosis refers to the natural curve of the lower spine, which helps to distribute forces and loads evenly throughout the vertebral column during various activities (20). When a vertebral compression fracture leads to a loss of lumbar lordosis, the normal alignment of the spine is disrupted. This altered spinal alignment can put uneven distribution of mechanical loads across the vertebral bodies, discs, and other supporting structures. Therefore, certain spine areas might experience higher stress concentrations, including von Mises stress (21,22). The loss of lumbar lordosis can lead to a more flattened or kyphotic alignment of the spine, which can affect the biomechanics of weight-bearing and movement (19). This change in alignment may cause the vertebral bodies to experience different loading patterns than they would in a healthy spine with the normal lumbar lordosis. Consequently, some regions of the spine may be subjected to higher stress levels due to the changed loading conditions, potentially leading to increased von Mises stress. An old vertebral compression fracture can lead to higher von Mises stress as a result of changes in the mechanical properties of the affected vertebral body (20,22). When a vertebral compression fracture occurs, the structural integrity of the vertebral body is compromised, lowering its capacity to withstand mechanical loads. As the vertebral body heals over time, it may become more rigid and less flexible compared to a healthy vertebral body. This altered mechanical behaviour can result in an uneven distribution of stress during weight-bearing activities. The surrounding areas of the healed fracture site might experience higher stress concentrations, which can lead to an accumulation of stress and ultimately result in higher von Mises stress (20-22) (Figure 3A-C).

Additionally, the altered geometry and biomechanics resulting from the healed fracture can disrupt the natural load distribution within the spine, causing neighbouring vertebral bodies to compensate for the compromised structural integrity. This compensation can further contribute to increased stress on certain regions of the spine, including the area of the old fracture. In summary, an old vertebral compression fracture can lead to higher von Mises stress caused by changes in the mechanical properties of the healed vertebral body, altered load distribution, and potential compensatory mechanisms within the spine (18,23).

Simulation analyses of Patient 2

Current literature indicates that lumbar spondylosis is commonly caused by intervertebral disc degeneration, which is characterized by narrowing of the intervertebral joint space. By looking at the value of the von Mises Stress in the intervertebral disc component, it has been noted that the von Mises stress value of intervertebral disc (annulus fibrosus and nucleus pulposus) has a reciprocal relationship with the value of the lumbar disc height parameter, where the most significant average von Mises stress is found in the discs of lumbar 1-2 with ventral parameters and dorsal disc height values of 7.53 mm and 3.22. The average von Mises stress value of the lumbar vertebra 2-3 disc decreased as far as the ventral parameters and the dorsal disc height increased to 8.32 mm and 3.32 mm. Continuing at the average of the von Mises stress value, the discs of the lumbar vertebrae 3-4 also decreased as many ventral parameters and dorsal disc height increased to 8.46 mm and 8.46 mm (24-26).

Patient 2 suffered from degenerative lumbar scoliosis, which is represented as asymmetry of the lateral curve of the lumbar segment. This condition is caused by disc degenerative disease with uneven load distribution leading to an unequal portion of the disc shape. In degenerative lumbar scoliosis caused by disc degenerative disease, the correlation with von Mises stress is complex and influenced by various biomechanical factors. It is interesting to note that the highest von Mises stress occurs in the upper lumbar region, while the apex of degenerative scoliosis curvature is in the L4-5 region (28,29) (Figure 3D-F).

This phenomenon can be explained by considering the altered biomechanics and load distribution associated with the scoliotic curvature as follows.

Altered alignment. The presence of a scoliotic curve resulting in an abnormal lateral curvature of the spine. This altered alignment shifts the load-bearing dynamics and introduces asymmetrical forces on the vertebral bodies, discs, and facet joints. The spine adapts to this misalignment by redistributing loads across different regions (28,30).

Compensation mechanisms. The spine has an inherent ability to compensate for misalignments. In the case of degenerative lumbar scoliosis with an apex at L4-L5, the upper lumbar region (above the apex) may undergo compensatory changes to maintain overall spinal balance. This can lead to increased stress in the upper lumbar vertebrae, as they work to support the imbalanced forces resulting from the scoliosis curve (28,31).

Changes in load distribution. The scoliotic curvature can cause the vertebral discs to experience uneven loading. The discs in the upper lumbar region might have to bear a larger portion of the load due to the curvature's effects. As a result, the von Mises stress may be higher in these upper lumbar discs, reflecting the increased mechanical demands placed upon them. This condition is synchronous with the condition occurring in Patient 2, where the intervertebral disc at the level L1-2 has less height than other levels (30,31).

Facet joint mechanics. The facet joints are important structures that guide spinal movement and distribute loads. In scoliosis, the facet joints may experience abnormal loading due to the altered alignment. This could increase stress concentrations, especially in regions where the alignment changes (28,29).

The limitations of this study were: Finite Element model used in this research does not include tendons, ligaments, and muscles. Further finite element analyses using a combination of lumbosacral MRI and CT scans are necessary. Hydrostatic pressure was not included in our analysis; therefore, the results of our study reflect changes in overall stress intensity rather than pure compressive–tensile loading. In addition, this study does not account for the anisotropy of bone tissue or the role

of interstitial fluid, which is an important factor in the mechanics of intervertebral discs. Future studies should incorporate these to accurately represent the mechanical response; the load applied in this simulation is based on a reference of 400Nm from a study conducted by Kang et al. Simulation using the patient's body weight as a load can be used to obtain more realistic results, perform finite element analyses involving a larger sample size and a greater variability of lumbar abnormalities; performing finite element analyses utilizing the entire vertebral segment from the cervical to the lumbosacral region.

In summary, the correlation between degenerative lumbar scoliosis and von Mises stress involves complex interactions between altered alignment, compensatory mechanisms, load distribution, and facet joint mechanics. The specific location of the highest von Mises stress (upper lumbar region) can be attributed to the adjustments the spine makes to accommodate the scoliotic curvature and maintain balance. It should be kept in mind that this explanation is a simplified overview, and the actual biomechanics involved can be more intricate and influenced by individual factors. (28-31).

In this study, the reconstruction of vertebra lumbar 1-sacral 1 has been carried out as well as a comprehensive analysis of finite elements for von Mises stress that occurs in the reconstruction results of lumbar 1 to sacral 1. The results of this study can be concluded as follows: finite element modelling can offer a more straightforward and more accurate method of analysis in finding mechanical changes due to lumbar curve alteration and/or intervertebral disc degeneration; patient 1 had an old compression fracture of the second and fourth lumbar, which can lead to higher von Mises stress due to changes in the mechanical properties of the healed vertebral body, altered load distribution, and potential compensatory mechanisms within the spine; patient 2 with degenerative lumbar scoliosis due to disc degenerative disease presents complex interactions between altered alignment, compensatory mechanisms, load distribution, and facet joint mechanics resulting in higher von Mises stress. The specific location of the highest von Mises stress (upper lumbar region) might be attributed to adjustments the spine makes to accommodate the scoliotic curvature and maintain balance; by determining the magnitude of von Mises stress obtained in each lumbosacral segment, we can estimate which segments are at the highest risk, and which can develop into lumbar spondylosis or further disc degenerative disorders.

AUTHOR CONTRIBUTIONS STATEMENT

Conceptualization, A.I.P.P. and A.S.; Methodology, A.I.P.P, and A.S.; Software, A.I.P.P, and A.S.; Validation, T.I.W. and J.J.; Formal Analysis, A.I.P.P. and A.S.; Investigation, A.I.P.P. and A.S.; Resources, T.I.W and J.J.; Data Curation, A.I.P.P. and A.S.; Writing (Original Draft), A.I.P.P.; Writing (Review & Editing), T.I.W., J.J., A.S.; Visualization, A.I.P.P.; Supervision, T.I.W., J.J., and A.S.; Project Administration T.I.W. and J.J.; Funding Acquisition T.I.W. and J.J.

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TRANSPARENCY DECLARATION

Conflicts of interest: None to declare

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ORIGINAL ARTICLE

An animal model in rat for secondary osteoporosis: the effect of gonadotropin-releasing hormone (GnRH) agonists, low calcium diet, and immobilization on tartrate-resistant acid phosphatase 5b (TRACP-5b) and procollagen type 1 N-terminal propeptide (PINP) levels

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ABSTRACT

Aim Osteoporosis is a global health concern characterized by reduced bone density, microarchitectural changes, and an increased risk of fractures. The prevalence of osteoporosis is rising, particularly in developing countries. In Indonesia, the incidence of upper femur fractures due to osteoporosis is notably high. As human studies are limited, animal models are crucial for investigating osteoporosis, with several methods used to induce the condition. This study proposes an alternative model using leuprolide acetate, gonadotropin-releasing hormone (GnRH) agonist, combined with a low-calcium diet and immobilization, to induce secondary osteoporosis in animals.

Methods This experimental study employed a post-test-only control group design with 12 groups of Wistar rats (*Rattus norvegicus*). Three control groups received no treatment, while nine experimental groups were administered leuprolide acetate along with a low-calcium diet and immobilization. The study measured tartrate-resistant acid phosphatase 5b (TRACP-5b) and procollagen type 1 N-terminal propeptide (PINP) levels in rat bone tissue.

Results Significant increase in TRACP-5b and decrease in PINP levels were observed on days 21, 28, and 35 in the treated groups. Post hoc analysis revealed significant differences between the treatment groups.

Conclusion The experimental model successfully demonstrated a reliable and reproducible method for inducing secondary osteoporosis. Elevated TRACP-5b and reduced PINP levels indicate increased osteoclast activity and decreased osteoblast activity resulting from excessive bone remodelling. The combination of leuprolide acetate, a low-calcium diet, and immobilization is an effective and alternative method for inducing secondary osteoporosis in experimental animals.

Keywords: calcium, immobilization, leuprolide, osteoporosis

INTRODUCTION

Osteoporosis is a significant global health issue characterized by decreased bone density and microarchitectural deterioration, leading to increased bone fragility, reduced bone strength, and a heightened risk of fractures (1,2). The prevalence of osteoporosis is rising, particularly with the growing elderly population, and is increasingly recognized as a significant public health concern, especially in developing countries (3). In Indonesia, for example, the Ministry of Health reported in 2010 that the incidence of upper femur fractures due to osteoporosis was 200 cases per 200,000 people by the age of 40 years (4).

Advances in genetic research have enabled the creation of animal models with specific hereditary traits by manipulating or disabling certain genes (5). Genetically modified mice, for instance, can replicate early onset osteoporosis, thus shortening experimental durations. However, the high cost, complexity, and technical requirements of such models limit their widespread use (6).

Another approach to inducing osteoporosis in animals is to simulate androgen deficiency, which has been shown to affect bone microarchitecture and density. In particular, treatments involving gonadotropin-releasing hormone (GnRH) agonists, such as leuprolide acetate, can cause osteopenia in mice (7). Immobilization is another method to induce bone loss in an-

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imals, where bone mass decreases due to a halt in periosteal bone formation and increased endosteal resorption (8).

Given the complex nature of osteoporosis, a combined treatment approach is often more effective than single methods in mimicking the condition's mechanisms.

The aim of this study was to explore an alternative model combining leuprolide acetate, a GnRH agonist, a low-calcium diet, and immobilization to induce secondary osteoporosis in rats. While each of these treatments has been shown to influence bone density independently, the combined effect is hypothesized to produce a more pronounced osteoporotic phenotype.

MATERIALS AND METHOD

Materials and study design

This study was conducted from January to March 2025 at the Animal Research Laboratory, Faculty of Medicine, and the Physiology Laboratory, Faculty of Medicine, Universitas Brawijaya.

This study employed an experimental design with a post-test-only control group approach. The primary objective was to evaluate the effects of administering GnRH agonists (leuprolide acetate) combined with a low-calcium diet and immobilization on the development of a secondary osteoporosis animal model in rats. The key parameters measured included tartrate-resistant acid phosphatase 5b (TRACP-5b) and procollagen Type 1 N-terminal propeptide (PINP) levels in bone tissue. The sample size was calculated using the Federer formula (9) to estimate the required number of subjects based on power analysis. The formula used is as follows:

$(n - 1)(t - 1) \geq 15$ where: n = required sample size per group, t = number of treatment groups

$$(n-1)(12-1)\geq 15$$
$$(n-1)(11)\geq 15$$
$$11n-11\geq 15$$
$$11n\geq 26$$
$$n\geq 26/11$$
$$n\geq 2.36$$

In this study, there were 12 treatment groups (Table 1). Solving the equation yields a required sample size of 3 rats per group. To avoid a lack of sample size, each group consisted of 4 rats. Therefore, a total of 48 rats were included in this study. The rats were randomly assigned to either the control or experimental groups. The inclusion criteria specified male Wistar rats, aged 12 weeks and weighing approximately 250 grams, which were in good health and showed no physical impairments. The exclusion criteria included male Wistar rats with extremity disabilities, infections, or those that died during the study.

All protocols were approved by the Health Research Ethics Commission of General Hospital Saiful Anwar, with ethical approval number 400/358/K.3/102.7/2024, which was issued on 9 December 2024.

Methods

The animal subjects that met the inclusion criteria were acclimatized for a period of 7 days in a controlled environment with a temperature of 22-24 °C, a 12-hour light/dark cycle, and access to standard laboratory food and water ad libitum.

Table 1. Study treatment groups and protocols

Group	Treatment	Duration (days)
Group 1 (Control)	Rats without treatment, fed a standard diet	21
Group 2 (Control)	Rats without treatment, fed a standard diet	28
Group 3 (Control)	Rats without treatment, fed a standard diet	35
Group 4	Rats injected with Leuprolide acetate	21
Group 5	Rats injected with Leuprolide acetate	28
Group 6	Rats injected with Leuprolide acetate	35
Group 7	Rats received Leuprolide acetate combined with a low-calcium diet	21
Group 8	Rats received Leuprolide acetate combined with a low-calcium diet	28
Group 9	Rats received Leuprolide acetate combined with a low-calcium diet	35
Group 10	Rats receiving Leuprolide acetate, a low-calcium diet, and limb immobilization	21
Group 11	Rats receiving Leuprolide acetate, a low-calcium diet, and limb immobilization	28
Group 12	Rats receiving Leuprolide acetate, a low-calcium diet, and limb immobilization	35

During the acclimatization period, the animals were monitored for health and behaviour to ensure they had adapted appropriately to the environment before commencing the experimental procedures. After acclimatization, the animals were assessed for overall health and subsequently assigned to their respective treatment groups as predefined in the study protocol.

GnRH agonist administration. Leuprolide acetate (KalbeMed, Indonesia) was administered subcutaneously at a dose of 75 mg/kg body weight. The administration schedule varied according to the treatment group. The fourth group received Leuprolide acetate for 21 days, the fifth group for 28 days, and the sixth group for 35 days. For the subsequent groups, Leuprolide acetate was combined with a low-calcium diet for 21, 28, or 35 days, in groups 7, 8, and 9, respectively. Lastly, in groups ten, eleven, and twelve, the same treatment of Leuprolide acetate, low-calcium diet, and limb immobilization was applied for 21, 28, or 35 days.

Low-calcium diet. A low-calcium diet containing <1% calcium to induce calcium deficiency, a known factor in the development of osteoporosis, was given to the treatment group, which was set to receive a low-calcium diet.

Limb immobilization. Limb immobilization was performed immediately after recovery from anaesthesia using plaster of Paris (POP). The left hind limb was flexed at the knee joint to approximately 90°, and POP was applied circumferentially from the groin to the ankle to restrict motion completely. The immobilization mimicked disuse osteoporosis, a well-established contributor to secondary bone loss in both animal and human studies. The fixation was allowed to harden for 10–15 minutes before returning the rat to its cage. To ensure consistency in immobilization throughout the study period, the casts were inspected daily for signs of loosening, cracking, or discomfort. If necessary, damaged casts were replaced under light ether anaesthesia. Body weight, limb colour, and mobility were monitored to detect circulatory or neurological complica-

tions. After each experimental endpoint (21, 28, and 35 days), the immobilization cast was carefully removed before sample collection.

Surgical procedure. A critical-sized bone defect (CSD) of 3 mm in diameter was surgically created in the mid-diaphyseal region of the femur for all experimental rats. The choice of a 3 mm defect was based on established preclinical models, which indicate that this dimension exceeds the natural bone healing capacity of rats within a typical observation period (4–6 weeks), thereby fulfilling the criteria for a CSD. Such a defect does not spontaneously heal without intervention, closely mimicking non-union or delayed union conditions observed in human long-bone fractures. This approach allows the study of bone remodelling dynamics and osteoporotic bone healing under controlled conditions. The defect also represents a clinically relevant scale of segmental bone loss, proportionally corresponding to defects of several centimetres in human femoral bone.

The surgical procedure was performed under general anaesthesia using a combination of ketamine hydrochloride and ether solution for induction and maintenance. After skin sterilization with povidone-iodine, a longitudinal incision was made over the lateral aspect of the femur to expose the bone. Using a low-speed microdrill (1.5 mm burr tip) under saline irrigation to prevent thermal necrosis, a circular 3 mm defect was created at the midshaft region. The defect site was treated according to the respective group protocols (e.g., synthetic bone graft, rh-BMP2, or combination treatment). The wound was irrigated with sterile saline and closed in layers using absorbable sutures. Postoperative care included a single dose of enrofloxacin (10 mg/kg, subcutaneously) and meloxicam (1 mg/kg, subcutaneously) to prevent infection and relieve pain.

Sample harvest and analysis. After the treatment period, rats were anesthetized using a combination of ketamine hydrochloride and ether solution to ensure pain relief. Once anesthetized, the animals were euthanized via an intraperitoneal injection of euthasol. The animals were then positioned in a supine position on a sterile surgical table, and the surgical site (the tibia) was cleaned with an antiseptic solution to prevent infection. A small incision was made over the tibia, and the surrounding soft tissue, including muscles and ligaments, was carefully dissected using sterile instruments. Once the tibia was exposed, a small section of the bone was excised using a sterile bone cutter or surgical scissors. The harvested tibial bone tissue was immediately placed in a sterile container on ice to preserve its integrity. The bone samples were then transferred to cold storage, typically in saline or PBS solution, to prevent enzymatic degradation. Following the preservation, the bone samples were cleaned of any remaining soft tissue and prepared for biochemical analysis. Bone fragments from the tibial diaphysis or metaphysis were processed for testing. TRACP-5b (Tartrate-resistant acid phosphatase 5b) and PINP (Procollagen Type 1 N-terminal propeptide) levels, markers for osteoclast and osteoblast activity, respectively, were measured using an ELISA Kit.

Statistical analysis

The collected data were organized, edited, and tabulated for analysis. A significance level of $p \leq 0.05$ was set, and a 95% confidence interval /CI) was used. One-way ANOVA was applied to compare the TRACP-5b and PINP levels across the different treatment groups and durations. Post-hoc tests were conducted to evaluate specific differences between the groups.

RESULTS

The data from the analysis of TRACP-5b and PINP levels from the 12 groups were first subjected to normality testing using the Shapiro-Wilk test and homogeneity testing using the Levene test to determine whether parametric or non-parametric statistical analysis should be applied. As the results of normality and homogeneity tests were met, an ANOVA was conducted for both TRACP-5b and PINP levels (Table 2). The Sum of Squares (SS) quantifies the total variation in the data. Specifically, the Sum of Squares Between Groups represents the variation due to the differences between the means of the 12 groups, while the Sum of Squares Within Groups reflects the variation within each group. The degrees of freedom (df) for between-groups was calculated as $k-1=12-1=11$, where k is the number of groups. The df within the groups was calculated as the total number of observations minus the number of groups, $n-k=48-12=36$, where n is the total number of data points. The df total is the total number of data points minus one, which is $n-1=48-1=47$.

Table 2. ANOVA test for procollagen Type 1 N-terminal propeptide (PINP) and tartrate-resistant acid phosphatase 5b (TRACP-5b) levels across treatment groups

Variable	Comparison	Sum of squares	df	Mean square	F	p
PINP	Between Groups	13042470.667	11	1185679.152	19.340	0.000
	Within Groups	2207088.000	36	61308.000		
	Total	5249558.667	47			
TRACP-5b	Between Groups	5.723	11	0.520	64.732	0.000
	Within Groups	0.289	36	0.008		
	Total	6.012	47			

Sum of squares, the total variation or dispersion in the data; df, degrees of freedom, refer to the number of independent values that can vary in the calculation without violating any given constraints; Mean square, an estimate of variance; F, the ratio of the variance between groups to the variance within groups, usually formulated as the mean square between groups divided by the mean square within groups;

The Mean Square (MS) is an estimate of the variance and it was calculated by dividing the Sum of Squares by the respective degrees of freedom. For example, MS Between Groups is the Sum of Squares Between Groups (13042470.667) divided by the df Between Groups (11), resulting in a Mean Square Between Groups (1185679.152) for PINP, and similarly for TRACP-5b. The F-value is the ratio of the variance between groups to the variance within groups, calculated as MS Between Groups / MS Within Groups. A larger F-value indicates that the variation between groups means is significantly greater than the variation within groups, suggesting a meaningful difference between the groups. For PINP, the F-value was 19.340, and for TRACP-5b 64.732, both with $p<0.05$, indicating that the differences between the treatment groups were statistically significant.

To specifically identify which groups exhibited statistically significant differences, a post hoc test was performed. Tukey HSD post hoc tests were chosen as they are most suitable for group comparisons.

The post hoc analysis comparing the TRACP-5b levels among

Table 3. Post Hoc tartrate-resistant acid phosphatase 5b (TRACP-5b)

Group for comparison	Comparing group	p	Group for comparison	Comparing group	p	Group for comparison	Comparing group	p	Group for comparison	Comparing group	p
C 21 days	C 28 days	0.900	G 21 days	C 21 days	0.001*	G+I 21 days	C 21 days	0.000*	G+I+CA 21 days	C 21 days	0.000*
	C 35 days	0.906		C 28 days	0.000*		C 28 days	0.000*		C 28 days	0.000*
	G 21 days	0.001*		C 35 days	0.000*		C 35 days	0.000*		C 35 days	0.000*
	G 28 days	0.000*		G 28 days	0.070		G 21 days	0.093		G 21 days	0.000*
	G 35 days	0.000*		G 35 days	0.012*		G 28 days	0.888		G 28 days	0.001*
	G+I 21 days	0.000*		G+I 21 days	0.093		G 35 days	0.360		G 35 days	0.006*
	G+I 28 days	0.000*		G+I 28 days	0.015*		G+I 28 days	0.413		G+I 21 days	0.000*
	G+I 35 days	0.000*		G+I 35 days	0.000*		G+I 35 days	0.001*		G+I 28 days	0.005*
	G+I+CA 21 days	0.000*		G+I+CA 21 days	0.000*		G+I+CA 21 days	0.000*		G+I 35 days	0.991
	G+I+CA 28 days	0.000*		G+I+CA 28 days	0.000*		G+I+CA 28 days	0.000*		G+I+CA 28 days	0.000*
	G+I+CA 35 days	0.000*		G+I+CA 35 days	0.000*		G+I+CA 35 days	0.000*		G+I+CA 35 days	0.000*
C 28 days	C 21 days	0.900	G 28 days	C 21 days	0.000*	G+I 28 days	C 21 days	0.000*	G+I+CA 28 days	C 21 days	0.000*
	C 35 days	0.994		C 28 days	0.000*		C 28 days	0.000*		C 28 days	0.000*
	G 21 days	0.000*		C 35 days	0.000*		C 35 days	0.000*		C 35 days	0.000*
	G 28 days	0.000*		G 21 days	0.070		G 21 days	0.015*		G 21 days	0.000*
	G 35 days	0.000*		G 35 days	0.438		G 28 days	0.497		G 28 days	0.000*
	G+I 21 days	0.000*		G+I 21 days	0.888		G 35 days	0.922		G 35 days	0.000*
	G+I 28 days	0.000*		G+I 28 days	0.497		G+I 21 days	0.413		G+I 21 days	0.000*
	G+I 35 days	0.000*		G+I 35 days	0.001*		G+I 35 days	0.005*		G+I 28 days	0.000*
	G+I+CA 28 days	0.000*		G+I+CA 21 days	0.001*		G+I+CA 21 days	0.005*		G+I 35 days	0.000*
	G+I+CA 35 days	0.000*		G+I+CA 28 days	0.000*		G+I+CA 28 days	0.000*		G+I+CA 21 days	0.000*
	G+I+CA 35 days	0.000*		G+I+CA 35 days	0.000*		G+I+CA 35 days	0.000*		G+I+CA 35 days	0.001*
C 35 days	C 21 days	0.906	G 35 days	C 21 days	0.000*	G+I 35 days	C 21 days	0.000*	G+I+CA 35 days	C 21 days	0.000*
	C 28 days	0.994		C 28 days	0.000*		C 28 days	0.000*		C 28 days	0.000*
	G 21 days	0.000*		C 35 days	0.000*		C 35 days	0.000*		C 35 days	0.000*
	G 28 days	0.000*		G 21 days	0.012*		G 21 days	0.000*		G 21 days	0.000*
	G 35 days	0.000*		G 28 days	0.438		G 28 days	0.001*		G 28 days	0.000*
	G+I 21 days	0.000*		G+I 21 days	0.360		G 35 days	0.006*		G 35 days	0.000*
	G+I 28 days	0.000*		G+I 28 days	0.922		G+I 21 days	0.001*		G+I 21 days	0.000*
	G+I 35 days	0.000*		G+I 35 days	0.006*		G+I 28 days	0.005*		G+I 28 days	0.000*
	G+I+CA 21 days	0.000*		G+I+CA 21 days	0.006*		G+I+CA 21 days	0.991		G+I 35 days	0.000*
	G+I+CA 28 days	0.000*		G+I+CA 28 days	0.000*		G+I+CA 28 days	0.000*		G+I+CA 21 days	0.000*
	G+I+CA 35 days	0.000*		G+I+CA 35 days	0.000*		G+I+CA 35 days	0.000*		G+I+CA 28 days	0.001*

*statistically significant difference;
C, Control; G: GnRH Agonist; I, immobilization; CA, low calcium intake

the treatment groups indicated that the combination of GnRH agonists, a low-calcium diet, and immobilization for 35 days yielded the most significant difference compared to the other groups (Table 3).

The post hoc results comparing each group to the others showed the combination of GnRH agonist, low calcium diet, and immobilization at 35 days showed the most significant difference when compared to others (Table 4).

DISCUSSION

The animal model used in this study combined the administration of a GnRH (Leuprolide acetate) agonist, a low-calcium diet, immobilization, and varying treatment durations. This combination is less commonly used, as most studies typically employ only one treatment per model. The administration of GnRH agonists can induce a hypogonadotropic-hypogonadal state, which accelerates bone loss (10). Additionally, adequate calcium intake is crucial for bone mass accumulation, and a calcium deficiency leads to reduced bone mass, often accompanied by a decrease in osteoblast numbers and an increase in osteoclast activity (11). Immobilization primarily results in bone loss in the hind limbs, which experience the most mechanical loading (12). The rate of bone loss is generally faster in cancellous bone than in cortical bone. Significant bone mass loss in the proximal and distal tibia metaphysis of this model typically occurs during the transition phase, between 14 and 30 days after immobilization (13).

Osteoclasts secrete TRACP into the circulation, making serum TRACP a valuable marker for osteoclast activity and bone resorption (14). In this study, TRACP-5b levels were significantly increased on days 21, 28, and 35. ANOVA analysis revealed a significant difference between the treatment groups in the TRACP-5b parameter. These findings align with previous studies on post-ovariectomy rats, which have shown increased TRACP-5b levels, indicating heightened osteoclast activity (15). The post hoc test revealed no significant difference in TRACP-5b levels between the GnRH agonist group on day 21 and the control group; however, a significant difference emerged on day 28. This is consistent with studies that show the effects of GnRH agonist treatment are typically observed from the second to the third month of administration (16-18). Statistically, no significant difference was observed between the GnRH agonist group and the group treated with GnRH agonist plus a low-calcium diet on day 21. However, immobilization significantly influenced the differ-

Table 4. Post Hoc procollagen type 1 N-terminal propeptide (PINP)

Group for comparison	Comparing group	p	Group for comparison	Comparing group	p	Group for comparison	Comparing group	p	Group for comparison	Comparing group	p
C 21 days	C 28 days	0.645	G 21 days	C 21 days	0.005*	G+I 21 days	C 21 days	0.000*	G+I+CA 21 days	C 21 days	0.000*
	C 35 days	0.581		C 28 days	0.015*		C 28 days	0.000*		C 28 days	0.000*
	G 21 days	0.005*		C 35 days	0.018*		C 35 days	0.000*		C 35 days	0.000*
	G 28 days	0.000*		G 28 days	0.039*		G 21 days	0.068		G 21 days	0.051
	G 35 days	0.000*		G 35 days	0.000*		G 28 days	0.790		G 28 days	0.896
	G+I 21 days	0.000*		G+I 21 days	0.068		G 35 days	0.012*		G 35 days	0.017*
	G+I 28 days	0.000*		G+I 28 days	0.034*		G+I 28 days	0.743		G+I 21 days	0.892
	G+I 35 days	0.000*		G+I 35 days	0.000*		G+I 35 days	0.013*		G+I 28 days	0.848
	G+I+CA 21 days	0.000*		G+I+CA 21 days	0.051		G+I+CA 21 days	0.892		G+I 35 days	0.019*
	G+I+CA 28 days	0.000*		G+I+CA 28 days	0.016*		G+I+CA 28 days	0.522		G+I+CA 28 days	0.613
	G+I+CA 35 days	0.000*		G+I+CA 35 days	0.000*		G+I+CA 35 days	0.000*		G+I+CA 35 days	0.000*
C 28 days	C 21 days	0.645	G 28 days	C 21 days	0.000*	G+I 28 days	C 21 days	0.000*	G+I+CA 28 days	C 21 days	0.000*
	C 35 days	0.927		C 28 days	0.000*		C 28 days	0.000*		C 28 days	0.000*
	G 21 days	0.015*		C 35 days	0.000*		C 35 days	0.000*		C 35 days	0.000*
	G 28 days	0.000*		G 21 days	0.039*		G 21 days	0.034*		G 21 days	0.016*
	G 35 days	0.000*		G 35 days	0.023*		G 28 days	0.951		G 28 days	0.707
	G+I 21 days	0.000*		G+I 21 days	0.790		G 35 days	0.027*		G 35 days	0.054
	G+I 28 days	0.000*		G+I 28 days	0.951		G+I 21 days	0.743		G+I 21 days	0.522
	G+I 35 days	0.000*		G+I 35 days	0.025*		G+I 35 days	0.029*		G+I 28 days	0.753
	G+I+CA 28 days	0.000*		G+I+CA 21 days	0.896		G+I+CA 21 days	0.848		G+I 35 days	0.058
	G+I+CA 35 days	0.000*		G+I+CA 28 days	0.707		G+I+CA 28 days	0.753		G+I+CA 21 days	0.613
	G+I+CA 35 days	0.000*		G+I+CA 35 days	0.000*		G+I+CA 35 days	0.000*		G+I+CA 35 days	0.000*
C 35 days	C 21 days	0.581	G 35 days	C 21 days	0.000*	G+I 35 days	C 21 days	0.000*	G+I+CA 35 days	C 21 days	0.000*
	C 28 days	0.927		C 28 days	0.000*		C 28 days	0.000*		C 28 days	0.000*
	G 21 days	0.018*		C 35 days	0.000*		C 35 days	0.000*		C 35 days	0.000*
	G 28 days	0.000*		G 21 days	0.000*		G 21 days	0.000*		G 21 days	0.000*
	G 35 days	0.000*		G 28 days	0.023*		G 28 days	0.025*		G 28 days	0.000*
	G+I 21 days	0.000*		G+I 21 days	0.012*		G 35 days	0.968		G 35 days	0.015*
	G+I 28 days	0.000*		G+I 28 days	0.027*		G+I 21 days	0.013*		G+I 21 days	0.000*
	G+I 35 days	0.000*		G+I 35 days	0.968		G+I 28 days	0.029*		G+I 28 days	0.000*
	G+I+CA 21 days	0.000*		G+I+CA 21 days	0.017*		G+I+CA 21 days	0.019*		G+I 35 days	0.014*
	G+I+CA 28 days	0.000*		G+I+CA 28 days	0.054		G+I+CA 28 days	0.058		G+I+CA 21 days	0.000*
	G+I+CA 35 days	0.000*		G+I+CA 35 days	0.015*		G+I+CA 35 days	0.014*		G+I+CA 28 days	0.000*

*statistically significant difference;
C, Control; G, GnRH Agonist; I, immobilization; CA, low calcium intake

ence, as it enhances the activation of the bone remodelling process and reduces osteoblast activity (19).

Procollagen-1 N-terminal peptide (PINP), a specific marker for type 1 collagen deposition, was consistently decreased in this study on days 21, 28, and 35. ANOVA analysis revealed a significant difference between the treatment groups in the PINP parameter ($p<0.05$). These results are consistent with previous research that demonstrated PINP as a predictor of bone loss, particularly in premenopausal women with systemic lupus erythematosus (20).

The biomarkers observed in this study indicate an increase in osteoclast activity and a decrease in osteoblast activity, suggesting an imbalance in bone remodelling. However, these findings should be further supported by X-ray and histopathological examinations. Dual-energy X-ray absorptiometry (DEXA) and histopathological analysis of rat bone tissue can help correlate laboratory biomarker titters with clinical imaging, providing a more comprehensive understanding of the bone loss process (21).

This study has several limitations. First, the use of a single animal model (Wistar rats) may not fully represent the complexity of human osteoporosis. The findings might not entirely translate to human conditions due to species differences. Additionally, the study duration (35 days) may be too short to observe the long-term effects of the combined treatment on bone density and microarchitecture. A small sample size may also limit the study's generalizability. Another limitation is that only biomarkers were used to assess bone turnover, which may not fully capture the structural changes occurring at the bone level. Radiological and histological confirmation would have provided a more comprehensive evaluation of the bone loss process.

Future studies should explore the long-term effects of GnRH agonists combined with a low-calcium diet and immobilization over a longer treatment period, extending the observation time to several months. Additionally, the use of different animal models, such as osteoporotic mice or large animal models could provide more insight into how these treatments affect various bone types and the structural integrity of bones. It would also be valuable to incorporate a combination of radiological imaging (such as DEXA and micro-CT scans) and histopathological examination to complement biomarker analysis, offering a more holistic view of bone remodelling. Furthermore, future research could investigate the effects of different dosages and combinations of treatments to identify the most effective regimen for inducing secondary osteoporosis. Ultimately, investigating the molecular

mechanisms underlying the changes in osteoclast and osteoblast activity in response to this combined treatment could provide deeper insights into the pathophysiology of osteoporosis.

In conclusion, this study successfully demonstrated that the combination of GnRH agonists (Leuprolide acetate), a low-calcium diet, and immobilization can effectively induce secondary osteoporosis in Wistar rats, as evidenced by significant changes in TRACP-5b and PINP levels. The results indicate an imbalance in bone remodelling, characterized by increased osteoclast activity and decreased osteoblast function. The findings suggest that this combined treatment approach provides a reliable and alternative method for creating experimental models of secondary osteoporosis. Future research should further investigate the long-term effects and molecular mechanisms of this model to understand its potential applications in osteoporosis studies better.

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TRANSPARENCY DECLARATION

Conflicts of interest: None to declare.

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ORIGINAL ARTICLE

Advanced volar locking plate fixation for intra and extra-articular distal radius fractures: a retrospective study

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ABSTRACT

Aim Distal radius fractures are among the most common orthopaedic injuries, with a high risk of functional impairment. Volar locking plates have become the standard treatment for unstable and extra-articular fractures, offering excellent outcomes compared to traditional techniques. This study aimed to evaluate clinical, radiographic, and functional outcomes of volar locking plate fixation in patients with intra- and extra-articular distal radius fractures.

Methods A retrospective analysis was conducted on 34 patients treated with volar locking plates between 2018 and 2024. Outcomes were assessed using the Disabilities of the Arm, Shoulder and Hand (DASH) score, Visual Analog Scale (VAS) for pain, range of motion, and radiographic consolidation. Complications were recorded and analysed.

Results At a mean follow-up of 24 months, the mean DASH score was 4.2±1.8 and VAS score was 0 in all cases. Range of motion (ROM) recovery ranged from 93% to 98% of the contralateral side. Radiographs confirmed complete healing in all patients. Only two experienced minor soft tissue discomfort.

Conclusion Volar locking plate fixation for distal radius fractures ensures excellent functional recovery, reliable bone healing, and minimal complications, making it a safe and effective treatment approach.

Keywords: bone plates, orthopaedic procedures, range of motion, articular, retrospective studies, treatment outcome

INTRODUCTION

Distal radius fractures represent approximately 15% of all extremity fractures and affect a wide age range (1). These injuries are particularly common in elderly patients due to osteoporotic bone and low-energy trauma, while younger individuals typically sustain them from high-energy mechanisms such as motor vehicle accidents or sports injuries (2). If not adequately treated, these fractures may lead to chronic pain, stiffness, reduced range of motion, and long-term disability (3).

Over the past two decades, surgical management has evolved considerably. Traditional methods such as cast immobilization and external fixation often failed to ensure anatomical reduction and stable fixation, resulting in malunion and suboptimal functional outcomes (4). Volar locking plates have since emerged as the gold standard for managing complex distal radius fractures, combining rigid internal fixation with the potential for early mobilization (5,6). Their anatomical, low-profile design reduces soft tissue irritation, particularly involving the flexor and extensor tendons (7), and provides biomechanical stability to restore radial height, inclination, and volar tilt (8). Despite these benefits, complications such as tendon injuries, hardware irritation, and infection still occur, with rates reported between 22% and 27% (9). Nonoperative treatment remains a reasonable alternative in selected elderly patients with low-risk fracture patterns (10). Another critical aspect is the distinction between extra-articular and intra-articular fractures: the former are typically more amenable to surgical fixation due to their simpler morphology, while the latter present greater challenges in restoring joint congruence and alignment (11). Recent studies advocate for individualized treatment based on fracture classification, patient characteristics, and functional goals (12–16).

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This study aims to evaluate clinical, radiographic, and functional outcomes of volar locking plate fixation in patients with both extra-articular and intra-articular distal radius fractures, contributing to current evidence on the effectiveness and safety of this surgical approach.

PATIENTS AND METHODS

Patients and study design

This retrospective study evaluated the clinical, radiographic, and functional outcomes of 34 patients treated for intra- and extra-articular distal radius fractures using the GEMES GR distal volar locking plate system. Surgical procedures were performed at the Giaccone University Hospital, Palermo, between 2018 and 2024 by experienced orthopaedic surgeons, with contributions from a multidisciplinary team involved in clinical follow-up, data analysis, and manuscript preparation. All patients provided an informed consent for the use of their anonymized clinical data.

The cohort included 18 males and 16 females, with a mean age of 58 years (range: 20–80 years).

Fractures were classified according to the Arbeitsgemeinschaft für Osteosynthesefragen/Orthopaedic Trauma Association (AO/OTA) system (1): 20 extra-articular fractures (types A2.2 and A3.1) and 14 intra-articular fractures (types C2.1 and C3.2). Fifteen fractures involved the left wrist and nineteen the right wrist. Plate selection was based on fracture morphology and patient anatomy: 12 small, 20 medium, and 2 large GEMES GR distal volar locking plate system plates were implanted (Table 1).

Inclusion criteria were: patients aged 18 years or older, diagnosed with intra- or extra-articular distal radius fractures, treated with the GEMES GR distal volar locking plate system, and followed clinically and radiographically for a minimum of 12 months. Exclusion criteria included polytrauma, pathological or open fractures classified as Gustilo–Anderson grade II or higher (the Gustilo–Anderson classification categorizes open fractures based on wound size, level of contamination, and soft-tissue damage).

Table 1. Patient demographics and fracture details

Patient ID	Age (years)	Sex	AO fracture*	Fracture type
P01	45	Male	A2.2	Extra-articular
P02	32	Male	C3.2	Intra-articular
P03	60	Male	A3.1	Extra-articular
P04	54	Male	C2.1	Intra-articular
P05	27	Male	A3.1	Extra-articular
P06	78	Male	A3.1	Extra-articular
P07	39	Male	C3.2	Intra-articular
P08	58	Male	A2.2	Extra-articular
P09	68	Male	A3.1	Extra-articular
P10	50	Male	C2.1	Intra-articular
P11	44	Male	A2.2	Extra-articular
P12	61	Male	C3.2	Intra-articular
P13	70	Male	A3.1	Extra-articular
P14	38	Male	A2.2	Extra-articular
P15	52	Male	C2.1	Intra-articular
P16	29	Male	C3.2	Intra-articular
P17	47	Male	A2.2	Extra-articular
P18	64	Male	A3.1	Extra-articular
P19	36	Male	C3.2	Intra-articular
P20	41	Female	A2.2	Extra-articular
P21	55	Female	C2.1	Intra-articular
P22	62	Female	A3.1	Extra-articular
P23	38	Female	A2.2	Extra-articular
P24	75	Female	C3.2	Intra-articular
P25	48	Female	A2.2	Extra-articular
P26	67	Female	C2.1	Intra-articular
P27	72	Female	A3.1	Extra-articular
P28	58	Female	A2.2	Extra-articular
P29	46	Female	C3.2	Intra-articular
P30	31	Female	A3.1	Extra-articular
P31	40	Female	A2.2	Extra-articular
P32	69	Female	C2.1	Intra-articular
P33	59	Female	A3.1	Extra-articular
P34	49	Female	A2.2	Extra-articular

* Osteosynthesefragen/Orthopaedic Trauma Association (AO/OTA) system

Methods

All procedures followed a standardized surgical protocol. Under axillary block or general anaesthesia, patients were positioned supine with the injured limb supported on a radiolucent hand table. A volar Henry approach (5,6) was used to expose the fracture site. Fractures were reduced under fluoroscopic control, and the appropriate plate size was selected and fixed with locking screws to restore alignment and stability. Final intraoperative fluoroscopy confirmed fracture reduction and implant positioning.

Postoperative management included wrist immobilization with a splint for two weeks. Passive mobilization was initiated after suture removal, followed by active range-of-motion exercises at four weeks and strengthening at six weeks. Follow-up visits were scheduled at 2 weeks, 6 weeks, 3 months, 6 months, and 12 months, with annual assessments thereafter.

Primary outcomes included: pain, measured by the Visual Analog Scale (VAS) (4), functional recovery, assessed using the Disabilities of the Arm, Shoulder, and Hand (DASH) (5) score, range of motion (ROM) (6) in flexion, extension, pronation, and supination.

Visual Analog Scale (VAS): The VAS is a validated 10-point scale used to quantify pain intensity, where 0 indicates no pain and 10 represents the worst imaginable pain. Patients mark their perceived pain level on a 10-cm line, providing a continuous and sensitive measure of postoperative pain.

The Disabilities of the Arm, Shoulder and Hand (DASH) (5) is a 30-item, patient-reported outcome measure evaluating upper-limb disability and symptoms. Each item was scored from 1 (no difficulty) to 5 (unable to perform), generating a final score ranging from 0 to 100, where: 0 = no disability, 100 = maximum disability. The DASH is widely used to assess functional recovery after wrist and upper-extremity injuries.

Radiographic evaluation focused on fracture consolidation, radial height, inclination, and volar tilt. All complications, including tendon irritation, hardware prominence, infection, malunion, and nonunion, were systematically recorded throughout follow-up.

RESULTS

A total of 34 patients with distal radius fracture were treated with volar locking plate fixation and followed for a mean pe-

riod of 24 months (range: 12–36 months). The study population included both extra-articular and intra-articular fracture types, classified according to the AO/OTA system: 20 extra-articular fractures (A2.2 and A3.1) and 14 intra-articular fractures (C2.1 and C3.2).

Fifteen fractures involved the left wrist and nineteen the right. Plate selection was based on fracture morphology and patient anatomy: 12 patients received small plates, 20 medium plates, and 2 large plates (Table 2).

Table 2. Patient demographics and procedural details

Category	Count
Total number of patients	34
Fracture side (left wrist)	15
Fracture side (right wrist)	19
Extra-articular fractures	20
Intra-articular fractures	14
Plate size (small)	12
Plate size (medium)	20
Plate size (large)	2

Pain outcomes were excellent: at final follow-up, all patients reported complete pain resolution. Functional evaluation using the DASH score showed minimal residual disability, with a mean final score of 4.2±1.8. Range of motion (ROM) measurements demonstrated near-complete restoration of wrist function, with mean flexion, extension, pronation, and supination values ranging from 70° to 85°, corresponding to 92–98% of the contralateral wrist's mobility (Table 3).

Table 3. Detailed Range of Motion (ROM) outcomes following volar locking plate fixation for distal radius fractures, including mean final joint angles and percentage recovery compared to the contralateral limb

ROM measurement	Mean final ROM (degrees)	Compared to contralateral (%)
Flexion	75	95%
Extension	70	93%
Pronation	85	97%
Supination	85	98%

Radiographic analysis confirmed complete fracture healing in all cases, with evidence of bridging callus and no instances of delayed union, malunion, or nonunion. Anatomical parameters including radial height, radial inclination, and volar tilt were successfully restored and maintained throughout the follow-up period (Figures 1 and 2).

No intraoperative complications such as reduction difficulties or hardware malposition were observed.

Postoperatively, no flexor tendon problems, infections, or hardware irritation occurred.

Only two patients (5.8%) reported transient soft-tissue discomfort, which was resolved with conservative measures. There were no cases of malunion, nonunion, delayed union, tendon irritation, or hardware-related problems throughout the follow-up.

These results confirm that volar locking plate fixation is a safe and effective technique for treating both extra-articular and intra-articular distal radius fractures. The combination of complete pain relief, excellent functional recovery, and favourable radiographic outcomes supports the clinical reliability of this surgical approach across different fracture types.

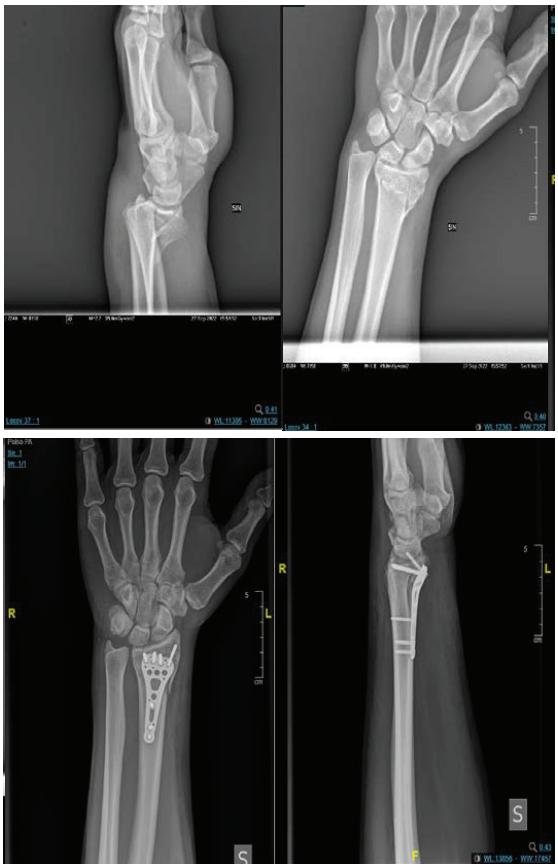

Figure 1. Radiographic progression of a distal radius fracture managed with volar locking plate fixation. A-B) Preoperative anteroposterior and lateral wrist radiographs showing an extra-articular distal radius fracture with loss of volar tilt and mild radial shortening; C-D) Postoperative anteroposterior and lateral radiographs demonstrating anatomic reduction of the fracture and stable internal fixation using a volar locking plate. Restoration of volar tilt, radial height, and inclination is clearly visible. No hardware malposition or screw penetration into the dorsal compartments is observed (Policlinico Universitario Giaccone, Palermo; 2024)

DISCUSSION

Distal radius fractures are among the most frequent injuries encountered in orthopaedic practice, representing a considerable clinical and economic burden. These fractures predominantly affect two patient groups: elderly individuals with osteoporotic bone who typically sustain low-energy injuries, such as falls, and younger patients involved in high-energy trauma, including motor vehicle accidents and sports-related incidents (1,2). The rising incidence, particularly within the aging population, highlights the need for effective treatment strategies to restore wrist function and minimize complications (3). Inadequate management of distal radius fractures may result in substantial morbidity, including malunion, joint stiffness, diminished grip strength, and chronic pain (17). These complications not only impair wrist function but also significantly affect patients' overall quality of life. Consequently, advances in surgical techniques and implant design have become essential for improving clinical outcomes.

Over the past two decades, volar locking plates have emerged as the gold standard for the treatment of complex and unstable distal radius fractures (18). Compared with traditional approaches such as casting or external fixation, volar locking

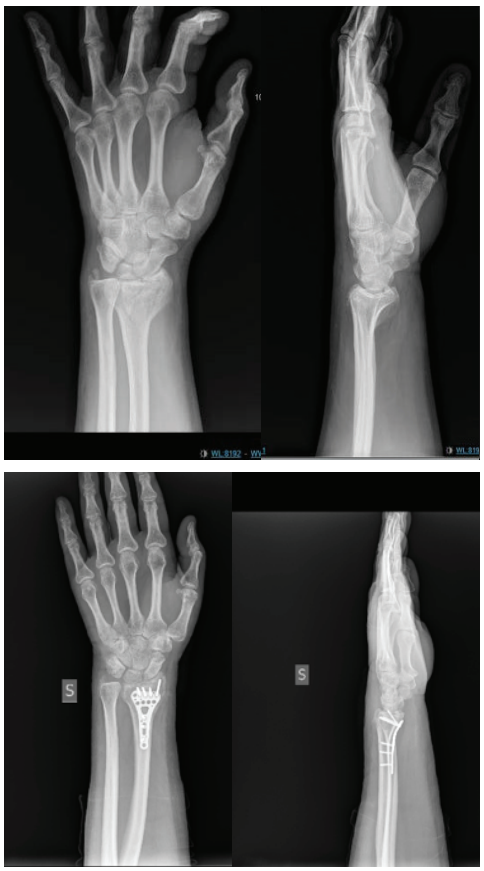

Figure 2. Radiographic sequence of a patient with a distal radius fracture treated with volar locking plate fixation. A-B) Preoperative anteroposterior and lateral wrist radiographs demonstrate an extra-articular distal radius fracture characterized by dorsal displacement, loss of volar tilt, and mild radial shortening; C-D) Postoperative anteroposterior and lateral radiographs show anatomic restoration of radial height, inclination, and volar tilt following internal fixation using a volar locking plate. The implant is correctly positioned, with no evidence of screw protrusion into the dorsal compartments or postoperative complications (Policlinico Universitario Giaccone, Palermo; 2024)

plates provide several key advantages, including stable internal fixation, precise anatomical reduction, and the possibility for early mobilization (5,18). These features collectively contribute to superior functional and radiographic outcomes, as supported by numerous clinical studies (8,19). Modern volar locking plates incorporate low-profile designs and anatomical contouring, significantly reducing soft-tissue complications—particularly flexor tendon irritation, extensor tendon attrition, and hardware prominence—that were common with earlier-generation implants (20).

The present study evaluated the GEMES GR distal volar locking plate system within this context. Although the detailed results are reported elsewhere in the manuscript, the findings were consistent with existing literature supporting the effectiveness of volar locking plates in ensuring stable fixation, reducing pain, and promoting early functional recovery (18,19). The low-profile configuration and anatomical design of the GEMES GR plate appear to have contributed to a reduced incidence of soft-tissue complications, improving both comfort and wrist mobility during rehabilitation (20). Moreover, the system's ability to restore anatomical alignment while minimizing soft-tissue disruption likely supports accelerated healing and optimized functional recovery (20).

Literature comparisons reinforce these observations. Diaz-Garcia and Chung (2012) demonstrated superior functional outcomes for volar plating relative to external fixation and casting, with notable improvements in pain scores and DASH outcomes (18). Similarly, Clement et al. (2013) reported faster recovery and fewer complications in patients treated with volar plates compared with traditional fixation methods (19). Mulders et al. (2018) found favourable range-of-motion outcomes, attributing these results to the mechanical stability provided by modern plate designs, which permit early mobilization and reduce postoperative stiffness (8).

The absence of complications such as flexor tendon irritation (particularly of the flexor pollicis longus), extensor tendon tenosynovitis or rupture (e.g., extensor pollicis longus), hardware prominence, infection, and symptomatic screw protrusion in the present study underscores the improved design characteristics of the GEMES GR system. Historically, such complications were frequently reported with older, bulkier volar plate models that lacked adequate contouring, had sharp edges, or required dorsal screw lengths with a higher risk of penetrating the extensor compartments (17–25). By minimizing these risks, the GEMES GR plate appears to enhance patient outcomes, particularly regarding wrist mobility and functional recovery.

Future research should include larger and multicentre cohorts to validate the present findings more comprehensively. Comparative studies evaluating the GEMES GR system against other modern volar locking plate designs may further clarify its clinical advantages. Additionally, long-term follow-up studies are needed to assess the durability of functional and radiographic outcomes over extended periods of time. Incorporating patient-reported outcome measures (PROMs) would provide valuable insight into subjective satisfaction and quality-of-life improvements. Finally, economic evaluations examining the cost-effectiveness of the GEMES GR distal volar locking plate system relative to alternative implants could inform clinical decision-making within value-based healthcare models.

In conclusion, this study affirms the GEMES GR distal volar locking plate system as a highly effective solution for managing intra and extra-articular distal radius fractures. The system's advanced design, characterized by its low-profile, anatomical contouring, and robust locking mechanism, successfully addresses the limitations of previous implant generations, yielding superior clinical outcomes with minimal complications. Thus, the GEMES GR distal volar locking plate system represents a significant advancement in fracture management, facilitating improved functional recovery and patient satisfaction.

Author Contributions Statement:

G.R., F.B., E.S and G.V.: conception, methodology, writing; A.P. and F.G.: software, validation; F.L and A.S.: investigation; F.D.M and A.Z.: resources; G.T., A.Z, L.C. and G.R.: data curation, consent for pf-visualization; G.R., GT, : supervision; L.C.: writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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TRANSPARENCY DECLARATION

Conflict of interests: None to declare**.**

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Med Glas (Zenica)

ORIGINAL ARTICLE

Reconstruction of a short tibia stump after forced shortening with subsequent lengthening using the Ilizarov method

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ABSTRACT

Aim To demonstrate the feasibility of reconstructing a prosthetic stump in a complex defect using staged osteotomy and lengthening, as sufficient tibial stump length is required for successful prosthetic fitting and is not always achievable.

Methods In this article, we present a 25-year-old man diagnosed with a non-prosthetic short stump of the right tibia with extensive necrotized scars of the anterior and end surfaces, closely soldered to the bone sawdust, which underwent lengthening of the short stump of the tibia using the Ilizarov method.

Results The uniqueness of the procedure lies in tibial shortening combined with excision of pathologically altered tissues, stump formation, and subsequent osteotomy and lengthening of the tibial stump. Reconstruction lasted 105 days (5 days of fixation, 25 days of lengthening, and 75 days of fixation), resulting in the formation of a functional stump.

Conclusions A stump that provided functional prosthetics was created. We believe that the technique can be successfully used in young people even in the absence of soft tissue reserve of the stump.

Keywords: amputation, Ilizarov technique, myocutaneous flap, osteotomy, tibia

INTRODUCTION

Transtibial amputation is the most common type of amputation performed after trauma (1). According to American statistics (2), 41.9% of patients required revision after primary amputation. The majority of patients underwent revision of the residual stump, while in other cases, due to the short stump, revision surgery was performed on the thigh. However, regardless of the length, the advantage of the lower leg stump is the preservation of the knee joint (3). Transfemoral amputations lead to loss of the knee joint, muscle imbalance, reduced functionality, and a 65% increase in energy expenditure during movement (1,4).

Another possible solution for restoring limb function could be osteointegration surgery – the direct fixation of an artificial implant into living bone (5-7). Osteointegration is performed in people with transfemoral amputation (8-10). It improves mobility and the ability to move, reducing energy consumption compared to the traditional prosthesis with a sleeve. However, after transtibial amputation, osteointegration is rarely performed and is even considered contraindicated (11). Furthermore, revision surgery, reamputation, bone fractures, implant failure and loosening are often required after this procedure. The risk of infectious complications is 18-63% (12). A disadvantage of potential osteointegration is geometric problems with instability due to a curved femur. The main reason for the lack of interest in transtibial osteointegration is better objective mobility after conventional transtibial amputation (10).

The desire to preserve the knee joint in a short stump led to the idea of its lengthening. The literature describes cases of lengthening such stumps using the Ilizarov technique (13-18). At the same time, certain problems associated with premature consolidation of the posterior corticotomy site and soft tissues were identified (19).

Preliminary preparation of the skin and muscles was proposed using tissue expanders or myocutaneous flaps (13). Later, improved methods of lengthening, methods of creating synostosis without shortening the bone lever (20-23), and reconstructive interventions to expand the distal end of the stump (23) were developed, which allowed for positive functional results.

In the case presented in the article, the situation was complicated by the presence of extensive immobile scars, tightly fused with protruding bones.

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Unlike known methods, the goal in the presented case was to develop a new method for reconstructing the non-prosthetic remainder of the tibia, which includes removing the defect at the end of the remainder with shortening of the tibia and its lengthening using the Ilizarov method.

PATIENT AND METHODS

Patient and study design

A young man aged 25 previously healthy with no significant medical history or comorbidities sustained a mine-blast wound to his right lower limb with shattered soft tissue and bones of the shin. He underwent amputation of the upper third of his tibia. He previously underwent necrectomy, vacuum therapy, medication, dressings, and physiotherapy. The postoperative period was complicated by massive necrosis of the skin and adjacent tissues. After healing, a tibia stump was formed with extensive immobile scars, fused with protruding bone fragments (Figure1).

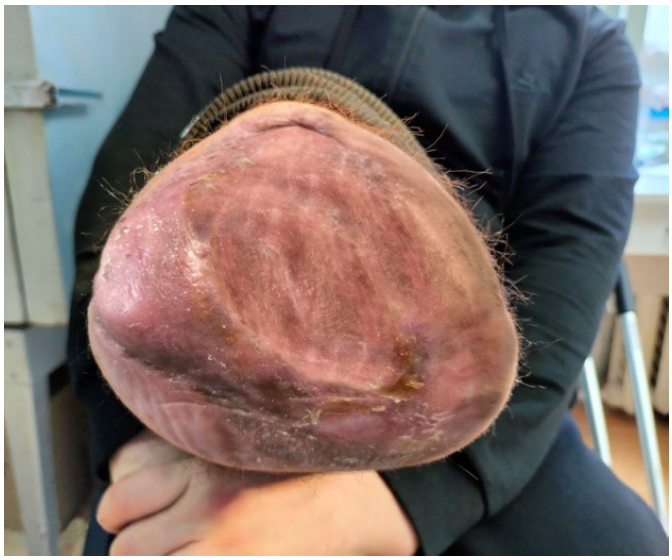

Figure 1.Tibial stump before surgery (Shevchuk V, 2021)

On the examination the prosthesis could not be loaded. Movement in the knee joint was full. The skin and muscles of the posterior and partially inner surface were preserved below the level of the bone amputation. A non-prosthetic short stump of the right tibia with extensive innervated scars of the anterior and end surfaces, closely soldered to the bone sawdust was found.
This study was approved by the Ethics Committee (Approval no: 24/2025, 30.06.2025). Written informed consent was obtained from the patient for the case details and images to be published.

Methods

After the flap incision with scar excision within healthy tissue was done, the bones were exposed. The preserved ends of the tibialis anterior, gastrocnemius, and long fibular muscles were isolated from the scars. The stumps of the tibia and fibula were truncated 6 cm proximal to the ends. Vessels were ligated, nerves were treated and shortened.
In the proximal metaphysis of the shortened tibia, 1.5 cm below the knee joint gap, perpendicular to the limb axis, without piercing the muscle tissue and taking into account the passage of the peroneal nerve, two cross pins were made, which were fixed in the ring of the Ilizarov apparatus (Federal State Budgetary Institution 'National Medical Research Centre of Traumatology and Orthopaedics named after Academician G.A. Ilizarov' of the Ministry of Health of the Russian Federation, Kurgan, Russia) (19). The *Pes anserinus* was separated from the tibia. The superficial medial collateral ligament was exposed. The distal part of the exposed ligament was separated from the bone. To protect the posterior neurovascular structures, a blunt retractor was inserted posteriorly to the medial collateral ligament and tibia. After isolating the medial border of the patellar tendon, it was cut subperiosteal from the tibial tuberosity. Under the intraoperative X-ray control with mobile C-arm imaging system control, two guide pins were placed 3.5 cm below the knee joint gap and parallel to it. Under the pins, a two-plane oblique-frontal and horizontal osteotomy of the tibia was performed directly under the pins using an oscillating saw so that the tuberosity remained on the proximal fragment (Figure2). Intraoperative control was performed using a mobile C-arm imaging system, and the mobility of the osteotomy site was checked. A self-tapping rod with a diameter of 3.5 mm in the sagittal plane and a spoke rod in the frontal plane were inserted through the distal tibia fragment perpendicular to the bone. The latter was fixed to the ring of the apparatus, which in turn was connected by threaded rods to the ring above.
Interfragmentary compression was performed in the osteotomy area. The antagonist muscles were stitched together. Active wound drainage was performed. The wound is sutured in layers.
The prosthesis quality index – The Locomotor Capabilities Index (LCI) as part of the Prosthetic Profile of the Amputee questionnaire was used to assess the patient's locomotor capabilities (24).

RESULTS

On the 5th day, distraction was started at 1 mm per day. Interfragmentary compression was continued for 4 days. X-ray control was performed every 15 days of fragment displacement. Distraction lasted for 75 days, fixation 75 days. The length of the regenerate obtained was 6 cm.
On the 15th day of distraction on control radiographs the height of the regenerate corresponded to the pace and rhythm of distraction. Cloud-like shadows of medium intensity were visible throughout the whole area. After 30 days, a normoplastic type regenerate was formed in the interfragmentary diastasis, evenly filling the diastasis (Figure2). After 60 days of distraction, the normoplastic regenerate was characterized by the presence of intense shadows of the proximal and distal sections and a medial zone of lucency. After 75 days of distraction, analogous results were observed. After 1 month of fixation, there was a fusion of the medial lucency zone. Homogeneous shadows of high intensity were determined. The cortical layer began to form on the posterior surface. After 2.5 months of fixation, there was fusion of bone trabeculae in the distal part of the regenerate. Well-formed cortical lamina was determined along the posterior and lateral edges. Scanning in the middle zone of the regenerate revealed vessels 0.28-32 cm in diameter with rheographicindices (RI) RI=3.61 and RI=1.4, indicating the maturity of the regenerate (Figure3). The Ilizarov apparatus was removed. The 6-centimetre regenerated bone had a homogeneous structure

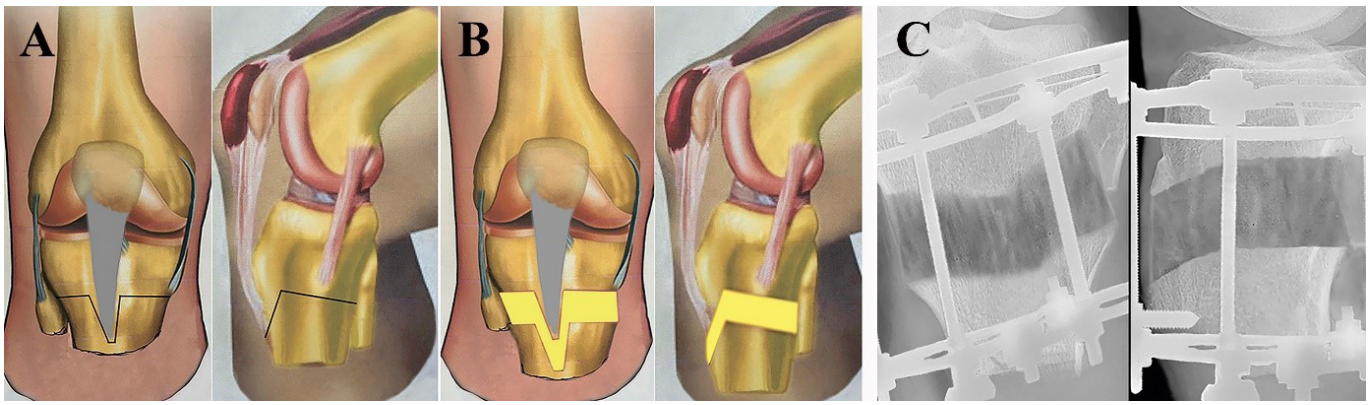

Figure 2. The osteotomy scheme and radiograph of distraction regenerate after 30 days after surgery. A) Schematic representation of the osteotomy site; B) Schematic representation of stump lengthening; C) X-ray image of the regenerated tibia 30 days after distraction (Shevchuk V, 2021)

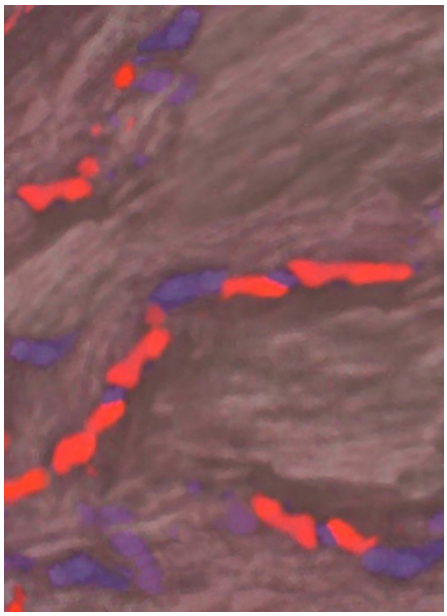

Figure 3. Scan of blood vessels in the regenerate 75 days after the surgery (Shevchuk V, 2021)

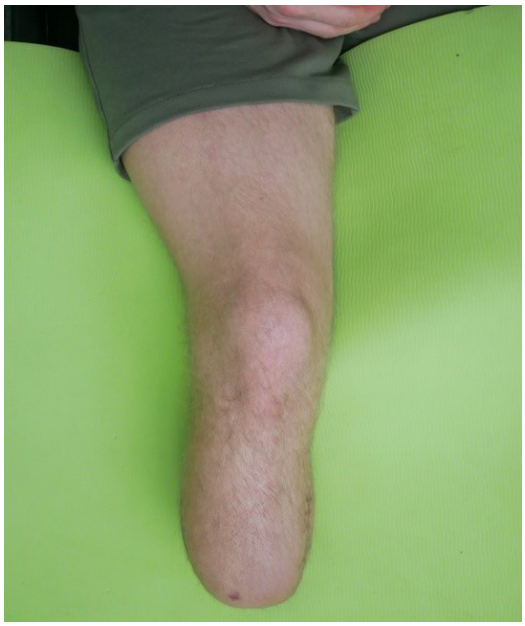

Figure 5. Moderately conical tibial stump one year after surgery (Shevchuk V, 2022)

of spongy bone tissue. In terms of density, it approached the adjacent areas of the mother bone (Figure 4). Nine and half months after the apparatus was removed, the density of the regenerate was slightly higher than that of the parent bone. The cortical diaphyseal layer was formed along the entire perimeter of the regenerate (Figure4). The shape of the stump was moderately conical (Figure5). After 3 years, the organotypic restructuring of the bone regenerate characteristic of this section was completed on radiographs in the lengthening zone (Figure4).

The prosthesis quality index – The Locomotor Capabilities Index (LCI) as part of the Prosthetic Profile of the Amputee questionnaire (24) after the operation was 55. The patient, who working as a mechanic in a garage used the prosthesis without additional means of support, and walked up to 12-13 km per day.

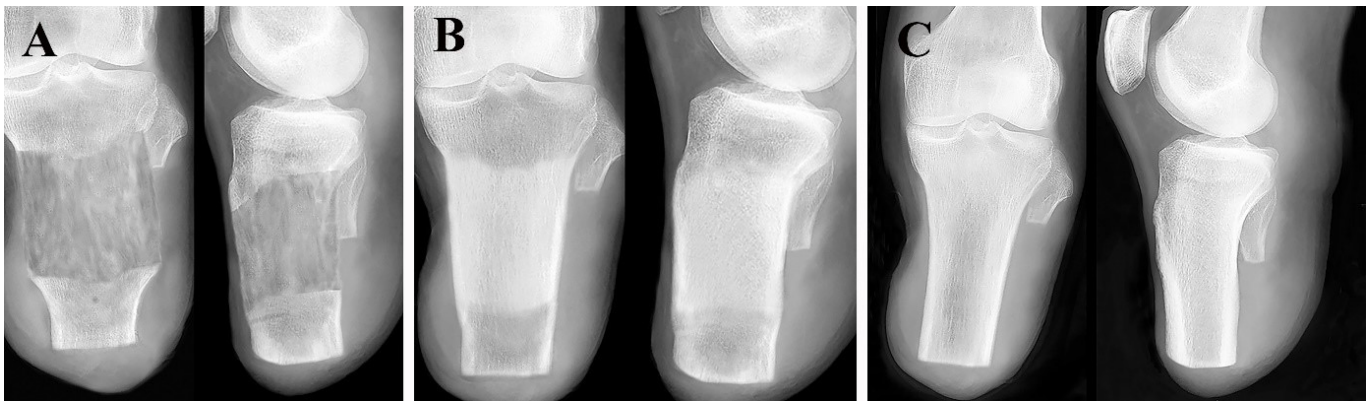

Figure 4. Radiographs presentation. A) distraction regeneration of the tibia after 75 days of lengthening (Shevchuk V, 2021); B) distraction regeneration of the tibia after 9.5 months; (Shevchuk V, 2021); C) tibial stump 3 years after surgery (Shevchuk V, 2023)

DISCUSSION

A transtibial amputation has definite advantages over a hip amputation in terms of greater functionality (3). However, prosthetics is sometimes complicated by a too short stump. There are isolated reports in the literature about the lengthening of the tibial stump (24). A prerequisite for stump lengthening is the creation of a soft tissue reserve, for which tissue expanders are used (13). In contrast to the known reports (13-17), the proposed technique involves removal of the defective end of the stump while preserving its length, which was achieved. Therefore, the first step of the operation was a high amputation of the tibia within healthy tissues with the formation of a soft tissue reserve with the expectation of subsequent lengthening. The second feature in our case was the performance of a two-plane osteotomy, which allowed us to obtain a fairly massive regenerate.

The presence of a dense capillary network in the epi-metaphyseal zone of the tibia creates favourable conditions for compensation of local microcirculatory disorders caused by osteotomy (25). Therefore, when compression was applied at the osteotomy site, an endosteal reparative reaction of the cancellous bone occurred, which made it possible to start distraction in 5 days (24).

As a result of traction forces application, conditions of tension with regenerate formation were created in the tissues. When the necessary length of the regenerate is reached, fixation conditions are created. During this period, the apparatus system was stabilized, performing only manipulations to ensure elastic tension of the fixation elements.

In the process of distraction, it may be necessary to prolong the action of tension forces. Technically, this is accomplished by further application of traction or compression forces, their sequential change, and recomposition of the apparatus.

The lengthening stage is divided into two periods: active lengthening, when compression and distraction are performed, and the fixation period, which is necessary for rebuilding the formed regenerate into mature bone tissue (24).

Possible complications of the **intervention** are most often associated with the insertion of the spokes (26,27). They can be divided into infectious (suppuration of soft tissues, purulent arthritis, dermatitis), non-infectious (reactive arthritis, vascular and nerve injury, joint contracture). When performing the intervention, complications may occur due to compression or distraction and improper application of the apparatus (impaired innervation, impaired trophism, joint contracture) (26,27). After removal of the apparatus, deformation of the regenerate or its fracture is possible (27). Observance of asepsis, uniform tension of the spokes, diagnosis and treatment of early and late inflammatory complications, timely removal and rewiring of the spokes will allow to stop the process. Knowledge of topographic anatomy and variants of location of the main neurovascular bundles excludes their damage. If a vessel is damaged, the spoke should be removed and a new one should be inserted next to it (26,27). Bleeding is stopped by pressing the puncture site with a tampon. If the peroneal nerve is damaged, the spoke must be removed (26,27).

According to Latimer (14), some patients had a significant loss of length of the regenerate after lengthening. The authors attribute these findings to early functional loading. In our opinion, to prevent shortening caused by compression during early loading, it is necessary to overstretch the regenerate by 1–2 cm in advance, which apparently was not considered by these and other researchers (2), as no reference to this approach is provided. In addition, after the end of distraction, the distance between the rings should be increased 1 mm every 5-7 days (13,14). None of the available reports (13,18) provided information on the exposure of the anterior medial ligament, severing its distal part from the bone, or sub periosteal severing of the medial part of the patellar tendon from the tibial tuberosity, which could also affect the shortening of the regenerate.

Our clinical experience shows that in case of sharply expressed osteoporosis with the formation of the cortical layer due to the possibility of crumpling or secondary resorption of the porous bone area under the influence of functional overload, it is necessary to provide the conditions of the most gentle mode of increasing the functional load.

After removing the appliance, conditions of regenerate deformation or fracture may occur. Therefore, the device can be removed when the density of the regenerate on the radiograph approaches the density of the surrounding bone.

These possible errors and complications arise due to incorrect tactics and techniques of using the Ilizarov apparatus and should not be a barrier when performing reconstructive interventions on the bone stump (27). Such interventions can improve the quality of life of patients, and knowledge of the causes and conditions of possible errors and complications opens real ways to their prevention (27).

The most important limitations of the study are: a single case which does not allow giving a number of theoretical and biological explanations; residual limb after amputation due to obliterating diseases of the peripheral vessels of the joints; festering inflammation of the skin; insufficient use of the apparatus of external fixation; a long period of time for obtaining the regenerate and its rebuilding.

On the example of the presented clinical observation, the authors demonstrated the saving tactics, real possibility, technical details and quite satisfactory results of a non-standard variant of surgical treatment of a patient with malformed stump of the tibia.

Stable fixation, fractional distraction, and maintenance of elastic tension of the fixation elements contribute to the formation of a newly formed bone area and high-quality prosthetics. Similar techniques can be used in young people after amputation with removal of extensive scars fused with protruding bone and end-stage osteomyelitis. To obtain optimal results, the technique can be adapted to individual cases.

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TRANSPARENCY DECLARATION

Conflicts of interest: None to declare.

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Med Glas (Zenica)

ORIGINAL ARTICLE

Outcomes and complications of exposed Kirschner wire fixation for metacarpal fractures: a retrospective study

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ABSTRACT

Aim Metacarpal fractures are common hand injuries, frequently treated with closed reduction and internal fixation (CRIF) using Kirschner wires (K-wires). While exposed K-wires allow for simple hardware removal and early mobilization, their use is traditionally associated with a higher risk of pin tract infections. There is a lack of studies focusing exclusively on exposed K-wires in metacarpal fractures.

Methods A total of 134 patients who underwent CRIF with exposed K-wires for metacarpal fractures between January 2023 and March 2024 were retrospectively reviewed. After applying inclusion and exclusion criteria, 118 patients were analysed. Demographics, fracture characteristics, complications, and follow-up data were collected. Functional outcomes were assessed using the QuickDASH and the Michigan Hand Outcomes Questionnaire (MHQ) before hardware removal.

Results Most patients were male, 88 (75%), with a mean age of 39.3 years. The little finger was the most frequently injured, 71 (60%). In 102 (86%) cases, a single metacarpal fracture was observed. The average number of wires used was 1.7, and hardware was removed after a mean of 40.1 days. The rate of pin tract infection was <4%, all classified as superficial and managed conservatively. Only one case required reoperation due to secondary displacement. The mean QuickDASH score was 25, and the average MHQ score was 70.15, indicating overall good functional and patient-reported outcomes.

Conclusion Exposed K-wire fixation for metacarpal fractures appears safe and reliable, with low complication rates and favourable patient-reported outcomes, supporting its use in outpatient pathways without compromising safety.

Keywords: device-related infections, hand bones, retrospective studies, treatment outcome

INTRODUCTION

Fractures of the metacarpal bones account for approximately 10% of all fractures, constituting more than one-third of all hand fractures (1). Most hand fractures can be treated conservatively, provided they are stable and show minimal shortening or malrotation (2-4). Unstable, closed, and reducible metacarpal fractures can be effectively treated with Kirschner wire (K-wire) fixation (5-6).

Closed reduction and internal fixation (CRIF) with K-wires provide functional outcomes and complication rates comparable to those of open reduction and internal fixation (ORIF) using mini-plates (7). Both techniques are reliable and should be chosen according to the fracture pattern and patients' characteristics (8).

K-wire fixation offers minimal soft-tissue damage and has the advantage of leaving the patient hardware-free after approximately 4 weeks (7). When used in fracture fixation, K-wires may be left buried under the skin or left exposed outside the skin surface. Buried wires reduce the risk of external contamination and allow earlier dressing removal; however, their removal typically requires an additional operating room procedure. In contrast, exposed wires allow for simple outpatient removal and obviate a secondary surgery, potentially facilitating early mobilization. The main concern traditionally raised against exposed wires has been pin tract infection, yet emerging data indicate that the practical advantages of exposed K-wires may outweigh this risk, especially in routine clinical settings (9–12).

Pin site infection is reported as the most common complication, with a prevalence ranging from 4% to 20% (13-14). K-wire site infections are typically managed with antibiotics and early pin removal, though in some cases surgical debridement may be required (11). Several studies have attempted to determine whether this complication is more likely to occur when K-wires are left exposed, but the debate remains open (9–11).

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A recent prospective study found no statistically significant difference in infection rates between exposed and buried K-wires for hand fractures, highlighting the practicality of exposed wires for outpatient management without compromising safety (23). Available studies are not conclusive, either due to the limited number of patients included (16,19) or, in comparative studies (buried vs exposed), because of unbalanced group sizes (10–11, 13). In other cases, the analysis was not confined to a single anatomical region, thereby reducing the specificity and applicability of the findings (11,13,17).
The aim of our study was to focus exclusively on metacarpal fractures, analysing both the post-operative complication rate and patient-reported satisfaction in cases treated with exposed K-wires.

MATERIALS AND METHODS

Patients and study design

A total of 134 patients with metacarpal fractures treated with closed reduction and internal fixation (CRIF) were retrospectively identified at the Department of Orthopaedics and Hand Surgery of the Policlinico Gemelli in Rome between 1 January 2023 and 1 March 2024.
Inclusion criteria were: patients of any age with one or more metacarpal fractures, treated with CRIF using K-wires, minimum follow-up available until hardware removal (at least 30 days), and availability of a Quick Disabilities of the Arm, Shoulder and Hand (QuickDASH) score (21). Exclusion criteria were: fractures of other bones, hardware removal performed at another institution, neurological impairments or any other neuromuscular conditions.
Patient age, gender, comorbidities, and date of primary surgery were recorded. Surgical indication, fracture location, K-wire placement, number of K-wires, and date of removal were collected through chart review, together with postoperative complications such as pin tract infection, secondary displacement, and the need for reoperation, which were systematically assessed at scheduled follow-up visits.
Patients completed the QuickDASH score (21) and the Michigan Hand Outcomes Questionnaire (MHQ) (18-22) either in clinic or by phone, referring to the period prior to hardware removal. Both instruments were selected because they are validated outcome measures for upper limb musculoskeletal conditions, providing reliable and reproducible data on function and patient-reported satisfaction.
The study was conducted in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments. Informed consent for data collection and analysis was obtained from all patients involved.

Methods

Surgical procedure. Surgery was performed by experienced orthopaedic surgeons from the same team. All patients received a single dose of intravenous cefazolin (2 g) 30 minutes prior to surgery. The surgical field was prepared and draped under sterile conditions.
The standard procedure consisted of closed reduction. In cases where closed reduction was not feasible or extremely difficult, a small incision cantered over the fracture site was made to perform a mini-open reduction. Internal fixation was achieved using K-wires of various diameters (ranging from 1.2 mm to 1.8 mm), under fluoroscopic guidance.
For fractures involving the head, diaphysis, and distal metaphysis, K-wires were inserted in a proximal-to-distal direction. For fractures of the base, with few exceptions, wires were inserted retrograde.
The wires were left exposed in all cases and bent at right angles near the skin surface to prevent migration and to create a tension-free interface between the skin and the K-wire. The exposed segment of wire was covered with diluted povidone-soaked ribbon gauze, and a light dressing was applied, leaving the metacarpophalangeal (MCP), proximal interphalangeal (PIP), and distal interphalangeal (DIP) joints free to move (Figure 1, 2).

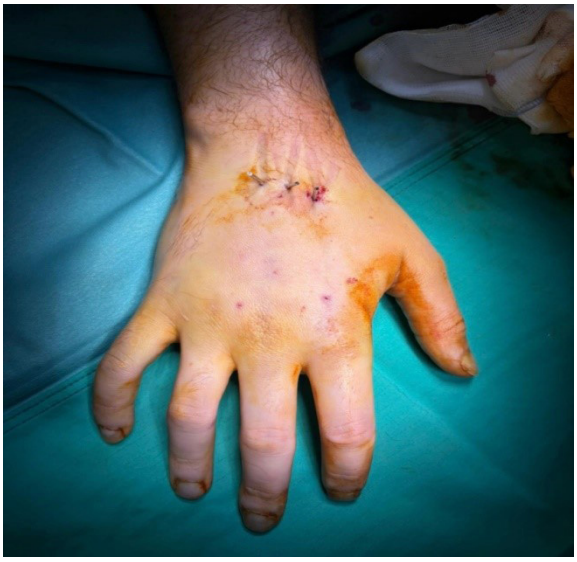

Figure 1. Intraoperative image demonstrating exposed Kirschner wire fixation for metacarpal fracture (Department of Orthopaedics and Hand Surgery, Fondazione Policlinico Universitario A. Gemelli IRCCS, 2023)

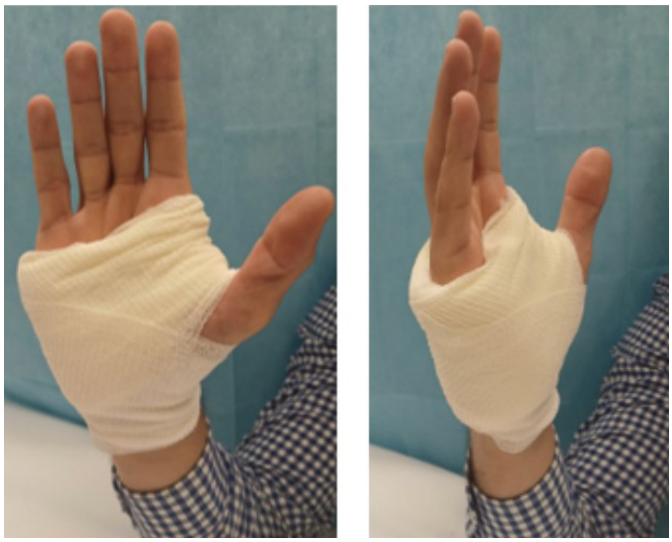

Figure 2. Postoperative view of the hand dressing (Department of Orthopaedics and Hand Surgery, Fondazione Policlinico Universitario A. Gemelli IRCCS, 2023)

Postoperative care. No antibiotics were administered during the standard postoperative period. Patients were instructed to maintain the dressing clean and dry, and were advised against changing it at home. No sutures were placed, except in cases where open reduction was required. All patients were evaluated in the clinic at 7 and 30 days postoperatively with X-ray imaging. An additional (intermediate) X-ray was requested for patients at risk of secondary fracture displacement.

The dressing was not changed until the day of hardware removal, unless clinical signs of infection were present or the dressing became soiled. Infection was defined clinically as erythema, tenderness of the surrounding soft tissues, or purulent discharge at the pin site. K-wire removal was performed between four and six weeks postoperatively.

The QuickDASH (21) is a shortened version of the original DASH Outcome Measure, consisting of 11 items (instead of 30 originally). It is designed to assess physical function and symptoms in individuals with upper limb musculoskeletal disorders. We used the Italian version of the MHQ (18, 22). The MHQ is a self-administered questionnaire composed of six subscales: overall hand function, activities of daily living, work performance, pain, aesthetics, and patient satisfaction. Items are rated on a 5-point scale, ranging from 1 (very good) to 5 (very poor). The scores are then converted to a 0–100 scale, where higher values indicate better hand function and greater patient satisfaction.

Statistical analysis

Data were analysed using descriptive statistics. Continuous variables were reported as means with standard deviations, while categorical variables were expressed as percentages. These methods were chosen to provide reliable and transparent reporting of clinical outcomes and to ensure comparability with previous studies on hand fractures.

RESULTS

A total of 118 patients (out of 134) with surgically treated metacarpal fractures were ultimately included. Five patients were excluded due to loss to follow-up or incomplete documentation. Two patients presented with neurological impairments, while three could not be reached by phone, preventing collection of the QuickDASH and MHQ scores. Six patients reported concurrent major trauma and were therefore excluded.

Eighty-eight (75%) patients were male. The mean age at the time of injury was 39.26 years (range: 14–82).

The right-to-left ratio of injured hands was 7:3; however, data on hand dominance were not collected.

The most frequently injured finger was the little finger, involved in 71 (60%) cases. Ring finger injuries accounted for 20 (17%), index finger for 15 (13%), and middle finger for 14 (12%). The remaining 11 (9%) cases involved the thumb.

In 102 (86%) patients, a single metacarpal bone was fractured; in the remaining cases, two or more digits were affected.

The mean number of K-wires used was 1.7. Hardware removal was performed at a mean of 40.1 days postoperatively (Table 1). Infection was observed in fewer than 4% of cases (4/118), all of which were characterized by soft tissue redness at the pin site with serous or purulent discharge. In one case, the fracture was open, while another patient presented with an extremely deteriorated and dirty dressing. One patient was 78 years old; the others were under 60. None of them had comorbidities known to increase infection risk, such as diabetes.

In all cases, the infection resolved with empiric antibiotic therapy (amoxicillin/clavulanic acid at standard dosage) and early hardware removal, followed by immobilization until complete healing was achieved. In all cases of infection, microbiological cultures were obtained from the pin tract. *Staphylococcus aureus* was identified as the causative pathogen in each case.

Loss of reduction occurred in only one patient (<1% of the cohort), requiring reoperation and new reduction.

Table 1. Characteristics of 118 patients with surgically treated metacarpal fractures

Variable	
Mean age (range±SD) (years)	39.26 (14-82±14.5)
	No (%) of patients
Gender	
Male	88 (74.6)
Female	30 (25.4)
Number of metacarpal fractures	
Single	102 (86.4)
Multiple	16 (13.6)
Distribution by digit	
I° MC	11 (9.3)
II° MC	15 (12.7)
III° MC	14 (11.9)
IV° MC	20 (16.9)
V° MC	71 (60.2)
Mean days before wire removal	40.1
Mean MHQ score	70.15
Mean QuickDASH score	25

In five patients, hardware removal was delayed due to slow fracture healing.

No cases of malrotation or unacceptable shortening were reported.

The mean QuickDASH score was 25, which corresponds to mild residual disability, indicating that patients were able to resume most daily-life activities with only minor limitations. The mean MHQ score was 70.15, reflecting good perceived function and high patient satisfaction. It should be noted that some questionnaire items were not entirely applicable to our patient population. Specifically, questions related to heavy activities (such as opening jars, carrying bags over 10 pounds) or washing could not be properly answered, as patients had been instructed to avoid such tasks during recovery. Conversely, patients engaged in office-based work reported being able to attend their workplace or work remotely (Table 2).

Table 2. Postoperative complications in 118 patients

Complication	**No (%) of patients**
Superficial infections	4 (3.4)
Deep infections	0 (0)
Reintervention	1 (0.85)
Loss of reduction	1 (0.85)
Hardware removal after expected period	5 (4.2)

DISCUSSION

This retrospective study was designed to confirm the reliability and safety of exposed Kirschner wires in the treatment of metacarpal bone fractures. The main focus was to debunk the idea that associates exposition of wires and higher pin-related infection (19). Most studies supporting high infection risks analyse distal radius fractures with longer hardware removal time (14,16,19) and do not take into account data regarding exclusively metacarpal bone fractures (13, 17, 19). Many studies compare the complications and infection rates between bur-

ied and exposed K-wires but most of the time there is unbalance between the two groups in favour of exposed K-wires (10,11,13). Some authors pointed out that despite the widespread use of K-wires for their ease and low cost, the risk of pin site infection remains a relevant issue, particularly when wires are left exposed, and current evidence does not clearly favour one technique over the other (15).

Our study aimed to provide a focused analysis of metacarpal bone fractures trying to give a clear overview on the complications of the treatment with exposed K-wires. Compared to previous reports in the literature, the demographic data we collected are overlapping. The first difference we noticed is the average hardware removal time. We recorded an average time of 40.05 days, which is higher than what is reported in literature (16, 19-20). These findings are favourable, as we observed a lower infection rate despite an extended hardware retention period. As regards infection, we recorded a rate of <4% of infection of the pin tract and all of them were superficial infections caused by the isolated pathogen *Staphylococcus aureus*. These data recall the lower end indicated in literature; the highest reported infection rate for exposed K-wires is 20% (14,19). However, this figure is not specific to metacarpal fractures and may therefore be biased. In other studies, focusing more specifically on the use of exposed K-wires in phalangeal and metacarpal fractures, the reported infection rate was 6.5% (13,17), which is more consistent with our findings.

One of the biggest advantages of K-wires is the possibility to perform hardware removal quickly. K-wires are usually removed in outpatient consultations. Reasons for reintervention could be unacceptable or loss of reduction, infection or difficult removal outside the operating room. While the first two complications are less likely in phalangeal and metacarpal fractures, the removal of buried K-wires can be challenging in an outpatient setting. According to Hargreaves et al. (19), up to 18% of buried K-wires require removal in the operating room, compared to exposed wires which are typically removed in clinic. In our series, only one patient required reintervention due to secondary loss of reduction.

One of the advantages of buried K-wires is the possibility to leave the hand free of dressing. Our personal preference when using exposed K-wires is to leave a soft dressing with the wrist and the metacarpal-phalangeal joints free until removal. In this way the patient is free to move immediately.

However, micromotion between the wire and the soft tissue interface may lead to excessive soft tissue injury, potentially resulting in deep-seated infections around the buried metacarpal wires, so it is important to properly bend the wires and cover them with abundant dressing. Patients are educated to perform finger exercises, so that mobility will be almost at full ROM after hardware removal, and to use the hand for basic daily life activities. We decided to administer our patients both QuickDASH and MHOQ to prove that even with K-wires they could manage basic daily life activities. As expected, all items related to strength and work-related activities received lower scores, given the activity restrictions imposed during the recovery period.

This study has some limitations, including the relatively small sample size, the retrospective design, and the absence of a control group treated with buried K-wires.

In conclusion, our results provide clinically relevant data, since no previous study has focused exclusively on metacarpal fractures treated with exposed K-wires. For surgeons, these findings highlight that exposed K-wire fixation can be considered a safe and effective option, especially in cases where outpatient management and simple hardware removal are desirable, without increasing the risk of severe complications. Future prospective and multicentre studies, ideally randomized and comparing exposed and buried techniques under uniform conditions, are needed to confirm these observations and to establish standardized treatment protocols.

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TRANSPARENCY DECLARATION

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ORIGINAL ARTICLE

A useful surgical landmark for the trapezio-scaphoid joint

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ABSTRACT

Aim Unintended scaphoid excision is a rare but serious complication that can occur during surgical procedures involving the trapezium, including but not limited to trapeziectomy. Although prosthetic replacement is increasingly favoured in the treatment of rhizarthrosis, trapeziectomy (with or without ligamentoplasty) remains a widely used and effective option. To reduce the risk of inadvertently removing the scaphoid, we rely on the anatomical intersection between the radial artery branch and the first dorsal compartment tendons as a landmark for identifying the scapho-trapezial joint.

Methods In our institution, Policlinico Universitario A. Gemelli IRCSS, we have been using a simple landmark to identify scapho-trapezial joint to teach residents: during surgery, after isolating the radial artery, a 16G needle is inserted at the intersection of the extensor tendons and the radial artery branch, followed by fluoroscopy to confirm needle placement in the scapho-trapezial joint. Patients were classified according to Eaton-Littler classification and the accuracy of the landmark was then assessed among groups.

Results So far we used the landmark on 212 patients. The distribution by Eaton-Littler stage was: 11 stage 1, 63 stage 2, 79 stage 3, and 33 stage 4. The reference point was accurate in 178 cases. No significant differences were found by sex or between stages 1, 2 and 3. However, accuracy in stage 4 was significantly lower ($p<0.00001$).

Conclusion Our results confirm reliability of this reference point, particularly in stages 1–3. While useful in stage 4, additional caution is required due to slightly reduced precision.

Keywords: C05 musculoskeletal diseases, E01 diagnosis, E04 surgical procedures

INTRODUCTION

Rizharthrosis, or thumb basal joint arthritis, is a pathology that involves the thumb's base joint (carpometacarpal joint; CMC joint). Rhizartrhrosis usually advances over time as the joint cartilage begins to degenerate, although some cases may stem from previous injuries or inflammatory processes (1).

The thumb basal joint is the second most frequent location of osteoarthritis (OA) in the hand after the distal interphalangeal (IP) joint of the forefinger with radiographic manifestation in almost 40% of females by age 80 (2). The female sex is a consistent risk factor for thumb CMC OA as it the likely outcome of increased ligamentous laxity (3-4). Other contributing factors are genetic, environmental, and preexisting comorbid conditions (5).

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Alterations in the clinical presentation may occur in varying degrees of severity as the disease is not always clinically evident (6). Symptoms increase in severity with activities demanding a pinching or gripping action like signing, fetching, or jar opening (7-8). Examination of the thumb shows first metacarpal adduction with accompanying metacarpophalangeal (MCP) hyperextension compensatory deformity (9).

The CMC grind test is mostly the one test performed to confirm the diagnosis (10).The diagnosis of rhizarthrosis relies mainly on clinical assessment combined with the patient's history, and, in some cases, X-rays are needed. Characterization is usually done by standard radiographs including postero-anterior (PA) view, lateral, and oblique views of the hand and wrist. CT scans are not in daily based diagnosis but can be useful for challenging pre-operative planning situations (12). Several classifications exist for describing arthritis of the thumb's basal joint using X-ray, with the most popular being Eaton-Littler's (12).

Treatment options for rhizarthrosis aim to alleviate pain, improve function, and slow down the progression of joint damage. Conservative treatments may include activity modification, splinting, pain medications and corticosteroid injections

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to reduce inflammation (13). Occupational therapy can also be beneficial for strengthening the surrounding muscles and improving thumb mobility (14).

In cases where conservative measures are ineffective, surgical intervention may be considered. Surgical options for rhizarthrosis include trapezial excision with or without ligament reconstruction, arthroplasty (joint replacement), arthroscopy, or arthrodesis (joint fusion), depending on the severity of the condition and the patient's individual needs (15).

The main procedure performed at our centre is trapeziectomy with tendon interposition. The main aim of the ligament reconstruction and tendon interposition (LRTI) is the reconstruction of anterior oblique ligament by using half of flexor carpi radialis tendon or abductor pollicis longus tendon. The key points of this surgery are the identification and protection of radial artery and the correct excision of the trapezium. In case of severe alternation of normal anatomy the joint line might not be clearly recognizable (16).

For patients undergoing surgery, complications such as infections, nerve or tendon injury, stiffness, joint instability, and reduced strength are well-recognized (17). However, among these, one particularly serious and underreported complication that deeply concerns especially young surgeons during their initial procedures is the incorrect removal of the scaphoid instead of the trapezium (18). This mistake can lead to severe functional impairment, prolonged disability, and complex revision surgeries (19).

Given the catastrophic consequences of scaphoid excision, the identification of a reliable and reproducible anatomical landmark to accurately locate the scapho-trapezial space is essential. According to Caggiano et al. (18), a clear intraoperative reference can drastically reduce this risk. In our experience, the intersection between the first extensor compartment tendons, abductor pollicis longus (APL) and extensor pollicis brevis (EPB) and the branch of the radial artery corresponding to the scapho-trapezial joint provides a highly dependable landmark. This landmark plays a critical role in guiding the surgeon, especially when progressive rhizarthrosis leads to distorted anatomy, altered bony landmarks, and extensive osteophyte formation, which can otherwise make the joint line difficult to discern (16).

By using this vascular-tendinous intersection as a reference point, surgeons can enhance the precision of trapeziectomy, avoid the devastating error of scaphoid removal, and ultimately improve surgical safety and patient outcome, as this reference is highly patient-specific beyond any geographic and environmental influence (18-19).To the best of our knowledge, there are no articles in the literature that describe a landmark meeting these requirements. The aim of this study is to define and validate a reproducible vascular–tendinous intraoperative landmark for precise identification of the scaphotrapezial (ST) joint during trapeziectomy for thumb CMC osteoarthritis, and to evaluate its reliability, accuracy, and safety in patients with basal thumb arthritis.

PATIENTS AND METHODS

Patient and study design

The landmark has been used in our institution, Policlinico Gemelli, Rome, Italy as teaching support for residents for about 3 years. Surgeries were performed on patients recruited from the hand surgery outpatient clinic on the Policlinico Gemelli Campus from 1 April 2021 to 1 April 2024.

Patients were considered eligible for landmark evaluation in case of: radiographic diagnosis of rhizarthrosis in any stage and indication to surgical treatment by trapeziectomy with or without tenoplasty. Patients younger than 45, with anatomical variations (such as trapezium dysplasia, presence of superficial dorsal branch of the radial artery, abductor pollicis longus insertion over the trapezium), history of previous hand surgeries as previous first metacarpal fractures or De Quervain syndrome surgery, vascular or nerve disorders (e.g. peripheral microvascular impairment and polyneuropathy), and history of treatment with injection therapy were not tested for the landmark.

The study complies with national ethical standards and the Declaration of Helsinki. According to institutional protocols, each patient was given informed consent for surgery and for the collection of clinical data for scientific purposes at admission and before the surgery.

Methods

The evaluation process begins with taking a thorough patient history, followed by a physical exam. Standard imaging is then performed using X-rays in both anteroposterior (AP) and lateral views, with particular focus on the trapeziometacarpal joint to check for signs of arthritis or degenerative changes. When indicated, additional X-rays of the base of the thumb are taken to get a clearer picture of joint alignment, especially in cases involving subluxation or the presence of osteophytes.

Surgeries were performed under locoregional anaesthesia with a tourniquet applied to the proximal upper arm. The classic surgical approach for performing trapeziectomy was used, with a longitudinal incision of approximately 4 cm at the base of the thumb. During the surgical approach for trapeziectomy, once the radial artery was freed, all patients had a 16G needle inserted into the capsule at the intersection between the extensor tendons of the first compartment and the branch of the radial artery (Figure 1).

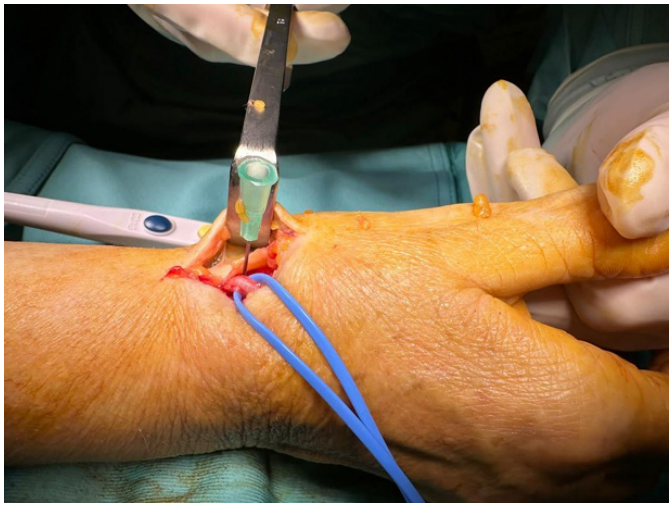

Figure 1. Image showing a 16G needle inserted at the intersection between the extensor tendons of the first compartment and the deep branch of the radial artery (property of Camillo Fulchignoni, 2024)

A fluoroscopic image (in Kapandji projections – antero-posterior and lateral) was taken to confirm that the needle was correctly placed in the scapho-trapezial joint (Figure 2).

Selected patients were divided into 4 groups according to Eaton-Littler classification, as described in Table 1 (12).

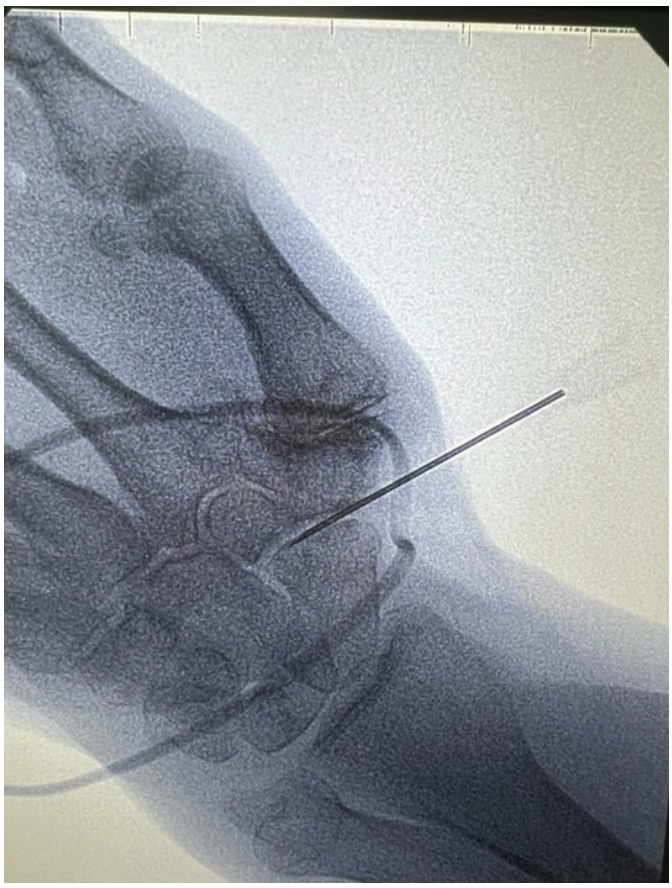

Figure 2. Fluoroscopy image showing the needle placed in the scapho-trapezial joint (property of Camillo Fulchignoni, 2024)

Table 1. Eaton-Littler classification

Eaton Littler's Grade	Definition
Grade I	Normal or slightly widened joint space (synovitis/ laxity), no osteophytes, minimal/no subluxation (<⅓ of the metacarpal base
Grade II	Mild joint-space narrowing, early subchondral sclerosis/cysts, osteophytes <2 mm, subluxation still <⅓
Grade III	Marked narrowing of the CMC joint, prominent sclerosis/cysts, osteophytes >2 mm, and subluxation >⅓ of the metacarpal base
Grade IV	Criteria of Stage III plus degenerative changes of the scaphotrapeziotrapezoid (STT) joint (pan-trapezial involvement)

Statistical analysis

The reliability of the landmark was presented as raw numbers and corresponding percentages to provide a clear overview of its consistency across observations. To evaluate the significance of changes in the distribution of bounded scores among the different groups (or stages), a two-sided $\chi 2$ test was employed, with the significance threshold set at $\alpha=0.05$. Categorical data are reported as n (%) with 95% confidence intervals (CI) for proportions (Wilson).This statistical approach was chosen due to its suitability for categorical data and its ability to detect differences in frequency distributions between independent groups. All statistical analyses, including the calculation of observed and expected frequencies, as well as p-values, were performed using Microsoft Excel 2003, which allowed for the organization, tabulation, and comparison of data in a structured format.

RESULTS

In the selected period of time, surgery was performed on 212 patients, of which 186 were tested for the landmark (Figure 3). A total of 127 patients were female (59.9%) and 59 (40.1%) male; the average age was 67.

Patients were allocated to groups according to the Eaton–Littler stage (12) determined on pre-operative radiographs. Two trained hand surgeons independently graded the images blinded to intraoperative findings and outcomes; disagreements were resolved by consensus. At the moment of selection, 11 patients presented with grade 1 tapezio-metacarpal (TM) arthrosis (six-females and five males). In all cases (100%) the intersection between the first extensor channel and the deep branch of the radial artery corresponded to the scaphotrapezial joint. The same rate was recorded for 63 patients in grade 2 (45 females and 18 males). Our landmark came up less accurately in patients belonging to grande III and IV (98.7% and 78.8%). Gender and age were not significantly associated with landmark accuracy or with disease grades I, II, and III. A significant difference between the accuracy of the landmark in the first three grades and grade IV was found ($p<0.00001$)

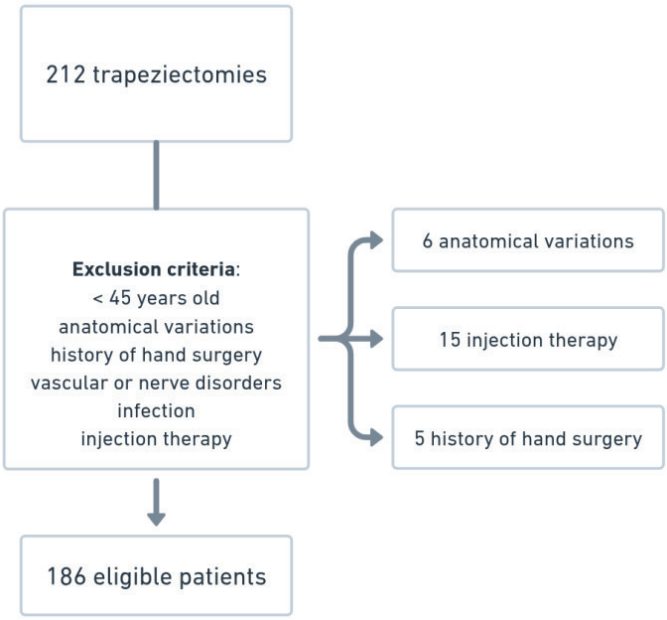

Figure 3. Flowchart showing patients selection

Table 2. demographic data and landmark accuracy

Eaton Littler's Grade	No (%) of patients	Female	Male	Accuracy (%) (CI)
Grade I	11 (5.91%)	6 (54.54%)	5 (45.45%)	100 (74.1 – 100)
Grade II	63 (33.87%)	45 (71.42%)	18 (28.57%)	100 (94.3-100)
Grade III	79 (42.47%)	56 (70.88%)	23 (29.11%)	98.7 (93.2-99.8)
Grade IV	33 (17.74%)	20 (60.60%)	13 (39.39%)	78.8 (62.2-89.3)

* Percentages by gender are calculated relative to each group's sample size (group n), not the overall cohort – CI = confidence interval

DISCUSSION

Trapeziectomy is the most performed surgery for arthritis of the trapeziometacarpal joint, both alone or followed by a suspension arthroplasty. Trapeziectomy has traditionally been the operation of choice after conservative failure, first performed in 1948 (19), and has since been modified to prevent excessive thumb shortening (20). The main complications of this technique are lesions of the dorsal branch of the radial nerve and lesions to the deep branch of the radial artery (21). The first step of this procedure is to identify these structures and protect them throughout the whole surgery. Despite appearing to be an easy and standardized procedure for expert surgeons, young surgeons and trainees might find it hard to identify the articular space, especially in cases of severe arthritis and dysplasia of the trapezium. According to Caggiano et al. (19), taking out the wrong bone in hand surgery is not so uncommon. The most commonly incorrectly excised bone is the scaphoid, with the most common reasons being inadequate visualization or inadequate localization (18-19). Our landmark could effectively solve part of this problem as it presents an easy and reproducible way to localize the articular line between scaphoid and trapezium.

From an educational standpoint, especially for junior surgeons, a consistent land-mark simplifies the process of learning trapeziectomy and builds confidence. However, it is also critical to acknowledge that anatomical landmarks can be influenced by individual variations, such as a bifurcation of the radial artery or an atypical split in the abductor pollicis longus tendon (22-23). In rare scenarios where the artery or tendon distribution is aberrant, relying solely on this landmark might lead to inaccuracies, underscoring the importance of always corroborating with visual inspection and imaging (24).

Our results confirm its reliability with an overall 95.7% accuracy that reaches 100% in grades I and II and 98.7% in grade III. These data lead us to affirm that the crossing of the first extensor channel and the deep branch of the radial artery is a true landmark for the articular space between scaphoid and trapezium. The lowest accuracy rate of 78.8% was recorded in grade IV patients, probably due to the severe dysplasia and degeneration of the trapezium, which alters the normal anatomy of the thumb. Moreover, the subluxation of the joint, the severe narrowing of the articular space, and the presence of sclerosis also at the scaphoid-trapezium joint constitute more elements disrupting the normal anatomy (11).

Despite some known anatomical landmarks that present differences between females and males (25), our landmark does not rely on gender or age.

To our knowledge, no other landmark has been described in this regard. The only study we found related to thumb anatomy discusses the anatomical course of the deep branch of the radial artery to identify and protect it during the procedure (26). Although the mean distance between the radial artery and the scaphoid-trapezium joint line is useful for predicting the artery's location and avoiding iatrogenic injury, it provides limited assistance in identifying the joint space. Another point in favour of our study is that it was conducted *in vivo*, while the study of Yildirim et al. (27) was entirely on cadavers.

An interesting observation was that, in the few cases requiring multiple needle placements, osteophytes around the trapezium were notably large or the joint surface was extensively degenerated. In such instances, small changes in needle angle improved landmark accuracy. Beyond the primary outcome of correct needle placement, we noted minimal differences in operative time among the different disease stages, though slightly longer surgeries were required in advanced cases due to the challenges posed by more deformed anatomy. No instances of inadvertent scaphoid removal were documented in this cohort, suggesting that integrating this reference point into the surgical workflow may enhance overall safety. A key contribution of our study is that the landmark we validate is patient-specific: it relies on the radial artery within the APL–EPB interval as it crosses the scaphotrapezial level—anatomical structures identified in vivo for each individual. Unlike protocol- or resource-dependent approaches (e.g., routine fluoroscopy or centre-specific navigation aids), this strategy does not depend on location, surgeon training pathway, or equipment and, by design, does not rely on patient sex, ethnicity, or age. In practical terms, a patient-specific landmark can standardize a critical step—localizing the joint line—across diverse settings, thereby reducing the scope for geography-driven or ethnicity-linked variability in technical accuracy.

In our cohort, landmark accuracy was consistently high across disease stages I–III and only declined in stage IV, where extreme deformity can obscure any bony cue; this pattern supports the notion that biologic, patient-level anatomy rather than external context primarily governs success. While broader treatment outcomes may still be influenced by access, rehabilitation, and social determinants, the intraoperative risk we target—wrong-bone excision—can be materially reduced by a patient-specific technique that is reproducible regardless of where or in whom the surgery is performed.

The principal limitations of our study include the relatively small proportion of stage IV cases, as well as the overall distribution of patients across the four Eaton-Littler grades. Additionally, our patient cohort was derived from a single institution, which might impact the generalizability of our findings. Future multicentre research involving a larger number of high-grade rhizarthrosis cases could provide a more robust validation of the landmark's accuracy. Similarly, the potential influence of anatomical variations or concurrent pathologies, such as longstanding rheumatoid arthritis, on this landmark remains an area ripe for further inquiry. Investigations combining ultrasound imaging or three-dimensional intraoperative navigation could also shed more light on the interplay between soft tissues and bony landmarks in advanced osteoarthritic conditions.

In conclusion, the intersection of the first extensor compartment tendons and the deep branch of the radial artery is a reliable intraoperative landmark for identifying the scapho-trapezial joint, particularly in early to moderate rhizarthrosis. Our results show high accuracy of the patient-specific landmark across disease stages I–III (and a measurable drop in stage IV). This supports a stage-aware operating protocol that standardizes joint-line identification while acknowledging when escalation is prudent: if the landmark is uncertain or distorted (osteophytes, gross subluxation), pause and corroborate with fluoroscopy (or ultrasound, if available). To collect further data and try to make the landmark reliable in more advanced stages, operating room feedbacks could be taken into account: (a) landmark identification rate, (b) time-to-exposure, (c) reliance on intraoperative imaging, (d) arterial/tendon injuries, (e) rate of wrong-bone excision (goal: 0 per 1,000 cases). A simple, patient-specific landmark combined with a stage-aware safety algorithm provides an immediate, scalable method to reduce technical disparities—independent of geogra-

phy, equipment, or patient ethnicity. Policy support (guidelines, training, reporting, rehab access) can amplify this effect, converting a procedural insight into the system-level improvement.

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TRANSPARENCY DECLARATION

Competing interests: None to declare

Abbreviations

The following abbreviations are used in this manuscript:

CMC	Carpometacarpal
OA	Osteoarthritis
IP	Interphalangeal
MCP	Metacarpophalangeal
PA	Postero-anterior

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ORIGINAL ARTICLE

The effect of liquid nitrogen exposure on the proliferative phase of Achilles tendon healing in *Rattus norvegicus* rats

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ABSTRACT

Aim Tendon healing involves a crucial proliferative phase, during which fibroblasts and fibrocytes orchestrate collagen deposition. The use of liquid nitrogen (LN) in orthopaedic oncology may inadvertently affect adjacent tendon tissues. This study aimed to evaluate the impact of LN exposure on the histological features of tendon healing.

Methods This experimental study employed a randomized post-test-only control group design involving 24 males *Rattus norvegicus*, randomly divided into four groups: control (no LN exposure) and three treatment groups exposed to LN for 1, 5, and 10 minutes, respectively, following Achilles tendon transection and repair. After a 21-day healing period, histological analysis was performed to assess the counts of fibroblasts, fibrocytes, and collagen content. Statistical analyses included one-way ANOVA, Post-hoc Tukey, and Pearson correlation ($p<0.05$ was considered significant).

Results LN exposure significantly reduced fibroblast, fibrocyte, and collagen levels compared to controls ($p<0.05$). The 10-minute group showed the lowest counts. A significant negative correlation was found between LN immersion duration and the number of fibroblasts ($r= -0.87$), fibrocytes ($r= -0.829$), and collagen content ($r= -0.83$) ($p<0.05$).

Conclusion Liquid nitrogen (LN) impairs tendon healing in a dose-dependent manner, likely due to cryo-induced cell death and disruption of blood flow. This results in an acellular and avascular tendon matrix, hindering the repair process. LN exposure negatively impacts the proliferative phase of tendon healing in rats, suggesting the need for caution in clinical use to prevent damage to surrounding tendinous tissues.

Keywords: Achilles tendon, cryosurgery, fibroblasts, tendon injuries, wound healing

INTRODUCTION

Tendon injuries heal through a coordinated process of inflammation, proliferation, and remodelling (1). In the proliferative phase of tendon healing, which occurs in the weeks following injury, fibroblasts actively proliferate and synthesize collagen to form the reparative scar tissue bridging the tendon ends. These fibroblasts eventually mature into fibrocytes as the tissue organizes and remodels. This cellular activity is crucial for the proper repair and restoration of tendon function (2).

A challenge in tendon healing arises when cryotherapy is used, particularly liquid nitrogen (LN) exposure, which is commonly employed in orthopaedic oncology to treat bone tumours (3). The LN, with its temperature reaching -196 °C, is used to induce deep tissue freezing and tumour cell necrosis. While effective in destroying cancerous tissue, LN exposure is not selective to tumour cells and can also damage surrounding healthy tissues, including tendons (4). A prior study demonstrated that LN exposure to bone causes total devitalization of bone cells (osteocytes, osteoblasts, osteoclasts) (5). However, the mineralized matrix remains to serve as a scaffold for new cell ingrowth. In bone, this allows a gradual regenerative process known as creeping substitution to restore the tissue despite the initial cell loss. Tendon tissue, on the other hand, is less mineralized and more avascular, raising the concern that LN exposure could severely impair its intrinsic healing capacity (6).

Tendon healing may be particularly vulnerable to cryo-induced cell loss. If LN eradicates tendon cells (tenocytes/fibroblasts), healing would have to rely entirely on extrinsic cell infiltration from surrounding tissues, a slower and less effective mechanism (7). This raises the question of whether LN exposure

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leads to complete tendon devitalization, or if some tendon cells can survive cryotherapy and contribute to healing. Despite its clinical applications in orthopaedic oncology, the unintended effects of LN on tendon healing have mainly been understudied. To date, few studies have specifically explored the impact of cryosurgery on tendon structure and its healing process (8). The aim of this study was to evaluate the effects of liquid nitrogen exposure on the proliferative phase of tendon healing, focusing on the Achilles tendon in a rat model, as well as to assess three key parameters of healing tissue quality: fibroblast count, fibrocyte count, and collagen content. Through this investigation, we aim to understand how LN affects tendon repair and potentially guide future cryosurgical practices to minimize unintended tendon damage.

MATERIALS AND METHODS

Materials and study design

This experimental study employed a randomized post-test-only control group design to assess the effects of liquid nitrogen (LN) exposure on Achilles tendon healing in *Rattus norvegicus*.

Six-month-old *Rattus norvegicus*, weighing approximately 500 grams, with no disabilities in their extremities and actively moving, were included in the study. They were chosen to ensure that the animals were in their adult stage, thus providing mature tendon tissue for evaluation, which closely mimics human tissue in orthopaedic research. Rats with pre-existing infections, disabilities in the extremities, and those experiencing compartment syndrome were excluded from the study. The rats were housed under controlled laboratory conditions with a 12-hour light/dark cycle and free access to food and water.

The sample size was determined using Federer's formula (9) for calculating the required sample size for each group as follows:

$(n - 1)(t - 1) \geq 15$

where: n = required sample size per group, t = number of treatment groups

$$(n-1)(4-1) \geq 15$$

$$(n-1)(3) \geq 15$$

$$3n - 3 \geq 15$$

$$3n \geq 18$$

$$n \geq \frac{18}{3}$$

$$n \geq 6$$

The number of treatment groups was four:

- Control Group: Rats underwent tendon transection, followed by suturing. Tissue samples were then collected for histopathological analysis after 21 days.
- Treatment Group 1 (labelled as P1): Rat tendons were transected, followed by immersion in liquid nitrogen for 1 minute. The tendons were then sutured back, and a cast was applied. Tissue samples were collected for histopathological analysis after 21 days.
- Treatment Group 2 (labelled as P2): Rat tendons were transected, followed by immersion in liquid nitrogen for 5 minutes. The tendons were then sutured back, and a cast was applied. Tissue samples were collected for histopathological analysis after 21 days.
- Treatment Group 3 (labelled as P3): Rat tendons were transected, followed by immersion in liquid nitrogen for 10 minutes. The tendons were then sutured back, and a cast was applied. Tissue samples were collected for histopathological analysis after 21 days.

The equation solving yields a required sample size of at least six rats per group: 24 male *Rattus norvegicus* were involved in the study.

Methods

The animals were acclimatized for 7 days in a controlled environment with ad libitum access to food and water. Anaesthesia was induced by administering ketamine (2 mg/kg body weight) intraperitoneally, followed by prophylactic antibiotics (cefazolin, 0.1 mg/kg) intramuscularly. The surgical site was shaved, disinfected with betadine, 70% alcohol, and povidone-iodine, and covered with a sterile dressing. A 3 cm incision was made along the skin to expose the Achilles tendon, which was fully transected 3 cm above its insertion point on the calcaneus. The tendon was then immersed in liquid nitrogen for 1 minute (P1), 5 minutes (P2), and 10 minutes (P3) for the respective experimental groups. One group was not exposed to liquid nitrogen and served as the control group. After freezing, the tendon was sutured back into position using 6/0 monofilament and a Kessler technique (10), followed by skin closure with 4/0 monofilament sutures. The animals were immobilized with a plaster cast and allowed to heal for 3 weeks. At the end of this period, tendon samples were collected for histopathological examination.

After a 21-day healing period, the tendon tissues were harvested and immediately fixed in 10% formaldehyde. The samples were then processed for histological evaluation, with a focus on fibroblast, fibrocyte, and collagen content. The tissues were stained using Hematoxylin and Eosin (H&E) to assess general tissue structure, and Masson's Trichrome staining to evaluate collagen content. For each tendon sample, 20 high-power fields (1000× magnification) were examined using an Olympus BX-51 microscope (Olympus Corporation, Tokyo, Japan).

Statistical analysis

Statistical analysis was conducted using one-way analysis of variance (ANOVA) to compare differences between the experimental groups. ANOVA was used to determine if there were any statistically significant differences in fibroblast count, fibrocyte count, and collagen content among the groups exposed to different durations of liquid nitrogen. If significant differences were found, post-hoc Tukey tests were performed to identify which specific groups differed from one another. Pearson correlation was used to assess the relationships between the duration of LN exposure and the measured healing parameters, providing insights into how the length of exposure affected the healing process. A significance level of $p<0.05$ was considered statistically significant for all analyses.

RESULTS

Tendon samples exposed to liquid nitrogen demonstrated markedly impaired healing parameters compared to controls. All LN-treated groups had significantly lower fibroblast counts, fi-

Table 1. Fibroblast, fibrocyte, and collagen counts in tendon samples exposed to liquid nitrogen for varying durations

Parameter		N	Mean (SD)
Fibroblast	Control	7	138.14 (30.03)
	P1	7	105.43 (22.48)
	P2	7	67.57 (12.26)
	P3	7	51.57 (25.69)
Fibrocytes	Control	7	174.14 (36.70)
	P1	7	125.14 (24.46)
	P2	7	73.14 (15.00)
	P3	7	60.43 (14.79)
Collagen	Control	7	157.00 (13.49)
	P1	7	130.62 (11.79)
	P2	7	122.30 (11.54)
	P3	7	108.96 (8.69)

SD, standard deviation; N, number of the sample.

brocyte counts, and collagen content than the uninjured control tendons p<0.05 for each). In the control group, histological analysis showed an average of approximately 138 fibroblasts and 174 fibrocytes per 20 high-power fields. In contrast, the group exposed to LN for 10 minutes showed a drastic reduction, with only approximately 51 fibroblasts and 60 fibrocytes remaining. The 1-minute and 5-minute LN exposure groups had intermediate cell counts (approximately 105 and 67 fibroblasts; 125 and 73 fibrocytes, respectively), indicating a progressive decline in cellularity with longer freeze duration (Table 1).

Collagen content in the tendon tissue also decreased correspondingly as the LN exposure time increased (Figures 1, 2). Statistical analysis confirmed that fibroblast count, fibrocyte count, and collagen content differences were significant (p<0.05). Post-hoc Tukey tests (Table 2) revealed significantly lower fibroblast and fibrocyte counts, as well as smaller collagen areas in the LN-exposed groups (1, 5, and 10 minutes) compared to the control group (p<0.05 for all comparisons).

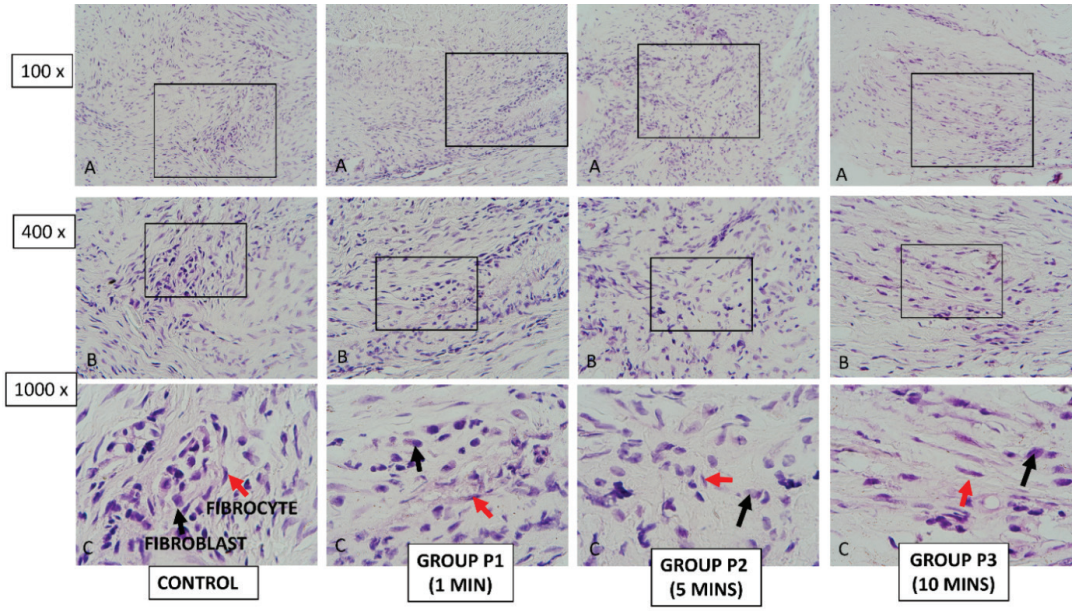

Figure 1. Fibroblast (black arrow) and fibrocytes (red arrow) counts under 1000x magnification (Faculty of Medicine, Universitas Brawijaya, 2025)

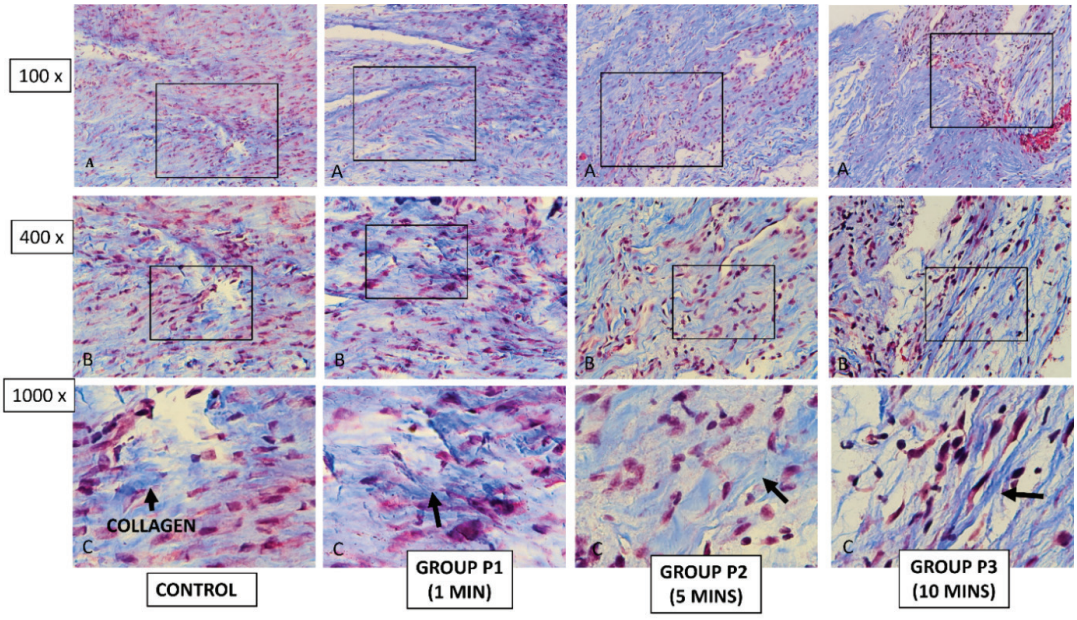

Figure 2. Collagen (black arrow) counts under 1000x magnification (Faculty of Medicine, Universitas Brawijaya, 2025)

Table 2. Post-hoc Tukey tests for fibroblast, fibrocyte, and collagen counts across experimental groups

Group comparison		Fibroblast	p*	Fibrocyte	p*	Collagen	p*
		Average difference		Average difference		Average difference	
Control	P1	32.714	0.070	49.000	**0.005**	26.378	**0.001**
	P2	70.571	**0.000**	101.000	**0.000**	34.703	**0.000**
	P3	86.571	**0.000**	113.714	**0.000**	48.037	**0.000**
P1	P2	37.857	**0.029**	52.000	**0.003**	8.325	0.540
	P3	53.857	**0.001**	64.714	**0.000**	21.658	**0.000**
P2	P3	16.000	0.589	12.714	0.766	13.333	0.161

*statistical significance in bold.

Table 3. Pearson correlation analysis between liquid nitrogen exposure duration and healing parameters (fibroblast, fibrocyte, and collagen)

Variables Relationship	r	p*
Exposure duration – Fibroblast	-0.829	**0.000**
Exposure duration – Fibrocyte	-0.871	**0.000**
Exposure duration – Collagen	-0.830	**0.001**

*statistical significance in bold;
r, correlation coefficient;

Pearson correlation analysis (Table 3) demonstrated strong negative correlations between LN exposure duration and healing parameter: fibroblast count ($r= –0.87$; $p<0.05$), fibrocyte count ($r= –0.829$; $p<0.05$), and collagen content ($r= –0.83$; $p<0.05$), indicating that longer LN exposure was associated with progressively fewer tendon cells and lower collagen deposition in the healing tissue.

DISCUSSION

The significant reduction in fibroblast and fibrocyte counts observed in LN-exposed tendons indicates that liquid nitrogen exposure severely impairs the proliferative phase of tendon healing (11). Fibroblasts, responsible for synthesizing collagen and forming the reparative matrix, are vital for tendon repair. A drastic decrease in fibroblast numbers suggests that much less collagen is produced, halting the normal healing process (12). The parallel reduction in fibrocytes further indicates that the overall cellularity of the healing tissue is diminished, which likely leads to the formation of a weaker repair scar with inferior biomechanical properties. This impairment could lead to poor functional outcomes, as tendon repairs typically result in fibrotic scar tissue that never achieves the strength of uninjured tendon tissue (13-15).

The biophysical injury mechanisms of extreme cold on biological tissues can explain the deleterious effect of liquid nitrogen on tendon healing. At –196 °C, LN causes almost instantaneous freezing of tissue fluid. Ice crystals form inside and outside cells, rupturing cell membranes and organelles, which leads to immediate cell death when a critical temperature threshold is passed. Experimental cryobiology studies have noted that cooling cells below roughly –40 °C results in irreversible cell injury for nearly all cell types (16). In our context, immersion of the Achilles tendon in LN would have rapidly brought the tissue well below –40 °C, ensuring that the majority of resident tendon cells (fibroblasts, tenocytes, and vascular cells) were irreparably damaged. In addition to this direct cryogenic cell destruction, the freeze-thaw process induces secondary ischemic injury. As the frozen tendon thaws, microvascular circulation may remain stagnant (vascular stasis) for some time, leading to hypoxia and further cell death due to nutrient deprivation (17,18). These processes likely explain the significantly reduced population of fibroblasts and fibrocytes observed in the LN-treated tendons.

From a clinical perspective, these results highlight significant risks associated with LN exposure during cryosurgery. While LN is effective for tumour ablation, its use in proximity to tendons and other critical soft tissues should be approached with caution (19). Unintended exposure of tendon tissue to LN during procedures, such as tumour removal where tendons are nearby, could lead to postoperative tendon damage and compromised healing. Surgeons should consider protective measures (e.g., insulating tendons with gauze or limiting the spread of local anaesthesia) and use the minimal effective freeze duration to minimize collateral damage. Previous clinical observations have reported complications, including non-union or pathological fractures, when LN cryotherapy is used aggressively on bone, highlighting the need to balance tumour control with preservation of healthy tissue (20,21). This study provides quantitative evidence that tendon tissue is highly susceptible to LN-induced freeze injury. Future research should focus on optimizing cryosurgery protocols, such as adjusting freeze times and developing strategies to protect tendons, ensuring tumour destruction without compromising tendon healing.

In conclusion, liquid nitrogen (LN) exposure impairs the proliferative phase of tendon healing in a dose-dependent manner. A significant decrease in fibroblast, fibrocyte, and collagen content was observed with longer LN exposure times. These findings underscore the importance of minimizing exposure to adjacent tendons during cryotherapy to prevent significant tendon damage. Surgeons should carefully consider reperfusion intervals and protective measures to limit the risks of ischemic reperfusion injury and promote better functional outcomes following orthopaedic procedures. Future research is needed to assess biomechanical strength post-cryotherapy, as well as the long-term effects of prolonged LN exposure, particularly regarding its impact on tendon healing beyond the Achilles tendon.

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Conflicts of interest: None to declare.

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ORIGINAL ARTICLE

Promising outcomes of medial unicompartmental knee arthroplasty in anterior cruciate ligament deficiency patients

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ABSTRACT

Aim Recent studies challenge the initial belief that medial unicompartmental knee arthroplasty (UKA) is contraindicated for patients with anterior cruciate ligament deficiency (ACLD) due to increased risk of periprosthetic tibial fractures, revealing promising outcomes with advancements in surgical techniques and patient selection. This study aimed to evaluate the outcomes of patients who received medial unicompartmental knee arthroplasty with anterior cruciate ligament deficiency.

Methods Five patients, aged 59-74, with knee pain, joint instability, and limited mobility, were treated for medial compartment osteoarthritis and ACLD using an Oxford design mobile-bearing unicompartmental knee prosthesis.

Results Medial UKA offers excellent clinical outcomes in ACL-deficient patients, improving knee function and reducing pain. It challenges the traditional view of ACLD as a contraindication, preserving knee kinematics and offering enhanced postoperative recovery. Advances in surgical techniques and prosthesis design expand their suitability.

Conclusion Medial UKA may be a viable treatment option for osteoarthritis patients with ACLD, potentially offering an alternative to total knee arthroplasty.

Keywords: anterior cruciate ligament injuries, knee replacement arthroplasty, osteoarthritis

INTRODUCTION

Osteoarthritis is the most prevalent form of arthritis, accounting for 62% of all arthritic conditions globally between 2017 and 2018. It commonly affects the hands, hips, knees, feet, and spine, making it a significant cause of disability among musculoskeletal disorders. Since OA primarily impacts older adults, approximately 70% of those affected are over the age of 55, the global prevalence of OA is expected to rise as populations age. Although OA typically begins in a person's late 40s to mid-50s, it can also affect younger individuals, particularly athletes or those who have suffered joint injuries or trauma (1).

Osteoarthritis (OA), a prevalent and progressive musculoskeletal disorder, significantly affects weight-bearing joints, such as the hips and knees, resulting in the structural deterioration of articular cartilage, subchondral bone, and adjacent structures (2). Joint cartilage degeneration and irregular bone formation cause severe pain, functional disability, and limited mobility, impacting 80% of affected patients and 25% experiencing complete incapacitation (3). Knee osteoarthritis (KOA), a global health issue primarily affecting individuals over 50, has increased due to obesity and aging populations, affecting around 250 million people globally (4,5). KOA is categorized into primary and secondary types, with primary KOA originating from mechanical stress, inflammation, and genetics, and secondary KOA linked to trauma or congenital abnormalities (6).

Varus alignment, a key factor in medial compartment OA, alters knee load distribution, leading to excessive stress and cartilage wear. Anterior cruciate ligament insufficiency (ACLD) further destabilizes the knee, exacerbating degenerative processes (7). KOA diagnosis involves clinical evaluation, imaging, and laboratory studies, with X-rays being the most commonly used diagnostic tool. The Kellgren and Lawrence grading system assesses severity, especially in advanced stages with severe joint space narrowing (8).

Functional assessments, such as Knee Injury and Osteoarthritis Outcome Score (KOOS) (9) and Lower Extremity Functional Scale (LEFS) (10), are commonly used to evaluate patient outcomes and monitor disease progression.

KOA treatment can involve non-surgical interventions or surgical procedures, with total knee arthroplasty (TKA) being the preferred method. Unicompartmental knee arthroplasty (UKA), an alternative for isolated medial compartment OA,

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has gained popularity (11). Historically, UKA was contraindicated for patients with ACLD due to knee instability and tibial fracture risk. However, recent advancements in surgical techniques and prosthesis design have expanded its application to ACL-deficient knees (12,13).

UKA, a minimally invasive alternative to TKA, was developed by Ahlback in 1968. Early milestones included McIntosh and Hunter's unicompartmental arthroplasty in 1954, McKeever's tibial prostheses in the 1960s, and the St. Georg Sled in the 1970s. However, concerns about survivability, patient selection, and technology remain, leading to the Ten Enigmas of UKA (14).

UKA offers faster recovery, shorter hospital stays, and superior knee kinematics compared to TKA. Its patient satisfaction is often higher than TKA due to its preservation of essential structures, leading to improved long-term outcomes and bone stock, which is beneficial for future revision surgery (11,15). UKA is recommended for unicondylar osteoarthritis or osteonecrosis, with specific parameters such as frontal deformity, flexion contracture, and an intact anterior cruciate ligament, as well as anteromedial osteoarthritis with fully functional stabilizing structures (16). UKA, once contraindicated for ACLD patients, can now be successful in ACL-deficient knees, achieving comparable survival rates to those in ACL-intact knees, due to advancements in prosthesis design and surgical techniques (17).

The aim of this study was to evaluate the outcome of patients who received medial UKA with anterior cruciate ligament deficiency.

PATIENT AND METHODS

Patient and study design

Five patients (three males and two females) underwent unicompartmental knee arthroplasty (UKA) for medial compartment knee osteoarthritis at Dr. Saiful Anwar General Hospital. The mean age of patients was 62 (range 59–74) years. Four patients had comorbidities, including obesity (BMI ≥30 kg/m²) in two, hypertension in three, and type 2 diabetes mellitus in one. Despite these conditions, all patients met the criteria for medial UKA based on symptom severity, radiographic findings, and ligamentous integrity.

All patients reported chronic medial knee pain persisting for almost two years, exacerbated by weight-bearing activities such as prolonged standing, walking on uneven surfaces, and stair descent. Three patients described episodes of knee instability, particularly when changing direction while walking or bearing weight on the affected limb. None exhibited features suggestive of inflammatory arthritis, systemic connective tissue disease, or major ligamentous insufficiency.

Radiographic assessments of the patients in this study revealed joint space narrowing, osteophyte formation, and varus deformities, indicating advanced-stage osteoarthritis.

The Kellgren and Lawrence grading system was used to evaluate the severity of OA, which ranges from Grade 0 (no signs of OA) to Grade 4 (severe joint space narrowing and bone sclerosis) (18). Grade 0 represents no evidence of OA, with normal joint space and no osteophytes or other abnormalities. Grade 1 is considered doubtful OA, where small osteophytes may be present, but no significant joint space narrowing or other signs are evident. Grade 2 is mild OA, characterized by definite osteophytes and slight joint space narrowing, with some subchondral sclerosis. Grade 3 indicates moderate OA, with more pronounced osteophytes, significant joint space narrowing, moderate subchondral sclerosis, and possible cyst formation. Grade 4 is severe OA, with complete joint space loss, large osteophytes, marked sclerosis, and substantial cyst formation, often leading to significant pain and functional impairment. This grading system is crucial for evaluating OA progression and informing treatment decisions (19).

Stability tests, including the Lachman and posterior drawer tests, were performed. The Lachman test was used to assess the integrity of the anterior cruciate ligament (ACL) by evaluating the tibia's forward movement relative to the femur. The patient lies supine with the knee flexed at 20-30 degrees, and the examiner applies an anterior force to the tibia. A positive test is indicated by excessive tibial translation, suggesting ACL instability. The Posterior Drawer test is used to assess the posterior cruciate ligament (PCL) by applying a posterior force to the tibia with the knee flexed at 90 degrees. A positive result, indicated by excessive posterior displacement of the tibia, suggests PCL injury. Both tests are critical in diagnosing ligament injuries in the knee, often guiding further imaging or surgical decisions (20).

Functional recovery was assessed using both the Knee Injury and Osteoarthritis Outcome Score (KOOS) (9) and the Lower Extremity Functional Scale (LEFS) (10) at four key time points: pre-operatively, and at 1 month, 3 months, and 6 months post-operatively (Table 1 and 2). The KOOS is a patient-reported measure that evaluates knee-related pain, symptoms, function, and quality of life. It comprises five subscales, with a higher score indicating better knee function. KOOS helps assess changes over time and is widely used in clinical practice and research for knee injuries and osteoarthritis (9). The LEFS is a validated questionnaire used to assess lower extremity function in individuals with musculoskeletal conditions. It consists of 20 items scored from 0 to 80, with a higher score indicating better functional ability. It is commonly used in rehabilitation settings (10).

Methods

Medial unicompartmental knee arthroplasty (UKA) was performed. First, the patient was positioned supine with the knee flexed to 110°, and a medial parapatellar incision was made, extending about 3 cm distal to the joint line. The patella was subluxed, and the medial meniscus was excised. The ACL was then inspected for integrity. After that, osteophytes from the medial femoral condyle and intercondylar notch were excised using a 6 mm narrow chisel. The tibial saw guide was applied with a 7° posterior slope and secured in place with pins. Next, vertical resection of the tibial plateau was performed, ensuring the cut was 2-3 mm below the deepest part of cartilage erosion. Following that, an intramedullary rod was inserted, and the femoral drill guide was aligned. Bone was drilled with 4 mm and 6 mm drills to ensure accurate component placement. Afterward, a posterior resection guide was inserted, and femoral condyle resection was performed with a 12 mm oscillating saw, ensuring the ACL and MCL were not damaged. Then, milling begins with the zero spigot to remove bone for the femoral component, followed by subsequent milling passes using spigots 3, 4, or 5, as needed to balance flexion and extension gaps. Subsequently, the tibial template and twin-peg femoral trial component were inserted at 100° knee flexion. Feeler gauges were

used to assess the gaps, and adjustments were made to ensure they are equal. After that, excess bone was removed with the anterior mill, and posterior osteophytes were excised to avoid impingement. Then, the tibial template was inserted and pinned, followed by performing the keel cut with the tibial groove cutter to prepare the tibial surface for the final component.

Following that, the femoral trial component and meniscal bearing were inserted. The knee was manipulated to check bearing and tracking, ensuring no impingement occurs, and reassessing joint stability and ligament tension. Finally, cement was applied to the tibial and femoral components, and they were impacted into place. Feeler gauges ensure proper cement pressure. After the cement had hardened, the trial components were removed, and the definitive meniscal bearing was inserted. The wound is closed using standard techniques.

Statistical analysis

Given the repeated-measures nature of the data, where the same group of patients was evaluated at multiple intervals, and the small sample size, the Friedman test was selected as the most appropriate statistical method. This non-parametric test does not assume normal distribution. It is specifically designed to detect differences in ordinal or interval-level data across related groups, making it suitable for clinical outcome scores like KOOS and LEFS.

RESULTS

Gait assessment revealed an antalgic pattern in all patients, with two demonstrating a slight varus thrust during ambulation. On inspection, mild quadriceps atrophy was noted in three patients. Medial joint line tenderness was a consistent finding, accompanied by crepitus during passive knee flexion. Varus malalignment was observed in three patients, with mechanical axis deviation averaging 5° (range: 3°–8°). Knee range of motion varied among patients, with flexion ranging from 110° to 130° and full extension preserved in all cases. No significant flexion contracture or recurvate was detected. Stability tests, including the Lachman and posterior drawer tests, were positive in two cases, confirming the instability of the anterior cruciate ligaments.

Radiographic findings, including weight-bearing anteroposterior, lateral, and Merchant views, confirmed medial compartment osteoarthritis in all patients. Three had Kellgren-Lawrence grade IV osteoarthritis, and two patients had grade III osteoarthritis but experienced persistent symptoms despite extensive conservative management. None exhibited severe patellofemoral arthritis or advanced lateral compartment degeneration, ensuring they remained suitable candidates for medial UKA. Two patients exhibited anterior tibial translation, as observed in the radiographic examination. Long-leg alignment radiographs in the standing position confirmed varus alignment in three patients.

Functional impairment was assessed using validated scoring systems. The mean KOOS in the sixth month was 73%, with a pre-operative score of 24.6% (Table 1). The preoperative LEFS score ranged from 10 to 17 and increased to 56-66 six months postoperatively, indicating a significant improvement in daily living and activity capabilities (Table 2).

For the KOOS scores, the Friedman test demonstrated a statistically significant difference across time points (p=0.0018), indicating consistent improvement in knee-specific function following surgery. Similarly, the LEFS scores also showed a statistically significant increase over time (p=0.0018), reflecting enhanced lower extremity functional performance throughout the postoperative period. These findings collectively support a positive and progressive recovery trajectory in joint-specific and overall limb function across the patient cohort.

The diversity in patient characteristics underscores the suitability of medial UKA for a broad spectrum of individuals, including those with obesity and metabolic conditions, provided that appropriate indications are met. None of the patients in this series experienced contraindications related to BMI or comorbidities, highlighting the feasibility of UKA beyond traditional patient selection criteria.

Table 1. Knee Injury and Osteoarthritis Outcome Score (KOOS) of five patients*

Patient's gender/ age	Knee Injury and Osteoarthritis Outcome Score (KOOS) (%)			
	Pre-operative	1-month post-operative	3 months post-operative	6 months post-operative
M/62	33	50.5	73.25	76.35
F/71	26.7	40.9	64	76.82
F/74	30.5	49.82	61	71
M/67	17.88	32.44	50.78	72
M/59	15	26.27	48.71	68.82
The mean score	24.6	40	59.5	73

p=0.0018 across time points
M, male; F, female

Table 2. Lower Extremity Functional Scale (LEFS) scoring of all patients*

Patient's gender/age	LEFS score			
	Pre-operative	1-month post-operative	3 months post-operative	6 months post-operative
M/62	14	26	60	66
F/71	13	24	50	62
F/74	17	25	41	56
M/67	11	19	43	58
M/59	10	29	51	63
The mean score	13	24.6	49	61

p=0.0018 increase over time
M, male; F, female

DISCUSSION

The study found that patients with severe knee pain, stiffness, and limited mobility, common symptoms of OA, were affected in their overall quality of life due to increased stiffness, fatigue, sleep disturbances, and reduced activity levels (18). Varus deformity, a common factor in medial compartment OA, shifts load distribution unevenly across the knee joint, leading to further degeneration and pain during weight-bearing activities (21).

Knee osteoarthritis patients often show tenderness along joint lines and patellar facets. Clinicians assess the patellofemoral compartment by moving the knee, checking for pain, crepitus, clicking, and locking. Normal knee ROM is 0 degrees for ex-

tension and 135 degrees for flexion (22). This ROM is often restricted in knee OA, so using a goniometer for precise measurement is crucial.

Clinical examinations for patients with OA reveal joint tenderness, patellar facets, and neurovascular issues. Assessments include quadriceps, hamstring strength, sensory, and vascular checks to rule out neurogenic or vascular problems (23). The study found significant improvements in pain relief and functional mobility post-surgery, as indicated by the KOOS and LEFS scores, with one patient's score increasing from 33% to 76.35% (24,25). UKA offers advantages over TKA in unicompartmental OA, including shorter surgery times, less blood loss, quicker recovery, and higher patient satisfaction, preserving bone stock and key structures (11). UKA's surgical techniques, including single-use instrumentation (SUI), have improved operational and economic efficiency. SUI reduces costs associated with instrument reprocessing and sterilization, resulting in improved operating room turnover times and increased patient throughput (26). Patients with medial UKA experience better range of motion, improved knee function, reduced future surgery risk, fewer complications, and higher satisfaction rates compared to TKA, even with well-positioned implants (14).

Osteoarthritis treatment typically involves nonoperative methods, but surgical options like arthroscopy, cartilage repair, osteotomy, and knee arthroplasty are considered when conservative treatments fail. UKA is increasingly preferred for isolated medial compartment OA due to its shorter hospital stays, quicker recovery, and improved knee kinematics (11,21). Recent advancements in prosthetic design and surgical techniques have expanded the use of unicompartmental knee arthroplasty (UKA) in patients with ACLD, yielding positive functional outcomes and significant pain relief comparable to those in ACL-intact knees (17). This study suggests that ACLD should no longer be a contraindication for UKA, as it offers advantages such as shorter hospital stays, faster recovery, and better preservation of knee kinematics (12,21).

UKA is recommended for patients with isolated unicompartmental osteonecrosis, a varus or valgus angular deformity of less than 15°, a flexion contracture of less than 5°, a knee flexion range of motion greater than 90°, and an intact anterior cruciate ligament (ACL) and peripheral knee ligaments over 60 years old (11,25,26). UKA postoperative outcomes and longevity are primarily influenced by alignment, with better outcomes associated with mechanical axis angles of ≤7°, which prevents excessive tibial component wear and reduces deformity recurrence (27,28).

Recent evidence challenges the traditional belief that ACLD is a contraindication for UKA, highlighting no significant differences in survivorship between ACL-deficient and ACL-intact knees (29). Additionally, another study reported a 96% combined survivorship rate at 10 years in both ACL-deficient and ACL-intact knees (17).

UKA's minimally invasive approach preserves joint kinematics and proprioception, contributing to better long-term outcomes. The Oxford Unicompartmental Knee Prosthesis, a widely studied and utilized system, aims to replicate natural knee motion and minimize polyethylene wear through improved congruity and reduced stress on the articular surface (13). The Oxford prosthesis also allows for a greater degree of knee flexion. It has been associated with better functional outcomes and faster recovery than fixed-bearing implants, especially in active patients.

UKA postoperative rehabilitation involves a brace-free approach, restoring passive and active range of motion. Isometric exercises begin immediately, partial weight-bearing is allowed for six weeks, proprioceptive exercises are introduced, and strength training is started at six weeks (30). A study of 1,000 Oxford UKA procedures found a 2.9% complication rate, with common causes being OA progression, mobile inlay dislocation, and unexplained pain. UKA failure is primarily caused by aseptic loosening and progression of osteoarthritis, accounting for over half of revisions within the first five years, with infection and polyethylene wear being less common (31).

UKA's revision-free survival rate has not improved as much as TKA, possibly due to a lower threshold for revision. However, evidence suggests UKA-to-TKA conversions may have similar complication and outcome profiles (32). The impact of ACLD on UKA outcomes is debated, with some studies suggesting increased failure rates, while others report no significant difference. Careful planning is required for severe varus deformities (27,33).

In conclusion, UKA is a viable surgical option for patients with knee OA and Anterior Cruciate Ligament Deficiency (ACLD), despite traditional concerns. It offers advantages over TKA, including the preservation of native knee structures, enhanced postoperative recovery, and maintenance of natural knee kinematics. The study supports the use of UKA in ACL-deficient patients when carefully selected and managed, challenging the notion of an intact ACL.

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Conflicts of interest: None to declare.

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ORIGINAL ARTICLE

Comparison of immune responses to zirconia, polyether ether ketone (PEEK), and stainless-steel in orthopaedic implants

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ABSTRACT

Aim Orthopaedic implants must meet specific criteria, including mechanical strength, durability, and biocompatibility. This study compares the immune response to zirconia, polyether ether ketone (PEEK), and stainless-steel implants *in vivo*, focusing on lymphocyte and fibroblast infiltration as indicators of immune activation.

Methods A total of 27 New Zealand white rabbits was used, with nine animals in each group. Implants of zirconia, PEEK, or stainless steel were surgically placed in the thigh and observed for 4 weeks. Histological analysis measured lymphocyte and fibroblast infiltration at the implant site using a microscope at 400x magnification. Statistical analysis included the Kruskal-Wallis test for group comparisons, followed by Mann-Whitney and Bonferroni correction for pairwise comparisons.

Results The Kruskal-Wallis test showed significant differences in lymphocyte ($p=0.002$) and fibroblast ($p=0.003$) counts among the groups. Zirconia exhibited significantly lower lymphocyte (median=0.5) and fibroblast (median=1.0) infiltration compared to stainless steel (lymphocytes: median=3.0, fibroblasts: median=2.0), and PEEK (lymphocytes: median=2.0, fibroblasts: median=3.0). Bonferroni correction confirmed that zirconia exhibited the least immune activation ($p<0.0167$).

Conclusion Zirconia offers superior biocompatibility with minimal immune response, making it an ideal material for orthopaedic implants, particularly for patients with metal sensitivities. PEEK showed moderate immune activation but is helpful for non-load-bearing applications. Stainless Steel induced the highest immune response due to the release of metal ions and corrosion. Zirconia is the most biocompatible material tested, making it a promising choice for orthopaedic implants.

Keywords: immunity, polyether ether ketone, prostheses and implants, stainless-steel, zirconia

INTRODUCTION

Orthopaedic implants are widely used in the treatment of various bone and joint disorders, including osteoarthritis, rheumatoid arthritis (RA), and fractures (1). As the global population ages, the demand for these implants, particularly joint replacement surgeries, has steadily increased. The materials used in orthopaedic implants must fulfil several critical criteria, including mechanical strength, durability, and, most importantly, biocompatibility (2). Biocompatibility refers to the ability of the implant material to interact with the surrounding tissues without inducing an adverse immune response or causing toxicity. A key determinant of biocompatibility is the material's ability to minimize inflammatory responses and promote tissue healing, which are essential for the long-term success of the implant (3).

One of the most significant factors influencing the long-term success of orthopaedic implants is the immune response to the implanted material (4). The immune system's response to foreign materials can lead to chronic inflammation, fibrosis, or even implant rejection. This response is particularly problematic with metal-based implants, such as those made from stainless steel, which can release metal ions into the surrounding tissue due to corrosion, potentially causing allergic reactions or irritation (4,5). Corrosion has been identified as a significant

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issue in metallic implants, as the release of metal ions (such as nickel, chromium, and cobalt) can stimulate a pro-inflammatory immune response and alter the material's biomechanical properties over time (6).

In contrast, alternative materials such as ceramics and polymers have garnered increasing attention in recent years. Zirconia (ZrO2) is a ceramic material known for its low cytotoxicity, excellent mechanical properties, and superior wear resistance. Zirconia's low immune reactivity makes it an attractive candidate for use in orthopaedic implants, especially in weight-bearing joints, as it minimizes immune activation and encourages better tissue integration (7,8). Furthermore, zirconia has a unique phase transformation ability that enhances its toughness, making it suitable for joint replacements that endure mechanical stress (9).

Polyether ether ketone (PEEK), a thermoplastic polymer, is valued for its chemical stability, low wear, and favourable mechanical properties. It is beneficial in non-weight-bearing applications such as spinal implants and trauma devices, where its radiolucency is advantageous for post-surgical imaging. While PEEK shows a moderate immune response, its biocompatibility has been well-documented, and surface modifications can further improve tissue integration and reduce immune activation (10-12).

Despite the promising properties of zirconia and PEEK, stainless steel remains one of the most widely used materials in orthopaedic implants due to its high mechanical strength and affordability. However, its use is often limited by corrosion issues, which can trigger local inflammation and immune activation. The release of metal ions from stainless steel implants has been shown to activate the complement system and induce chronic inflammation, ultimately compromising implant stability and patient outcome (1,13).

Given these concerns, this study was initiated to evaluate the immune responses triggered by different orthopaedic implant materials. Specifically, the aim was to compare the immune response to zirconia, PEEK, and stainless-steel implants *in vivo*, using a rabbit model. This comparison is crucial for understanding how these materials interact with the immune system, particularly in terms of lymphocyte and fibroblast responses around the implants. Understanding these immune mechanisms will provide valuable insights into the long-term success and complications associated with each material, ultimately guiding the development of more biocompatible and effective orthopaedic implants.

MATERIALS AND METHODS

Materials and study design

The study was conducted at the Animal Research Laboratory, Faculty of Medicine, Universitas Brawijaya, where the animal subjects were maintained, treated, sampled, and subsequently euthanized from July to September 2024. The research continued at the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Brawijaya, where measurements of lymphocyte and fibroblast cell parameters were performed from September to October 2024.

This study employed a post-test-only control group design to compare the immune response induced by zirconia, PEEK, and stainless-steel orthopaedic implants *in vivo*. The manufacture of implants in the form of chips follows ISO 19227:2018 and is assisted by PT. EKA ORMED INDONESIA.

The three materials used in the study were:

Zirconia (ZrO2): a bioceramic material with low cytotoxicity, excellent mechanical properties, and known for its superior wear resistance and biocompatibility.

Polyether Ether Ketone (PEEK): a thermoplastic polymer known for its chemical stability, radiolucency, and good mechanical properties.

Stainless-steel: a traditional material used in orthopaedic implants with good mechanical strength but associated with metal ion release and corrosion.

The New Zealand white rabbit was chosen as the animal model due to its reliable anatomical similarity to human tissue, particularly in the subcutaneous layer, and its widely accepted use in preclinical studies involving biocompatibility and immune response assessments. The inclusion criteria for the rabbits in this study were that they were healthy and actively moving, with no disabilities in their extremities, and weighed between 1.5 and 2.5 kg. Rabbits with extremity disabilities, infection, and those that died before the study began were excluded. All rabbits were housed in controlled conditions with a temperature of 22°C±2°C, a humidity of 60%±5%, and a 12-hour light/dark cycle. The animals were allowed to acclimate to the environment for 7 days before the procedure. They were fed standard rabbit chow and given access to clean water ad libitum.

This study was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Brawijaya, under approval number 113/EC/KEPK-PSPDS/05/2024 in May 2024.

Methods

Sample size calculation. The sample size was determined using the Federer formula (14), a commonly applied method in experimental designs to ensure sufficient statistical power while controlling for variability between groups. The Federer formula is suitable here, as it strikes a balance between providing an adequate sample size and minimizing animal use. The formula used is as follows:

(n - 1) (t - 1)≥15 where: n = required sample size per group, t = number of treatment groups

$$(n-1)(3-1) \geq 15$$

$$(n-1)(2) \geq 15$$

$$2n-2 \geq 15$$

$$2n \geq 17$$

$$n \geq \frac{17}{2}$$

$$n \geq 8.5$$

In this study, the number of treatment groups was three: Group 1, zirconia; Group 2, PEEK; and Group 3, stainless steel. Solving the equation yields required sample size of 9 rabbits per group. Therefore, a total of 27 rabbits were included in this study.

Surgical procedure. The surgical procedure was performed under general anaesthesia using intramuscular injection of 200 mg ketamine and 20 mg xylazine each. The thighs, legs, and upper back were shaved, and each lower extremity was prepared with 70% alcohol and 7.5% Povidone Iodine solution. Throughout the procedure, the animals breathed spontaneously and were monitored by a veterinary technician. The lower extremities of

each animal were covered with sterile adhesive surgical drapes and secured with towel clips. All surgical procedures were performed under sterile conditions, using a separate set of surgical instruments for each site. To minimize variability and ensure standardized surgical procedures, the same surgeon performed all operations. A 2 cm longitudinal incision was made over the lateral aspect of each thigh and extended to the muscle layer until the bone was reached. Group 1: rabbits implanted with a set of zirconia-based chips, Group 2 with a set of stainless steel-based chips, and Group 3 with a set of PEEK-based chips. The skin was approximated using a subcuticular stitch with non-absorbable 3-0 monofilament nylon sutures. Postoperatively, each animal was placed in a warm, oxygenated recovery room until complete recovery from anaesthesia was observed. Once in their cages, each animal was provided access to water and rabbit food. The animals were monitored daily for food intake, faeces, urine output, and behaviour. Observation of the animals continued for up to 4 weeks post-operation.

Tissue preparation for histological examination. After 4 weeks, euthanasia was performed using a lethal dose of 200 mg/kg intramuscular ketamine on the animals. Subsequently, the muscle and bone tissues of the rabbits were excised and prepared as samples for further microscopic histological analysis. The samples were stored in formalin before histological analysis. The rabbits, from which the thigh tissues were collected for the study, were then buried according to standard animal disposal procedures. The excised thigh muscles were stored in formalin bottles for the preparation of microscopic slides. Tissue processing involved immersion in graded alcohols for approximately 21 hours. The tissue was then embedded in paraffin. The paraffin-embedded tissues were sectioned using a microtome. The tissue sections were then mounted onto glass slides. Staining was performed using Harris Hematoxylin and Eosin (HE) stain. The sections on the slides were sequentially immersed in the following solutions: Xylene, Xylene, Xylene, 100% Alcohol, 100% Alcohol, Distilled Water, Hematoxylin, Distilled Water, Eosin, 96% Alcohol, 96% Alcohol, 96% Alcohol, 100% Alcohol, 100% Alcohol, Xylene, and Xylene. Mounting was performed using entellan to adhere the cover glass to the slide, and the slides were then stored in an incubator for approximately 24 hours.

The counting of lymphocytes and fibroblast cells around the implantation site was observed under a microscope with 400x magnification using a semi-quantitative counting method.

Statistical analysis

A descriptive analysis was conducted to obtain a general description of the research variables. The median value was chosen as the measure of central tendency because it is more representative of the data, especially when the distribution is not normal or contains outliers. The number of infiltrating immune cells (lymphocytes and fibroblasts) was compared among the three groups using the Kruskal-Wallis test. A $p<0.05$ was considered statistically significant. For post-hoc pairwise comparisons to determine the significant differences between the groups, the Mann-Whitney test was performed. Since multiple pairwise comparisons were made, a Bonferroni correction was applied to adjust the significance level.

RESULTS

The median value was chosen as the measure of central tendency because it is more representative of the data, especially when the distribution is not normal or contains outliers. As the data distribution in this study was not normal, the median was preferred over the mean for presenting the central tendency (Table 1).

Table 1. Median lymphocyte and fibroblast cell counts in stainless steel, zirconia, and polyether ether ketone (PEEK)

Groups (number of animals)	**Lymphocyte cell counts** (median)	**Fibroblast cell counts** (median)
Stainless steel (9)	3.0	2.0
Zirconia (9)	0.5	1.0
PEEK (9)	2.0	3.0

A statistically significant difference was found for lymphocyte ($p=0.002$) and fibroblast counts ($p=0.003$) between the groups. Post-hoc pairwise comparisons were conducted using the Mann-Whitney test to determine which specific group comparisons were significantly different. All comparisons between Stainless Steel, Zirconia, and PEEK showed statistically significant differences in both lymphocyte and fibroblast responses (Table 2).

Table 2. Statistical comparison of lymphocyte and fibroblast across stainless steel, zirconia, and polyether ether ketone (PEEK)

Group	**Lymphocytes**	**Fibroblast**
	p	**p**
Stainless steel - zirconia	0.003	0.018
Stainless steel - PEEK	0.042	0.027
Zirconia - PEEK	0.015	0.004

Since all group comparisons were significant, the Bonferroni correction was applied to determine which material performed best. Bonferroni correction was performed by dividing the desired significance level (α) by the number of comparisons being made. If a significance level of 0.05 was used and three comparisons were made, the corrected significance level was 0.0167. Following the application of the Bonferroni correction, the adjusted significance level was 0.0167 (Table 3). After applying the Bonferroni correction, zirconia showed significant differences compared to stainless-steel and PEEK for both lymphocytes and fibroblasts. Specifically, zirconia exhibited the least immune response, with significantly lower lymphocyte infiltration and minimal fibrosis compared to the other materials. On the other hand, stainless steel showed the highest immune response, while PEEK exhibited moderate immune activation (Table 3).

Table 3. Bonferroni correction

Group	**Comparisons of original significance with adjusted significance**	**Significance**
Lymphocytes		
Stainless steel - zirconia	0.003 < 0.0167	Significant
Stainless steel - PEEK	0.042 > 0.0167	Not significant
Zirconia – PEEK	0.015 < 0.0167	Significant
Fibroblast		
Stainless steel - zirconia	0.018 > 0.0167	Not significant
Stainless steel - PEEK	0.027 > 0.0167	Not significant
Zirconia - PEEK	0.004 < 0.0167	Significant

DISCUSSION

The results of this study demonstrate that zirconia implants induced the least immune response, with significantly lower lymphocyte infiltration and minimal fibrosis around the implant site. These findings align with previous research highlighting zirconia's excellent biocompatibility and low cytotoxicity in orthopaedic applications (15,16). Zirconia's bioinert nature contributes to its suitability for joint replacements, as it minimizes immune activation and encourages better tissue integration. Its fracture toughness and mechanical properties also make it an ideal choice for load-bearing applications, such as joint prostheses. Moreover, zirconia's ability to suppress the TH1 pathway may contribute to its reduced immune response, limiting macrophage activation and pro-inflammatory cytokine production, which are associated with chronic inflammation (17,18).

While PEEK showed a moderate immune response, it exhibited more favourable tissue integration than stainless steel. The material's chemical stability, radiolucency, and favourable mechanical properties, including high tensile strength and elasticity, make it particularly advantageous in non-load-bearing applications, such as spinal implants and trauma devices, where post-surgical imaging is crucial (19). Despite moderate immune activation, PEEK remains a viable material for applications that require high strength without load-bearing needs. Furthermore, surface modifications, such as plasma treatment or bioactive coatings, have been shown to enhance PEEK's biocompatibility and reduce immune activation, positioning it as a promising candidate for broader orthopaedic use in the future (20).

In contrast, stainless steel implants induced the most significant immune response, characterized by increased lymphocyte infiltration and extensive fibrosis around the implant sites. This is primarily due to the release of metal ions, such as nickel, chromium, and cobalt, which occur as a result of corrosion. These metal ions stimulate a pro-inflammatory immune response, leading to chronic inflammation, fibrosis, and poor osseointegration, which negatively affect the long-term performance of metallic implants. These findings underscore the challenges associated with using stainless steel in orthopaedic implants, particularly in terms of corrosion and long-term implant stability (21,22). Despite the widespread use of stainless steel due to its strength and affordability, the persistent issue of corrosion underscores the need for alternatives such as zirconia and PEEK, which offer improved biocompatibility and reduced immune activation.

The comparative analysis suggests that zirconia is the superior choice in terms of immune compatibility and tissue healing, especially for weight-bearing applications. Its low immune activation, excellent mechanical properties, and reduced risk of corrosion make it a promising alternative to stainless steel, particularly for patients with metal sensitivities or those at risk for chronic inflammation (15). However, PEEK remains a valuable option for non-load-bearing applications, where its favourable biomechanical properties and the potential for surface modifications provide significant advantages, particularly for spinal and trauma implants (10,23).

While the findings of this study are promising, several limitations should be addressed. The subcutaneous model used in this study may not fully replicate the dynamic mechanical forces or biological conditions that implants experience within joint spaces. Future studies could consider using joint-specific models, such as those simulating weight-bearing or dynamic loading conditions, to more accurately reflect the real-world conditions of implants. Additionally, this study observed the implants only for 4 weeks, which may not be sufficient to assess long-term wear, mechanical degradation, or chronic inflammatory effects. More extended observation periods would provide more insight into the long-term performance and potential complications associated with these implant materials, especially stainless steel. Furthermore, surface modification techniques for PEEK, such as bio functionalization or coating with bioactive agents, should be further explored to enhance its biocompatibility and minimize immune responses.

In conclusion, zirconia demonstrated superior biocompatibility compared to PEEK and stainless-steel, with a minimal immune response. PEEK showed moderate immune activation, while stainless-steel induced the most significant immune response due to metal ion release and corrosion. These findings suggest that zirconia is a promising material for orthopaedic implants, particularly for patients prone to metal sensitivities, while PEEK remains suitable for non-load-bearing applications.

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TRANSPARENCY DECLARATION

Conflicts of interest: None to declare.

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ORIGINAL ARTICLE

The effect of tourniquet reperfusion interval on malondialdehyde (MDA) level and skeletal muscle damage in the treatment of long bone fractures

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ABSTRACT

Aim Tourniquets are commonly used during surgery to control bleeding, however, their application can lead to complications, including ischemic reperfusion injury. Post-tourniquet syndromes such as pain, swelling, and muscle weakness may persist for up to six weeks. The aim of this study was to investigate the reperfusion interval duration to prevent muscle tissue damage.

Methods This experimental study involved 48 male white rats (*Rattus norvegicus*) with induced tibial fracture. The rats were divided into two groups, A and B, with each group receiving four different treatments, resulting in a total of eight groups. The control groups (A1 and B1) had the tourniquet applied for 3 hours, while other groups had it used for 2 hours, followed by 5, 10, and 15-minute reperfusion intervals. Group A was sacrificed one hour post-deflation, and Group B was sacrificed on day 14. Malondialdehyde (MDA) level and muscle histology were analysed using one-way ANOVA.

Results In group A, significant difference was found between the 10 and 15-minute reperfusion intervals. In group B, the 10-minute reperfusion interval showed the most favourable outcome, with a 42.63% reduction in injury for group A and a 32.27% reduction for group B. Significant differences in MDA level were found between the control and reperfusion groups in both groups A and B.

Conclusion The 10-minute reperfusion interval effectively reduces ischemic reperfusion injury in muscle tissue, as indicated by lower MDA level and less muscle damage. This interval optimally restores aerobic metabolism and prevents excessive reactive oxygen species (ROS) production.

Keywords: malondialdehyde, reperfusion injury, tourniquet

INTRODUCTION

Harvey Cushing introduced the pneumatic tourniquet in 1904, revolutionizing orthopaedic surgery by providing a method to maintain a clean surgical field and enhance precision during procedures on the extremities (1). While tourniquet use has clear benefits in facilitating surgical access and minimizing blood loss, it is not without complications. Among the most concerning is ischemic reperfusion injury, which occurs when blood flow is restored to previously ischemic tissues, triggering inflammation, oxidative stress, and tissue damage (2,3). Clinically, ischemic reperfusion injury is a significant concern, occurring in approximately 1 in 6,000 cases of upper extremity surgery and 1 in 3,700 lower extremity surgeries (4). This underscores the need to optimize tourniquet protocols to minimize patient morbidity.

The pathophysiology of ischemic reperfusion injury involves a cascade of metabolic disruptions, oxidative stress, and cellular damage. Muscle tissue is particularly vulnerable due to its high oxygen demand, with irreversible injury beginning after just 3 hours of ischemia, compared to nerves (8 hours) and bone (4 days) (5,6). During ischemia, adenosine triphosphate (ATP) depletion and lactate accumulation initiate cellular damage and activate inflammatory mediators (7,8). Upon reperfusion, the sudden influx of oxygen triggers reactive oxygen species (ROS) formation, which exacerbates tissue injury through lipid peroxidation (9). Malondialdehyde (MDA), a secondary product of lipid peroxidation, is commonly used as a biomarker to assess oxidative stress and tissue damage (10,11).

Ischemic reperfusion injury is not limited to muscle tissue. Endothelial cells and microvasculature are highly susceptible, leading to increased vascular permeability, enema, and leukocyte adhesion, which further exacerbate tissue injury and delay recovery (12,13). Prolonged ischemia has been shown to cause

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significant microvascular damage, observable upon reperfusion (14,15). These findings highlight the importance of not only controlling the duration of ischemia but also managing the reperfusion interval, which may critically influence the extent of tissue damage.

Although much of the clinical focus has historically been on ischemia duration, recent studies suggest that the timing of reperfusion itself can significantly affect muscle recovery (16). Short reperfusion intervals, such as 10 minutes, have been reported to enhance metabolic recovery, reduce ROS formation, and maintain ATP levels, thereby mitigating ischemic reperfusion injury (17,18). Despite these promising findings, the optimal reperfusion interval for reducing oxidative damage in skeletal muscle remains unclear, and evidence directly comparing different intervals is limited.

The aim of this study was to investigate the effects of different reperfusion intervals on skeletal muscle MDA levels and histopathological changes following tourniquet-induced ischemia in a rat model assessing 5-, 10-, and 15-minute reperfusion intervals to determine how variations in reperfusion timing influence oxidative stress and muscle tissue recovery, providing novel insights that may guide safer tourniquet application in clinical practice.

MATERIALS AND METHODS

Materials and study design

This research was conducted between January and March 2023 in several locations. We raise, treat, and sacrifice the experimental animals at the Parasitology Laboratory, Faculty of Medicine, Universitas Brawijaya, Indonesia. The MDA level measuring was performed at the Physiology Laboratory, Faculty of Medicine, Universitas Brawijaya. The Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Brawijaya, was used for the staining process and organ histology assessment.

An experimental study on male white rats (*Rattus norvegicus*) was used to see the effect of reperfusion interval on the use of tourniquets on the systemic effect of reperfusion ischemic injury in skeletal muscle in the treatment of long bone fractures by analysing MDA biomarkers and histopathological observations of skeletal muscle.

The sample size was calculated using the Federer formula (19) to estimate the required number of subjects based on power analysis. The formula used was as follows:

(n - 1) (t - 1)≥15

where: n = required sample size per group, t = number of treatment groups

$$(n-1)\,(8-1)15$$

$$(n-1)\,(7)15$$

$$7n-715$$

$$7n22$$

$$n \geq \frac{22}{7}$$

$$n3.14$$

Eight groups in this study included:

- Group P1A-O1A: No reperfusion interval, the rats were sacrificed 1 hour after tourniquet deflation
- Group P2A-O2A: A 5-minute reperfusion interval, the rats were sacrificed 1 hour after tourniquet deflation.
- Group P3A-O3A: A 10-minute reperfusion interval, the rats were sacrificed 1 hour after tourniquet deflation.
- Group P4A-O4A: A 15-minute reperfusion interval, the rats were sacrificed 1 hour after tourniquet deflation.
- Group P1B-O1B: No reperfusion interval, the rats were sacrificed 14 days after the treatment.
- Group P2B-O2B: A 5-minute reperfusion interval, the rats were sacrificed 14 days after the treatment.
- Group P3B-O3B: A 10-minute reperfusion interval, the rats were sacrificed 14 days after the treatment.
- Group P4B-O4B: A 15-minute reperfusion interval, the rats were sacrificed 14 days after the treatment.

To avoid a lack of sample size, each group consists of 6 rats, therefore 48 rats were included in the study.

The inclusion criteria were as follows: male *Wistar* strain white rats (*Rattus norvegicus*), aged 3 to 4 months, with a body weight between 180 and 200 grams, and in good health, as indicated by active movement and the absence of any extremity deformities. The exclusion criteria included rats that were ill or showed signs of compartment syndrome.

All protocols were approved by the Health Research Ethics Commission of the Faculty of Medicine, Universitas Brawijaya Malang, with ethical approval number 70B/EC/KEPK-PPDS/02/2023.

Methods

Acclimatization. Rats were acclimatized for seven days in laboratory conditions, housed in standard cages (30x20 cm). The animals were given access to food and distilled water ad libitum, with the bedding changed daily to maintain cleanliness.

Fracture. The experimental animals fasted for 3 hours before termination. Anaesthesia was first administered through ketamine hydrochloride (Ketalar) at a dosage of 40 mg/kg, followed by the prophylactic antibiotic cefazolin at 5 mg/kg intramuscularly. The operating field was prepared by shaving the area, followed by cleaning with Savlon, 70% alcohol, and povidone-iodine, and then covered with a sterile sheet. Once anaesthesia was achieved, indicated by the rat's eyes beginning to close and its movements slowing, the surgery proceeded. The operator, wearing sterile gloves and a gown, ensured aseptic conditions throughout the procedure. A closed fracture of the middle segment of the tibia was treated. Following the fracture, a mini circular cast was applied to immobilize the injured segment.

Tourniquet. To induce ischemia, an orthodontic rubber band (4.5 oz) was tied around the groin of each rat's leg. The tourniquet was applied uniformly to all rats, ensuring consistent ischemia. Rats in the control group were subjected to a 3-hour ischemic period without reperfusion, while rats in the experimental group received different reperfusion intervals of 5, 10, or 15 minutes after 2 hours of ischemia. A 1-hour reapplication followed the initial reperfusion period to simulate the clinical use of a tourniquet during surgeries with varied reperfusion intervals.

Fixation and post-surgical care. Plaster of Paris was applied to the tibia (lower leg) using the long leg cast method (20), extending from one-third of the middle of the thigh to the an-

kle, ensuring that the leg was in a straight position with full extension. Following the procedure, the experimental animals were placed into their respective cages and provided daily food according to their usual feeding habits. If any signs of lethargy, difficulty eating, or shivering were observed, the rats were administered paracetamol at a dosage of 100 mg/kg as an anti-pain medication.

Specimen collection. In group A, rats were sacrificed 1 hour after tourniquet deflation, in group B rats were sacrificed 14 days post-treatment using the cervical dislocation technique. Following this, skeletal muscle samples were taken distal to the tourniquet location for tissue MDA examination and anatomical pathology histology preparations. Once the organs for the study were removed, the rats were confirmed dead. The carcasses were then placed in a basin, and subsequently buried in the ground at a minimum depth of 50 cm, with an area of 0.25 m^2 per burial site. To prevent the carcasses from being unearthed by other animals, such as cats, each hole was used to bury no more than ten rats. The hole was then covered with soil and compacted to ensure the odour of the carcasses did not escape. The specimen replication obtained from these procedures was then examined for measurements at the Physiology Laboratory, Faculty of Medicine, Universitas Brawijaya.

Measurements of malondialdehyde (MDA) level. Skeletal muscle samples were ground with the assistance of liquid nitrogen and weighed to 50-100 mg. The samples were then placed in a Petri dish, and one cc of phosphate buffer was added. Following this, one cc of 100% TCA (Trichloroacetic Acid), one cc of 1 N HCl (Normal Hydrochloric Acid), and one cc of 1% Na-thiobarbiturate were added sequentially. The mixture was heated in a water bath at 100 °C for 25 minutes. After heating, the solution was centrifuged at 2000-3000 rpm for 15 minutes. The supernatant was then collected and diluted with water to a final volume of 3 cc. The sample was then analysed using spectrophotometry at a wavelength of 532 nm (Shimadzu Corporation, Kyoto, Japan).

Histological examination. After taking the rat's skeletal muscle tissue, the tissue was taken to the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Brawijaya, to prepare for staining and histological examination. The tissue samples were soaked in a medium containing 10% formaldehyde. Dehydration was then performed using a series of alcohol solutions: 70% alcohol for 1 hour, followed by 80% alcohol for 1 hour, 90% alcohol for 1 hour, 95% alcohol for 1 hour, 99% alcohol for 1 hour, and 100% alcohol for 1 hour. The clearing process was conducted by placing the dehydrated material in xylol solution for two 30-minute intervals. Following this, the embedding process was carried out. The block was then placed on the rotary microtome, and thin longitudinal cuts of 3-5 µm were made. The cut sections were transferred to a water bath to allow proper expansion, after which they were placed onto a labelled glass slide. Staining was performed with Hematoxylin & Eosin (HE), and the slides were covered with a cover glass. Histological observations of the skeletal muscles were carried out by quantitatively counting the fields of view in 10 small sections using an Olympus BX-51 dot Slide microscope, equipped with an Olympus XC10 camera (Olympus Corporation, Tokyo, Japan), at 400x magnification. The results were then analysed for further evaluation.

Statistical analysis

The normality of the data was assessed using the Kolmogorov-Smirnov test. If the data were normally distributed, a parametric test performed; otherwise, non-parametric tests were used. Homogeneity of variances was assessed using Levene's test to ensure equal variances between the groups. For comparing between-group differences, a one-way analysis of variance (ANOVA) was conducted. This test assessed whether there were significant differences in the measured outcomes. If ANOVA indicated significant differences, Tukey's HSD test was used for pairwise comparisons between the groups, allowing for identification of which groups significantly differed from each other. A paired t-test was used for within-group comparisons at the two time points (1 hour and 14 days) to assess changes over time within each group.

RESULTS

The results of the normality test using the Kolmogorov-Smirnov test showed significance values of p=0.280 and p=0.251 for the percentage of injury, and p=0.291 and p=0.287 for MDA. Since the p-value was greater than 0.05, we accepted Ho, indicating that the data followed a normal distribution. Before testing using ANOVA, the data obtained for each treatment were analysed for homogeneity of variance using the homogeneity of variance test (Levene test) to determine whether the data used had the same variance (Table 1).

Table 1. Homogeneity of variance test using Levene for the percentage of injury (PI) and malondialdehyde (MDA) levels across different groups

Parameter	Levene statistic	Significance (p-value)
PI	0.395	0.758
PI-14	0.196	0.754
MDA	0.187	0.058
MDA-14	2.819	0.065

The test results showed the value of the Levene test for the Percentage of Injury, with p=0.758 and p=0.754, while MDA had p=0.058 and p=0.065. Because the p-value was greater than 0.05, Ho was accepted, and it could be concluded that the data used had a homogeneous variance. Thus, ANOVA could be used to test. A one-way ANOVA test was conducted to determine whether there was a significant difference between the treatments (Table 2). Based on the ANOVA analysis, the p-values for the percentage of injuries, the 14-day period, and MDA were all p=0.000.

Table 2. One-way ANOVA test between percentage of injury (PI) and malondialdehyde (MDA) levels

Parameter	p	Description
PI	0.000	Significance
PI-14	0.000	Significance
MDA	0.000	Significance
MDA-14	0.000	Significance

The results of comparing each group after the One-way ANOVA test were further analysed using the Tukey test. The results of the Tukey test that had different group mean values were shown if they had a $p<0.05$. Tukey's test results on percentage of injury

Table 3. Paired t-test percentage of injury (PI) and malondialdehyde (MDA) levels between the first day and the 14th day

Parameter	Mean	p	Description
PI	58.90	0.000	Significance
PI-14	47.87		
MDA	261.70	0.000	Significance
MDA-14	361.67		

(PI) showed that the control group differed significantly from the 10-minute and 15-minute groups. A comparison between the 5-minute reperfusion interval groups showed a significant difference between the 10-minute and 15-minute groups. Meanwhile, the 10-minute reperfusion interval group showed no difference from the 15-minute reperfusion interval group.

Table 3 presents the results of testing the percentage of injuries on the first day showed p=0.000 for 14 (Table 3). Thus, it could be concluded that there was a significant difference between the percentage of injuries on the first and 14th day, where the percentage of injuries on the first day was higher than on day 14. The results of testing the levels of MDA on the first and 14th days showed p=0.000 which was <0.05. Thus, it could be concluded that there was a significant difference between the MDA on the first day and the MDA on day 14, where the MDA level on day 14 was higher than on the first day (Figures 1, 2).

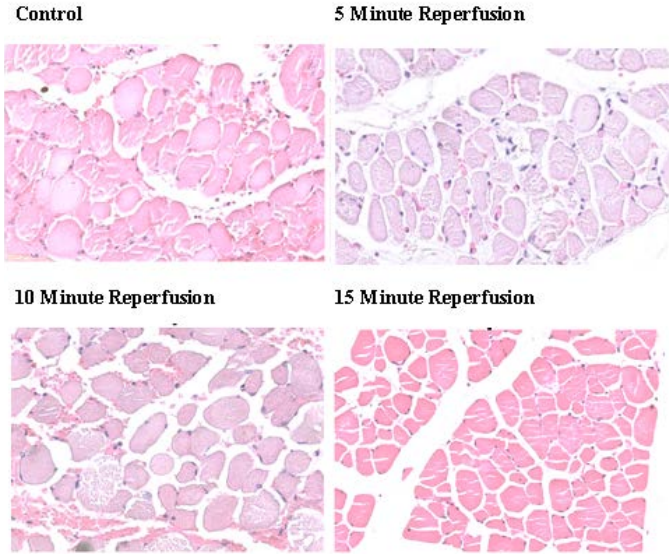

Figure 1. Histology of percentage injury in Control, 5, 10, and 15-minute reperfusion groups (Faculty of Medicine, Universitas Brawijaya, 2023)

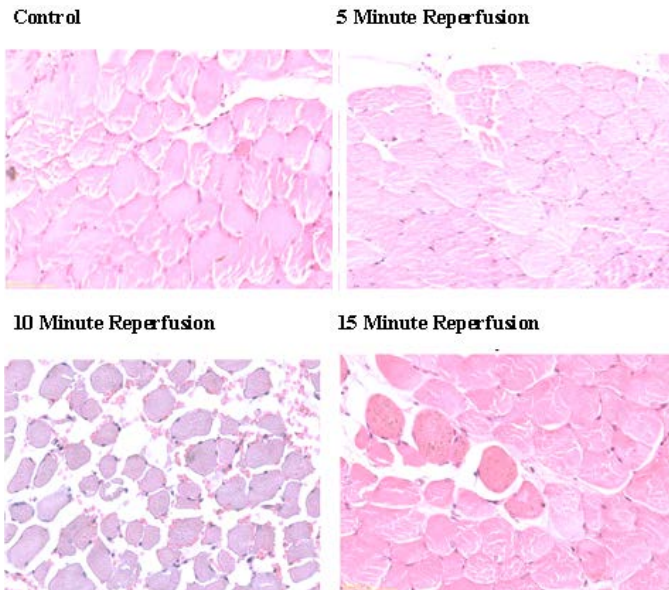

Figure 2. Histology of percentage injury day-14 in Control, 5, 10 and 15- minute reperfusion groups (Faculty of Medicine, Universitas Brawijaya, 2023)

DISCUSSION

Tourniquets to assist haemostasis have been commonly used clinically in trauma cases (1). Initially used in wartime to stop bleeding from war wounds, tourniquets are now standard in orthopaedics to reduce bleeding and maintain a clear operating field during surgeries for fractures (21). However, the return of blood circulation to ischemic tissue after tourniquet deflation can lead to ischemic reperfusion injury (5). Previous research using Wistar rats in a 3-hour leg ischemia model demonstrated an increase in serum levels of urea, creatinine, SGOT, SGPT, LDH, TNF-α, and IL-6 in the group given reperfusion for 1, 2, and 3 hours (22).

In our study, the measurement of MDA levels in Group A, in which rats were sacrificed 1 hour after tourniquet deflation, revealed a significant difference between the control group and those receiving reperfusion intervals of 5 minutes, 10 minutes, and 15 minutes. Interestingly, while there were no significant differences between the 5- and 15-minute reperfusion groups, the 10-minute reperfusion interval showed significantly lower MDA level when compared to both the 5-minute and 15-minute reperfusion groups. This finding is consistent with previous studies, which have shown that shorter reperfusion intervals may not provide sufficient recovery, leading to higher oxidative stress levels and muscle damage (18).

Our results align with those of a study investigating circulatory discontinuation and its impact on oxidation during healing in rats. Femoral artery clamping for 4.5 hours after tibial fracture significantly increased MDA levels on days 3, 7, and 14 (23). This is in line with our findings, where a longer reperfusion interval (10 minutes) improved recovery compared to shorter intervals, further reducing oxidative stress as measured by MDA level. Our study also supports the idea that a 10-minute reperfusion interval facilitates better metabolic recovery compared to 5-minute reperfusion intervals, which have been shown to result in extensive metabolic breakdown and poor recovery (13).

Furthermore, several studies suggest that reperfusion intervals help reduce muscle damage when used after prolonged ischemia (more than 2 hours) (17). Other research showed that the presence of a reperfusion interval in the experimental group of rats, for 20 minutes after experiencing ischemia for 1.5 hours, showed better blood flow in skeletal muscle than the group without a reperfusion interval (24). It was shown that a group of experimental animals that had received reperfusion intervals showed a decrease in the degree of cell damage and a decrease in serum creatinine phosphokinase levels (16). A study suggested that to reduce the complications of using a tourniquet, you can give a perfusion time interval with tourniquet deflation for a specific duration, with a reperfusion interval of 10 minutes or 30 minutes after using a tourniquet for 2 hours (13).

Ischemic conditions, caused by tourniquet use, disrupt the bloodstream, leading to tissue damage and organ dysfunction (25). The tissue undergoes hypoxia, resulting in decreased ATP

levels and activation of anaerobic glycolysis, which accumulates lactate and other toxic byproducts (6). These changes activate phospholipase A2, which converts phospholipids into arachidonic acid, leading to the production of inflammatory mediators such as leukotrienes and prostaglandins (26). These metabolites increase leukocyte adhesion to the vascular endothelium and enhance permeability of post-capillary venules (27). Additionally, ischemia induces the conversion of xanthine dehydrogenase (XDH) to xanthine oxidase (XO), which, along with hypoxanthine accumulation, produces oxidants (28). The reduction in ATP also impairs calcium pumps, raising intracellular calcium levels and causing mitochondrial damage (6). During the reperfusion phase, the return of blood flow provides an adequate blood supply.

Oxygen in ischemic tissue through blood vessels restores tissue metabolism to aerobic, but at the same time, triggers the formation of ROS. The formation of excess ROS due to the interaction of XO and hypoxanthine causes oxidative stress and lipid peroxidation, activation of transcription factors such as NF-B, and increased proinflammatory mediators. Lipid peroxidation is produced by the product malondialdehyde (MDA) (3). In addition, antibody complexes also appear in the reperfusion phase, which will damage cell membranes (3). Complement activation that occurs also causes degranulation of mast cells and the release of histamine and other chemical mediators that can lead to multiple organ failure, resulting in postoperative mortality and morbidity (8).

Oxidative stress due to increased ROS in the reperfusion phase is triggered by the return of oxygen supply (reoxygenation), which can cause damage to cellular macromolecules such as nucleic acids, proteins, and lipids, especially in endothelial cells (7). Under physiological conditions (low lipid peroxidase level), cells can maintain balance through the antioxidant defence system (29). On the other hand, in conditions of high lipid peroxidase level, the oxidative damage that occurs exceeds cell repair ability, so cells will begin to undergo apoptosis or necrosis (30). Hydrogen peroxide forms in tissues exposed to ischemic reperfusion injury and is a relatively stable oxidant. At concentrations of 10-100 M, H_2O_2 can cause endothelial dysfunction, increased expression of adhesive molecules against leukocytes, and increased inflammatory mediators. One of the parameters that can be used to assess oxidative stress conditions is measuring MDA levels (31).

This study demonstrated that ischemic-reperfusion injury causes damage to muscle organs, primarily due to the return of an abundant oxygen supply during reperfusion, which triggers a surge in ROS production and leads to oxidative stress. This condition is capable of damaging muscle cell structure, as previously described (32). Statistical tests showed higher MDA levels in group B (the group that was sacrificed on day 14); in that group, the MDA levels in the control group were much higher than the treatment group; this indicates that the reperfusion interval can reduce oxidative stress conditions. The injury process is still ongoing until day 14; it can be correlated with the post-tourniquet syndrome process in patients who have undergone surgery using tourniquets, which can occur up to the sixth week postoperatively. Histopathological analysis of muscle organs in group B showed a significantly lower PI value than in group A, indicating the reversibility of the injury and the process of repair and regeneration of skeletal muscle cells (4).

The 10-minute reperfusion interval in this study was proven to prevent excessive ROS formation, thereby reducing muscle tissue damage in the distal part of the tourniquet. However, further research is necessary to investigate oxygen levels in the distal tortuous during both ischemia and reperfusion in real-time. Additionally, studies are needed on the oxygen levels required by distal tourniquet tissue to maintain vital functions, as well as on the use of antioxidants as initial therapy to prevent the effects of ROS. Furthermore, additional research is needed to investigate the effects of longer reperfusion intervals and study durations on the persistence of oxidative stress following tourniquet use.

This study demonstrates that administering reperfusion intervals during tourniquet use significantly reduces MDA levels in skeletal muscle on both the 1st and 14th days post-injury. The application of reperfusion intervals also alleviates ischemic reperfusion injury, as evidenced by the reduction in skeletal muscle damage (percentage injury) at both time points. These findings suggest that incorporating a 10-minute reperfusion interval following tourniquet application can help mitigate muscle damage and improve tissue recovery during and after orthopaedic surgeries. In clinical practice, these results could guide surgeons in adjusting reperfusion strategies during surgeries to minimize ischemic reperfusion injury and enhance postoperative recovery, particularly in patients undergoing procedures involving tourniquets.

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Conflicts of interest: None to declare.

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ORIGINAL ARTICLE

Impact of geographic location and place of surgery on treatment outcomes of total hip replacement

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ABSTRACT

Aim Environmental factors may influence postoperative outcome and quality of life following total hip replacement (THR). The aim of this study was to investigate the impact of the geographical location of the surgical site, as well as the patient's place of birth and residence, on treatment outcome in individuals with artificial hips.

Methods A prospective study was conducted involving 280 patients (both genders; mean age 62±8.8 years) who underwent THR due to primary or secondary hip osteoarthritis. The patients were divided into two groups: Group A (N=64) included individuals who were not operated in their place of birth and residence, and Group B (N=216) consisted of those who were born, resided, and underwent surgery in the same geographical location. Outcome was assessed using the EQ-5D questionnaire (covering mobility, self-care, usual activities, pain/discomfort, and anxiety/depression), the Visual Analogue Scale (VAS) for pain, and a VAS-based treatment satisfaction scale, administered preoperatively and one year postoperatively. Statistical analysis was performed using Fisher's exact test ($p<0.05$).

Results Only 64 (22.9%) of all patients underwent surgery in their place of birth and residence, mostly for primary hip osteoarthritis. Preoperatively, Group A reported significantly greater limitations in self-care ($p<0.05$). One year postoperatively, Group B showed a significantly higher VAS score for treatment satisfaction ($p<0.05$).

Conclusion Patients who underwent total hip replacement in their place of birth and residence demonstrated a better postoperative outcome compared to those who had relocated.

Keywords: arthroplasty, immigrants, quality of life

INTRODUCTION

The prevalence of degenerative hip disease is steadily rising, with a tendency toward further growth. This condition causes significant disability in patients and markedly reduces their quality of life (1). Due to its numerous benefits, hip arthroplasty has been declared the operation of the 20th century (2). However, some studies have shown that hip-related pain was reported in 28.1% of patients 12 to 18 months after primary total hip replacement (THR), and that 7% of patients were dissatisfied or very dissatisfied one year after THR (3,4).

Surgical approach, implant design, method of fixation, type of implant, femoral head size, hospital size, and fast-track protocols can all influence THR outcome. Additional factors such as age and gender, comorbidities, medication use, alcohol consumption, smoking habits, and activity level may also affect the outcome of THR (5,6).

During the 1990s, Bosnia and Herzegovina (B&H) experienced significant migration and displacement. Regarding the status of immigrants and its impact on THR outcome, we found only studies comparing immigrants and native-born patients within the same countries (7-10).

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The aim of this study was to evaluate whether patients born and undergoing THR surgery in their hometowns in B&H reported comparable clinical outcomes to those born in one city but receiving surgical treatment in another.

PATIENTS AND METHODS

Patients and study design

This study was conducted at two university hospitals in B&H, Tuzla and Banja Luka. Patient data from these two centres were obtained from the EQ-5D health questionnaire form (11) completed by the patients. Data were collected both preoperatively and one year postoperatively.

For the purpose of this study, an immigrant was defined as an individual born in one location but residing and undergoing surgery in another, while still within the territory of B&H. A total of 64 patients (Group A), who underwent surgery in Tuzla and Banja Luka but were born in other cities, were identified and included in the immigrant group.

The main difference between the two comparison groups is the patients' region of origin. Patients treated in Banja Luka generally came from nearby municipalities surrounding the city, whereas those treated in Tuzla were from cities and cantons within the wider Tuzla region (Group B; N=216).

Data collection for all patients undergoing total hip replacement (THR) in Tuzla and Banja Luka was conducted in several stages. The last author of the study coordinated the official translation of the EQ-5D form into all legally recognized languages of B&H by engaging a certified translator.

Subsequently, arthroplasty surgeons in Banja Luka and Tuzla were contacted to coordinate the data collection process, which was conducted from January 2016 to December 2023. Patient-reported outcome measures (PROMs) were collected preoperatively and one year after THA, under the supervision of a dedicated nurse at each hospital, who ensured that the completed forms were accurately entered into a centralized database. Preoperatively, patients completed the forms within four weeks prior to surgery. Data collection and analysis were finalized in 2023. The extended duration of the study was primarily due to interruptions caused by the COVID-19 pandemic.

The inclusion criteria were as follows: provision of written informed consent to participate, patients either from their home cities or born in one city and operated in another, and completion of a preoperative standard-of-care patient-reported outcome measures (PROMs) questionnaire (12). Exclusion criteria included patients with malignancy, those scheduled for one-stage bilateral total THA, patients undergoing reoperations during the study period, and the first hip surgery in patients who received a second THA during the study.

Data from 80 patients were collected between January 2016 and December 2018 in Tuzla, whom 39 (48%) were born in cities other than Tuzla. In Banja Luka, data from 200 patients were collected, with 25 (12.5%) born outside Banja Luka. In total, 280 patients were included, with 64 (22.8%) born in cities or cantons other than their place of surgery (Group A). The remaining 216 patients (87.2%) were operated in their native city (Group B). All patients completed PROMs both preoperatively and at 1-year follow-up.

Data collected from patients in Tuzla and Banja Luka included age, gender, education level, civil status, place of birth, family type, Charnley classification (13), type of implant fixation, and type of surgical incision. Information on hip disease diagnosis was obtained from medical records. Educational level (categorized as low or middle/high) and cohabitation status (YES/NO) were self-reported via questionnaires. The simplified classification of education was based primarily on the International Standard Classification of Education (ISCED) developed by UNESCO (14).

The study received ethical approval from the Regional Ethical Review Board in Banja Luka, Bosnia and Herzegovina (Protocol No.: 116-16-8017/2016), as well as from the Regional Ethical Review Board in Tuzla, Bosnia and Herzegovina (Protocol No.: 02-09/2-4/2016).

Methods

A nurse in both cities contacted patients approximately one month before surgery to inform them about the study and to invite their participation. Patients who consented to participate were provided with the EQ-5D questionnaire (11), along with questions regarding demographic and clinical variables, including age (<60 or ≥60 years), gender (male/female), diagnosis (primary osteoarthritis or secondary osteoarthritis), Charnley classification (class A/B or C) (13), education level (low/middle/high), cohabitation status (YES/NO), type of surgical incision (lateral or posterior), and choice of implant fixation (cemented or uncemented). Patients completed the questionnaires independently, and the nurse subsequently entered the responses into an Excel database.

Each patient was contacted for a follow-up one year postoperatively, at which time all patients were asked to complete the patient-reported outcome measures (PROMs) questionnaire (12). The PROM protocol included the health-related quality of life (HRQoL) measure EQ-5D (11), a visual analogue scale (VAS) for pain (15), the Charnley classification survey (classes A + B, C) (13), and a VAS assessing patient satisfaction after surgery (11). The EQ-5D (11) form used was translated into the official languages of the peoples of Bosnia and Herzegovina.

The EQ-VAS for general health ranges from 0 (worst imaginable health state) to 100 (best imaginable health state). The Pain-VAS ranges from 0 (no pain) to 100 (unbearable pain). The Satisfaction-VAS used at follow-up measures satisfaction with the total hip replacement outcome, where 0 represents complete satisfaction and 100 indicates maximum dissatisfaction. To assess patient-reported comorbidity status, patients completed the Charnley classification survey.

Prior to surgery, patients attended a preoperative visit during which they were examined by an orthopaedic surgeon (typically the surgeon who would perform the operation), an anaesthesiologist, a physiotherapist, and a nurse. During this visit, patients received detailed information about the type of anaesthesia and surgical procedure, expected outcome, potential risks and complications, and the postoperative rehabilitation process.

Each participant was identified using a unique personal identity number. One-year postoperative data collection was conducted during routine follow-up visits at the two hospitals. All data collection in B&H was performed using paper form. Patients were contacted in the clinic preoperatively and again one year postoperatively to complete the survey.

The questionnaire has also been adapted for preoperative use as an internet-based touchscreen application in hospital clinics and is optionally available via the Web.

The three-level EQ-5D form was utilized, defining each di-

mension as no problems, some or moderate problems, or extreme problems. The British tariff (16) was applied to calculate the EQ-5D index score for this population.

Statistical analysis

To assess whether any of the five EQ-5D dimensions (mobility, self-care, usual activities, pain/discomfort, anxiety/depression) differed between patients operated outside their home city and those operated within it, responses were dichotomized into "no problems" versus "moderate/severe problems" for each dimension.

Differences between the two groups were tested using Fisher's exact test for dichotomous variables and Fisher's non-parametric permutation test for continuous variables. To evaluate the influence of covariates, binary logistic regression analyses were performed using the dichotomized outcomes for each EQ-5D dimension. The effects of covariates on each EQ-5D dimension were examined by calculating unadjusted and adjusted odds ratios (ORs), with each covariate included separately in the models. Covariates that altered the unadjusted OR by more than 10% were retained in adjusted analyses. Covariates included age, gender (male/female), diagnosis (primary osteoarthritis (OA), secondary OA), Charnley class (A/B or C), education level (low, middle/high), cohabitation status (yes/no), and type of incision (lateral, posterior). At the 1-year follow-up, preoperative values for each dimension were also analysed as potential predictors.

Visual Analog Scales for pain, satisfaction, and general health (EQ-VAS) were analysed using Fisher's non-parametric permutation test and linear or logistic regression models to adjust for covariates. Variables not meeting normality assumptions were dichotomized and analysed via logistic regression.

All statistical tests were two-sided and conducted at a significance level of 0.05.

Table 1. Demographic characteristics of the participants

Variable	Patients operated outside of home city (N=64)	Patients operated in home city (N=216)	p
Age (years)			
Mean±SD	60.2±11.8	60.7 (12.3)	
Median (Min - Max)	59 (29 - 81)	63 (26 - 89)	0.80
	No (%) of patients		
Gender			
Male	23 (35.9)	70 (32.4)	0.70
Female	41 (64.1)	146 (67.6)	
Age group (years)			
<50	11 (17.2)	37 (17.1)	
50-59	23 (35.9)	53 (24.5)	
60-75	20 (31.3)	109 (50.5)	0.64
>75	10 (15.6)	17 (7.9)	
Diagnosis			
Primary OA	10 (15.6)	43 (19.9)	0.57
Secondary OA	54 (84.4)	173 (80.1)	
Charnley class			
A+B	54 (84.4)	186 (86.1)	0.86
C	10 (15.6)	30 (13.9)	
Cohabiting			
NO	4 (6.3)	25 (11.6)	0.32
YES	60 (93.8)	191 (88.4)	
Education			
Low	17 (26.6)	46 (21.3)	0.47
Middle / High	47 (73.4)	170 (78.7)	
Type of fixation			
Uncemented	64 (100.0)	216 (100.0)	0.29
Surgical incision			
Lateral	16 (25.0)	55 (25.5)	1.00
Posterior	48 (75.0)	161 (74.5)	

RESULTS

Out of the total 280, 64 patients underwent hip arthroplasty outside their home city; 23 were male, with a mean age of 59 years (Table 1), and the most common indication for surgery in this group was secondary hip osteoarthritis. Among patients who were operated on in their home city, primary hip osteoarthritis was the predominant reason for total hip replacement.

Table 2. Preoperative and 1-year postoperative data of the pain/satisfaction, EuroQol Visual Analogue Scale (EQVAS)

Parameter	Preoperatively		p	One year postoperatively		p
	Values	Difference		Values	Difference	
Pain VAS						
Mean (SD)						
Home city	41.5 (15.4)	0.72		15.2 (8.8)	0.78	
Outside home city	40.8 (16.4)			16.0 (11.4)		0.58
Median (min-Max)			0.79			
Home city	40 (0-100)	0.79		10 (10-70)	(-2-3.3)	
Outside home city	40 (10- -5.2)			10 (10-60)		
Satisfaction VAS						
Mean (SD)						
Home city	-			12.3 (4.5)	2.5	
Outside home city	-			14.8 (6.9)		
Median (min-Max)						0.0016
Home city	-			10 (10-30)	(1.02-3.9)	
Outside home city	-			10 (10-40)		
EQVAS						
Mean (SD)						
Home city	44.0 (15.7)	0.71		90.5 (10.4)	-0.06	
Outside home city	44.7 (15.7)			90.5 (10.9)		
Median (min-Max)			0.79			0.98
Home city	50 (10-80)	(-37.5 - 5.1)		90 (40-100)	(-3.0 - 3.0)	
Outside home city	50 (10-80)			90 (55-100)		

VAS, Visual Analogue Scale for pain;

Table 3. Preoperative and 1-year postoperative data of the EuroQol 5 Dimension (EQ5D) questions

EQ5D questions Moderate or severe problems	No (%) of patients	95% CI	p	No (%) of patients	95% CI	p
Mobility						
Home city	206 (95.4)	3.10 (-2.1% - 8.2)	0.48	22 (10.2)	-3.90 (-12.1 - 4.2)	0.49
Outside home city	63 (98.4)			4 (6.3)		
Self-care						
Home city	161 (74.5)	-21.40 (-36.0 - -6.9)	0.002	36 (16.7)	-1.00 (-12.2 - 10.2)	1
Outside home city	34 (53.1)			10 (15.6)		
Usual activities						
Home city	189 (87.5)	-7.80 (-19.6 - 4.0)	0.18	29 (13.4)	-4.10 (-13.5 - 5.4)	0.53
Outside home city	51 (79.7)			6 (9.4)		
Pain/discomfort						
Home city	211 (97.7)	-3.90 (-11.2 - 3.3)	0.25	34 (15.7)	-3.20 (-13.7 - 7.2)	0.68
Outside home city	60 (93.8)			8 (12.5)		
Anxiety/Depression						
Home city	114 (52.8)	9.70 (-4.9 -24.3)	0.22	51 (23.6)	-1.70 (-14.4 - 10.9)	0.92
Outside home city	40 (62.5)			14 (21.9)		

Table 4. Odds Ratio (OR) of moderate or severe problems for the five EuroQol Visual Analogue Scale (EQVAS) questions for patients operated outside of home city compared to patients operated in home city

EQ5D questions Moderate or severe problems	Preoperatively OR (95% CI)	p	One year follow-up OR (95% CI)	p
Mobility				
Unadjusted	3.06 (0.38-24.36)	*0.29	0.59 (0.19-1.77)	0.35
Self-care				
Unadjusted	0.39 (0.22-0.69)	*0.0013	0.93 (0.43-1.99)	0.84
Usual activities				
Unadjusted	0.56 (0.27-1.16)	*0.12	0.67 (0.26-1.69)	0.39
Pain/discomfort				
Unadjusted	0.36 (0.09-1.37)	*0.13	0.76 (0.33-1.75)	0.52
Adjusted*	0.32 (0.08-1.27)	*0.11		
Anxiety/Depression				
Unadjusted	1.49 (0.84-2.64)	*0.17	0.91 (0.46-1.77)	0.77

*Adjusted for cohabitation

There was no statistically significant difference between Group A and Group B in terms of education level or living arrangements (living alone or with family) (p=0.32).

Patients who underwent surgery outside their home city reported slightly higher level of anxiety and depression (Table 3) compared to those operated in their hometown (p=0.22). Pain levels, as measured by both the Visual Analogue Scale (VAS) and EQ-VAS, were similar between the two surgical groups (Table 2).

No covariates were found to significantly influence the odds ratios of the preoperative EQ-5D dimensions, except for cohabitation status, which affected the pain/discomfort domain. The odds ratio for pain/discomfort changed after adjusting for cohabitation status (Table 5).

Patients operated outside their home city reported significantly higher average satisfaction score on VAS (p=0.0016) (Table 2). They also had significantly lower odds of reporting a perfect satisfaction score of 10 on VAS (p=0.005). This result remained statistically significant even after adjusting for covariates (p=0.0037) (Table 5).

Postoperatively, no meaningful differences were observed between the groups in Pain VAS or EQ-VAS score. On average, Pain VAS scores were slightly higher in patients operated outside their home city (p=0.58) (Table 2). Nevertheless, the odds of reporting a maximum Pain VAS score of 10 were also slightly higher in this group (p=0.49) (Table 4).

The EQ-5D domains postoperatively did not show significant differences between the groups. A notable preoperative difference in the self-care domain diminished postoperatively, reaching non-significance (p=1.0) (Table 3). Furthermore, no covariates, including undergoing surgery outside the patient's home city compared to surgery in the home city, were found to significantly influence the odds ratios for any of the postoperative EQ-5D dimensions. (Table 5)

Table 5. Mean change and odds ratio of pain-satisfaction and EuroQol Visual Analogue Scale (EQVAS) for patients operated outside of home city compared to patients operated in their home city

Parameter	Preoperatively OR Mean change (95% CI)	p	One year follow-up OR Mean change (95% CI)	p
Pain VAS				
Unadjusted	-0.72 (-5.10 - 3.65)	0.75	1.23† (0.68 - 2.22)	0.49
Adjusted*	-0.12 (-3.89 - 3.66)	0.95	1.26† (0.67 - 2.34)	0.47
Satisfaction VAS				
Unadjusted	-	-	0.43‡ (0.24 - 0.78)	0.0050
Adjusted*	-	-	0.41‡ (0.22 - 0.75)	0.0037
EQVAS				
Unadjusted	0.71 (-3.70 - 5.11)	0.75	1.18¶ (0.67- 2.07)	0.56
Adjusted*	0.78 (-3.64 - 5.20)	0.73	1.16¶ (0.66 -2.05)	0.61

*Adjusted for age, gender, diagnosis, Charnley class, cohabitation and incision. At one year preoperatively values were also adjusted for; †Odds ratio (OR) for scoring 10; ‡OR for scoring 10; ¶OR for scoring 100
The preoperative results for VAS Satisfaction were marked with (-) because all patients are dissatisfied with the condition.
VAS, Visual Analogue Scale for pain;

DISSCUSION

The quality of life in patients with total hip replacement is commonly assessed using various standardized questionnaires, among which the EQ-5D is one of those employed (11). Several studies have investigated the impact of sociodemograph-

ic and socioeconomic factors on the quality of life in patients with hip arthroplasty (17-22).

This study found that a higher proportion of patients operated outside their home city suffered from secondary hip osteoarthritis. One possible explanation for this finding is the necessity for such patients to seek treatment in highly specialized clinics, often located outside their local communities. Additionally, patients with secondary osteoarthritis typically experience long-standing symptoms that may lead to chronic exhaustion and an increased prevalence of anxiety or depression, which could explain the outcome observed in this study. On the other hand, patients who underwent surgery in their hometown may have benefited from greater stability, ranging from emotional and familial support to financial security, since the overall treatment costs are usually lower when managed locally.

There is a lack of studies specifically addressing this topic. One study, published in 2024, demonstrated a significant difference in quality of life among patients who underwent surgery abroad and had migrated from their country of origin, compared to those who were operated on within their local healthcare systems (23).

There is a clear need for more methodologically sound research to further elucidate and explain the influence of sociodemographic factors on quality-of-life outcome in patients following total hip replacement. Studies with more holistic approach to the management of hip osteoarthritis yield important data with particular emphasis on environmental determinants that may impact the quality of life in affected individuals. Such studies are of predictive value, since they also take into account the geographical factor when assessing the chances of a desired outcome of surgery.

In conclusion, our study did not confirm a significant association between the geographic location of treatment and residence and the quality-of-life outcome following total hip replacement. Similar future studies with larger sample sizes may help to further elucidate the outcome and characteristics of this patient group.

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TRANSPARENCY DECLARATION

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Med Glas (Zenica)

ORIGINAL ARTICLE

Knowledge of general medicine students from the medical faculties in Tuzla and Zenica about lung cancer prevention

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ABSTRACT

Aim To assess the level of knowledge about risk factors and prevention of lung cancer among medical students, and to identify differences in knowledge based on the year of study and previous secondary education background.

Methods The study was conducted among 223 students of School of Medicine, University of Zenica, and School of Medicine, University Clinical Centre Tuzla using an anonymous online survey via the Google Forms platform. The collected data were analysed using descriptive statistics and the $\chi 2$ test to assess statistical significance.

Results The majority of students identified smoking as the main risk factor for lung cancer, while air pollution was rated as the most overlooked risk factor. There were significant differences in the perception of neglected risk factors between years of study ($p<0.05$). Most students from Zenica acquire their knowledge through formal education, while students from Tuzla more often rely on the internet and media ($p<0.05$). Additionally, 82.5% of students believe that passive smoking is equally harmful as active smoking, with no significant differences between the groups.

Conclusion Medical students demonstrate a good level of awareness regarding risk factors and prevention of lung cancer; however, there is a need for greater emphasis on environmental risks and passive smoking in their education. The results highlight the importance of continuous education to ensure that future healthcare professionals are equipped to effectively promote health and prevent this disease.

Keywords: air pollution, health knowledge, attitudes, practice, lung neoplasms / prevention & control, medical students, smoking

INTRODUCTION

Insufficient prevention, increasingly widespread air pollution, and smoking as the main cause of lung cancer are the reasons for the rising incidence of lung cancer. An estimated 2.48 million new cases of lung cancer occurred worldwide in 2022 (1). Prevention and early detection of lung cancer remain challenging (2).

Smoking is the leading risk factor for lung cancer, accounting for approximately 90% of all cases. A person who smokes one pack of cigarettes per day has a 20 times higher risk of developing lung cancer compared to a non-smoker (3). Age, sex, and heredity also play a major role in the development of this disease (4). It is estimated that quitting smoking after a lung cancer diagnosis improves 5-year survival by approximately 35%. Passive smoking causes preventable mortality and morbidity (3). Prevention strategies to reduce cigarette smoking in public places should be part of public health policy (5). Approximately 10-15% of lung cancers in Western countries occur in people who have never smoked (6). These cancers are explained by other occupational and environmental risk factors, such as passive smoking, radon exposure, air pollution, and genetic factors (7).

Between 9% and 15% of diagnosed lung cancers in men and about 5% in females can be attributed to inhalation of carcinogenic agents at work. According to the Francis Crick Institute and University College London, funded by Cancer Research UK, particles commonly found in vehicle exhaust and fossil fuel smoke are associated with the risk of non-small cell lung cancer (NSCLC), which accounts for over 250,000 lung cancer deaths globally each year (8).

Lung cancer incidence is stagnating or slightly decreasing in men, but increasing in women, which is linked to higher smoking prevalence among women (9). Higher cancer mortality is linked to higher smoking prevalence, as well as better or worse access to health insurance, possibilities for early diagnosis, and therapeutic options (10).

According to the British Thoracic Society (BTS) smoking cessation report, only 28% of smokers wanted to quit, and only 6% of them received advice on how to do so (11). Almost 90% of smokers aged 15 or older registered with general practitioners had records of offered support or treatment (1).

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Education on lung cancer prevention and early detection is of great importance, especially among medical students. Through education and future professional work, they can raise public awareness about the importance of lung cancer prevention, thus enabling earlier diagnosis (4).

Despite the growing burden of this disease (9), there is a noticeable lack of studies examining the level of knowledge and awareness about lung cancer prevention among future healthcare professionals in Bosnia and Herzegovina (B&H). To the best of our knowledge, no previous research of this kind has been conducted in B&H. The decision to focus on medical students stems from their dual role – as individuals who must adopt healthy lifestyle habits themselves and as future physicians responsible for educating patients and promoting preventive health behaviours. By identifying knowledge gaps and attitudes toward lung cancer prevention, the findings of this research can serve as a foundation for improving the medical curriculum, designing targeted educational interventions, and strengthening public health strategies aimed at reducing the burden of lung cancer in the population.

The aim of this study was to investigate the level of awareness about risk factors and lung cancer prevention among medical students of two medical faculties to determine possible differences in knowledge about lung cancer prevention among students who completed medical high school, general high school (gymnasium), or other types of secondary education, and to assess medical awareness of the importance of eliminating risk factors and preventing lung cancer in relation to their lifestyle.

MATERIALS AND METHODS

Participants and study design

This prospective, descriptive, and epidemiological investigation aimed at assessing the level of knowledge and awareness about lung cancer prevention among medical students.

The inclusion criterion for participants was enrolment in the general medicine study program at School of Medicine, University of Zenica, and School of Medicine at the University Clinical Centre in Tuzla (B&H). All students were invited to participate voluntarily. The exclusion criterion was refusal to participate in the study. In total, 223 students from the two faculties participated in the study. The sample included students born between 1994 and 2004, representing different academic years of the study. Participation was entirely voluntary and anonymous, and each respondent was informed about the purpose of the research before completing the questionnaire.

Methods

Data collection was carried out using a structured questionnaire distributed through the Google Forms platform. The online survey was available for completion in the areas of Zenica and Tuzla from 31 March 2023 to 7 April 2023.

The questionnaire consisted of two main sections: sociodemographic data including variables such as age, sex, faculty, and year of study, and knowledge and awareness of lung cancer prevention, with questions designed to assess students' understanding of risk factors, prevention strategies, and early detection methods.

The instrument was self-administered and designed to ensure confidentiality and ease access for participants. Data were automatically collected through an online form and exported for statistical analysis.

Statistical analysis

Descriptive statistics (frequencies, percentages, means, and standard deviations) were used to summarize sociodemographic characteristics and knowledge levels. Crosstabulation analyses were conducted to examine potential associations between variables such as sex, year of study, and knowledge scores. For data visualization, graphs and tables were generated using Microsoft Excel to facilitate the interpretation of findings and to illustrate trends within the dataset. Value of $p<0.05$ was considered statistically significant.

RESULTS

A total of 223 students were surveyed: 126 (56.5%) from School of Medicine in Zenica and 97 (43.5%) from School of Medicine in Tuzla; 151 (67.7%) were females and 72 (32.3%) were males. The distribution by year of study was as follows: 36 (16.1%) first-year students, 55 (24.7%) second-year students, 28 (12.6%) third-year, 26 (11.7%) fourth-year, 46 (20.6%) fifth-year, and 32 (14.3%) sixth-year students.

The largest percentage of respondents had previously completed medical high school 125 (56.1%), followed by those who attended a general high school 85 (38.1%), and those who completed another type of secondary school 13 (5.8%).

The highest percentage of medical students in Zenica and Tuzla who felt they had received adequate education on lung cancer prevention through their current studies was among students in higher years, particularly the fifth and sixth years. This trend was more pronounced among students from Tuzla. In total, 29.1% of respondents indicated the fifth year as the period in which they received sufficient education, while the lowest percentage of such responses came from first-year students (8.2%) ($p>0.05$). Additionally, a higher prevalence of students from Zenica (53.2%) reported feeling adequately educated than those from Tuzla (44.3%) ($p>0.05$).

The highest number of students came from medical high schools (56.1%), followed by general high schools (38%), while 13 students (5.8%) came from other types of secondary schools. The highest perceived level of education was among students from general high schools (51.8%), and the lowest among those from other secondary schools (46.2%), without statistical significance ($p>0.05$) (Table 1).

Table 1. Perception of the level of education in relation to the previously completed secondary school

Previously completed secondary school	No (%) of students with self-assessed adequacy of education on lung cancer prevention		Total
	Adequate	Not adequate	
Medical high school	61 (48.8)	64 (51.2)	125
General high schools	44 (51.8)	41 (48.2)	85
Other types of secondary schools	6 (46.2)	7 (53.8)	13
Total	111 (49.8)	112 (50.2)	223

Out of the 223 students, a higher prevalence of male (54.2%) than female (47.7%) students felt they were adequately educated, with no statistically significant difference ($p>0.05$) (Table 2).

Table 2. Perception of the level of education in relation to sex

Sex	No (%) of students with self-assessed adequacy of education on lung cancer prevention		Total
	Adequate	Not adequate	
Female	72 (47.7)	79 (52.3)	151
Male	39 (54.2)	33 (45.8)	72
Total	111 (49.8)	112 (50.2)	223

The proportion of students who stated that they "do not consider themselves smokers but occasionally smoke" was highest in the second and third years (21.8% and 21.4%, respectively), which may indicate transitional phases in smoking behaviour ($p<0.01$) (Table 3).

Table 3. Smoking status of students in relation to the year of study

Year of study	No (%) of students with smoking status				Total
	Smoker	Non-smoker	Former smoker	Non-smokers but occasionally smoke	
First	9 (25)	24 (66.7)	2 (5.6)	1 (2.8)	36
Second	3 (5.5)	37 (67.3)	3 (5.5)	12 (21.8)	55
Third	3 (10.7)	16 (57.1)	3 (10.7)	6 (21.4)	28
Fourth	8 (30.8)	13 (50)	3 (11.5)	2 (7.7)	26
Fifth	7 (15.2)	35 (76.1)	2 (4.3)	2 (4.3)	46
Sixth	8 (25)	20 (62.5)	4 (12.5)	0	32
Total	38 (17)	145 (65)	17 (7.6)	23 (10.3)	223

The year of study significantly affected the perception of neglected factors: students from different years did not equally perceive the importance of smoking (by years of study, it ranges from 12.5-47.2%), diet (11.5-25.5%), pollution (17.9-50%), genetics (0-26.9%), or other overlooked risk factors ($p<0.05$) (Table 4).

Table 4. Students' views on the neglect of risk factors

Year of study	No (%) of students about the most neglected risk factors					Total
	Smoking	Diet	Air pollution	Genetics	No neglected factors	
First	17 (47.2)	5 (13.9)	11 (30.6)	0	3 (8.3)	36
Second	10 (18.2)	14 (25.5)	16 (29.1)	6 (10.9)	9 (16.4)	55
Third	7 (25)	4 (14.3)	5 (17.9)	6 (21.4)	6 (21.4)	28
Fourth	7 (26.9)	3 (11.5)	7 (26.9)	7 (26.9)	2 (7.7)	26
Fifth	11 (23.9)	10 (21.7)	18 (39.1)	3 (6.5)	4 (8.7)	46
Sixth	4 (12.5)	5 (15.6)	16 (50)	5 (15.6)	2 (6.3)	32
Total	56 (25.1)	41 (18.4)	73 (32.7)	27 (12.1)	26 (11.7)	223

A statistically significant difference in the method of acquiring knowledge about lung cancer prevention between students from School of Medicine in Zenica and those in Tuzla was found ($p<0.05$) (Figure 1).

The majority of students (82.5%) believe that passive smoking is equally harmful as active smoking, while 17.5% consider passive smoking to be less harmful. The highest proportion of students who perceive passive smoking as equally harmful is recorded in the second (85.5%) and sixth year (87.5%) of studying with no statistically significant difference.

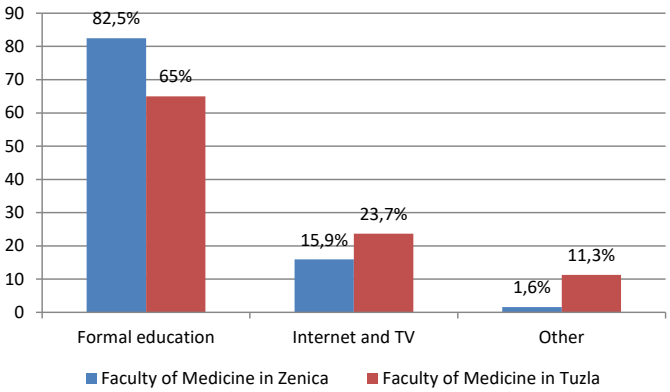

Figure 1. Students' knowledge about risk factors and prevention of lung cancer according to the method of acquiring knowledge

DISCUSSION

The results of our study showed that the majority of students possess a basic understanding of risk factors, particularly smoking and air pollution, which is consistent with findings from other similar studies (12). Similarly, a study from Malaysia found 93% of respondents were aware of lung cancer and all identified smoking as the main risk factor. This high level of awareness may be the result of effective public health campaigns and prevention programs in Malaysia (13). Our findings align with these results, especially in terms of recognizing smoking as a key risk factor, identified by 100% of our participants.

Passive smoking was recognized as a serious health risk by 82.5% of respondents, which is in line with similar research (14). However, a proportion of respondents still considered passive smoking to be less harmful, indicating the need for additional educational efforts. In a study from India, only 35.6% of participants were aware of secondhand smoke, and only 23.3% were aware of thirdhand smoke (14). Secondhand smoke is a recognized public health concern with high exposure rates, which aligns with our finding that medical students acknowledge passive smoking as an important, yet often overlooked risk factor for lung cancer (15).

Air pollution was identified as the most neglected risk factor by the majority of our respondents (32.7%), which corresponds with other studies emphasizing the importance of environmental factors in the development of lung cancer (16). This suggests that medical students are becoming increasingly aware of less obvious risk factors, likely due to the progression of their education during years of study (17).

Our study also showed a statistically significant difference in students' perceptions across different years of studying regarding the neglect of certain risk factors. This is expected, as senior students – with more clinical experience and theoretical knowledge – tend to have a broader perspective on the complexity of risk factors and lung cancer prevention (18,19).

In our study, a diet was recognized as a neglected risk factor, which is significant as it points to a potential need to incorporate dietary advice into lung cancer prevention programs. Our students demonstrated relatively greater awareness of the importance of nutrition comparing with some other studies where only 31.5% of respondents believed certain dietary habits could be a risk factor (16). Reportedly, a significant negative correlation between Mediterranean diet patterns and the risk of lung cancer in the general population was found (20). The findings from the UK Biobank study, which included 416,588 participants, confirmed that diet plays a significant role in in-

fluencing lung cancer risk (21). Lifestyle modification should include changing eating habits based on a healthy diet, which may be an additional factor in reducing the risk of developing cancer (22).

In our study, students in higher years, especially fifth- and sixth-year students, most often reported receiving adequate education on lung cancer prevention, which is in line with other studies (23).

In our study, a slightly higher percentage of male students than female students felt adequately educated about lung cancer prevention, which is consistent with previous findings (24), although the knowledge about cancer risk factors among medical students remains suboptimal across both sexes (25).

This study is limited to two medical faculties, which may restrict the generalizability of the findings, and the use of a questionnaire may reflect subjective perceptions rather than actual knowledge or behaviour. Also, the cross-sectional design precludes any inference of causality, and potential response and recall biases may have influenced the results. Finally, the relatively small sample size may reduce statistical power and limit the possibility of detailed subgroup analyses.

In conclusion, this study provides the first systematic assessment of medical students' knowledge of lung cancer prevention in Bosnia and Herzegovina, incorporating comparisons across secondary education types and exploring students' perceptions of lifestyle- and environment-related determinants. These findings offer novel, context-specific evidence that can support curriculum enhancement and inform local public health strategies. Despite acceptable overall knowledge, gaps in awareness of passive smoking and environmental risks highlight the need for more targeted and continuous educational activities. As future healthcare providers, medical students will play a key role in health promotion and prevention, underscoring the importance of strengthening cancer prevention topics early in medical education.

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