EABR
Experimental and Applied Biomedical Research
ISSN 2956-0454
eISSN 2956-2090
VOL 27 No 3
September, 2026

REVIEW PAPER

Shared inflammatory pathways in atopic dermatitis and Alzheimer's disease

Development of simulation methods in biomedical sciences

CASE REPORT

A case of biphasic tick-borne encephalitis virus meningitis in a presumed low-risk region of central Serbia

Challenges in removing a broken abutment screw: a case report

ORIGINAL SCIENTIFIC ARTICLE

Predictive effectiveness of body mass index, waist-to-height ratio and body roundness index in categorizing metabolic syndrome severity

Folate-related genetic risk and associations of betaine-homocysteine methyltransferase and plasminogen activator inhibitor-1 gene variants with coagulation parameters in pediatric patients with thrombophilia

The frequency of certain genetic polymorphisms and their influence on the therapeutic response of patients treated with olanzapine

Correlation of WOMAC index and cytokine values in patients with OA knee osteoarthritis

Development and stability evaluation of natural topical formulations containing Pinus sibirica essential oil

Factors associated with the occurrence of depressive symptoms among patients undergoing magnetic resonance imaging

GAP (Glasgow Coma Score – GCS, age, arterial pressure), MGAP (mechanism of injury, GCS, age, arterial pressure) and RTS (Revised Trauma Score) as predictors of poo outcomes and mortality after injury in the prehospital setting

Temporal kinetics of urinary versus serum amylase for prognosis and severity stratification and their correlation with CT severity index and clinical outcomes in acut pancreatitis: a prospective cohort study

ISSN 2956-0454

General Manager
Vladimir Janjic

Editor in Chief
Olgica Mihaljevic

Editorial board
Olgica Mihaljevic, Faculty of Medical Sciences, University of Kragujevac, Serbia
Milica Borovcanin, Faculty of Medical Sciences, University of Kragujevac, Serbia
Dejan Aleksic, Faculty of Medical Sciences, University of Kragujevac, Serbia
Milos Milosavljevic, Faculty of Medical Sciences, University of Kragujevac, Serbia
Valentina Opancina, Faculty of Medical Sciences, University of Kragujevac, Serbia

Editorial Advisory Board
(Surnames are given in alphabetical order)

Antovic J (Stockholm, Sweden), Bosnakovski D (Stip, FYR Macedonia), Chaldakov G (Varna, Bulgaria), Conlon M (Ulster, UK), Dhalla NS (Winnipeg, Canada), Djuric D (Belgrade, Serbia), Fountoulakis N (Thessaloniki, Greece), Kozlov R (Smolensk, Russian Federation), Kusljic S (Melbourne, Australia), Lako M (Newcastle, UK), Mitrovic I (San Francisco, USA), Muntean D (Timisoara, Romania), Paessler S (Galvestone, USA), Pechanova O (Bratislava, Slovakia), Serra P (Rome, Italy), Strbak V (Bratislava, Slovakia), Svrakic D (St. Louis, USA), Tester R (Glasgow, UK), Vlaisavljevic V (Maribor, Slovenia), Vujanovic N (Pittsburgh, USA)

Editorial Staff
Ana Miloradovic, Milan Milojevic

Publishers

Faculty of Medical Sciences University of Kragujevac
69 Svetozara Markovica Street, 34000 Kragujevac, Serbia

De Gruyter Brill Sp. z. o. o.
Nowogordska 4/3
00-513 Warsaw, Poland

Indexed in

EMBASE/Excerpta Medica, Index Copernicus, BioMedWorld, KoBSON, SCIndeks, Chemical Abstracts Service, Cabell's Directory, Celdes, CNKI Scholar (China National Knowledge Infrastructure), CNPIEC, EBSCO Discovery Service, Elsevier - SCOPUS, Google Scholar, J-Gate, Naviga (Softweco), Primo Central (ExLibris), ReadCube, SCImago (SJR), Summon (Serials Solutions/ProQuest), TDOne (TDNet), WorldCat (OCLC)

Address:

Experimental and Applied Biomedical Research, Faculty of Medical Sciences,
University of Kragujevac 69 Svetozara Markovica Street, 34000 Kragujevac, PO Box 124, Serbia

Contact:

eabr@fmn.kg.ac.rs

http:/medf.kg.ac.rs/eabr
https://reference-global.com/journal/SJECR

EABR is published four times annually

Experimental and Applied Biomedical Research is categorized as a scientific journal of M51 category by the Ministry of Education, Science and Technological Development of the Republic of Serbia

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

61

EABR : Experimental and Applied Biomedical Research / editor in chief Olgica Mihaljevic. - Vol. 27, no. 3 (sept 2026)- . - Kragujevac : Faculty of Medical Sciences, University of Kragujevac, 2024- (Kragujevac : Faculty of Medical Sciences, University of Kragujevac). - 30 cm

Tromesečno. - Je nastavak: Serbian Journal of Experimental and Clinical Research = ISSN 1820-8665
ISSN 2956-0454 = EABR. Experimental and Applied Biomedical Research
COBISS.SR-ID 81208329

TABLE OF CONTENTS

Review Paper
SHARED INFLAMMATORY PATHWAYS IN ATOPIC DERMATITIS AND ALZHEIMER'S DISEASE 211

Original Scientific Article
PREDICTIVE EFFECTIVENESS OF BODY MASS INDEX, WAIST-TO-HEIGHT RATIO AND BODY ROUNDNESS INDEX IN CATEGORIZING METABOLIC SYNDROME SEVERITY 221

Original Scientific Article
FOLATE-RELATED GENETIC RISK AND ASSOCIATIONS OF BETAINE-HOMOCYSTEINE METHYLTRANSFERASE AND PLASMINOGEN ACTIVATOR INHIBITOR-1 GENE VARIANTS WITH COAGULATION PARAMETERS IN PEDIATRIC PATIENTS WITH THROMBOPHILIA 229

Original Scientific Article
THE FREQUENCY OF CERTAIN GENETIC POLYMORPHISMS AND THEIR INFLUENCE ON THE THERAPEUTIC RESPONSE OF PATIENTS TREATED WITH OLANZAPINE 239

Original Scientific Article
CORRELATION OF WOMAC INDEX AND CYTOKINE VALUES IN PATIENTS WITH OA KNEE 249

Original Scientific Article
DEVELOPEMENT AND STABILITY EVALUATION OF NATURAL TOPICAL FORMULATIONS CONTAINING *PINUS SIBIRICA* ESSENTIAL OIL 255

Original Scientific Article
FACTORS ASSOCIATED WITH OCCURRENCE OF DEPRESSIVE SYMPTOMS AMONG PATIENTS UNDERGOING MAGNETIC RESONANCE IMAGING 265

Original Scientific Article
GAP (GLASGOW COMA SCORE - GCS, AGE, ARTERIAL PRESSURE), MGAP (MECHANISM OF INJURY, GCS, AGE, ARTERIAL PRESSURE) AND RTS (REVISED TRAUMA SCORE) AS PREDICTORS OF POOR OUTCOMES AND MORTALITY AFTER INJURY IN THE PREHOSPITAL SETTING 275

Original Scientific Article
TEMPORAL KINETICS OF URINARY VERSUS SERUM AMYLASE FOR PROGNOSIS AND SEVERITY STRATIFICATION AND THEIR CORRELATION WITH CT SEVERITY INDEX AND CLINICAL OUTCOMES IN ACUTE PANCREATITIS: A PROSPECTIVE COHORT STUDY 281

Review Paper
DEVELOPMENT OF SIMULATION METHODS IN BIOMEDICAL SCIENCES 297

Case Report
A CASE OF BIPHASIC TICK-BORNE ENCEPHALITIS VIRUS MENINGITIS IN A PRESUMED LOW-RISK REGION OF CENTRAL SERBIA 305

Case Report
CHALLENGES IN REMOVING A BROKEN ABUTMENT SCREW: A CASE REPORT 311

SHARED INFLAMMATORY PATHWAYS IN ATOPIC DERMATITIS AND ALZHEIMER'S DISEASE

Faique Tanvir Falke[1], Lubna Khondker[2], Tejaswi Kothapally[3], Shanthi Ramesh[4], Shamina S[5] and Sridevi Visvanathan[6*]

[1]*Burjeel Medical City, Abu Dhabi, UAE*
[2]*Bangladesh Medical University (BMU), Department of Dermatology and Venereology, Dhaka, Bangladesh*
[3]*Apollo Institute of Medical Sciences and Research, Department of DVL, Hyderabad, India*
[4]*Sree Balaji Medical College & Hospital, Department of Pediatrics, Chennai, India*
[5]*RVS College of Arts and Science, Department of Biochemistry, Sulur, Coimbatore, Tamil Nadu, India*
[6]*AIMST University, Faculty of Medicine, Unit of Biochemistry, Semeling, Bedong 08100, Kedah, Malaysia*

Received: 18.04.2026.
Accepted: 27.05.2026.

Corresponding author:

Sridevi Visvanathan

Phone: 7339431139

E-mail: srideviayyanar18@gmail.com

ABSTRACT

Atopic dermatitis (ADerm) is a chronic inflammatory skin disease characterized by immune dysregulation, epidermal barrier dysfunction, and genetic susceptibility. Emerging evidence suggests that ADerm and Alzheimer's disease (ALZ) may share overlapping inflammatory and immunological pathways. Transcriptomic and proteomic analyses in ALZ further support the existence of molecular subtypes, similar to biomarker defined endotypes in ADerm. However, early research gaps remain significant: current biomarkers lack sufficient accuracy to predict therapeutic response across endotypes, and longitudinal validation across diverse populations is limited. ADerm Understanding these shared inflammatory mechanisms may provide insights into potential therapeutic targets, although their applicability to ALZ management remains to be validated. Such cross-disease insights may eventually contribute to improved risk stratification and hypothesis-driven personalized approaches, pending further validation aimed at modifying chronic neuroinflammation. The review narratively highlights immune pathway specific indicators across T helper 2 (Th2), T helper 17 (Th17), and T helper 22 (Th22) axes, along with predictive biomarkers for targeted therapies. Aderm ALZ These observations highlight potential areas for future research, including the exploration of biomarkers that may reflect systemic inflammatory states. At present, their role in screening, risk stratification, or precision-based interventions remains exploratory and requires further validation. This narrative review also examines endotype based biomarkers, paediatric and ethnic variability, and markers of disease progression and therapeutic response. Although epidemiological studies suggest an association between ADerm and ALZ, the underlying molecular and immunological mechanisms remain poorly defined. This review addresses this gap by integrating current evidence on shared inflammatory pathways, systemic immune dysregulation, barrier dysfunction, and emerging biomarkers that may link chronic atopic inflammation with neuroinflammatory processes and neurodegeneration.

Keywords: *Atopic dermatitis, Alzheimer's disease, Biomarkers, Immune endotypes, Precision medicine, Proteogenomics.*

UDK:

Eabr 2026; 27(3): 211-220
DOI: 10.2478/eabr-2026-0028

INTRODUCTION

Atopic dermatitis (ADerm) is a chronic inflammatory skin condition characterized by immune dysregulation and elevated systemic inflammation, involving cytokines like IL-4, IL-13, and TNF-α (1). Chronic inflammation is also a key pathological feature in ALZ, suggesting shared immunological pathways between ALZ and ADerm. Over recent decades, the prevalence of ADerm has risen markedly in industrialized countries, reflecting the influence of environmental and lifestyle factors. Genetic mutations, particularly in the filaggrin (FLG) gene, along with immune abnormalities and structural barrier defects, contribute to impaired keratinocyte differentiation, increased transepidermal water loss (TEWL), and diminished epidermal lipids (2). These alterations result in chronic inflammation, epidermal hyperplasia, and increased susceptibility to microbial infections. The "atopic march" describes the tendency for ADerm to present in early childhood and potentially precede other atopic disorders, including food sensitivities, allergic rhinitis, and asthma (3). The variable clinical course and diverse manifestations of ADerm present ongoing challenges for accurate disease assessment and management. The Scoring Atopic Dermatitis (SCORAD) index, the Eczema Area and Severity Index (EASI), the Investigator's Global Assessment (IGA), and pruritus scales are among the clinical scoring systems that are frequently employed to quantify the severity of the disease (4). It is challenging to quantify the subtle improvements in erythema or lichenification, particularly after short-term interventions, which frequently result in high placebo rates in clinical trials. Genetic factors, particularly FLG mutations, disrupt the skin microbiome and compromise the epidermal barrier, which may facilitate the activation of type 2 immune responses (5). The itch-scratch cycle is also perpetuated by neuroimmune interactions, which exacerbate inflammation. The course of the disease is highly variable; some individuals experience transient episodes, while others develop chronic relapsing forms that are accompanied by additional allergic comorbidities. Ethnic and racial differences influence clinical phenotype, immunologic pathways, and therapeutic response. Recent developments in molecular biology and biotechnology, including genomics, transcriptomics, metabolomics, lipidomics, and proteomics, have significantly improved our comprehension of the complex biology of ADerm. Proteomic and gene-expression analyses have revealed that the T helper 2 (Th2) and T helper 22 (Th22) immune pathways are the most frequently activated, with variable contributions from T helper 1 (Th1) and T helper 17 (Th17) responses (6). Genetic expression alone is insufficient to adequately represent disease activity, as protein function is influenced by post-transcriptional and post-translational modifications (e.g., methylation, acetylation, ubiquitination). Consequently, integrated proteogenomic approaches provide a more thorough comprehension of therapeutic targets and disease mechanisms. ADerm ALZ Chronic systemic inflammation represents a potential shared mechanism between ADerm and ALZ. Persistent Th2 driven cytokines in ADerm and sustained elevations of Interleukin-1 beta (IL-1β) , Interleukin-6 (IL-6), and Tumor Necrosis Factor-alpha (TNF-α) in ALZ collectively promote long-term immune activation, which may influence neuroinflammatory pathways; however, the extent to which these processes contribute to neurodegeneration remains to be fully established (7,8). Th2 cytokines such as Interleukin-4 (IL-4) and Interleukin-13 (IL-13) are central drivers of inflammatory responses in ADerm. In contrast, elevated levels of IL-1β, IL-6, and TNF-α in the brain are strongly associated with microglial activation and neuroinflammatory processes implicated in ALZ progression (9). Both ADerm and ALZ exhibit an imbalance between pro- and anti-inflammatory cytokines, leading to persistent immune activation rather than effective resolution. Elevated mediators such as TNF-α and IL-6 are implicated in chronic inflammatory processes that may contribute to tissue damage and disease progression (10). Thus, chronic systemic inflammation in ADerm ALZ may be associated with alterations in neuroinflammatory pathways implicated in ALZ, although a direct causal relationship has not been established. Systemic inflammation has been proposed to compromise the integrity of the blood-brain barrier, facilitating the entry of neurotoxic substances and inflammatory mediators into the central nervous system. Barrier dysfunction represents a potential mechanistic bridge between ADerm and ALZ. Cytokines such as TNF-α and IL-1β are known to disrupt BBB integrity by altering endothelial signaling and increasing vascular permeability (11). In ADerm, systemic cytokines increase vascular permeability, while in ALZ, blood-brain barrier disruption facilitates immune cell infiltration and amyloid accumulation, suggesting a possible connection between chronic peripheral inflammation and central neuroimmune activation (12). ALZ Such processes are implicated in ALZ pathogenesis, although their direct relationship with ADerm remains speculative. Persistent systemic inflammation has been proposed as a potential contributor to neuroinflammatory processes, including effects on blood-brain barrier integrity. These observations suggest biological plausibility; however, direct mechanistic links between ADerm-related inflammation and neurodegenerative processes remain unconfirmed. ADerm Epidemiological data suggest a modest association between ADerm and increased risk of cognitive decline or dementia. However, such findings reflect correlation rather than causation and may be influenced by confounding factors including comorbidities, systemic inflammation, and treatment exposures. At present, these observations should not be interpreted as evidence of a direct disease trajectory. However, their potential role as early indicators of neurodegeneration has not yet been established and requires further investigation. Despite advances in elucidating the underlying mechanisms, current therapeutic guidelines largely rely on clinical severity scores rather than targeting specific molecular or etiological subtypes. Given this heterogeneity, it is unlikely that a single treatment modality would be universally effective across all patients. Dysregulated innate immune

activation represents a shared inflammatory feature of ADerm and ALZ. In ADerm, dendritic cells and macrophages sustain cutaneous inflammation, while in ALZ, persistent microglial activation has been associated with neuroinflammation, synaptic dysfunction, and neuronal injury in experimental and clinical studies. The identification and validation of biomarkers have become indispensable for the advancement of ALZ research and patient stratification. Genomic, transcriptomic, or proteomic data can be used to identify biomarkers in tissues, blood, secretions, or urine. In the diagnostic assessment of ADerm, clinical criteria that are supported by laboratory tests, such as serum immunoglobulin E (IgE) levels, eosinophil counts, and allergen sensitivity assays, are frequently implemented. The strongest association with disease severity and activity is demonstrated by the Thymus and Activation-Regulated Chemokine (TARC/CCL17). In paediatric populations, elevated serum TARC/CCL17 levels consistently reflect Th2-mediated inflammation and function as predictive indicators of disease severity in ADerm (13). In addition to their potential for disease monitoring, these biomarkers may also contribute to the personalization of treatment approaches and therapeutic decision-making. In general, ADerm is a multifaceted and diverse inflammatory disorder that is influenced by genetic, immunologic, and environmental factors. It is imperative to integrate clinical, genomic, and proteomic data in order to create precise diagnostic tools and personalized therapeutic strategies. To further strengthen the conceptual framework of this review, it is essential to ADerm explore whether neuroinflammation in ALZ may share features with chronic peripheral immune activation observed in ADerm. Exploring these shared molecular signatures could clarify whether long-standing atopic inflammation contributes to blood-brain barrier disruption and microglial activation. Therefore, integrating dermatologic biomarkers with neurodegenerative risk profiling may represent a potential area for future translational research in early detection, stratified prevention, and immunomodulatory intervention across both conditions. Despite increasing evidence linking chronic inflammatory disorders with neurodegeneration, the potential relationship between ADerm and ALZ remains insufficiently explored. Existing studies are fragmented and largely epidemiological, with limited integration of immunological, genomic, and biomarker data. This review addresses these gaps by synthesizing current evidence on shared inflammatory pathways, barrier dysfunction, and potential translational biomarkers connecting ADerm and ALZ. This narrative review aims to explore the potential immunological and inflammatory links between ADerm and ALZ. It focuses on shared cytokine pathways, systemic inflammation, and barrier dysfunction, while highlighting emerging biomarkers and molecular mechanisms that may connect chronic atopic inflammation with neuroinflammatory processes and neurodegeneration. Although epidemiological and mechanistic associations are emerging, current evidence does not establish a direct causal relationship between ADerm and ALZ.

SEARCH STRATEGY

A comprehensive narrative literature review was conducted to explore the emerging evidence linking ADerm and ALZ through shared inflammatory, immunological, and biomarker pathways. Relevant studies were identified through systematic searches of electronic databases, including PubMed/MEDLINE, Scopus, Web of Science, and Google Scholar. The search covered publications from January 2000 to January 2026, capturing key advances in immunology, neuroinflammation, and proteomic biomarker research. Search terms were developed using combinations of Medical Subject Headings (MeSH) and free-text keywords, including “atopic dermatitis,” “Alzheimer’s disease,” “neuroinflammation,” “systemic inflammation,” “cytokines,” “immune dysregulation,” “biomarkers,” “Th2 immune response,” “Th17 pathway,” “proteomics,” “OLINK,” “SOMAscan,” and “precision medicine.” Boolean operators (AND, OR) were applied to refine search results. Artificial intelligence (AI)-assisted tools were utilized to support keyword optimization, literature organization, and refinement of the search strategy. However, all articles were independently screened, critically appraised, and selected by the authors to ensure scientific accuracy and relevance. Eligible studies included original research, clinical cohort studies, translational investigations, and high-quality reviews focusing on inflammatory mediators, immune pathways, and biomarker signatures related to ADerm or ALZ. Studies unrelated to the topic, conference abstracts without full data, and non-English publications were excluded. Reference lists of key articles were manually screened to ensure comprehensive coverage. The selected literature was synthesized to highlight shared immunological mechanisms, overlapping biomarker profiles, and their potential implications for biomarker-driven risk stratification and precision medicine approaches.

ENDOTYPE-BASED BIOMARKERS OVERLAPPING ADerm AND ALZ

Emerging evidence suggests that the immunologic heterogeneity observed in ADerm may share mechanistic parallels with systemic neuroinflammatory processes implicated in ALZ, including dysregulated chemokine signaling and cytokine networks, suggesting potential mechanistic overlap between ADerm and ALZ ALZ (14,15). Therapeutic response has also been linked to biomarker expression where elevated cutaneous IL-22 expression predicts responsiveness to anti-IL-22 therapy (fezakinumab), whereas higher serum levels of IL-13 related markers such as dipeptidyl peptidase-4 (DPP4) and periostin are associated with improved outcomes following tralokinumab treatment (16). Dyjack et al., identified a type 2-high inflammatory signature in ADerm, indicating potential responsiveness to targeted cytokine therapies, where IL-4/IL-13-directed biologics such as dupilumab and tralokinumab have demonstrated substantial clinical efficacy in modulating this pathway (17). Pediatric patients exhibited increased expression of innate cytokines, including TH17-, TH9/IL-9-, and IL-33-related pathways, whereas older adults display increased inflammatory activity with relatively

attenuated TH2/TH22 polarization (18). Chronic T helper 2 (TH2)/T helper 2 (TH17) skewing, dysregulation of regulatory T-cell function, and persistent cytokine elevation contribute to systemic inflammatory priming, potentially influencing neuroimmune interactions (19). In ALZ, neuroinflammation is a central pathological feature, characterized by microglial activation, elevated pro-inflammatory cytokines, and dysregulated immune signaling pathways. Biomarkers such as IL-6, IL-1β, TNF-α, and altered chemokine profiles overlap with inflammatory pathways that were identified in ADerm endotypes (20). Proteomic approaches used to define ADerm subtypes parallel biomarker-driven stratification strategies in ALZ, including the assessment of amyloid-β, phosphorylated tau, and inflammatory mediators in cerebrospinal fluid and plasma (21). ALZ is characterized by the progressive accumulation of amyloid-β (Aβ) peptides, which deposit as extracellular plaques, and the abnormal hyperphosphorylation of tau protein, leading to the formation of intracellular neurofibrillary tangles. Hyperphosphorylated tau loses its capacity to stabilize neuronal microtubules, resulting in cytoskeletal disruption, impaired axonal transport, and progressive neuronal dysfunction. (22). These shared cytokine signatures indicate overlapping immune pathways between peripheral and central inflammatory processes. However, such overlap reflects biological similarity rather than a direct causal relationship.(23). In ALZ, similar cytokine-driven endotypes have been proposed, where distinct inflammatory profiles correlate with disease progression and cognitive decline, further supporting the concept of biomarker-defined subtypes across both conditions. In ADerm, elevated levels of cytokines including IL-4, IL-13, IL-22, and chemokines such as CCL17 (TARC) reflect a dominant Th2/Th17-skewed immune profile. These cytokines are not confined to local tissue inflammation, but can enter systemic circulation, contributing to a chronic low-grade inflammatory state. This systemic immune activation may influence the brain through several mechanisms, such as disruption of the (BBB), allowing peripheral cytokines and immune cells to access the CNS, Activation of microglia and astrocytes, leading to sustained neuroinflammatory responses. Th17-related cytokines like IL-22 and Th2 mediators such as IL-4 and IL-13 can modulate both peripheral and central immune responses, suggesting that peripheral inflammatory biomarkers act as early indicators of systemic immune dysregulation (24). This dysregulated immune environment ALZ may be associated with increased vulnerability to neuroinflammatory changes, although evidence for progression to ALZ is limited. Therefore, integrated biomarker panels combining dermatological and neuroinflammatory indicators may inform future approaches to risk stratification and precision diagnostics, pending validation.

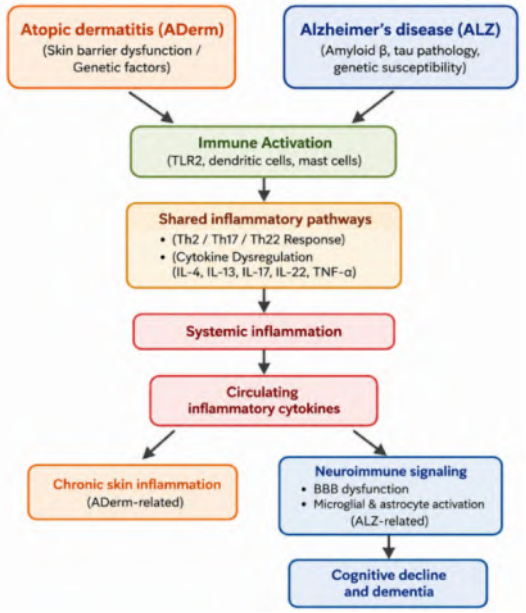
Early life Determinants Linking ADerm and ALZ Pathogenesis

Prenatal and early postnatal exposure to air pollutants, including carbon monoxide (CO), nitrogen dioxide (NO_2), nitric oxide (NO), and ozone (O_3), has been associated with an increased risk of ADerm, asthma, and other allergic conditions in children which induces oxidative stress and systemic inflammatory responses, activating cytokine pathways such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α), and contribute to immune dysregulation in ADerm (25). These induced systemic cytokine elevation, oxidative stress, and immune dysregulation, can disrupt blood-brain barrier integrity and activate microglia (26).These processes have been associated with amyloid-β accumulation and neuroinflammation, ALZ suggesting potential overlap between early-life exposures, atopic immune responses, and pathways implicated in ALZ . Epidemiological and experimental studies suggest that early-life systemic inflammation and environmental exposures may increase long-term susceptibility to neurodegenerative diseases, including ALZ, by priming microglial responses and altering neuroimmune regulation. Maternal allergic predisposition further modulates neonatal immune programming, as evidenced by elevated IL-4^+ $CD4^+$ T-cell levels and a reduced regulatory T cell (Treg)/T helper 2 (Th2) ratio in umbilical cord blood (27). This early skewing toward a Th2-dominant immune response promotes systemic inflammatio and increases susceptibility to ADerm. Skin barrier dysfunction in early life, reflected by increased transepidermal water loss (TEWL), may contribute to systemic immune activation through enhanced allergen penetration and microbial dysbiosis (28). This can promote chronic cytokine release and immune priming, potentially influencing neuroinflammatory pathways, microglial activation, and ALZ which may have implications for long-term neuroinflammatory processes, though direct links to ALZ remain unproven Genetic susceptibility further contributes to disease risk, particularly loss-of-function mutations in the FLG gene, which impair epidermal barrier function. The FLG P478S GG variant has been associated with an increased risk of ADerm (29). These Filaggrin (FLG) promote chronic systemic inflammation with elevated cytokines which can disrupt the BBB and activate microglia, leading to neuroinflammation (30). ALZSuch immune dysregulation may potentially impair amyloid-β clearance, which has been implicated in ALZ progression. Chronic systemic inflammation, cytokine imbalance, impaired skin barrier integrity, and reduced regulatory T-cell activity observed in ADerm may also contribute to long-term neuroinflammatory processes associated with ALZ (31). The convergence of Th2/Th17 immune polarization, lipid metabolic dysregulation, and immune regulatory defects suggests the presence of overlapping biomarker pathways linking ADerm and ALZ (32). From a clinical perspective, integrating peripheral inflammatory markers, genetic susceptibility variants, and barrier-function indices may provide a framework for future investigation into early identification of individuals at potential risk for both ADerm progression and later neuroinflammatory vulnerability. Furthermore, biomarker-driven therapeutic strategies targeting shared inflammatory pathways may not only improve precision management of ADerm but also may have indirect effects on systemic inflammation, which could be relevant to neuroinflammatory processes and warrant further investigation.

BIOMARKERS FOR PROGNOSIS AND SCREENING OF ADerm

The most definitive predisposing factor for ADerm is a positive family history of ADerm. The identification of reliable prognostic and screening biomarkers could facilitate early detection and timely intervention among individuals at elevated risk. Despite mutations in the FLG gene are strongly linked to severe and early-onset forms of ADerm that persist into maturity, not all individuals who are affected carry these mutations, and not all carriers develop the disease. FLG mutations may be utilized as valuable biomarkers for the prediction and screening of individuals who are predisposed to severe or early-manifesting ADerm. Food allergies that develop during the ADerm are associated with increased immunoglobulin E (IgE) levels and reduced FLG expression in the context of ADerm-related comorbidities (33). Emerging evidence indicates that early-onset ADerm are associated with several biomarkers, including elevated TEWL, diminished levels of natural moisturizing factor (NMF), increased umbilical cord serum IgE, and increased epidermal expression of thymic stromal lymphopoietin (TSLP) (34). ATP screening strategies include these TEWL measurement, NMF assessment, cord blood IgE levels, and biomarker profiling to enable early identification, risk stratification, and timely preventive or therapeutic interventions. Persistent ADerm during infancy has been correlated with reduced serum vascular endothelial growth factor (VEGF) levels, while upregulation of indoleamine 2,3-dioxygenase-1 (IDO1) has been proposed as a potential predictive indicator (Figure 1). These systemic biomarkers may extend beyond dermatological relevance, as chronic peripheral inflammation and immune dysregulation have been implicated in neuroinflammatory processes associated with ALZ. In particular, inflammatory mediators and vascular factors such as VEGF are known to influence blood-brain barrier integrity and microglial activation, which are key features in ALZ pathogenesis.

Figure 1. Shared Biomarker Pathways for ADerm and ALZ



BIOMARKERS FOR THE SEVERITY OF ADerm AND ALZ

The 16934A>T polymorphism in the Toll-like receptor 2 (TLR2) locus is a potential indicator of ADerm severity, due to its association with the SCORAD index and its impact on transcriptional regulation (35). Serum eosinophil-derived neurotoxin (EDN) has emerged as a predictive biomarker for recurrence and disease intensity in severe, treatment-resistant ADerm. Similarly, serum squamous cell carcinoma antigen 2 (SCCA2) levels are significantly elevated in ADerm compared to healthy individuals and psoriasis patients, indicating its diagnostic value (36). These peripheral inflammatory biomarkers may may have broader implications beyond dermatological disease, although this remains under future investigation, as systemic immune activation and eosinophil-associated mediators have been implicated in neuroinflammatory pathways contributing to neurodegenerative ALZ, where chronic inflammation and immune dysregulation play a central role in disease progression (37). Similar inflammatory mediators, including TNF-α, IL-6, and eosinophil-associated proteins, have been implicated in ALZ, where they contribute to chronic neuroinflammation, synaptic dysfunction, and neuronal injury. CTACK (cutaneous T-cell-attracting chemokine) showed a strong association with pruritus severity, while CCL17 (TARC), CCL22, and CCL27 correlate positively with SCORAD scores (38). ADerm patients exhibited elevated levels of antimicrobial peptides such as human neutrophil defensins and dermcidin, along with Th2-associated chemokines (39). These antimicrobial peptides also modulate amyloid aggregation, neuroinflammation, and innate immune responses, ALZsuggesting possible mechanistic parallels between chronic cutaneous inflammation and neurodegenerative processes (40). Although these biomarkers are primarily associated with ADerm, overlapping pathways such as innate immune activation, cytokine dysregulation, and chemokine signaling contribute to neuroinflammatory ALZ mechanisms.

BIOMARKERS ASSOCIATED WITH SYSTEMIC INFLAMMATION IN ADerm AND ALZ

The systemic nature of ADerm was demonstrated by proteomic analyses of blood, particularly in adults with moderate-to-severe disease, which reveal elevated levels of proinflammatory cytokines such as Interleukin-36 gamma (IL-36γ), Interleukin-1 family member 9 (IL-1F9) (41). Pavel et al. utilized OLINK proteomic technology to analyze serum samples and skin biopsies from individuals with moderate-to-severe ADerm (42). Their results demonstrated a substantial increase in the expression of inflammatory markers in nonlesional epidermis. Such circulating inflammatory mediators, including cytokine-driven pathways and vascular inflammatory markers, ALZhave also been implicated in neuroinflammatory processes observed in ALZ, where chronic peripheral inflammation contribute to microglial activation and neurodegeneration. These findings suggest partially overlapping systemic inflammatory and immune signaling

pathways between ALZ and ADerm (43). The integrated OLINK-based proteomic profiling revealed that patients with mild or limited ADerm primarily exhibited localized Th2/Th22 pathway activation (e.g., IL-13, CCL-17, IL-22) that are restricted to lesional skin. This activation is characterized by minimal systemic inflammation and comparatively lower alterations in atherosclerosis or cardiovascular associated proteins. Inflammatory signaling pathways characterized by those cytokines, such as IL-6, IL-1β, and TNF-α, are increasingly recognized as central mediators of ALZ neuroinflammation. Persistent immune activation promotes microglial activation, endothelial dysfunction, and disruption of the neurovascular unit, contributing to blood-brain barrier impairment and progressive neuronal injury. This IL-16 and IL-36γ indicate systemic immune activation and oxidative stress and are also recognized contributors to neuroinflammation, endothelial injury, and neurovascular impairment in ALZ. This may inform future risk stratification approaches and guide the development of targeted therapies, that modulate cytokine signalling, vascular inflammation, and oxidative stress, potentially benefiting both dermatologic and neurodegenerative disease management.

ETHNIC VARIABILITY IN BIOMARKERS ACROSS ADerm AND ALZ

The immunological phenotypes and IgE sensitization patterns of ADerm patients across diverse ethnic populations have been investigated using proteomic approaches, taking into account substantial geographical and genetic heterogeneity (44). In sub-Saharan African cohorts, particularly from Tanzania, individuals with ADerm exhibited elevated serum levels of pro-inflammatory Th1/Th17 cytokines and chemokines, along with reduced expression of molecules involved in apoptotic regulation and cellular growth signaling (45). Mikhaylov et al., analyzed serum samples from Asian patients with ADerm using Meso-Scale Discovery Electrochemiluminescence (MSD-ECL) and Singulex Erenna immunoassays, comparing the findings with previously reported data from European American ADerm cohorts (46). Their results demonstrated a significant upregulation of Th2 associated immune markers, including IL-13 and CCL26, as well as Th22 associated cytokines such as IL-22. Strong correlations were observed between serum and nonlesional skin levels of Th2 and Th22 mediators, indicating systemic immune activation beyond lesional sites and aligning with prior observations in European American populations. Elevated Th2 associated cytokines and chemokines in ADerm reflects systemic immune activation. Hulshof et al., conducted a proteomic comparison of pediatric ADerm patients with different skin phototypes (Fitzpatrick type II versus type VI), reporting lower levels of NMF in type II skin (47). Elevated cytokines, including IL-1β, IL-18, C-X-C motif chemokine ligand 8 (CXCL8), C-C motif chemokine ligand 17 (CCL17), C-C motif chemokine ligand 22(CCL22), and C-C motif chemokine ligand 2(CCL2) were identified in lesional ADerm skin, reflecting activation of inflammatory pathways that may overlap with those implicated in ALZ neuroinflammatory processes (48). However, this study did not directly investigate associations with ALZ, and any proposed link remains hypothetical, based on shared immune and inflammatory mechanisms rather than direct clinical evidence. Ethnic diversity plays a critical role in shaping disease prevalence, immune signatures, and clinical manifestations of ADerm. African American patients with ADerm often exhibit greater disease severity, altered epidermal lipid composition, increased TEWL, and a lower prevalence of FLG mutations compared with Caucasian populations (49). Variations in the epidemiology and progression of ALZ have also been reported among different ethnic groups, including Asian and Hispanic populations (50). Studies have identified associations between ALZ, central adiposity, and systolic hypertension in pediatric cohorts of Asian and Hispanic ancestry (51). Collectively, these findings stress the importance of considering population-specific genetic, immunological, and metabolic factors when interpreting systemic biomarker profiles across diseases. These observations provide a theoretical basis for exploring possible neuroimmune interactions, although direct evidence linking these variations to ALZ remains limited. Integrating molecular, cellular, and biomarker-based analyses across diverse populationsmay be important for identifying disease subtypes and could contribute to improving early diagnosis, and advancing precision medicine strategies targeting both dermatological and neurodegenerative conditions. Ethnic variability has also been observed in ALZ, with differences in disease prevalence, progression, and biomarker expression across populations, suggesting that population-specific immune and genetic factors may influence both dermatological and neurodegenerative disease pathways.

EPIDEMIOLOGICAL ASSOCIATION, MECHANISTIC OVERLAP, AND CAUSAL INFERENCE

The relationship between Atopic Dermatitis and Alzheimer's Disease has been explored across epidemiological, immunological, and mechanistic domains. However, these lines of evidence represent distinct levels of inference and should not be interpreted interchangeably. Population-based and observational studies suggest that individuals with atopic dermatitis may exhibit a modestly increased risk of cognitive decline or dementia. These findings support an association, but they do not establish causality. Potential confounders—including age, comorbid allergic conditions, systemic inflammation, medication exposure, and lifestyle factors—may contribute to the observed relationships. Therefore, epidemiological evidence should be interpreted as indicative of correlation rather than a direct disease trajectory. Both conditions exhibit alterations in immune signaling, including dysregulation of cytokines such as interleukin (IL)-1β, IL-6, IL-4, IL-13, and tumor necrosis factor-α (TNF-α). These mediators have been implicated in systemic inflammation and may influence central nervous system processes. For instance, peripheral inflammatory signals have been hypothesized to affect the blood-brain barrier and modulate the activity of microglia. Such findings suggest mechanistic overlap and biological plausibility; however, they remain largely

derived from experimental models or indirect clinical observations. Several conceptual frameworks propose that chronic systemic inflammation associated with atopic dermatitis may contribute to neuroinflammatory processes relevant to ALZ. These models describe a potential sequence involving peripheral immune activation, altered blood-brain barrier integrity, microglial activation, and downstream neuronal dysfunction. Importantly, these pathways should be regarded as hypothesis-generating constructs, rather than confirmed biological cascades. Current evidence does not demonstrate that atopic dermatitis directly initiates or drives these processes in humans. At present, there is no definitive causal or interventional evidence demonstrating that atopic dermatitis leads to ALZ or that treatment of atopic dermatitis modifies the risk or progression of neurodegeneration. Establishing causality would require longitudinal studies with robust control of confounding variables, as well as interventional trials demonstrating that modulation of atopic inflammation alters neurological outcomes. Such evidence remains limited.

SOMAscan AND OLINK FOR BIOMARKER DETECTION IN ADerm AND ALZ

Comprehensive proteomic investigations have significantly advanced the understanding of the molecular mechanisms underlying both ADerm and ALZ. High-throughput platforms such as OLINK proximity extension assays and SOMA scan have enabled the large-scale identification of dysregulated immune mediators, barrier-associated proteins, and neuroinflammatory markers involved in these disorders. In ADerm, proteomic profiling has revealed alterations in Th2 associated cytokines, chemokines, and barrier-related proteins, reflecting systemic immune activation beyond skin (52). Similarly, proteomic analyses in ALZ have identified inflammatory mediators, complement proteins, oxidative stress markers, and vascular-related molecules that contribute to neurodegeneration and neuroinflammation. These findings highlight the role of systemic immune dysregulation and chronic inflammatory signaling as shared mechanistic features linking dermatological inflammation with neurodegenerative pathology. Among the available technologies, mass spectrometry-based proteomics detection typically involves peptide separation through liquid chromatography or two-dimensional gel electrophoresis, followed by ionization using electrospray or MALDI and analysis through instruments such as time-of-flight or quadrupole-Orbitrap analyzers. Mass spectrometry provides unbiased and sequence-specific protein characterization, allowing researchers to detect new inflammatory mediators involved in both skin and neural tissue pathology. However, the technique requires specialized expertise, and can be affected by interference from highly abundant proteins such as albumin or immunoglobulins, and often demonstrates limited sensitivity for low-abundance proteins unless enrichment procedures are applied. Targeted high-sensitivity platforms complement discovery-based proteomics. The OLINK proximity extension assay employs pairs of DNA-labeled antibodies that hybridize upon binding to a specific protein, enabling highly sensitive quantification through quantitative PCR. This approach requires only minimal sample volumes, typically about 10 μL of serum or less amounts of tissue and are particularly effective for detecting low-abundance inflammatory mediators implicated in immune dysregulation. Similarly, SOMAscan, an aptamer-based proteomic platform, utilizes SOMAmer reagents that bind selectively to target proteins and convert protein signals into measurable nucleotide signals detected via microarray fluorescence. This system supports extensive multiplexing and detect proteins at extremely low concentrations, making it valuable for identifying circulating biomarkers associated with systemic inflammation, vascular dysfunction, and neurodegenerative pathways. Ultrasensitive immunoassay technologies further expand biomarker detection capabilities. The Erenna immunoassay quantifies extremely low-abundance proteins through single-molecule counting within bead-based sandwich complexes analyzed by laser confocal microscopy, enabling detection at sub-picogram levels from blood samples. An oligomer-specific ELISA performed on the Erenna platform detected soluble amyloid-β oligomers in CSF samples, providing a sensitive approach for studying early Alzheimer's pathology (53). Erenna technology enables the quantification of low-abundance inflammatory mediators implicated in ADerm, such as IL-4, IL-13, IL-31, CCL17 (TARC), and CCL22, which may support the identification of systemic immune signatures associated with disease severity (54). Luminex bead-based multiplex assays allow simultaneous quantification of numerous cytokines, chemokines, and inflammatory mediators using dual fluorescent detection through flow cytometry-based instrumentation. Luminex panels for ADerm have been used to quantify Th2-associated cytokines and chemokines such as IL-4, IL-13, CCL17 (TARC), CCL22 (MDC), and IL-31, which correlate with disease severity and pruritus intensity (55). Similarly, multiplex Luminex assays have been applied in ALZ research to measure inflammatory mediators, including IL-6, TNF-α, IL-1β, MCP-1 (CCL2), and vascular factors such as VEGF (56). The ability of Luminex technology to profile multiple immune mediators simultaneously makes it valuable for identifying shared inflammatory signatures and potential diagnostic biomarkers linking systemic inflammatory disorders and neurodegenerative diseases. Collectively, the integration of these advanced proteomic technologies has enabled the systematic identification of inflammatory and immune-regulatory biomarkers that may bridge peripheral inflammatory diseases such as ADerm with neuroinflammatory mechanisms of ALZ. Such multi-omics approaches are crucial for discovering shared molecular pathways, improving biomarker-based diagnostics, and guiding the development of targeted therapeutic strategies aimed at modulating systemic immune dysregulation and chronic inflammation.

NOVEL TARGETED THERAPEUTICS

The development of novel targeted therapeutics in ADerm increasingly relies on biomarker-guided precision medicine approaches that identify patient subgroups most likely to benefit from specific immunomodulatory interventions. Targeted inhibition of key cytokine pathways has

shown promising clinical outcomes. Therapeutic responses to the anti-IL-13 monoclonal antibody Tralokinumab have been associated with elevated baseline serum levels of periostin and dipeptidyl peptidase-4 (DPP-4) (57), indicating their potential utility as predictive biomarkers of treatment response. Similarly, enhanced clinical efficacy of anti-IL-22 monoclonal antibody Fezakinumab has been observed in patients with higher baseline tissue expression of IL-22, suggesting that cytokine-driven molecular endotypes can guide therapeutic selection (58). Macrophage-derived chemokine (MDC/CCL22) has been proposed as a dynamic indicator of disease activity and treatment response across multiple therapeutic strategies (59). Furthermore, nitric oxide synthase-2 (NOS2) and CTACK/CCL27 have emerged as diagnostic biomarkers that aid in distinguishing ADerm from psoriasis in patients presenting with psoriasiform dermatitis, potentially improving diagnostic accuracy and therapeutic targeting. Many of these immune mediators are also implicated in systemic inflammatory and neuroimmune processes associated with ALZ. NOS2-mediated oxidative stress and periostin-driven inflammatory signaling have been linked to neuronal injury, microglial activation, and amyloid-associated pathology in neurodegenerative conditions (60). These overlapping biomarker pathways highlight the potential for targeted cytokine-modulating therapies in ADerm may improve dermatological outcomes, with potential indirect effects on systemic inflammation that warrant further investigation in neuroinflammatory contexts. Targeting inflammatory cytokine pathways is also an emerging therapeutic strategy in ALZ, where modulation of TNF-α, IL-1β, and IL-6 signaling is being explored to reduce neuroinflammation and slow disease progression.

CONCLUSION

The identification and validation of robust biomarkers have revolutionized the conceptualization and management of ADerm, transforming it from a symptom-based to a mechanism-driven disease model. Biomarker research has delineated distinct molecular endotypes offering potential insight into disease progression, remission, and treatment responsiveness. Integrative multi-omics approaches have provided a comprehensive understanding of epidermal barrier dysfunction, systemic inflammation, and immune dysregulation, supporting the development of approaches aimed at earlier diagnosis and targeted intervention. Proteomic technologies such as OLINK and SOMAscan have enhanced sensitivity in detecting low-abundance biomarkers, highlighting their potential clinical applicability, pending further validation. The identification of TARC/CCL17, periostin, IL-22, and DPP-4 as reliable biomarkers stresses the potential for precision-guided therapeutics. Understanding overlapping pathways may contribute to future risk prediction models, pending validation and the development of targeted therapeutic strategies addressing both conditions. Furthermore, consideration of ethnic, genetic, and environmental diversity remains essential in developing equitable treatment frameworks. A biomarker-oriented clinical paradigm wouldmay facilitate the transition toward more personalized and predictive dermatologic care in the future, reducing disease burden and improving patient quality of life through biologically informed precision medicine. While shared inflammatory pathways offer a biologically plausible link, prospective longitudinal and translational studies are needed before clinical extrapolation can be justified. Overall, the current body of evidence primarily supports biological plausibility and epidemiological associations between ADerm and ALZ. However, these findings do not establish causality or support clinically actionable prediction. Future longitudinal, mechanistic, and interventional studies are required to determine whether these associations translate into meaningful clinical applications.

ACKNOWLEDGMENTS

None.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to report.

ARTIFICIAL INTELLIGENCE UTILIZATION FOR ARTICLE WRITING

The study's conduct and manuscript preparation were conducted in accordance with ethical principles and guidelines for the use of artificial intelligence.

REFERENCES

1. Zhang Z, Chang C, Xiao L, Su H, Lyu Y, Zhao J, et al. The neuroimmune axis in atopic dermatitis: from pathogenic mechanisms to targeted neuroimmunotherapy. *J Inflamm Res.* 2025;18079-18113.
2. Flohr C, England K, Radulovic S, McLean WH, Campbell LE, Barker J, et al. Filaggrin loss-of-function mutations are associated with early-onset eczema and severity. *Br J Dermatol.* 2010;163(6):1333-1336.
3. Dierick BJ, van der Molen T, Flokstra-de Blok BM, Muraro A, Postma MJ, Kocks JW, et al. Burden and socioeconomics of allergic diseases. *Expert Rev Pharmacoecon Outcomes Res.* 2020;20(5):437-453.
4. Chopra R, Vakharia PP, Sacotte R, Patel N, Immaneni S, White T, et al. Severity strata for atopic dermatitis indices. *Br J Dermatol.* 2017;177(5):1316-1321.
5. Olah P, Szlavicz E, Kuchner M, Nemmer J, Zeeuwen P, Lefevre-Utile A, et al. FLG mutations in host-microbe interactions. *J Dermatol Sci.* 2022;106(3):132-140.
6. Hung CY, Gonzalez A, Wüthrich M, Klein BS, Cole GT. Vaccine immunity and T helper pathways. *Infect Immun.* 2011;79(11):4511-4522.
7. Zhou Q, Yang D, Xiong C, Li X. Atopic dermatitis and cognitive dysfunction: meta-analysis. *PLoS One.* 2023;18(10):e0292987.
8. Nakajima S, Tie D, Nomura T, Kabashima K. Cytokine-based pathogenesis of atopic dermatitis. *Cytokine.* 2021;148:155664. doi:10.1016/j.cyto.2021.155664.

9. Chen Z, Balachandran YL, Chong WP, Chan KWY. Roles of cytokines in Alzheimer's disease. *Int J Mol Sci.* 2024;25(11):5803. doi:10.3390/ijms25115803.
10. Ng A, Tam WW, Zhang MW, Ho CS, Husain SF, McIntyre RS, et al. Cytokines in depression and Alzheimer's disease. *Sci Rep.* 2018;8:12050. doi:10.1038/s41598-018-30487-6.
11. Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, et al. Neuroinflammation in Alzheimer's disease. *Lancet Neurol.* 2015;14(4):388-405. doi:10.1016/S1474-4422(15)70016-5.
12. Yang J, Ran M, Li H, Lin Y, Ma K, Yang Y, et al. Inflammatory cytokines and blood-brain barrier in neurodegeneration. *Front Mol Neurosci.* 2022;15:1013933. doi:10.3389/fnmol.2022.1013933.
13. Nagao M, Fujisawa T, Kato Y, Noma Y, Katsumata H, Nishimori H, et al. Serum TARC/CCL17 levels in atopic dermatitis. *J Allergy Clin Immunol.* 2005;115(2):S272. doi:10.1016/j.jaci.2004.12.1097.
14. Heppner FL, Ransohoff RM, Becher B. Role of inflammation in Alzheimer disease. *Nat Rev Neurosci.* 2015;16(6):358-372. doi:10.1038/nrn3880.
15. Interleukin-17 plays a crucial role in atopic dermatitis-associated BBB disruption. *Brain Behav Immun.* 2026;106564. doi:10.1016/j.bbi.2026.106564.
16. Pavel A, Brunner PM, Khattri S, Malik K, Fuentes-Duculan J, Garcet S, et al. IL-22 expression and therapeutic response. *J Invest Dermatol.* 2018;138(5):S74.
17. Dyjack N, Goleva E, Rios C, Kim BE, Bin L, Taylor P, et al. Tape strip RNA sequencing in atopic dermatitis. *J Allergy Clin Immunol.* 2018;141(4):1298-1309. doi:10.1016/j.jaci.2017.10.046.
18. Brunner PM, Israel A, Zhang N, Leonard A, Wen HC, Huynh T, et al. Pediatric atopic dermatitis inflammation. *J Allergy Clin Immunol.* 2018;141(6):2094-2106. doi:10.1016/j.jaci.2018.02.040.
19. Shi Y, Wei B, Li L, Wang B, Sun M. Th17 cells in neurological disorders. *Front Immunol.* 2022;13:932152. doi:10.3389/fimmu.2022.932152.
20. Nedoszytko B, Sokołowska-Wojdyło M, Ruckemann-Dziurdzińska K, Roszkiewicz J, Nowicki RJ. Cytokine network in inflammatory skin diseases. *Postepy Dermatol Alergol.* 2014;31(2):84-91. doi:10.5114/pdia.2014.40920
21. Kang JH, Korecka M, Lee EB, Cousins KA, Tropea TF, Chen-Plotkin AA, et al. Alzheimer biomarkers from CSF to plasma. *Clin Chem.* 2023;69(11):1247-1259. doi:10.1093/clinchem/hvad139.
22. Wang Y, Mandelkow E. Tau in physiology and pathology. *Nat Rev Neurosci.* 2016;17(1):22-35. doi:10.1038/nrn.2015.1.
23. Brunner PM, Guttman-Yassky E, Leung DY. Immunology of atopic dermatitis. *J Allergy Clin Immunol.* 2017;139(4):S65-S76. doi:10.1016/j.jaci.2017.01.011
24. Guttman-Yassky E, Krueger JG. Atopic dermatitis and psoriasis spectrum. *Curr Opin Immunol.* 2017;48:68-73. doi:10.1016/j.coi.2017.08.008.
25. Fadadu RP, Abuabara K, Balmes JR, Hanifin JM, Wei ML. Air pollution and atopic dermatitis. *Int J Environ Res Public Health.* 2023;20(3):2526. doi:10.3390/ijerph20032526.
26. Costa LG, Cole TB, Dao K, Chang YC, Coburn J, Garrick J. Neurotoxicity of air pollution. *Adv Neurotoxicol.* 2019;3:195-221.
27. Schaub B, Liu J, Höppler S, et al. Maternal farm exposure and neonatal immunity. *J Allergy Clin Immunol.* 2009;123(4):774-782.
28. Kelleher MM, Galvin AD, Murray D, Irvine AD, Hourihane JO, et al. TEWL predicts atopic dermatitis. *J Allergy Clin Immunol.* 2013;131(2):AB102.
29. Kono M, Nomura T, Ohguchi Y, et al. FLG mutations in atopic dermatitis. *J Invest Dermatol.* 2014;134(4):939-943.
30. Block ML, Calderón-Garcidueñas L. Air pollution and neuroinflammation. *Trends Neurosci.* 2009;32(9):506-516. doi:10.1016/j.tins.2009.05.009.
31. Holmes C, Cunningham C, Zotova E, Woolford J, Dean C, Kerr SU, et al. Systemic inflammation and Alzheimer progression. *Neurology.* 2009;73(10):768-774. doi:10.1212/WNL.0b013e3181b6bb95.
32. Meesters LD, Roubroeks JAY, Gerritsen A, et al. TH2 and TH17 cytokines in atopic dermatitis. *J Allergy Clin Immunol.* 2025;156(3):690-704. doi:10.1016/j.jaci.2025.05.007.
33. Wang IJ, Lin TJ, Kuo CF, Lin SL, Lee YL, Chen PC. Filaggrin polymorphism and atopic phenotypes. *Br J Dermatol.* 2011;164:791-796.
34. Ziegler SF. TSLP and atopic march. *Ann Allergy Asthma Immunol.* 2021;127(3):306-311. doi:10.1016/j.anai.2021.06.004.
35. Ahmad-Nejad P, Mrabet-Dahbi S, Breuer K, et al. TLR2 polymorphism in atopic dermatitis. *J Allergy Clin Immunol.* 2004;113(3):565-567.
36. Schmid B, Künstner A, Fähnrich A, Bersuch E, Schmid-Grendelmeier P, Busch H, et al. Skin microbiota dysbiosis in atopic dermatitis. *J Eur Acad Dermatol Venereol.* 2022;36(10):1811-1819.
37. Kim HS, Kim JH, Seo YM, Chun YH, Yoon JS, Kim HH, et al. Eosinophil-derived neurotoxin as biomarker. *Ann Allergy Asthma Immunol.* 2017;119(5):441-445.
38. Fujisawa T, Nagao M, Hiraguchi Y, Katsumata H, Nishimori H, Iguchi K, et al. CCL17 levels in atopic dermatitis. *Pediatr Allergy Immunol.* 2009;20(7):633-641.
39. Harder J, Schröder JM. Antimicrobial peptides in skin. *Chem Immunol Allergy.* 2005;86:22-41.
40. Williams WM, Torres S, Siedlak SL, et al. β-defensin-1 in Alzheimer's brain. *J Neuroinflammation.* 2013;10:127.
41. D'Erme AM, Wilsmann-Theis D, Wagenpfeil J, Hölzel M, Ferring-Schmitt S, Sternberg S, et al. IL-36γ as psoriasis biomarker. *J Invest Dermatol.* 2015;135:1025-1032.
42. Pavel AB, Zhou L, Diaz A, et al. Proteomic skin profile in atopic dermatitis. *J Am Acad Dermatol.* 2019;82(3):690-699.
43. Grech VS, Lotsaris K, Kefala V, Rallis E. Genetic mediators linking skin and brain diseases. *Genes.* 2025;16(12):1463.

44. Kaufman BP, Guttman-Yassky E, Alexis AF. Atopic dermatitis in diverse populations. *Exp Dermatol.* 2018;27(4):340-357.
45. Lang CC, Renert-Yuval Y, Del Duca E, Pavel AB, Wu J, Zhang N, et al. Atopic dermatitis in Tanzanian patients. *Ann Allergy Asthma Immunol.* 2021;127(3):334-341.
46. Mikhaylov D, Del Duca E, Guttman-Yassky E. Proteomic signatures in skin diseases. *Expert Rev Proteomics.* 2021;18(5):345-361.
47. Hulshof L, De Wijs LEM, Bosma AL, et al. Biomarker profiles in children with atopic dermatitis. *J Allergy Clin Immunol.* 2021;147(6):2240-2249.
48. Nograles KE, Zaba LC, Guttman-Yassky E, et al. IL-17 and IL-22 pathways. *J Biol Chem.* 2008;283(29):20307-20317.
49. Polcari I, Becker L, Stein SL, Smith MS, Paller AS. Filaggrin mutations in African Americans. *Pediatr Dermatol.* 2014;31(4):489-492.
50. Steenland K, Goldstein FC, Levey A, Wharton W. A meta-analysis of Alzheimer's disease incidence and prevalence comparing African-Americans and Caucasians. *J Alzheimers Dis.* 2016;50(1):71-76. doi:10.3233/JAD-150778.
51. Wang S, Zhang Q, Hou T, Wang Y, Han X, Song L, et al. Adiposity indices and dementia. *J Am Med Dir Assoc.* 2023;24(9):1412-1419.
52. Brunner PM, Suárez-Fariñas M, He H, Malik K, Wen HC, Gonzalez J, et al. Blood signature in atopic dermatitis. *Sci Rep.* 2017; 7:8707.
53. Yang T, O'Malley TT, Kanmert D, et al. Detection of Aβ oligomers. *Alzheimers Res Ther.* 2015; 7:14.
54. Orayj K, Samuel VP, Saleem S, Ravula SR, Ali H, Gupta G, et al. Biomarkers in atopic dermatitis. *Clin Chim Acta.* 2026; 579:120634.
55. Gittler JK, Shemer A, Suárez-Fariñas M, et al. Th2/Th22 cytokine activation. *J Allergy Clin Immunol.* 2012; 130:1344-1354.
56. Ray S, Britschgi M, Herbert C, et al. Plasma biomarkers for Alzheimer diagnosis. *Nat Med.* 2007; 13:1359-1362.
57. Wollenberg A, Blauvelt A, Guttman-Yassky E, Worm M, Lynde C, Lacour JP, et al. Tralokinumab trials in atopic dermatitis. *Br J Dermatol.* 2021;184(3):437-449.
58. Brunner PM, Pavel AB, Khattri S, Leonard A, Malik K, Rose S, et al. IL-22 and fezakinumab response. *J Allergy Clin Immunol.* 2019;143(1):142-154.
59. Hashimoto S, Nakamura K, Oyama N, Kaneko F, Tsunemi Y, Saeki H, et al. CCL22 and disease activity. *J Dermatol Sci.* 2006;44(2):93-99.
60. Heneka MT, Kummer MP, Latz E. Innate immune activation in neurodegeneration. *Nat Rev Immunol.* 2014;14(7):463-477.

PREDICTIVE EFFECTIVENESS OF BODY MASS INDEX, WAIST-TO-HEIGHT RATIO AND BODY ROUNDNESS INDEX IN CATEGORIZING METABOLIC SYNDROME SEVERITY

Biljana Tomovic[1,2], Petar Gogic[1,2*] and Ivica Petrovic[3]

[1]*University of Kragujevac, Faculty of Medical Sciences, Serbia*
[2]*University Clinical Center Kragujevac, Clinic for Endocrinology, Diabetes and Metabolic Diseases, Serbia*
[3]*University of Kragujevac, Faculty of Medical Sciences, Department of Pathophysiology, Serbia*

Received: 23.02.2026.
Accepted: 27.05.2026.

Corresponding author:

Petar Gogic

Phone: +381 65 9681104

E-mail: petargogic13@gmail.com

UDK:

Eabr 2026; 27(3): 221-228
DOI: 10.2478/eabr-2026-0029

ABSTRACT

Metabolic syndrome (MetS) is a major public health challenge, defined by the co-occurrence of central obesity, insulin resistance, dyslipidemia, and elevated blood pressure, which increases the risk of cardiometabolic complications. Therefore, this study aimed to evaluate the predictive ability of Body Mass Index (BMI), Waist-to-height ratio (WHtR) and Body Roundness Index (BRI) in identifying individuals with more severe manifestations of MetS. This cross-sectional retrospective study included 63 patients diagnosed with metabolic MetS with clearly defined inclusion and exclusion criteria, conducted at the University Clinical Centre Kragujevac. The severity of metabolic syndrome was defined using a wide range of variables. A standardized 75g oral glucose tolerance test (OGTT) was performed on the entire cohort. Categorical variables were analyzed using the χ^2 (Chi-square) test and Fisher's exact test, statistical significance was set at p-value ≤ 0.05. Our analysis demonstrated that WHtR was the most effective index for identifying individuals with insulin resistance, impaired glucose tolerance, elevated fasting glucose levels and hyperinsulinemia. Body Roundness Index emerged as the strongest predictor of hyperinsulinemia at 120 minutes during the OGTT, highlighting its relevance in detecting increased cardiometabolic risk, while BMI showed the predictive capacity in identifying metainflammation, defined as a CRP concentration >2.5 mg/L. Waist-to-height ratio was the most effective and practical index for identifying individuals with insulin resistance, impaired glucose tolerance, and elevated cardiometabolic risk, whereas both BRI and BMI demonstrated a similarly limited predictive capacity among the analysed indices, each showing utility only for specific metabolic parameters.

Keywords: *Metabolic syndrome, obesity, body mass index, waist-to-height ratio, body roundness index.*

INTRODUCTION

Metabolic syndrome (MetS) represents a significant contemporary clinical and public health challenge characterized by the simultaneous presence of several metabolic abnormalities, most commonly central obesity, insulin resistance (IR), atherogenic dyslipidemia (elevated triglycerides and reduced levels of high-density lipoproteins) and high blood pressure (1,2,3). Defined by an increased tendency toward thrombotic and inflammatory activity, MetS is now viewed as a condition in which each component independently elevates the risk of cardiovascular disease and overall mortality (2).

Obesity has been steadily increasing worldwide, representing one of the leading public health challenges of the 21st century. The rising incidence of obesity is closely linked to a higher prevalence of metabolic disorders. Body weight on its own provides only a limited view of an individual's health status, but central obesity is the key feature of MetS (4). Early detection of this pattern matters, because simple lifestyle and clinical interventions can markedly reduce long-term harm when applied before irreversible disease develops (5). The ability to understand and identify MetS at an early stage depends on accurate and affordable assessments of body composition and fat distribution.

Among various anthropometric indices, body mass index (BMI) remains one of the most frequently utilized. Despite its widespread acceptance as a measure of obesity, it has notable limitations. Body mass index has been questioned for its inability to differentiate between adipose tissue and muscle mass, as well as for failing to capture the pattern of fat distribution in an individual (6). Quantitative and qualitative evaluation of body composition demonstrates that characterizing obesity based exclusively on BMI and fat accumulation provides an incomplete view of obesity-related health risks (7).

Several newly proposed measures have been introduced as indices of adiposity. Simple anthropometric ratios, especially waist-to-height ratio (WHtR), are easy to measure. This anthropometric index, calculated as waist circumference (WC) divided by height, has become increasingly recognized as a valuable marker of central adiposity and the visceral fat accumulation. Compared to BMI, WHtR offers greater sensitivity for detecting health risks (5). Waist-to-height ratio can assess cardiovascular risk factors and reliably predict the development of MetS (8,9). Body roundness index (BRI), a recently developed index based on WC and height, is considered as a potentially superior predictor of total and visceral fat compared with conventional anthropometric indices including BMI, WC, and hip circumference (8,10). Among individuals diagnosed with type 2 diabetes mellitus (T2DM), BRI demonstrated a strong association with both MetS and various indicators of metabolic dysfunction (4).

AIM

The aim of this study is to evaluate the predictive ability of BMI, WHtR, and BRI in identifying individuals with significant severity of MetS. Additionally, the research aims to clarify whether these simple, non-invasive measurements can be used as reliable early screening tools for high-risk patients.

MATERIALS AND METHODS

Patients and study design

The research was conducted throughout the course of 2016 within the department of the Daily hospital of the Clinic for Endocrinology, Diabetes and Metabolic Diseases of the University Clinical Centre Kragujevac. This investigation was designed as a cross-sectional retrospective study and included 63 patients diagnosed with MetS exhibiting a mean age of 40.21 ± 10.36 years (range: 19-60 years). Of the total study population, females represented 61.9% of the study population, compared to 38.1% of males. To be enrolled in the study, each participant had to meet the group-specific inclusion criteria and did not meet any exclusion criteria. The diagnosis of MetS was established based on the International Diabetes Federation consensus criteria (11). Inclusion criteria required participants to be over 18 years, signed an informed consent form and met the diagnostic criteria for MetS. Exclusion criteria included the use of anti-obesity medications, corticosteroids, antidepressants, or antipsychotics, as well as a history of acute infection within the previous two weeks, the presence of kidney or liver dysfunction or malignant disease within the last five years. Ethical conduct of the study was ensured in accordance with the Declaration of Helsinki on Ethical Principles for Medical Research Involving Humans and approved by the Ethics Committee of the University Clinical Centre Kragujevac, Serbia (number 01/2154).

Clinical evaluation and sampling

Anthropometric parameters such as body weight, height, WC, and hip circumference (HC), were performed during the clinical examination. Venous blood samples were collected after a 12h fasting period for biochemical analysis including lipid profile (total cholesterol, LDL, HDL, triglycerides, non-HDL cholesterol and remnant cholesterol) and glycosylated hemoglobin A1C (HbA1C). All analyses were conducted at the laboratory of the University Clinical Centre Kragujevac.

Oral glucose tolerance test was performed after an overnight fast, participants ingested 75g of glucose dissolved in 250ml of water. Venous blood samples were collected before glucose intake, 60 minutes and 120 minutes post-ingestion for plasma glucose determination. Insulin concentrations were determined using the identical venous blood samples drawn for glucose analysis at baseline and 120 minutes post-ingestion. Impaired glucose homeostasis was defined as fasting blood glucose levels above 5.6 mmol/L, glucose levels

above 7.8 mmol/L at 120-minute of OGTT, or the combination of both criteria. Atherogenic dyslipidemia was defined as the coexistence of elevated LDL cholesterol and reduced HDL cholesterol levels, with thresholds adjusted according to gender. Insulin resistance was assessed by the Homeostatic Model Assessment (HOMA-IR), with values derived from the formula presented below:

$$HOMA-IR = (Fasting\ glucose\ [mmol/L] \times Fasting\ insulin\ [\mu U/mL])/22.5$$

For the purposes of this study, metainflammation was defined as a chronic low-grade inflammation triggered by metabolic factors and marked by C-reactive protein (CRP) levels >2.5 mg/L. While Dinh et al. utilized a CRP range of 3–10 mg/L to define low-grade inflammation and exclude acute infections (12), Onat et al. established a lower cut-off of >2.5 mg/L to identify metabolic and lipid dysfunction (13). To capture the earliest stages of inflammatory activity in our specific population, we adopted this lower threshold of >2.5 mg/L. Severe MetS was characterized by marked insulin resistance (demonstrated by elevated HOMA-IR >2.5), chronic low-grade inflammation (confirmed by CRP levels greater than 2.5 mg/L), fasting, as well as 120-minute hyperinsulinemia during the OGTT and dyslipidemia defined as HDL <1.3.

Body mass index has its own formula (14):

$$BMI = Weight(kg) / [Height\ (m)]^2$$

The waist-to-height ratio was determined by dividing WC (cm) by height (cm), according to the following formula:

$$WHtR = Waist\ circumference(cm) / Height(cm)$$

Height was measured without shoes, using a stadiometer. Waist circumference was measured at the end of the expirium in the middle of the distance between the lowest rib and the iliac crest, using a measuring tape. Higher WHtR values indicate a greater degree of central obesity. We have used a value of WHtR ≥ 0.5 as a cut off for increased cardiometabolic risk.

The recently developed BRI, introduced by Thomas et al. (15), based on WC and height is a useful predictor of total and visceral body fat. Body Roundness Index based on specific cut-off values (scores below 4.4 were indicative of a favorable body composition, values ranging from 4.4 to 6.9 suggested an increased metabolic risk, while values exceeding 6.9 were classified as significant central obesity) was calculated using the corresponding formula as follows:

$$BRI = 364.2 - 365.5 \times \sqrt{1 - \left(\frac{WC}{2\pi}\right)^2} \div (0.5 \times height)^2$$

Statistics

The survey data collected were analyzed using the commercial software package SPSS version 22. For the analysis of categorical variables, the χ^2 (Chi-square) test and Fisher's exact test was utilized to investigate the capacity of the evaluated anthropometric parameters to identify advanced manifestations of MetS. Statistical significance will be considered at a p-value ≤ 0.05.

RESULTS

We evaluated the comparative performance of BMI, WHtR, and BRI in detecting patients with IR, defined as HOMA-IR >2.5. Our analysis revealed that WHtR demonstrated superior diagnostic accuracy, outperforming both BMI and BRI in the effective identification of patients with IR (p=0.001).

Table 1. *Evaluation of BMI, WHtR, and BRI in classifying individuals with IR determined by HOMA-IR status.*

Anthropometric indices		Number of participants: HOMA IR < 2.5	Number of participants: HOMA IR > 2.5	p
BMI	Normal weight	3 (9.4%)	2 (6.7%)	0.215
	Overweight	14 (43.8%)	7 (23.3%)	
	Obesity class I	9 (28.1%)	9 (30.0%)	
	Obesity class II	6 (18.8%)	12 (40.0%)	
WHtR	< 0.5	25 (78.1%)	11 (36.7%)	**0.001***
	≥ 0.5	7 (21.9%)	19 (63.3%)	
BRI	< 4.4	9 (30.0%)	3 (10.7%)	0.140
	4.4 - 6.9	15 (50,0 %)	15 (53,6 %)	
	> 6.9	6 (20,0 %)	10 (35,7 %)	

* Statistical significance p ≤ 0,05; BMI- body mass index; WHtR- waist to height ratio; BRI- body roundness index

We compared the discriminatory capacity of BMI, WHtR, and BRI in identifying metainflammation, defined as a CRP concentration exceeding 2.5 mg/L. Our findings indicate that both BMI and WHtR provide high efficacy in identifying patients with metainflammation (0.002 and 0.016).

Table 2. *Comparative evaluation of BMI, WHtR, and BRI for identifying individuals with metainflammation, defined by CRP levels.*

Anthropometric indices		Number of participants		p
		CRP < 2.5 mg/L	CRP > 2.5 mg/L	
BMI	Normal weight	3 (12 %)	2 (5,4 %)	**0.002***
	Overweight	14 (56 %)	7 (18,9 %)	
	Obesity class I	7 (28,0 %)	12 (32,4 %)	
	Obesity class II	1 (5,9 %)	16 (43,2 %)	
WHtR	< 0.5	20 (80,0 %)	17 (45,9 %)	**0.016***
	≥ 0.5	5 (20,0 %)	20 (54,1 %)	
BRI	< 4.4	7 (29,2 %)	6 (17,6 %)	0.135
	4.4 - 6.9	14 (58,3 %)	16 (47,1 %)	
	> 6.9	3 (12,5 %)	12 (35,3 %)	

* Statistical significance p ≤ 0,05; BMI- body mass index; WHtR- waist to height ratio; BRI- body roundness index

Evaluation of the effectiveness of BMI, WHtR, and BRI in detecting and distinguishing individuals with increased fasting insulin levels of the OGTT. Among the three anthropometric indices examined, WHtR proved to be the most reliable predictor of fasting hyperinsulinemia (p=0.037).

Table 3. *Discriminative ability of BMI, WHtR, and BRI in identifying hyperinsulinemia based on fasting insulin levels.*

Anthropometric indices		Number of participants		p
		Fasting insulin levels < 9,9 µIU/mL	Fasting insulin levels > 9,9 µIU/mL	
BMI	Normal weight	4 (14,3 %)	1 (2,9 %)	0.163
	Overweight	9 (32,1 %)	12 (34,3 %)	
	Obesity class I	10 (35.7%)	9 (25.7%)	
	Obesity class II	5 (17.9%)	13 (37.1%)	
WHtR	< 0.5	21 (75.0%)	16 (45.7%)	**0.037***
	≥ 0.5	7 (25.0%)	19 (54.3%)	
BRI	< 4.4	9 (33.3%)	4 (12.5%)	0.142
	4.4 - 6.9	11 (40.7%)	19 (59.4%)	
	> 6.9	7 (25.9%)	9 (28.1%)	

* Statistical significance p ≤ 0,05; BMI- body mass index; WHtR- waist to height ratio; BRI- body roundness index

Among participants with elevated insulin levels at 120 minutes of the OGTT (> 40 µIU/mL) the vast majority presented with moderately increased risk (BRI 4.4 - 6.9) and high risk (BRI > 6.9) of cardiometabolic diseases. According to Table 4, these findings indicate that BRI is superior anthropometric index for identifying hyperinsulinemia at the 120-minute of the OGTT (p=0.040).

Table 4. *Comparison of BMI, WHtR, and BRI in detecting individuals with elevated insulin levels at 120 minutes of the OGTT.*

Anthropometric indices		Number of participants: Insulin levels at 120 min (OGTT) < 40 µIU/mL	Number of participants: Insulin levels at 120 min (OGTT) > 40 µIU/mL	p
BMI	Normal weight	3 (9.1%)	2 (7.1%)	0.852
	Overweight	11 (33.3%)	10 (35.7%)	
	Obesity class I	11 (33.3%)	7 (25.0%)	
	Obesity class II	8 (24.2%)	9 (32.1%)	
WHtR	< 0.5	23 (69.7%)	14 (50.0%)	0.191
	≥ 0.5	10 (30.3%)	14 (50.0%)	
BRI	< 4.4	11 (35.5%)	2 (7.7%)	**0.040***
	4.4 - 6.9	12 (38.7%)	16 (61.5%)	
	> 6.9	8 (25.8%)	8 (30.8%)	

*Statistical significance p ≤ 0,05; BMI- body mass index; WHtR- waist to height ratio; BRI- body roundness index

We evaluated the capacity of BMI, WHtR, and BRI to identify individuals with impaired glucose tolerance, based on glucose levels measured at 120 minutes of the OGTT (Table 5). Among the indices analyzed, WHtR emerged as the most effective predictor, exhibiting superior discriminative power in identifying individuals with elevated glucose concentrations. (p=0.022).

Table 5. *Assessment of BMI, WHtR, and BRI as predictors of impaired glucose tolerance based on glucose levels at the 120 minutes of the OGTT.*

Anthropometric indices		Number of participants: Glycemia at 120 min (OGTT) < 7.8 mmol/L	Number of participants: Glycemia at 120 min (OGTT) > 7.8 mmol/L	p
BMI	Normal weight	4 (8.5%)	1 (6.3%)	0.489
	Overweight	18 (38.3%)	3 (18.8%)	
	Obesity class I	13 (27.7%)	6 (37.5%)	
	Obesity class II	12 (25.5%)	6 (37.5%)	
WHtR	< 0.5	32 (68.1%)	5 (31.3%)	**0.022***
	≥ 0.5	15 (31.9%)	11 (68.8%)	
BRI	< 4.4	11 (25.0%)	2 (13.3%)	0.606
	4.4 - 6.9	22 (50.0%)	8 (53.3%)	
	> 6.9	11 (25.0%)	5 (33.3%)	

*Statistical significance p ≤ 0,05; BMI- body mass index; WHtR- waist to height ratio; BRI- body roundness index

We also evaluated the discriminative capacity of BMI, WHtR, and BRI to distinguish between participants with normal and impaired glucose regulation. The results demonstrated that none of these anthropometric markers could effectively stratify patients based on their FPG and PG 60' levels (Supplement Table 1,2).

This study assessed whether BMI, WHtR, and BRI could serve as reliable predictors of impaired glucose tolerance. Nevertheless, the results indicate that these anthropometric measures lack the necessary sensitivity to differentiate between individuals with normal and impaired glucose regulation within this population (Supplement Table 3).

The discriminative capacity of BMI, WHtR, and BRI was evaluated to determine their effectiveness in identifying patients with HbA1c levels > 5.7%. The results indicate that these anthropometric indices lack sufficient predictive utility

to distinguish between patients with normal and elevated HbA1c values within this cohort (Supplement Table 4).

None of the evaluated indices demonstrated significant discriminative power in identifying patients with HDL cholesterol levels below 1.3 mmol/L. These indices failed to effectively stratify the cohort based on their lipid profile (Supplement Table 5).

The anthropometric indices BMI, WHtR, and BRI did not show a statistically significant ability to categorize patients based on their atherogenic dyslipidemia status. These results suggest that these measurements are not dependable predictors in the observed population (Supplement Table 6).

DISCUSSION

Our study was conducted to evaluate the predictive value of BMI, WHtR, and BRI in individuals diagnosed with MetS, aiming to identify which anthropometric indices most accurately indicate high metabolic risk. Overall, the findings suggest that central adiposity may provide superior clinical insight compared with traditional anthropometric indices, supporting their use in the assessment and management of MetS.

Our findings indicate that BMI demonstrated significant predictive power solely for metainflammatory status determined by CRP values, showing its role as an effective tool for identifying individuals with low-grade inflammation. On the contrary, BMI failed to demonstrate significant predictive utility for metabolic markers such as HOMA-IR, HbA1c, insulin, or glucose levels. These findings suggest that BMI categories do not effectively stratify metabolic risk within the MetS population, which is consistent with recent evidence. A study conducted by Nasrabadi M. et al. shown significantly elevated hsCRP levels in participants with overweight or obesity compared to normalweight in both sexes, but there was a significant association between BMI and hs-CRP only in the obese group ($p < 0.001$) (16). Contrariwise**,** a study published by R Liu-Galvin et al. (17) demonstrated that participants with elevated BMI but normal body fat percentage (BF%) did not show significantly higher hsCRP levels, suggesting that increased body mass mediated by muscle rather than adipose tissue does not trigger systemic inflammation. Similarly, research conducted by Kim BM et al. (18) indicated that after adjusting for WC, a marker of central adiposity, the association between BMI and CRP was no longer statistically significant. This further confirms that total body mass is merely a confounding factor, whereas localized central adiposity is the actual biological promoter of metabolic inflammation.

In our study, the statistically significant predictive capacity of BRI regarding markers of metabolic dysregulation, specifically elevated insulin levels at 120 minutes of the OGTT supports the growing number of data that identify BRI as a valuable tool for metabolic risk stratification. A cross-sectional study conducted by de Luis D. et al. (19) observed that in postmenopausal women with obesity have demonstrated significant associations between BRI, HOMA-IR, and CRP levels, highlighting the role of BRI as a predictor of metabolic risk beyond classical measures. Notably, Eng PC et al. (20) showed that BRI is strongly associated with visceral adipose tissue and inversely correlated with insulin sensitivity in healthy, young adult males. Fahami M. et al. (21) reported that BRI outperformed traditional anthropometric measures, including BMI and WC, in predicting MetS and related risk factors. Contrary to previous studies (19-21) our findings suggest that BRI possesses limited discriminative utility, as it distinguished patients solely based on 120-minute insulin levels, while remaining non-significant for all other metabolic parameters.

The strong predictive performance of WHtR in this study likely reflects ability to capture patterns of central adiposity more precisely than BMI or BRI, supported by the fact that increases in waist circumference proportionally elevate the ratio, thereby enabling higher index values to precisely reflect a greater volume of visceral adipose tissue. Exceeding the WHtR threshold demonstrate significant discriminatory power in identifying patients with elevated glucose levels at 120 minutes during OGTT, IR, fasting hyperinsulinemia and elevated CRP values suggest that individuals with higher cut-off values of WHtR indicate early disruptions in glucose homeostasis and the existence of metainflammation. Visceral adiposity is a key contributor to chronic low-grade inflammation, which often precedes manifest metabolic disturbances. Zhu M. et al. (22) demonstrated that WHtR serves as the primary indicator for detecting IR in women with Polycystic Ovary Syndrome (PCOS). Importantly, even among individuals with normal BMI, normal WC, or both, an elevated WHtR was significantly associated with IR. According to Jamar et al. (23), among the different anthropometric indices for assessing obesity, WHtR showed the strongest correlation with IR and proved to be a better predictor of diabetes onset in obese individuals than other measures. Among each clinical parameter evaluated in study conducted by Kholmatova K. et al. (24) hs-CRP demonstrated the strongest positive association with WHtR in both sexes, whereas HDL exhibited the most pronounced negative correlation with WHtR, which is in alignment with the findings of our study.

STRENGTH AND LIMITATION

The principal strength of this study is the comprehensive evaluation of three commonly utilized anthropometric indices WHtR, BMI, and BRI, specifically their capacity to stratify participants based on the severity of metabolic disturbances. The main limitation of the study that we conducted is a relatively small sample size (n=63), which may limit the statistical power of the findings. However, this cohort provides valuable preliminary insights into the superiority of WHtR, demonstrating its clinical relevance despite the small sample size and minor data gaps.

CONCLUSION

Our analysis of anthropometric indices demonstrated that BMI and BRI possess parameter-specific predictive values. While BMI was specifically associated with metainflammation, BRI proved to be the strongest predictor of hyperinsulinemia at 120 minutes during the OGTT, although its capacity to reflect generalized metabolic risk remained limited. In contrast, WHtR emerged as simple, but the most effective index, offering superior accuracy in assessing cardiometabolic risk factors within our study population, thereby highlights the necessity for larger, prospective and longitudinal studies in the future to firmly confirm these conclusions.

REFERENCES

1. Kobo O, Leiba R, Avizohar O, Karban A. Normal body mass index (BMI) can rule out metabolic syndrome: An Israeli cohort study. Medicine (Baltimore). 2019;98(9): e14712.
2. Ma YL, Jin CH, Zhao CC, Ke JF, Wang JW, Wang YJ, Lu JX, Huang GZ, Li LX. Waist-to-height ratio is a simple and practical alternative to waist circumference to diagnose metabolic syndrome in type 2 diabetes. Front Nutr. 2022;9:986090.
3. Vasquez F, Correa-Burrows P, Blanco E, Gahagan S, Burrows R. A waist-to-height ratio of 0.54 is a good predictor of metabolic syndrome in 16-year-old male and female adolescents. Pediatr Res. 2019;85(3):269-74.
4. Liu B, Liu B, Wu G, Yin F. Relationship between body-roundness index and metabolic syndrome in type 2 diabetes. Diabetes Metab Syndr Obes. 2019;12:931-5.
5. Yoo EG. Waist-to-height ratio as a screening tool for obesity and cardiometabolic risk. Korean J Pediatr. 2016;59(11):425-31.
6. Abolhasani M, Maghbouli N, Sazgara F, Karbalai Saleh S, Tahmasebi M, Ashraf H. Evaluation of Several Anthropometric and Metabolic Indices as Correlates of Hyperglycemia in Overweight/Obese Adults. Diabetes Metab Syndr Obes. 2020;13:2327-36.
7. Bosy-Westphal A, Müller MJ. Diagnosis of obesity based on body composition-associated health risks-Time for a change in paradigm. Obes Rev. 2021;22 Suppl 2:13190.
8. Liu PJ, Ma F, Lou HP, Zhu YN. Comparison of the ability to identify cardiometabolic risk factors between two new body indices and waist-to-height ratio among Chinese adults with normal BMI and waist circumference. Public Health Nutr. 2017;20(6):984-91.
9. Rajput R, Rajput M, Bairwa M, Singh J, Saini O, Shankar V. Waist height ratio: A universal screening tool for prediction of metabolic syndrome in urban and rural population of Haryana. Indian J Endocrinol Metab. 2014;18(3):394-9.
10. Yang M, Liu J, Shen Q, Chen H, Liu Y, Wang N, Yang Z, Zhu X, Zhang S, Li X, Qian Y. Body Roundness Index Trajectories and the Incidence of Cardiovascular Disease: Evidence From the China Health and Retirement Longitudinal Study. J Am Heart Assoc. 2024;13(19):e034768.
11. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome - a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabet Med 2006; 23(5): 469-80.
12. Dinh KM, Kaspersen KA, Mikkelsen S, Pedersen OB, Petersen MS, Thørner LW, Hjalgrim H, Rostgaard K, Ullum H, Erikstrup C. Low-grade inflammation is negatively associated with physical Health-Related Quality of Life in healthy individuals: Results from The Danish Blood Donor Study (DBDS). PLoS One. 2019;14(3): e0214468.
13. Onat A, Can G, Murat S, Çiçek G, Örnek E, Yüksel H. Clinical biomarkers of high-density lipoprotein dysfunction among middle-aged Turks. Anatol J Cardiol. 2012 ;12(8):628-636.
14. Keys A, Fidanza F, Karvonen MJ, et al. Indices of relative weight and obesity. J Chronic Dis 1972;25:329-43.
15. Thomas DM, Bredlau C, Bosy-Westphal A, Mueller M, Shen W, Gallagher D, Maeda Y, McDougall A, Peterson CM, Ravussin E, et al. Relationships between body roundness with body fat and visceral adipose tissue emerging from a new geometrical model. Obesity (Silver Spring). 2013;21:2264-71.
16. Nasrabadi M, Hadiloo K, Andalibi NS, Azmon M, Tavallaei S, Timar A, Ferns GA, Ghayour-Mobarhan M. The associations of oxidative stress and inflammatory markers with obesity in Iranian population: MASHAD cohort study. BMC Endocr Disord. 2024;24(1):56.
17. Liu-Galvin R, Orlando FA, Saguil AA, Jo A, Smith KB, Miller AM, Nelson DS, Sanders EC, Mainous III AG. More evidence of the health risks of normal weight obesity: the association with systemic inflammation. Front. Endocrinol. 2025; 12: doi.org/10.3389/fmed.2025.1695 935.
18. Kim BM, Ryu SY, Han MA, Choi SW. Loss of significant association between high-sensitivity C-reactive protein (hs-CRP) and metabolic syndrome after adjustment for waist circumference found in 2022 Korea National Health and Nutrition Examination Survey data. J Physiol Anthropol. 2025;44(1):16.
19. De Luis D, Muñoz M, Izaola O, Lopez Gomez JJ, Rico D, Primo D. Body Roundness Index (BRI) Predicts Metabolic Syndrome in Postmenopausal Women with Obesity Better than Insulin Resistance. Diabetol. 2025;6(7): 60.
20. Eng PC, Teo AE, Leow MKS, Tai ES, Khoo CM. Body roundness index (BRI) and obesity-related anthropometrics: Relationship to visceral adiposity, insulin sensitivity index and cardiometabolic risk. Diabetes Obes Metab. 2025;27(10):5554-65.
21. Stefanescu A, Revilla L, Lopez T, Sanchez SE, Williams MA, Gelaye B. Using A Body Shape Index (ABSI) and Body Roundness Index (BRI) to predict risk of metabolic syndrome in Peruvian adults. J Int Med Res. 2020;48(1): 300060519848854.
22. Zhu M, Wang K, Feng J, Liu Y, Guan M, Wang Y, Wu X. The waist-to-height ratio is a good predictor for insulin resistance in women with polycystic ovary syndrome. Front Endocrinol (Lausanne). 2024;15:1502321.
23. Hend S, Samah A, Amany I. Waist-to-height ratio as a clinical predictor for cardiovascular risks and insulin

resistance in children and adolescents with exogenous obesity. Gaz. Egypt. Paediatr. Assoc. 2021; 69:38. doi:10.11 86/s43054-021-00085-9.

24. Kholmatova K, Krettek A, Dvoryashina IV, Malyutina S, Cook S, Avdeeva E, Kudryavtsev AV. Waist-to-Height Ratio - Reference Values and Associations with Cardiovascular Risk Factors in a Russian Adult Population. Diabetes Metab Syndr Obes. 2025;18:2641-53.

ORIGINAL SCIENTIFIC ARTICLE

FOLATE-RELATED GENETIC RISK AND ASSOCIATIONS OF BETAINE-HOMOCYSTEINE METHYLTRANSFERASE AND PLASMINOGEN ACTIVATOR INHIBITOR-1 GENE VARIANTS WITH COAGULATION PARAMETERS IN PEDIATRIC PATIENTS WITH THROMBOPHILIA

Liliia Fishchuk[1,2*], Kateryna Vilchevska[3], Viktoriia Ianovska[3], Iryna Bakhchyvandzhy[3], Andrii Zakuslo[3], Inna Parkhomenko[3], Khrystyna Yeroshkina[1], Larysa Sheiko[1,2], Nataliya Smulska[4], Zoia Rossokha[1] and Natalia Gorovenko[1,2]

[1]*Shupyk National Healthcare University of Ukraine, Kyiv, Ukraine*
[2]*SI "National Scientific Center named after M.D. Strazhesko NAMS of Ukraine", Kyiv, Ukraine*
[3]*National Specialized Children's Hospital OKHMATDYT under the Ministry of Health of Ukraine, Kyiv, Ukraine*
[4]*Kyiv City Pediatric Hospital 1, Kyiv, Ukraine*

Received: 16.02.2026.
Accepted: 02.06.2026.

Corresponding author:

Liliia Fishchuk

E-mail: medgen@ukr.net

ABSTRACT

The presence of thrombophilia in children is a risk factor for strokes, venous thromboembolic disease, heart attacks, etc. Genetic factors are considered to contribute to thrombophilia susceptibility. The aim of our study was to explore potential associations of gene variants with thrombophilic disorders in children. The study involved 116 children (mean age 9.44±5.01 years). All pediatric patients underwent standard hemostasis examination. The frequencies of CC and GG genotypes of the MTHFR (rs1801133) and SLC19A1 (rs1051266) genes were reduced in patients with diagnosed thrombophilic disorders. The prevalence of the AA and 5G4G genotypes of the SLC19A1 (G80A) and PAI1 (4G/5G) genes were increased in patients diagnosed thrombophilic disorders. D-dimer levels were significantly lower in patients with GG genotype of the BHMT gene. Patients with the 4G4G genotype had the highest levels of plasminogen. The results suggest possible associations of MTHFR, SLC19A1, PAI1 and BHMT gene variants with thrombophilic disorders and coagulation markers in children. However, these findings should be interpreted cautiously and confirmed in larger controlled studies.

Keywords: *Thrombophilia, Children, Gene variants, D-dimer, Plasminogen.*

UDK:

Eabr 2026; 27(3): 229-237
DOI: 10.2478/eabr-2026-0030

INTRODUCTION

Many diseases, especially those classified as rare or genetic, occur during childhood and adolescence, with their progression often resulting in a deterioration in quality of life, increased disability, and shorter life expectancy (1, 2). The evolution of laboratory and medical genetics has led to significant advances in the diagnosis and treatment of pathology. Consequently, this advancement has played a pivotal role in early disease detection, identifying high-risk groups, and implementing preventive measures. One such complex condition is thrombophilia, characterized by a predisposition to develop clots due to genetic factors, acquired changes in the coagulation system, or, more commonly, an interaction between genetic and acquired factors (3). The identification of hereditary factors, including deficiencies in protein C, protein S, antithrombin, variations in Factor II (*F2*) and Factor V (*F5*) genes, elevated levels of Factor VIII, IX, XI, hyperhomocysteinemia, and methylenetetrahydrofolate reductase (*MTHFR*) gene variants, is commonly evaluated in the diagnosis of thrombophilia (3-5). This list, though condensed, is subject to supplementation in certain national protocols and guidelines, incorporating additional coagulation parameters and gene variants. Over the past decade, some countries, particularly those with fortified food practices, have revised their standards, omitting the necessity for homocysteine level and *MTHFR* gene variant assessments (6, 7). While thrombophilia may remain asymptomatic for an extended period, underlying changes in hemostatic parameters are discernible. Often, thrombophilia is detected with a burdened family history (8).

Despite the fact that thrombotic conditions are much more common in adults, the presence of thrombophilia as a symptom complex can occur in childhood, starting as early as the newborn period. Data on the incidence of thrombophilia in children are varied and not comparable due to the lack of appropriate registries. It has already been proven that the presence of thrombophilia in children is a risk factor for strokes, venous thromboembolic disease, heart attack, etc. (9-12). Until recently, the diagnosis of stroke in a child was a rare occurrence in pediatric practice. However, there has been a statistically significant surge in the number of diagnosed strokes in children in recent times (13). Therefore, establishing the diagnosis of thrombophilia is very important, primarily for the prevention of clinical presentation of the disease, repeated clinical episodes of thrombosis, and an increased risk of developing further cardiovascular diseases in children.

It is important to further analyze the relationship between common changes in blood coagulation parameters and new candidate genes that may be associated with an increased risk of the clinical presentation of thrombophilia. To date, many results of such studies have been published in the scientific literature (14-17). However, all of them were conducted in adults, and we found only a few studies for the pediatric cohort. Identification of associations between gene variants with thrombophilic disorders is necessary to develop personalized approaches to the prevention and treatment of thrombophilia in children. Therefore, the aim of our study was to explore potential associations of gene variants with thrombophilic disorders in children.

MATERIALS AND METHODS

The study involved 116 children (aged 1 to 16 years, mean age – 9.44±5.01 years, including 67 boys and 49 girls) who were examined between June 2018 and November 2019 at the Center for Hemostasis Pathology of the National Specialized Children's Hospital "OKHMATDYT", a national tertiary referral pediatric center in Kyiv, Ukraine. Children with suspected thrombophilia were examined at the Center for Hemostasis Pathology: 21 (18.1%) children exhibited venous thrombosis of the extremities of various localizations, 36 (31.0%) had a history of ischemic stroke and were referred for thrombophilia evaluation, 43 (37.1%) showed no abnormalities but had a family history of thrombosis in first or second-degree relatives at a young age, and 16 (13.8%) children were born to mothers with thrombophilia during pregnancy.

The study adhered to the principles outlined in the Declaration of Helsinki and received approval from the Ethics Committee of the Institute of Genetic and Regenerative Medicine (Approval number 4, as of 14 May 2018). Prior to the study, informed consent was obtained from the parents of the participating children.

The exclusion criteria for the study were as follows: the presence of congenital malformations, oncological and endocrinological pathology, taking drugs that affect coagulation parameters 2 weeks before the examination, and lack of informed consent.

All patients underwent a hemostasiological examination: activated partial thromboplastin time (APTT, sec), thrombin time (TT, sec), prothrombin time (PT, sec), fibrinogen (Fbg, g/l), soluble fibrin monomer complexes (SFMC, mg/%), prothrombin according to Quick (%), D-dimer (μg/ml), coagulation factor 2 (FII, %), coagulation factor 5 (FV, %), coagulation factor 7 (FVII, %), coagulation factor 8 (FVIII, %), coagulation factor 9 (FIX, coagulation factor 10 (FX, %), coagulation factor 11 (FXI, %), coagulation factor 12 (FXII, %), von Willebrand factor antigen (vWF, %), active von Willebrand factor (vWF:RCo, %), protein C (%), protein S (%), plasminogen (%), plasminogen inhibitor (%), measured using an ACL TOP 300 analyzer (Instrumentation Laboratory, USA). Folate metabolism parameters were also determined in children: homocysteine in blood plasma, folic acid and vitamin B12 in blood serum.

To determine the genetic determinant in the development of thrombophilia in children, we expanded the list of genetic

tests. We selected variants of genes associated with both folate metabolism and hemostatic disorders. The characteristics of the studied gene variants are shown in Table 1. Genomic DNA was extracted from buccal epithelial cells preserved in DNA/RNA Shield (ZymoResearch, USA) using the "Quick-DNA MiniPrep Plus Kit" (ZymoResearch, USA). Genotyping of the *MTHFR* gene A1298C variant was performed by allele-specific polymerase chain reaction, while the other variants were detected by polymerase chain reaction-restriction fragment length polymorphism analysis. The amplification reactions were carried out with specific oligonucleotide primers (Metabion, Germany) and "DreamTaq Green PCR Master Mix (2X)" (ThermoScientific, USA). Restriction analysis was performed with specific restriction endonucleases (ThermoScientific, USA). The amplified/restricted fragments were separated by agarose gel electrophoresis and visualized using a UV transilluminator.

Table 1. Characteristics of studied gene variants of associated with increased susceptibility to disorders of folate metabolism and thrombophilic disorders

Ген	Name	rsID number	Gene region	Protein variant	Characteristic	Research protocol
PAI1	4G/5G	rs1799889	promoter	-	affects plasma levels of plasminogen activator inhibitor 1	(18)
F5	Leiden mutation or G1601A	rs6025	exon 10	Arg534Gln	causes FV resistant to activated protein C	(19)
F2	G20210A	rs1799963	3'UTR	-	affects plasma prothrombin concentrations	(19)
FGB	C148T	rs1800787	promoter	-	affects plasma fibrinogen concentration	(19)
FGB	G455A	rs1800790	promoter	-	affects plasma fibrinogen concentration	(19)
ITGA2	C807T	rs1126643	exon 7	Phe253Phe	enhancing platelet aggregation function	(19)
ITGB3	PlA1/A2 or T1565C	rs5918	exon 2	Leu33Pro	influence both platelet activation and aggregation	(19)
MTHFR	C677T	rs1801133	exon 4	Ala222Val	affects methylenetetrahydrofolate reductase enzyme activity	(20)
MTHFR	A1298C	rs1801131	exon 7	Glu429Ala	affects methylenetetrahydrofolate reductase enzyme activity	(20)
MTRR	A66G	rs1801394	exon 1	Ile22Met	affects methionine synthase reductase enzyme activity	(20)
MTR	A2756G	rs1805087	exon 25	Asp919Gly	affects methionine synthase enzyme activity	(20)
SLC19A1	G80A	rs1051266	exon 2	Arg27His	affects affinity for folic acid	(20)
BHMT	G742A	rs3733890	exon 6	Arg239Gln	induces change in the protein activity	(21)

Statistical analysis was performed using Microsoft Excel Pro Plus 2016 and SPSS v.27 software packages. In the analysis of the basic clinical characteristics, the mean value ± standard deviation was calculated. Correlation analysis was performed between the studied indicators using the nonparametric Spearman test. Statistical power indices (sp) were calculated for the detected significant correlations. The probability of differences in quantitative results was determined using the Kruskal-Wallis test followed by post-hoc analysis with the Bonferroni correction. Differences were considered significant for all types of analysis at a p level of less than 0.05.

RESULTS

According to the results of a comprehensive examination, 47 (40.5%) children were diagnosed with thrombophilia, which was accompanied by an increase in the level of homocysteine, and in 15 (12.9%) cases, thrombophilia was not accompanied by hyperhomocysteinemia. Twenty-nine (25.0%) patients had hyperhomocysteinemia, which was determined to be hereditary based on a positive family history, the presence of folate-related mutations, and the clinical presentation of the disease. Among the total number of examined children, 25 (21.6%) had no abnormalities in the hemostasiogram and folate metabolism. When analyzing hemostasiological parameters, it was found that in the general group of patients – 31 (26.7%) patients had signs of increased blood coagulation, 18 (15.5%) patients had signs of hypocoagulation, and 16 (13.8%) patients had signs of hyper- and hypocoagulation.

Within the overall subject cohort, abnormalities in hemostatic parameters, indicative of hereditary thrombophilia, were found as follows: protein C deficiency in 4 (6.5%), protein S deficiency in 7 (11.3%), antithrombin III deficiency in 2 (3.2%), *F5* Leiden mutation in 3 (4.8%), *F2* mutation in 3 (4.8%), and elevated FVIII, FIX and FXI levels were documented in others.

Upon assessing the calculated average values of the hemostatic system in the study group (Table 2), deviations

beyond the normal range were identified in prothrombin time, D-dimer, and FVIII. Notably, elevated FVIII levels were observed in 22 (19.0%) children, serving as a diagnostic criterion for thrombophilia.

Table 2. Indicators of the hemostasis system in the study group

Parameter	Study group (n=116)	Reference ranges
APTT activated partial thromboplastin time, sec	32.5±7.4	24,0-34,0
TT Thrombin time, sec	15.1±2.9	13,0-18,0
Fbg fibrinogen, g/L	3.1±1.2	2,0-4,0
PT prothrombin time, sec	16.1±3.4	11,5-15,1
SFMC Soluble fibrin monomer complexes, mg/%	4.7±5.2	3.38±0.2
Prothrombin according to Kwik, %	83.1±22.4	80.0-120.0
D-dimer, µg/mL	0.7±1.2	<0.5
FII, %	105.3±29.1	79.0-131.0
V5, %	97.6±31.0	62.0-139.0
FVII, %	108.2±32.1	50.0-129.0
FVIII, %	154.8±125.7	50.0-150.0
FIX, %	93.0±42.1	65.0-150.0
FX, %	98.1±32.1	77.0-131.0
FXI, %	94.5±33.2	65.0-150.0
FXII, %	98.7±39.4	50.0-150.0
Von Willebrand factor antigen, %	138.1±45.3	61.0-157.0
Active von Willebrand factor, %	117.5±40.1	49.0-169.0
Protein C, %	91.9±26.4	70.0-140.0
Protein S, %	93.5±23.4	74.0-146.0
Plasminogen, %	100.1±21.9	72.9-126.9
Plasminogen inhibitor 1, %	111.9±17.2	10.0-150.0

Folate metabolism indicators were also assessed within the overall pediatric cohort (Table 3). The detected hyperhomocysteinemia in the general study group (according to the average homocysteine level) was mild, and was accompanied by an average vitamin B12 level within the normal range and an average serum folic acid level slightly above the deficiency limit (according to the reference value).

Table 3. Average folate metabolism in the study group

Parameter		Study group (n=116)	Reference ranges
Average homocysteine level, µmol/l		10.1±2.9	< 5.0
Average homocysteine level, µmol/l	Children under 10 years of age	8.5±2.5	Up to 10 years <5.8, 11-16 years old <6.6
	Children aged 11-16 years	11.6±2.4	
Average folic acid level, ng/ml		6.6±2.5	>6.0
Average level of vitamin B12, pg/ml		382.9±153.1	187-883

We have divided the examined children into two groups – children with (n=91) and without thrombophilic disorders (n=25). The group of children with thrombophilic disorders (n=91) was further divided into three mutually exclusive subgroups: hyperhomocysteinemia (HHC, n=29), thrombophilia (HT, n=15), thrombophilia combined with hyperhomocysteinemia (HT+HHC, n=47). Thus, these subgroups do not overlap and together comprise the entire thrombophilic group. Table 4 summarizes the distribution of genotypes across the study groups.

Table 4. Significant differences in the prevalence of genotypes for the studied gene variants

Gene variant	Genotype	Children without thrombophilic disorders (n=25)	Children with thrombophilic disorders (n=91)	HHC (n=29)	HT (n=15)	HT+HHC (n=47)	Results of statistical analysis
PAI1 4G/5G	5G5G	24.0%	17.00%	17.9%	14.3%	17.4%	NS
	5G4G	28.0%	52.30%	42.9%	50.0%	58.7%*	*$\chi2$=5.31, p=0.0212, OR=3.79 (1.33-10.82)
	4G4G	48.0%	30.70%	39.3%	35.7%	23.9%	NS
MTHFR C677T	CC	72.7%	40.9%*	44.8%	33.3%**	40.4%***	*$\chi2$=6.52, p=0.0107, OR=0.27 (0.10-0.70) ** $\chi2$=4.26, p=0.039, OR=0.19 (0.05-0.78) *** $\chi2$=5.31, p=0.0212, OR=0.26 (0.09-0.75)
	CT	27.3%	47.0%	41.4%	60.0%	44.7%	NS
	TT	0.0%	12.2%	13.8%	6.7%	14.9%	NS
SLC19A1 G80A	GG	52.4%	19.7%*	16.9%**	52.6%	19.4%***	*$\chi2$=8.82, p=0.003 OR=0.23 (0.09-0.58) **$\chi2$=5.82, p=0.016 OR=0.19 (0.06-0.67) ***$\chi2$=6.82, p=0.009 OR=0.22 (0.08-0.64)
	GA	42.9%	55.7%	41.5%	26.3%	71.0%	NS
	AA	4.8%	24.6%	41.5%*	21.1%	9.7%	$\chi2$=8.32, p=0.004 OR=16.94 (2.01-142.92)

The analysis was carried out by comparing the indicated frequencies with the frequencies determined for the group of children without thrombophilic disorders;
HHC – children with hyperhomocysteinemia;
HT – children with thrombophilia;
HT+HHC – children with combined thrombophilia and hyperhomocysteinemia
NS – not significant

Significant differences were found for *MTHFR*, solute carrier family 19 member 1 (*SLC19A1*) and plasminogen activator inhibitor-1 (*PAI1*) gene variants. The frequencies of CC and GG genotypes for *MTHFR* (C677T) and *SLC19A1* (G80A) genes were significantly reduced in patients with diagnosed thrombophilic disorders. The prevalence of the AA genotype of the *SLC19A1* (G80A) gene was significantly increased in patients with hyperhomocysteinemia. In patients with combined thrombophilia with hyperhomocysteinemia, the frequency of the 5G4G genotype of the *PAI1* gene was significantly increased compared to children without thrombophilic disorders. These findings suggest a possible association between *MTHFR*, *SLC19A1* and *PAI1* genes variants and thrombophilic phenotypes. No significant differences were found for other studied gene variants (not presented in the table).

Correlation analysis was performed to establish the relationship between laboratory parameters and variants of the studied genes. Significant differences found in the calculations are shown in Table 5. We did not find a significant correlation with variants of the "classic genes" of hereditary thrombophilia – genes that encode FII and FV.

Table 5. Associations of investigated genes variants with blood parameters

Gene variant	Prothrombin according to Kwik	D-dimer	F9	F11	Von Willebrand factor antigen	Plasminogen	Plasminogen inhibitor
PAI1 4G/5G	NS	NS	NS	rs=0.377 p=0.023 sp=46%	NS	rs=0.708 p=0.003 sp=88%	rs=0.702 p=0.016 sp=78%
MTHFR C677T	NS	NS	rs=0.342 p=0.031 sp=42%	NS	NS	NS	NS
MTHFR A1298C	rs=0.414 p=0.040 sp=37%	NS	NS	NS	NS	NS	NS
ITGA2 C807T	rs=0.441 p=0.027 sp=42%	NS	NS	NS	rs=0.390 p=0.021 sp=20%	NS	NS
BHMT G742A	NS	rs=0.608 p=0.003 sp=89%	NS	NS	NS	NS	NS

sp – statistical power
NS – not significant

At the next stage of statistical analysis, the obtained significant correlations with a statistical power exceeding 80% were further analyzed. The plasminogen level was significantly lower in patients with the 5G5G genotype of the *PAI1* gene compared with patients with the 4G4G genotype: 88.6±14.5% vs. 132.0±17.1% (p=0.029), respectively. It should be noted that the patient with the lowest plasminogen level (65.2%) was determined to have the 5G5G genotype of the *PAI1* gene. As is known, plasminogen is the main component of the fibrinolytic system – it forms plasmin under the influence of activators, which destroys fibrin clots and helps restore blood flow. Therefore, the 4G5G and 4G4G genotypes of the *PAI1* gene in a patient may be associated with alterations in the fibrinolytic system.

Equally significant characteristics were pinpointed concerning the D-dimer index. The concentration of D-dimer was significantly lower in individuals with the GG genotype for the G742A variant of the betaine-homocysteine methyltransferase (*BHMT*) gene: GG vs. AA – 0.7±0.8 vs. 3.7±1.1 (p=0.019). That is, in the presence of the major genotype at variant G742A of the *BHMT* gene in the examined patients, the D-dimer was significantly reduced – closer to the reference values. D-dimer is a fragment formed during the breakdown of a fibrin clot. Elevated levels of D-dimer indicate a disorder in the blood coagulation system. Therefore, the presence of the G742A variant of the *BHMT* gene may be associated with altered coagulation parameters and a potential predisposition to thrombophilic manifestations.

DISCUSSION

Hemostasis is a complex biological process that regulates the formation and dissolution of a fibrin clot to preserve the integrity of blood vessels. The different phases of hemostasis are coordinated in such a way that effective blood clotting occurs only at the site of the vessel injury, while maintaining blood flow in other parts of the circulation.

To date, it has already been proven that there is a big difference between the hemostatic systems of newborns, children and adults. The concept of developmental hemostasis is universally acknowledged as paramount in ensuring optimal prevention, diagnosis, and treatment of thrombotic and hemorrhagic diseases in children (22).

Currently, the scientific literature presents the results of numerous studies on the associations of genetic factors and individual indicators of the hemostatic system. However, the majority of these studies have primarily focused on adults (14-17). In contrast, research specifically dedicated to children predominantly revolves around understanding how genetic factors are linked to diseases stemming from hemostatic disorders (23, 24).

The results of present study suggest possible associations between the variants of the *MTHFR* (C677T), *SLC19A1* (G80A), and *PAI1* (4G/5G) genes and thrombophilic phenotypes in children.

Additionally, we observed positive correlations between hemostatic system indicators in children and variations in the *PAI1* (4G/5G), *ITGA2* (C807T), *MTHFR* (C677T, A1298C), and *BHMT* (G742A) genes. However, the most significant correlations were as follows: *PAI1* (4G/5G) with plasminogen concentration and *BHMT* (G742A) with D-dimer concentration.

PAI1 is a gene that encodes a plasminogen activator inhibitor 1 – a critical factor of the fibrinolytic system, which is an integral part of the hemostasis system and always accompanies the blood clotting process. The most studied and significant variant is the 4G/5G variant at the 675th position in the *PAI1* gene promoter region. The wild-type genotype contains 5 nucleotide bases of guanine (5G), and the deletion genotype contains 4G. The 4G allele cells produce up to 6 times more mRNA in vitro. In vivo, it is associated with

higher plasma plasminogen activator inhibitor 1 levels in the blood (25).

We discovered that the 5G4G genotype was more prevalent in the subgroup patients with combined hrombophilia with hyperhomocysteinemia. It's important to highlight that we didn't find similar results in the scientific literature. However, there are results suggesting an association between the 4G/5G variant of the *PAI1* gene and such thrombophilic conditions like venous thromboembolism, ischemic stroke, acute coronary syndrome (26-28).

In our study, a positive correlation was found between genotypes for the 4G/5G variant of the *PAI1* gene and plasminogen activator inhibitor 1 levels, but the statistical power of the association was below 80%. It should be noted that our results are similar to those of other research groups (29). Instead, we found a significant correlation between *PAI1* variants and plasma plasminogen levels in children. Plasminogen is a plasma protein, a proenzyme that under certain conditions turns into the active form (plasmin), and is a component of the fibrinolytic system, which is necessary to prevent excessive formation of blood clots during blood clotting. Our results indicate that the presence of the 4G/5G variant of the *PAI1* gene in children may indicate a disorder in the fibrinolysis system. The exact effects of this influence are unknown, so the results require further research.

The *MTHFR* gene encoded the enzyme, that serves as a pivotal regulatory factor in the metabolism of homocysteine that plays a crucial role in vascular function. The most common *MTHFR* variant is a substitution of cytosine (C) with thymine (T) at position 677, causing the conversion of alanine to valine at amino acid 222 of the enzyme. This variant is associated with decreased activity of 5,10-methylenetetrahydrofolate reductase, resulting in elevated levels of homocysteine (30). We observed a lower frequency of CC genotype of the *MTHFR* (C677T) gene among children with thrombophilic disorders, suggesting a possible protective association. A similar finding was shown by other research groups (13, 31).

The *SLC19A1* gene encodes an integral membrane glycoprotein that provides a system of intracellular folate transport. Among the variants of the *SLC19A1* gene one of the most studied and widespread is the G80A variant (30). Data from Chango et al. suggests that this variant, when paired with a variant C677T of the *MTHFR* gene, influences levels of folate and homocysteine (32). We observed a potential protective association of the GG genotype of the *SLC19A1* gene in relation to the risk of developing thrombophilic disorders. In addition, the AA genotype was more frequent among children with hyperhomocysteinemia. It's important to mention that we didn't find similar studies. The related data pertained to the influence of variant G80A of the *SLC19A1* gene on the severe neonatal period in preterm infants with intraventricular haemorrhages (33).

BHMT is a gene encoding an enzyme that catalyzes the transfer of a methyl group from betaine to homocysteine to form dimethylglycine and methionine (B12-independent homocysteine remethylation) (34). Transition (G742A) occurs at nucleotide 742 in exon 6 of the gene which replaces an arginine with a glutamine at codon 239 (R239Q). Li et al. observed that this variant produces two distinct alloenzymes, which exhibit significant differences in K_m values for homocysteine and betaine, which is lower for the variant alloenzyme compared to the wild type (35). Also, a Norwegian study reported that plasma dimethylglycine (a byproduct of homocysteine metabolism) decreased with an increasing number of A alleles (36). Therefore, this variant may contribute to homocysteine homeostasis and protection against thrombophilia while increased homocysteine level potentiates hypercoagulation.

There were found a positive correlation between the above-mentioned *BHMT* gene variant and D-dimer concentration. To our knowledge, this association has not been previously reported. We have not found similar results in the scientific literature. However, there are research results indicating that serum homocysteine was positively correlated with serum D-dimer concentration (37). Therefore, these results indirectly confirm our findings. Considering both prior research findings and theoretical foundations, observations may provide a rationale for further investigation of personalized approaches aimed at modulating D-dimer levels through supplementary nutrient intake, particularly betaine (38-40).

We would like to note that this study examined the prevalence of hereditary thrombophilia genes among children with suspected thrombophilia and investigated possible associations between the gene variants and indicators of the hemostasis system and folate metabolism. We believe that the results may contribute to a better understanding of the potential role of genetic factors in pediatric thrombophilic disorders and support future research in this area. In this study, we did not study/assess the need for genetic testing in children with suspected thrombophilia.

It should be noted that this study has some limitations. The study did not include a healthy control group without a family history of thrombosis, which may limit the generalizability of the findings. Being a single-center study, the results should be interpreted with caution. Additionally, residual confounding cannot be excluded (e.g., inflammatory status, nutrition, and other inherited thrombophilic factors), and the 2-week drug washout period may be insufficient for some medications. Nevertheless, the results of this study provide valuable insights into the role of genetic variants in pediatric thrombophilic disorders and may be used in further studies.

CONCLUSIONS

The results indicate the variants of *MTHFR*, *SLC19A1* and *PAI1* genes may be associated with the development risk of thrombophilic disorders in children. In addition, there are significant positive correlations between *PAI1* (4G/5G) and

BHMT (G742A) genes variants and certain blood markers such as plasminogen and D-dimer. These findings support possible associations between selected genetic markers and thrombophilic disorders in children; however, the results should be interpreted cautiously and confirmed in larger controlled studies.

CONFLICTS OF INTEREST

The authors declare no competing or financial interest in this study.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

1. Nguengang Wakap S, Lambert DM, Olry A, Rodwell C, Gueydan C, Lanneau V, et al. Estimating cumulative point prevalence of rare diseases: analysis of the Orphanet database. Eur J Hum Genet. 2020;28(2):165-173. https://doi.org/10.1038/s41431-019-0508-0.
2. Domaradzki J, Jabkowski P, Walkowiak D. Rare disease, common struggles: quality of life, caregiver burden and financial wellbeing of family caregivers in Poland. Sci Rep. 2025;15, 22678. https://doi.org/10.1038/s41598-025-08866-7.
3. Khan S, Dickerman JD. Hereditary thrombophilia. Thromb J. 2006;4:15. https://doi.org/10.1186/1477-9560-4-15.
4. Dicks AB, Moussallem E, Stanbro M, Walls J, Gandhi S, Gray BH. A Comprehensive review of risk factors and thrombophilia evaluation in venous thromboembolism. J Clin Med. 2024;13(2):362. https://doi.org/10.3390/jcm13020362.
5. Campello E, Spiezia L, Adamo A, Simioni P. Thrombophilia, risk factors and prevention. Expert Rev Hematol. 2019;12(3):147-158. https://doi.org/10.1080/17474086.2019.1583555.
6. Scorsatto M, Uehara SK, Luiz RR, de Oliveira GM, Rosa G. Fortification of flours with folic acid reduces homocysteine levels in Brazilian women. Nutr Res. 2011;31(12):889-95. https://doi.org/10.1016/j.nutres.2011.10.003.
7. Papoutsakis C, Manios Y, Magkos F, Papaconstantinou E, Schulpis KH, Zampelas A, et al. Effect of the methylenetetrahydrofolate reductase (MTHFR 677C>T) polymorphism on plasma homocysteine concentrations in healthy children is influenced by consumption of folate-fortified foods. Nutrition. 2010;26(10):969-74. https://doi.org/10.1016/j.nut.2009.09.022.
8. van Sluis GL, Söhne M, El Kheir DY, Tanck MW, Gerdes VE, Büller HR. Family history and inherited thrombophilia. J Thromb Haemost. 2006;4(10): 2182-7. https://doi.org/10.1111/j.1538-7836.2006.02139.x.
9. Radulescu VC. Management of venous thrombosis in the pediatric patient. Pediatric Health Med Ther. 2015; 6: 111-9. https://doi.org/10.2147/PHMT.S65697.
10. Celkan T, Dikme G. Thrombosis in children: Which test to whom, when and how much necessary? Turk Pediatri Ars. 2018; 53(1): 1-9. https://doi.org/10.5152/TurkPediatriArs.2018.2586.
11. Carano N, Agnetti A, Hagler DJ, Tchana B, Squarcia U, Bernasconi S. Acute myocardial infarction in a child: possible pathogenic role of patent foramen ovale associated with heritable thrombophilia. Pediatrics. 2004; 114(2): e255-8. https://doi.org/10.1542/peds.114.2.e255.
12. Lehman LL, Khoury JC, Taylor JM, Yeramaneni S, Sucharew H, Alwell K, et al. Pediatric Stroke Rates Over 17 Years: Report From a Population-Based Study. J Child Neurol. 2018; 33(7): 463-7. https://doi.org/10.1177/0883073818767039.
13. Smulska N, Rossokha Z, Fishchuk L, Gorovenko N, Nechai A, Nicolaenko I, et al. Polymorphisms variants of the MTHFR C677T and PAI-1 5G/4G genes and their combinations in the group of children with arterial ischemic stroke. Child Neurol. 2020; 29(58): 27-32. https://doi.org/10.20966/chn.2020.58.455.
14. Luo Y, Wang C, Tu H. Impact of the 4G/5G polymorphism in the plasminogen activator inhibitor-1 gene on primary nephrotic syndrome. Mol Med Rep. 2014; 9(3): 894-8. https://doi.org/10.3892/mmr.2014.1903.
15. Tang W, Schwienbacher C, Lopez LM, Ben-Shlomo Y, Oudot-Mellakh T, Johnson AD, et al. Genetic associations for activated partial thromboplastin time and prothrombin time, their gene expression profiles, and risk of coronary artery disease. Am J Hum Genet. 2012; 91(1): 152-62. https://doi.org/10.1016/j.ajhg.2012.05.009.
16. Weng LC, Cushman M, Pankow JS, Basu S, Boerwinkle E, Folsom AR, et al. A genetic association study of activated partial thromboplastin time in European Americans and African Americans: the ARIC Study. Hum Mol Genet. 2014; 24(8): 2401-8. https://doi.org/10.1093/hmg/ddu732.
17. Sennblad B, Basu S, Mazur J, Suchon P, Martinez-Perez A, van Hylckama Vlieg A, et al. Genome-wide association study with additional genetic and post-transcriptional analyses reveals novel regulators of plasma factor XI levels. Hum Mol Genet. 2017; 26(3): 637-49. https://doi.org/10.1093/hmg/ddw401.
18. Emirogullari EF, Saatci C, Unal A, Sahin A, Ozkul Y. Protrombin factor-V Leiden and plasminogen activator ingibitor type 1 gene polymorphisms in hemodialysis patients with/without arteriovenous fistula thrombosis. Int J Nephrol Urol. 2010; 2(2): 314-319.
19. Fishchuk L, Rossokha Z, Medvedieva N, Vershyhora V, Sheyko L, Brisevac L, et al. Effect of polymorphic variants of hereditary thrombophilia genes on the risk of early pregnancy loss for married couples. Meta Gene. 2021; 29: 100902 https://doi.org/10.1016/j.mgene.2021.100902.
20. Fishchuk L, Rossokha Z, Pokhylko V, Cherniavska Y, Popova O, Tsvirenko S, et al. Associations of variants of folate cycle genes with features of the clinical course of severe intraventricular hemorrhages in premature infants.

J Neonatal Perinatal Med. 2022; 15(3): 545-551. https://doi.org/10.3233/NPM-210962.
21. Ananth CV, Elsasser DA, Kinzler WL, Peltier MR, Getahun D, Leclerc D, et al. Polymorphisms in methionine synthase reductase and betaine-homocysteine S-methyltransferase genes: risk of placental abruption. Mol Genet Metab. 2007; 91(1): 104-10. https://doi.org/10.1016/j.ymgme.2007.02.004.
22. Toulon P. Developmental hemostasis: laboratory and clinical implications. Int J. Lab Hematol. 2016; 38: 66-77. https://doi.org/10.1111/ijlh.12531.
23. Sarecka-Hujar B, Kopyta I, Skrzypek M. Lack of Associations Between PAI-1 and FXIII Polymorphisms and Arterial Ischemic Stroke in Children: A Systematic Review and Meta-Analysis. Clin Appl Thromb Hemost. 2019; 25: 1076029619869500. https://doi.org/10.1177/1076029619869500.
24. Sarecka-Hujar B, Kopyta I, Skrzypek M. Is the 1298A>C polymorphism in the MTHFR gene a risk factor for arterial ischaemic stroke in children? The results of meta-analysis. Clin Exp Med. 2018; 18(3): 337-45. https://doi.org/10.1007/s10238-018-0489-2.
25. Jafari M, Jarahzadeh MH, Dastgheib SA, Seifi-Shalamzari N, Raee-Ezzabadi A, Sadeghizadeh-Yazdi J, et al. Association of PAI-1 rs1799889 Polymorphism with Susceptibility to Ischemic Stroke: a Huge Meta-Analysis based on 44 Studies. Acta Medica (Hradec Kralove). 2020; 63(1): 31-42. https://doi.org/10.14712/18059694.2020.13.
26. Zhang Q, Jin Y, Li X, Peng X, Peng N, Song J, et al. Plasminogen activator inhibitor-1 (PAI-1) 4G/5G promoter polymorphisms and risk of venous thromboembolism - a meta-analysis and systematic review. Vasa. 2020; 49(2): 141-6. https://doi.org/10.1024/0301-1526/a000839.
27. Akhter MS, Biswas A, Abdullah SM, Behari M, Saxena R. The Role of PAI-1 4G/5G Promoter Polymorphism and Its Levels in the Development of Ischemic Stroke in Young Indian Population. Clin Appl Thromb Hemost. 2017; 23(8): 1071-6. https://doi.org/10.1177/1076029617705728.
28. Iwai N, Shimoike H, Nakamura Y, Tamaki S, Kinoshita M. The 4G/5G polymorphism of the plasminogen activator inhibitor gene is associated with the time course of progression to acute coronary syndromes. Atherosclerosis. 1998; 136(1): 109-14. https://doi.org/10.1016/s0021-9150(97)00191-3.
29. Komitopoulou A, Platokouki H, Kapsimali Z, Pergantou H, Adamtziki E, Aronis S. Mutations and polymorphisms in genes affecting hemostasis proteins and homocysteine metabolism in children with arterial ischemic stroke. Cerebrovasc Dis. 2006; 22(1): 13-20. https://doi.org/10.1159/000092332.
30. Fishchuk L, Skavinska O, Ievseienkova O, Rossokha Z, Sheiko L. Genetic predictors of toxic effects of methotrexate in cancer patients. Exp Oncol. 2024; 45(4): 399-408. https://doi.org/10.15407/exp-oncology.2023.04.399
31. Hashemi SM, Ramroodi N, Amiri Fard H, Talebian S, Haghighi Rohani M, Rezaei M, et al. Common Variations in Prothrombotic Genes and Susceptibility to Ischemic Stroke in Young Patients: A Case-Control Study in Southeast Iran. Medicina (Kaunas). 2019; 55(2): 47. https://doi.org/10.3390/medicina55020047.
32. Chango A, Emery-Fillon N, de Courcy GP, Lambert D, Pfister M, Rosenblatt DS, et al. A polymorphism (80G->A) in the reduced folate carrier gene and its associations with folate status and homocysteinemia. Mol Genet Metab. 2000; 70(4): 310-5. https://doi.org/10.1006/mgme.2000.3034.
33. Rossokha Z, Fishchuk L, Pokhylko V, Cherniavska Y, Gorovenko N. [Relationship between RFC gene variants (rs1051266) and clinical features of severe intraventricular hemorrhage in preterm infants]. Act Probl of the Modern Med. 2021; 21(3): 30-4. Ukrainian. https://doi.org/10.31718/2077-1096.21.3.30.
34. Amorim MR, Moura CM, Gomes AD, Barboza HN, Lopes RB, Ribeiro MG, et al. Betaine-homocysteine methyltransferase 742G>A polymorphism and risk of down syndrome offspring in a Brazilian population. Mol Biol Rep. 2013; 40(8): 4685-9. https://doi.org/10.1007/s11033-013-2563-x.
35. Li F, Feng Q, Lee C, Wang S, Pelleymounter LL, Moon I, et al. Human betaine-homocysteine methyltransferase (BHMT) and BHMT2: common gene sequence variation and functional characterization. Mol Genet Metab. 2008; 94(3): 326-35. https://doi.org/10.1016/j.ymgme.2008.03.013.
36. Fredriksen A, Meyer K, Ueland PM, Vollset SE, Grotmol T, Schneede J. Large-scale population-based metabolic phenotyping of thirteen genetic polymorphisms related to one-carbon metabolism. Hum Mutat. 2007; 28(9): 856-65. https://doi.org/10.1002/humu.20522.
37. Chen X, Zhang W, Huang J. Homocysteine is potential serological marker for predicting the risk of deep venous thrombosis of the lower extremities in patients received operation of lower limb fracture. Pteridines. 2021; 32: 33-8. https://doi.org/10.1515/pteridines-2020-0027.
38. Rossokha Z, Fishchuk L, Sheyko L, Medvedieva N, Gorovenko N. Positive effect of betaine-arginine supplement on improved hyperhomocysteinemia treatment in married couples with reproductive disorders. Georgian Med News. 2020; (309): 22-28.
39. Yeroshkina K, Rossokha Z, Fishchuk L, Gorovenko N. Betaine consumption as a new clinical approach to treatment and prophylaxis of folate-related pathologies. Nutr Rev. 2023; 81(6): 716-26. https://doi.org/10.1093/nutrit/nuac084.
40. Mangoni AA, Arya R, Ford E, Asonganyi B, Sherwood RA, Ouldred E, et al. Effects of folic acid supplementation on inflammatory and thrombogenic markers in chronic smokers. A randomised controlled trial. Thromb Res. 2003; 110(1): 13-7. https://doi.org/10.1016/s0049-3848(03)00295-0.

.

THE FREQUENCY OF CERTAIN GENETIC POLYMORPHISMS AND THEIR INFLUENCE ON THE THERAPEUTIC RESPONSE OF PATIENTS TREATED WITH OLANZAPINE

Sasa Babic[1], Milena Stojkovic[2], Elvis Mahmutovic[3], Teodora Safiye[4], Sladjana Veselinovic[5], Milan Djordjic[5], Petar Canovic[6], Olivera Radmanovic[7], Vladimir Gavrilovic[8], Maida Burdzovic[9] and Branimir Radmanovic[2]

[1]*Psychiatric Clinic, University Clinical Center Kragujevac, Kragujevac, Serbia*
[2]*University of Kragujevac, Faculty of Medical Sciences, Department of Psychiatry, Kragujevac, Serbia*
[3]*State University of Novi Pazar, Department of Biomedical Sciences, Novi Pazar, Serbia*
[4]*State University of Novi Pazar, Department of Psychology, Novi Pazar, Serbia*
[5]*University of Kragujevac, Faculty of Medical Sciences, Department of Communication Skills, Ethics, and Psychology, Kragujevac, Serbia*
[6]*University of Kragujevac, Faculty of Medical Sciences, Department of Biochemistry, Kragujevac, Serbia*
[7]*Clinic for Rheumatology and Allergology, University Clinical Center Kragujevac, Kragujevac, Serbia*
[8]*Department of Radiology, Angiography and Interventional Radiology Unit, Azienda Sanitari Universitaria Friuli Centrale, Italy*
[9]*Center for Mental Health, Primary Health Care Center, Podgorica, Montenegro*

Received: 01.05.2024.
Accepted: 16.10.2024.

Corresponding author:

Dr. Teodora Safiye

E-mail: teodoras0306@gmail.com

UDK:

Eabr 2026; 27(3): 239-248
DOI: 10.2478/sjecr-2024-0018

ABSTRACT

*Olanzapine is an atypical antipsychotic that serves as the first line of schizophrenia treatment. The metabolism of olanzapine takes place with the leading participation of two enzymes, CYP1A2 and CYP2D6. The CYP450 enzyme activity can be changed under the influence of many drugs, which results in potentially significant interactions in which one drug can increase the toxicity (inhibition of cytochrome) or reduce the second drug's therapeutic effect. The aim of this study was to examine the frequency of certain genetic polymorphisms and their impact on the therapeutic response of patients treated with olanzapine. This research was conducted according to the design of a prospective, interventional, clinical study of phase IV by type of case series, where the stratification of the subjects was performed according to the obtained types of tested genotypes. Patients (N=120) were recruited at the Clinic of Psychiatry, University Clinical Center Kragujevac, in Serbia. The primary endpoint to assess the therapeutic response in this study was PANSS. In our study, the presence of the investigated gene variations (UGT1A4, CYP1A2, FMO3, and CYP2D6) does not affect the clinical response to olanzapine therapy in patients suffering from schizophrenia, compared to patients who are carriers of the wild-type gene. The presence of genes of CYP1A2*1C (rs2069514, -3860G>A), CYP1A2 (rs2472297, 74735539C>T), FMO3 E158K (rs2266782, 15167G>A), FMO3 V257M (rs1736557, 18281G>A), FMO3 E308G (rs2266780, 21443A>G), CYP2D6*3 (rs35742686, 2549delA), CYP2D6*4 (rs3892097, 1846G>A), CYP2D6*6 (rs5030655, 1707delT) does not change the clinical response to olanzapine therapy in patients suffering from schizophrenia, compared to patients who are carriers of the wild-type gene.*

Keywords: *Olanzapine, genetic polymorphism, CYP1A2, CYP2D6.*

INTRODUCTION

Olanzapine is an atypical antipsychotic that serves as the first line of defense against schizophrenia (1). However, patients appear to not react equally to this therapy, most likely due to significant inter-individual variations in plasma drug concentrations (2, 3).

Olanzapine metabolism takes place with the leading participation of two enzymes, CYP1A2 and CYP2D6. The activity of CYP450 enzymes is known to be under the influence of many drugs and substances from the environment that result in potentially significant interactions in which one drug may intensify the toxicity (inhibition of cytochrome) or reduce the therapeutic effect of another drug (induction of cytochrome) (4, 5).

A large number of genetic variations of CYP1A2 are known, but only one allele, CYP1A2*1F, defined by polymorphism -163A (rs76251C>A), has proven to have a significant impact on the concentration of metabolites and olanzapine in white people in terms of activities. For another variation, rs2472297C>T, located within the CYP1A1/1A2 gene cluster, is shown to speed up olanzapine metabolism in the synthesis of n-desmethyl metabolite synthesis. Cytochrome P450 CYP1A2 is very susceptible to induction, which causes great interindividual variations in enzyme activity (6, 7). The expression of CYP1A2 strongly regulates the aryl hydrocarbon receptor (AHR) gene locus (8). It has been shown that one of the polymorphisms in this locus (rs4410790C>T) reduces the synthesis of n-desmethyl olanzapine in humans. However, the quantitative share of the influence of these variations on the systematic exposure to olanzapine in patients is practically negligible; their presence can explain only about 2% of the concentration of this drug in body fluids (9).

The aim of this study was to examine the frequency of certain genetic polymorphisms as well as their influence on the therapeutic response of patients treated with olanzapine.

MATERIALS AND METHODS

This research was conducted according to the design of a prospective, interventional, clinical study of phase IV by type of case series, where the stratification of the subjects was carried out according to the obtained types of tested genotypes.

This case-control study was carried out between 2014 and 2016. A total of 120 respondents were recruited in this study. Patients were recruited at the Clinic of Psychiatry, University Clinical Center Kragujevac in Serbia, who, according to research criteria (DSM-V) (10), had a diagnosis of schizophrenia (11). Participation in the study was voluntary; patients were included in the study after obtaining their consent to participate in the study, and the study was approved by the Ethics Committee of the University Clinical Center Kragujevac in Serbia (approval No. 01/5273; date of approval: 8th of July, 2009). The study was conducted in accordance with the Principles of Good Clinical Practice and the Declaration of Helsinki (12). There was no conflict of interest in the study related to the study drug or other possible commercial aspects of the study.

The criteria for inclusion in the study were:

1. The patient was capable of understanding the nature of the study and signed an informed patient consent form before any study procedure was performed.
2. The patient is of Serbian nationality, male or female, of adult age, 18 years or older.
3. Previously diagnosed with schizophrenia (minimum 2 years before inclusion in the study).
4. Hospitalized or outpatient patients currently in the phase of exacerbation of psychotic symptoms who, on the BPRS (Brief Psychiatric Rating Scale) (13), have scores of 4 or more on two of the four items (9, 10, 11, and 15).
5. Sexually active participants in the study who have committed to using medically acceptable forms of contraception during the duration of the study.
6. Stable social environment for patients treated on an outpatient basis, including the presence of a person from the environment in order to obtain reliable data.

The exclusion criteria were:

1. On axis 1 of the DSM-V, a diagnosis of a mental disorder other than schizophrenia was made.
2. High suicidal risk or risk of heteroaggressive behavior as assessed by the researcher.
3. Current diagnosis of comorbid alcoholism or abuse of other psychoactive substances
4. Pregnancy or breastfeeding.
5. Findings of clinically significant abnormalities on physical examination, electrocardiography (ECG), or laboratory analyses, determined or taken at the initial visit, as well as the presence of significant somatic diseases that, in the opinion of the researcher, prevent the subject's participation in the study.
6. Patients who had neuroleptic malignant syndrome.
7. Previous allergic reactions to olanzapine.
8. Use of other drugs known to affect the metabolism of olanzapine, including but not limited to the following: aprepitant, ciprofloxacin, clarithromycin, diltiazem, erythromycin, fluconazole, imatinib, itraconazole, carbamazepine, ketoconazole, modafinil, nefazodone, rifampicin, verapamil, voriconazole, and phenobarbitone.
9. Electroconvulsive therapy was applied in the last year.

A sample for genotyping in the form of 10 ml of whole blood was taken from each subject in a vacuum tube with EDTA and stored at -20ºC until analysis. Samples were taken once, at the beginning or end of the study, as appropriate. The most important scale for assessing therapeutic response in this study was the PANSS, which, next to the BPRS, is the most widely used scale (14).

The PANSS scale was designed by Kay in 1987 and consists of three subscales (positive symptoms, negative symptoms, and general psychopathology). The PANSS has a total of 30 items graded from 1 to 7 (1-absent, 2-minimally present, 3-weakly present, 4-moderately present, 5-moderately very present, 6-strongly present, and 7-extremely present). The scale includes all items from the BPRS scale and selected items from the Psychopathology Rating Scale. The PANSS measurement scale is characterized by high reliability, construct validity, and objectivity (14). Andreasen and others published a paper in the American Journal of Psychiatry in 2005 titled Remission in schizophrenia: proposed criteria and rationale for consensus (15). Consensus determined 8 PANSS items for symptomatic remission criteria: P1-delusions, P2-conceptual disorganization, P3-hallucinations, N1-affective dullness, N4-passivity or apathetic social withdrawal, N6-lack of spontaneity and smooth flow of conversation, G5-mannerisms and unusual body posture, and G9-unusual thought content. Symptomatic remission criteria are met if the listed items are rated 3 or less and must last at least 6 months. Researchers who used this scale were previously trained and certified in the administration of the PANSS.

Statistical analysis

Statistical analysis of the gathered data was performed using SPSS Statistics Software (IBM SPSS Statistics for Windows, Version 22.0, Armonk, NY, USA). The statistical analysis of the data included methods of descriptive statistics and hypothesis testing. The basic statistical test was Analysis of Variance (ANOVA) for parametric data and the Kruskal-Wallis test for data with a non-parametric distribution, which examined the influence of independent categorical factors (presence of metabolizing enzyme polymorphisms UGT1A4, CYP1A2, FMO3, CYP2D6) individually on the change in the PANSS score. The influence of individual independent variables on the dependent variables (psychometric scale score value) was examined by a Student's t-test. The statistically significant level of probability was $p<0.05$.

RESULTS

A total of 217 people were considered for participation in the study, of which 120 were included in the research. All subjects who were included completed the study. The research examined the frequency of certain genetic polymorphisms as well as their influence on the therapeutic response.

Frequency of genetic polymorphism

The frequency of genetic polymorphism was examined for each genotype separately, which is shown in tables 1–4.

UGT1A4

Table 1. Frequency of *UGT1A4* alleles and genotypes in patients on olanzapine therapy

Variation	Frequency	95% Confidence Interval
Alleles		
rs6755571 (70C>A, *UGT1A4*2*)		
70A	0.033 (8/240)	0.016; 0.066
Genotypes		
rs6755571 (70C>A, *UGT1A4*2*)		
CC	0.933 (112/120)	0.871; 0.967
CA	0.067 (58/120)	0.033; 0.129
AA	0.000 (0/120)	0.000; 0.038

CYP1A2

Table 2. Frequency of *CYP1A2* alleles and genotypes in patients on olanzapine therapy

Alleles, frequency, 95% confidence interval		
rs2069514 (-3860G>A, *CYP1A2*1C*)		
-3860A	0.000 (0/240)	0.000; 0.020
rs762551 (-163C>A, *CYP1A2*1F*)		
-163A	0.658 (158/240)	0.596; 0.715
rs2472297 (74735539C>T, *CYP1A1/1A2*)		
74735539T	0.154 (37/240)	0.114; 0.206
Genotypes		
rs2069514 (-3860G>A, *CYP1A2*1C*)		

Alleles, frequency, 95% confidence interval			
	GG	1.000 (120/120)	0.962; 1.006
	GA	0.000 (0/120)	0.000; 0.038
	AA	0.000 (0/120)	0.000; 0.038
rs762551 (-163C>A, *CYP1A2*1F*)			
	CC	0.108 (13/120)	0.064; 0.178
	CA	0.467 (56/120)	0.380; 0.556
	AA	0.425 (51/120)	0.340; 0.514
rs2472297 (74735539C>T, *CYP1A1/1A2*)			
	CC	0.717 (86/120)	0.630; 0.790
	CT	0.258 (31/120)	0.188; 0.344
	TT	0.025 (3/120)	0.006; 0.075

FMO3

Table 3. Frequency of *FMO3* alleles and genotypes in patients on olanzapine therapy

Variation		Observed frequency
Alleles		
rs2266782 (15167G>A, E158K)		
15167A		0.371 (89/240)
rs1736557 (18281G>A, V257M)		
18281A		0.021 (5/240)
rs2266780 (21443A>G, E308G)		
21443G		0.146 (35/240)
Genotypes		
rs2266782 (15167G>A, E158K)		
	GG	0.400 (48/120)
	GA	0.458 (55/120)
	AA	0.142 (17/120)
rs1736557 (18281G>A, V257M)		
	GG	0.958 (115/120)
	GA	0.042 (5/120)
	AA	0.000 (0/120)
rs2266780 (21443A>G, E308G)		
	AA	0.725 (87/120)
	AG	0.258 (31/120)
	GG	0.017 (2/120)

CYP2D6

Table 4. Frequency of *CYP2D6* alleles and genotypes in patients on olanzapine therapy

Variation		Observed frequency
Alleles		
rs35742686 (2549delA, *CYP2D6*3*)		
2549delA		0.0080 (2/240)
rs3892097 (1846G>A, *CYP2D6*4*)		
1846A		0.188 (45/240)
rs5030655 (1707delT, *CYP2D6*6*)		
1707delT		0.021 (5/240)
Genotypes		
rs35742686 (2549delA, *CYP2D6*3*)		
	AA	0.975 (117/120)
	A delA	0.017 (2/120)

Variation		Observed frequency
	delA delA	0.000 (0/120)
rs3892097 (1846G>A, *CYP2D6*4*)		
	GG	0.650 (78/120)
	GA	0.3257 (39/120)
	AA	0.025 (31/120)
rs5030655 (1707delT, *CYP2D6*6*)		
	TT	0.942 (113/120)
	T delT	0.025 (3/120)
	delT delT	0.008 (1/120)

The influence of genetic polymorphism on therapeutic response

The analysis of the distribution of the frequency of individual genotypes of the tested enzymes and the value of the change in PANSS scale scores showed that there was no statistically significant difference between the mentioned variables ($\chi 2$, $p>0.05$) for any combination of changes in PANSS score and enzyme genotypes (tables 5–13).

UGT1A4

Table 5. Influence of UG1A4*2 genotype variation on therapeutic response measured by the PANSS scale

UG1A4*2					
UG1A4		N	Mean	Standard Deviation	T-test, df, p
PANSS_delta	0	112	15.1	4.7	0.8, 118, 0.418
	1	8	16.5	3.6	

0-wild-type homozygote; 1-heterozygous

CYP1A2

Table 6. Influence of *CYP1A2*1F* genotype variation on therapeutic response measured by the PANSS scale

*CYP1A2*1F*						
PANSS_delta						
Variation	N	Mean	Standard Deviation	Minimum	Maximum	ANOVA, df, p
0	14	14.8	4.3	6.3	22.2	0.1, 2, 0.920
1	55	15.3	4.7	2.0	23.8	
2	51	15.3	4.8	3.7	25.0	
Total	120	15.2	4.7	2.0	25.0	

0-wild-type homozygote; 1-heterozygous; 2-variant homozygote

Table 7. Influence of *CYP1A2 A1/A2* genotype variation on therapeutic response measured by the PANSS scale

CYP1A2 A1/A2						
PANSS_delta						
Variation	N	Mean	Standard Deviation	Minimum	Maximum	ANOVA, df, p
0	86	15.3	4.8	2.0	25.0	0.4, 2, 0.691
1	31	15.1	4.5	5.3	23.9	
2	3	13.0	4.8	7.7	17.2	
Total	120	15.2	4.7	2.0	25.0	

0-wild-type homozygote; 1-heterozygous; 2-variant homozygote

FMO3

Table 8. Effect of *FMO3* E158K genotype variation on therapeutic response measured by the PANSS scale

E158K						
PANSS_delta						
Variation	N	Mean	Standard Deviation	Minimum	Maximum	ANOVA, df, p
0	48	15.0	4.6	5.7	25.0	0.5, 2, 0.608
1	55	15.7	4.5	3.7	23.9	
2	17	14.5	5.6	2.0	21.1	
Total	120	15.2	4.7	2.0	25.0	

0-wild-type homozygote; 1-heterozygous; 2-variant homozygote

Table 9. Effect of *FMO3* V257M genotype variation on therapeutic response measured by the PANSS scale

V257M						
PANSS_delta						
Variation	N	Mean	Standard Deviation	Minimum	Maximum	ANOVA, df, p
0	115	15.2	4.6	2.0	25.0	0.2, 2, 0.797
1	4	16.6	6.7	7.7	22.1	
2	1	16.7	n.a.	16.7	16.7	
Total	120	15.2	4.6	2.1	25.0	

0-wild-type homozygote; 1-heterozygous; 2-variant homozygote; n.a.-not applicable

Table 10. Influence of the *FMO3* E308G genotype variation on the therapeutic response measured by the PANSS scale

E308G						
PANSS_delta						
Variation	N	Mean	Standard Deviation	Minimum	Maximum	ANOVA, df, p
0	87	15.3	4.5	2.0	25.0	0.2, 2, 0.819
1	31	15.1	5.1	3.8	23.9	
2	2	13.3	4.5	10.1	16.5	
Total	120	15.2	4.7	2.0	25.0	

0-wild-type homozygote; 1-heterozygous; 2-variant homozygote

CYP2D6

Table 11. Influence of *CYP2D6*3* genotype variation on therapeutic response measured by the PANSS scale

*CYP2D6*3*					
PANSS_delta					
CYP2D6*3		N	Mean	Standard Deviation	T-test, df, p
PANSS_delta	0	118	15.2	4.7	0.67, 118, 0.504
	1	2	17.4	1.3	

0-wild-type homozygote; 1-heterozygous; 2-variant homozygote

Table 12. Influence of *CYP2D6*4* genotype variation on therapeutic response measured by the PANSS scale

*CYP2D6*4*						
PANSS_delta						
Variation	N	Mean	Standard Deviation	Minimum	Maximum	ANOVA, df, p
0	77	15.0	4.8	2.0	23.9	0.6, 2, 0.549
1	40	15.4	4.6	5.3	25.0	
2	3	17.9	2.3	16.5	20.6	
Total	120	15.2	4.7	2.0	25.0	

0-wild-type homozygote; 1-heterozygous; 2-variant homozygote

Table 13. Influence of *CYP2D6*6* genotype variation on therapeutic response measured by the PANSS scale

*CYP2D6*6*						
PANSS_delta						
Variation	N	Mean	Standard Deviation	Minimum	Maximum	ANOVA, df, p
0	116	15.2	4.7	2.0	25.0	1.1, 2, 0.317
1	3	13.2	3.5	10.3	17.2	
2	1	21.4	n.a.	21.4	21.4	
Total	120	15.2	4.7	2.0	25.0	

0-wild-type homozygote; 1-heterozygous; 2-variant homozygote

DISCUSSION

In our study, 6.7% of the subjects were heterozygous carriers. Variant alleles normally occur in Caucasians in about 8% of cases and are very rare or absent in homovariant form (16), which is the case with the patients in our study. In a 2005 study that included the Japanese population, the presence of neither homozygous nor heterozygous variants of this gene were detected (17). The extremely rare presence of this variant, in addition to Caucasians, is also confirmed by Lopez et al. in 2013, where they even state the differences within the Caucasian race, i.e., that Mexicans have a lower presence of this variant compared to Europeans, but still more often than Asian peoples (18).

As for CYP1A2*1C, only the wild type of the enzyme genotype was detected in the studied population. These results differ from those obtained in some other populations. In a study conducted on the Turkish population, it was found that one in ten carriers of the variant allele (as many as 2/3 of them are of the homovariant type). The representation of the variation varies from region to region, so the CYP1A2*1C allelic frequency in East Asia is present in 28% of the population, compared to only 2% in white Europeans. An even higher frequency of variation was found in Africa (31%) and in African Americans (36%). It is slightly lower in South Asia and China (20–24%), significantly lower than in these populations, but still four times more common in Hispanics (8%) (19, 20).

The representation of CYP1A2*1F was significantly more dispersible, and the presence of both wild and variant homozygotes as well as heterozygotes was recorded, where almost 90% were carriers of the variation, which is otherwise a characteristic of the white European population (21, 22). A somewhat smaller representation of the variant allele is among Asian peoples, while it is rarest, but again in half of the cases, found in Africa (19).

CYP1A1/A2 was investigated somewhat less frequently, possibly because the SNP is located between the CYP1A1 and CYP1A2 genes on chromosome 15q24 (23). In the population of patients who participated in our study, a quarter were carriers of the variant allele, among which only a few were carriers of the homovariant type. In other European nations, the prevalence of this polymorphism is even rarer and ranges from 10 to 19%. It is somewhat more common (in a similar percentage as in our study) in Hispanic Americans, while in Asian peoples it exceeds 30%, and in the Chinese population even 50% (24).

In our research, three genotypes were monitored: E158K, V257M, and E308G. All three forms of the E158K genotype were detected in the examined patient population. Almost ¾ of the patients are carriers of the variant allele, and among them, 17% (of the total population) are carriers of the homovariant type. This distribution of variant alleles is characteristic of the white European population. An example is a study from 2014 where the percentage of the variant

allele in the population of Russians from Kursk and Belgorod is almost identical to that of our study (25), while the homovariant type is much more common, even up to half in some Asian peoples (26).

The results obtained in our study show that the presence of the V257M variant allele in our population is rare, i.e., below 5%, and only as heterozygous carriers. Such a rare presence of this polymorphism is normally a characteristic of the white European race, although some studies have shown that the percentage exceeds 10% (e.g., in Ireland) with the presence of the homovariant type (27). The presence of the E308G genotype polymorphism in our study population was present in every fourth subject, but only two were carriers of the homovariant form. In other European nations, the variation is slightly more common and ranges from 30 to 35%, with the presence of the homovariant type also being more frequent (3–6%, while in our country it was less than 2%) (27). The presence of variations is significantly more common in Asian peoples and, for example, reaches 46% in the Korean population (28).

CYP2D6*3 was a very rare variation in the studied population, i.e., only two patients (1.7%) were registered as heterozygous carriers. The presence of variant carriers is much more common in some peoples, and in Africa, it ranges up to 9% in some parts of Zimbabwe (29). While in Europe and Asia, the presence of variations is much lower and does not exceed 3% (30).

In the case of the CYP2D6*4 genotype, variations were more frequent and were detected in a third of subjects. The homovariant type was extremely rare and was detected in only 3 patients. It is known that the presence of variant alleles is much more common in European nations and ranges from 18.5 to 26.3%, while it is far less common in Asians (0.42–7.7%) (31). Proof of this is a study from Russia, where this polymorphism is 12.2 times more common in Russians living in the European part compared to smaller ethnic groups (Nanai people) that inhabit the far east of the Asian part of the country (32). As far as other areas are concerned, a study from Brazil showed that the presence of the variant allele is about 13%, which is expected due to the different racial origin or mixing of the inhabitants of this country (33).

The presence of CYP2D6*6 variations was extremely rare in our studied population. Three heterovariants and one homovariant carrier were detected (3.3%). This variation is otherwise rare. Current knowledge indicates that it has reached the highest percentage in the European population and is around 2%, while in other parts of the world (Asia, Africa, and America), it is even rarer and is found only in a few per thousand inhabitants (30).

Polymorphism of metabolic enzymes and olanzapine. In our study, we tested polymorphisms of the olanzapine metabolizing enzymes UGT1A4, CYP1A2, FMO3, and CYP2D6; they did not, on the whole, affect the clinical response to olanzapine therapy. Previous research was mostly focused on the influence of the polymorphism of these enzymes on the concentration of olanzapine in the blood and/or its metabolites and not on the clinical outcomes themselves, where some variations were shown to be able to change the biotransformation of the drug, independently or in the presence of inducers. Acceleration of the metabolism of olazapine or a decrease in concentrations has previously been found in carriers of UGT1A4*3, CYP1A2*1F, and CYP1A2A1/A2 rs2472297C>T variants (9, 34, 35), while a decrease in the metabolic reactions of olanzapine has been shown for carriers of UGT2B10*2 (36), FMO1*6, and FMO3 E308G variations (9).

However, the influence of individual polymorphisms on the metabolism, concentrations, and clinical response of olanzapine is very complex. The main reason for this is the fact that the elimination of olanzapine is mediated by a larger number of enzymes that make up two metabolic pathways, one of which is the main one. Additionally, the metabolism of olanzapine is significantly influenced by other, non-genetic factors, of which the effect of exogenous inducers and inhibitors is one of the most pronounced. Methodological aspects of the design of individual studies are also important. Such circumstances are probably one of the important reasons for different findings when analyzing the same genetic variations.

Limitations of the study and future directions

Interpretation and extrapolation of the results of our study to a wider population of patients treated with olanzapine should be considered in light of possible methodological limitations. First of all, the total study sample is not too large. Many similar studies had the same approximate number of respondents as our research, and some were significantly smaller. However, due to the relatively low frequency of individual variations of the tested genes, significant differences that would exist in their carriers and whose influence would be more significantly observed could not be ruled out in a larger sample. More valid results in this sense could be obtained by including the widest population (genome-wide studies).

Our research has brought certain new findings that justify and theoretically support further research in this area. First of all, it is necessary to continue studying the influence of other, insufficiently tested polymorphisms, not only of genes for metabolizing enzymes but also of other molecules that are important for the therapeutic effects of olanzapine (target receptors, transporters).

CONCLUSIONS

Presence of gene variations CYP1A2*1C (rs2069514, -3860G>A), CYP1A2 (rs2472297, 74735539C>T), FMO3 E158K (rs2266782, 15167G>A), FMO3 V257M (rs1736557, 18281G>A), FMO3 E30 8G (rs2266780, 21443A>G), CYP2D6*3 (rs35742686, 2549delA), CYP2D6*4 (rs3892097, 1846G>A), CYP2D6*6 (rs5030655, 1707delT) does not change the clinical response to

olanzapine therapy in patients with schizophrenia, compared to patients who are carriers of the wild-type gene.

In our study, the presence of the investigated gene variations (UGT1A4, CYP1A2, FMO3, and CYP2D6) does not affect the clinical response to olanzapine therapy in patients suffering from schizophrenia, compared to patients who are carriers of the wild-type gene.

The results of our study, regarding the overall impact of individual tested polymorphisms in the metabolizing enzymes of olanzapine, are in high agreement with the previous findings. The influence of individual polymorphisms on the metabolism, concentrations, and clinical response of olanzapine is very complex. Additionally, the metabolism of olanzapine is significantly influenced by other, non-genetic factors, of which the effect of exogenous inducers and inhibitors is one of the most pronounced.

ACKNOWLEDGMENTS

The authors would like to express their deepest gratitude to all those who, with their misdeeds, encouraged them to work even harder and achieve scientific success.

DISCLOSURE STATEMENT

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

This research received no external funding.

REFERENCES

1. Hálfdánarson Ó, Zoëga H, Aagaard L, Bernardo M, Brandt L, Fusté AC, et al. International trends in antipsychotic use: A study in 16 countries, 2005-2014. Eur Neuropsychopharmacol. 2017; 27(10): 1064-76.
2. Skogh E, Reis M, Dahl ML, Lundmark J, Bengtsson F. Therapeutic drug monitoring data on olanzapine and its N-demethyl metabolite in the naturalistic clinical setting. Ther Drug Monit. 2002; 24(4): 518-26.
3. Batail JM, Langrée B, Robert G, Bleher S, Verdier MC, Bellissant E, et al. Use of very-high-dose olanzapine in treatment-resistant schizophrenia. Schizophr Res. 2014; 159(2-3): 411-4.
4. Kroon LA. Drug interactions with smoking. Am J Health Syst Pharm. 2007; 64(18): 1917-21.
5. Murray M. Role of CYP pharmacogenetics and drug-drug interactions in the efficacy and safety of atypical and other antipsychotic agents. J Pharm Pharmacol. 2006; 58(7): 871-85.
6. Sachse C, Brockmöller J, Bauer S, Roots I. Functional significance of a C-->A polymorphism in intron 1 of the cytochrome P450 CYP1A2 gene tested with caffeine. Br J Clin Pharmacol. 1999; 47(4): 445-9.
7. Ghotbi R, Christensen M, Roh HK, Ingelman-Sundberg M, Aklillu E, Bertilsson L. Comparisons of CYP1A2 genetic polymorphisms, enzyme activity and the genotype-phenotype relationship in Swedes and Koreans. Eur J Clin Pharmacol. 2007; 63(6): 537-46.
8. Patel MX, Bowskill S, Couchman L, Lay V, Taylor D, Spencer EP, et al. Plasma olanzapine in relation to prescribed dose and other factors: data from a therapeutic drug monitoring service, 1999-2009. J Clin Psychopharmacol. 2011; 31(4): 411-7.
9. Söderberg MM, Haslemo T, Molden E, Dahl ML. Influence of FMO1 and 3 polymorphisms on serum olanzapine and its N-oxide metabolite in psychiatric patients. Pharmacogenomics J. 2013; 13(6): 544-50.
10. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, Washington, DC, American Psychiatric Association, 2013.
11. Taylor D, Paton C, Kapur S. The Maudsley Prescribing Guidelines in Psychiatry, 12th Edition. London, Wiley-Blackwell, April 2015, pp. 90-143.
12. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013; 310(20): 2191-4.
13. Overall JE, Gorham DR. The Brief Psychiatric Rating Scale. Psychol Rep. 1962; 10: 799-812.
14. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull. 1987; 13(2): 261-76.
15. Andreasen NC, Carpenter WT Jr, Kane JM, Lasser RA, Marder SR, Weinberger DR. Remission in schizophrenia: proposed criteria and rationale for consensus. Am J Psychiatry. 2005; 162(3): 441-9.
16. Ehmer U, Vogel A, Schütte JK, Krone B, Manns MP, Strassburg CP. Variation of hepatic glucuronidation: Novel functional polymorphisms of the UDP-glucuronosyltransferase UGT1A4. Hepatology. 2004; 39(4): 970-7.
17. Saeki M, Saito Y, Jinno H, Sai K, Hachisuka A, Kaniwa N, et al. Genetic variations and haplotypes of UGT1A4 in a Japanese population. Drug Metab Pharmacokinet. 2005; 20(2): 144-51.
18. López M, Dorado P, Ortega A, Peñas-Lledó E, Monroy N, Silva-Zolezzi I, et al. Interethnic differences in UGT1A4 genetic polymorphisms between Mexican Mestizo and Spanish populations. Mol Biol Rep. 2013; 40(4): 3187-92.
19. International HapMap Project. Available online: https://catalog.coriell.org/1/NHGRI/Collections/HapMap-Collections/HapMap-Project (accessed on 1 April 2024).
20. Arici M, Özhan G. The genetic profiles of CYP1A1, CYP1A2 and CYP2E1 enzymes as susceptibility factor in xenobiotic toxicity in Turkish population Saudi Pharm J. 2017; 25(2): 294-7.
21. Salinero JJ, Lara B, Ruiz-Vicente D, Areces F, Puente-Torres C, Gallo-Salazar C, et al. CYP1A2 Genotype

Variations Do Not Modify the Benefits and Drawbacks of Caffeine during Exercise: A Pilot Study. Nutrients. 2017; 9(3): 269.
22. Urry E, Jetter A, Landolt HP. Assessment of CYP1A2 enzyme activity in relation to type-2 diabetes and habitual caffeine intake. Nutr Metab (Lond). 2016; 13: 66.
23. Sulem P, Gudbjartsson DF, Geller F, Prokopenko I, Feenstra B, Aben KK, et al. Sequence variants at CYP1A1-CYP1A2 and AHR associate with coffee consumption. Mol Genet. 2011; 20(10): 2071-7.
24. Barbieri RB, Bufalo NE, Cunha LL, Assumpção LV, Maciel RM, Cerutti JM, et al. Genes of detoxification are important modulators of hereditary medullary thyroid carcinoma risk. Clin Endocrinol (Oxf). 2013; 79(2): 288-93.
25. Bushueva O, Solodilova M, Churnosov M, Ivanov V, Polonikov A. The Flavin-Containing Monooxygenase 3 Gene and Essential Hypertension: The Joint Effect of Polymorphism E158K and Cigarette Smoking on Disease Susceptibility. Int J Hypertens. 2014; 2014: 712169.
26. Tang Y, Hu K, Huang W, Wang C, Liu Z, Chen Y, et al. Effects of FMO3 Polymorphisms on Pharmacokinetics of Sulindac in Chinese Healthy Male Volunteers. Biomed Res Int. 2017; 2017: 4189678.
27. Dolan C, Shields DC, Stanton A, O'Brien E, Lambert DM, O'Brien JK, et al. Polymorphisms of the Flavin containing monooxygenase 3 (FMO3) gene do not predispose to essential hypertension in Caucasians. BMC Med Genet. 2005; 6: 41.
28. Park CS, Kang JH, Chung WG, Yi HG, Pie JE, Park DK, et al. Ethnic differences in allelic frequency of two flavin-containing monooxygenase 3 (FMO3) polymorphisms: linkage and effects on in vivo and in vitro FMO activities. Pharmacogenetics. 2002; 12(1): 77-80.
29. Rajman I, Knapp L, Morgan T, Masimirembwa C. African Genetic Diversity: Implications for Cytochrome P450-mediated Drug Metabolism and Drug Development. EBioMedicine. 2017; 17: 67-74.
30. Dean L. Tamoxifen therapy and CYP2D6 genotype. In: Medical Genetics Summaries. National Center for Biotechnology Information (US): Bethesda; 2016.
31. LLerena A, Naranjo ME, Rodrigues-Soares F, Penas-LLedó EM, Fariñas H, Tarazona-Santos E. Interethnic variability of CYP2D6 alleles and of predicted and measured metabolic phenotypes across world populations. Expert Opin Drug Metab Toxicol. 2014; 10(11): 1569-83.
32. Sychev DA, Shuev GN, Suleymanov SS, Ryzhikova KA, Mirzaev KB, Grishina EA, et al. Comparison of CYP2C9, CYP2C19, CYP2D6, ABCB1, and SLCO1B1 gene-polymorphism frequency in Russian and Nanai populations. Pharmgenomics Pers Med. 2017; 10: 93-9.
33. De Ameida Melo M, De Vasconcelos-Valença R, Neto F, Borges R, Costa-Silva D, Da Conceição Barros-Oliveira M, et al. CYP2D6 gene polymorphisms in Brazilian patients with breast cancer treated with adjuvant tamoxifen and its association with disease recurrence. Biomed Rep. 2016; 5(5): 574-8.
34. Haslemo T, Loryan I, Ueda N, Mannheimer B, Bertilsson L, Ingelman-Sundberg M, et al. UGT1A4*3 encodes significantly increased glucuronidation of olanzapine in patients on maintenance treatment and in recombinant systems. Clin Pharmacol Ther. 2012; 92(2): 221-7.
35. Czerwensky F, Leucht S, Steimer W. CYP1A2*1D and *1F polymorphisms have a significant impact on olanzapine serum concentrations. Ther Drug Monit. 2015; 37(2): 152-60.
36. Erickson-Ridout KK, Zhu J, Lazarus P. Olanzapine metabolism and the significance of UGT1A448V and UGT2B1067Y variants. Pharmacogenet Genomics. 2011; 21(9): 539-51.

CORRELATION OF WOMAC INDEX AND CYTOKINE VALUES IN PATIENTS WITH OA KNEE

Jelena Miloševic[12], Vesna Grbović[13] and Aleksandra Jurišić-Škevin[13]

[1] *University of Kragujevac, Faculty of Medical Sciences, Department of Physical medicine and rehabilitation, Serbia*
[2] *University Cinical Center of Kragujevac, Clinic of Orthopedics and Traumatology, Serbia*
[3] *University Clinical Center of Kragujevac, Department of Physical medicine and rehabilitation, Serbia*

Received: 01.02.2022.
Accepted: 01.03.2022.

Corresponding author:

Jelena Milosevic

University of Kragujevac, Faculty of Medical Sciences, Department of Physical medicine and rehabilitation and Clinic of Orthopedics and Traumatology, University Cinical Center of Kragujevac, Serbia
Svetozara Markovica 69, 34000 Kragujevac, Serbia

Phone: + 381 64 2750927

E-mail: jecas0109@gmail.com

UDK:

Eabr 2026; 27(3): 249-254
DOI: 10.2478/sjecr-2022-0036

ABSTRACT

Osteoarthritis (OA) of the knee (gonarthrosis) is a degenerative rheumatic disease, which is the most common rheumatic disease. Numerous factors, such as mechanical, enzymatic, and biological, are responsible for the development of this complex, multifactorial disease. The connection between inflammation and the development of knee OA is being investigated more and more. Cytokines are thought to be one of the main causes of disturbances in normal cartilage metabolism.

This study, designed as a prospective cohort study included 60 patients with knee OA. The examinees were recruited from total population of patients with knee OA, hospitalized or treated as outpatients at Department of Physical Medicine and Rehabilitation, University Clinical Centre Kragujevac. After the consent was obtained, anamnestic data were collected, blood samples were taken and functional testing was done together with anthropometric measurements. The values of the following cytokines were determined from a blood sample: IL-6, TNF-α, IL-10, IL-17. The average age of patients was 69.10±9.06 years, most of whom were females (78,3%). Both IL-6 and TNF-α correlated positively with WOMAC scores. WOMAC index subscales showed different association with cytokines. In our studies the level of cytocines in the serum of pacients with OA knee is show not correlation with radiographic image.

The present study demonstrated than the concentrations of proinflammatory cytokines (TNF-α IL-6) can be correlated with WOMAC scores of knee OA patients. The results indicate that concentrations of TNF-α and IL-6 may affect knee joint function in patients with knee OA.

Keywords: *Knee osteoarthritis, WOMC index, cytokines, TNF-α IL-6, IL-10, IL-17.*

INTRODUCTION

Osteoarthritis (OA) of the knee is a degenerative rheumatic disease, which is the most common rheumatic disease. The changes in the OA of the knee primarily affect the articular cartilage, we must not forget that the changes also occur in the synovium and subchondral part of the bone (1, 2, 3, 4). Numerous factors, such as mechanical, enzymatic, and biological, are responsible for the development of this complex, multifactorial disease. The connection between inflammation and the development of knee OA is being investigated more and more (4, 5). In healthy individuals, there is a balance between wear and tear and cartilage regeneration. Cytokines are thought to be one of the main causes of disturbances in normal cartilage metabolism (4). In the process of inflammation chondrocytes become the target of catabolic cytokines (IL-1β, tumor necrosis factor (TNF) -α, IL-6), so cytokines are important in the development of inflammation in OA of the knee (6, 7, 8). As the OA knee complex is a disease of probably several different cytokines, in the form of a cytokine network it acts together in initiating the pathogenesis of the OA knee (9).

Tumor necrosis factor (TNF) - α is considered to be the major cytokine involved in the pathophysiological processes that occur in OA of the knee. Increased concentrations of TNF - α in synovial fluid, synovial membrane, cartilage and subchondral bone layer have been demonstrated in patients with knee OA (10).

IL-6 is considered a cytokine that strongly activates the immune system and enhances the response to inflammation (7,10). The effect of IL-6 together with other cytokines causes a decrease in the production of type II collagen, and is considered a key cytokine that causes changes in the subchondral part of the bone. With the synergistic effect of IL-6 and TNF-α, they lead to accelerated osteoclast formation, and thus bone reabsorption (10).

IL-10 has numerous immune functions, inhibits the synthesis of proinflammatory cytokines IL-2, IL-4, TNF-α, has the ability to affect the suppression of antigen presenting cells, affects the differentiation of B cells (11). IL-10 can affect chondrocyte proliferation and differentiation thereby contributing to enchondral bone formation (8). Thus, it is known that IL-10 has a chondroprotective effect in chondrocytes and cartilage tissue, not only anti-inflammatory, anticatabolic and anti-apoptotic effect. Increased values of TNF - α and IL-10 can be interpreted as a compensatory attempt to slow the onset of inflammation in OA (8).

There is a growing number of reports in clinical and animal studies that support a role for IL/17 cytokines in OA. IL-17 can affect cartilage destruction, bone remodeling, and pain sensitization (5, 12).

Many tests are used today to evaluate the functional capabilities of patients with OA of the knee, the most well-known and most commonly used test being the WOMAC index.

There are few studies comparing cytokine values with the functional capacity of patients with OA of the knee.

AIM

The main aim of the research is to examine the connection between cytokine values and the functional ability of patients with OA of the knee. In line with this basic aim of the study, secondary objectives are to determine the association between cytokine values and radiographic characteristics, C reactive protein (CRP) values as well as disease duration, Body Mass Index (BMI) of patients with OA of the knee.

MATERIAL AND METHODS

This study, designed as a prospective cohort study, was conducted in the Department of Physical Medicine and Rehabilitation of the University Clinical Center in Kragujevac (UCC) in the period from February to December 2018. The protocol of this study was approved by the Ethics Committee of UCC Kragujevac. A total of 60 respondents for the study were recruited from the total population of patients with OA of the knee who were treated on an outpatient or inpatient basis in the Department of Physical Medicine and Rehabilitation of the University Medical Center "Kragujevac".

Inclusion criteria were: subjects of both sexes aged 40-70 years, with a confirmed diagnosis of OA of the knee according to the criteria of the American College of Rheumatology (13), Kellgren and Lawrence (KL) radiographic osteoarthritis grade 2 and 3 to moderate radiographic osteoarthritis) (14), pain in the knee joint for 3 months and longer. All respondents signed a consent form to participate in the study. Criteria for exclusion from the study were as follows: subjects who had implanted knee endoprosthesis, knee joint injury during the last 6 months, secondary rheumatoid or septic arthrosis or systemic disease involving the knee joint, active OA of the knee with intra-articular effusion, as well as subjects who in parallel intervention studies.

Before joining the study, all respondents first signed an informed consent to participate in the study. After that, anamnestic data were collected, and then a blood sample was taken from the subjects and functional testing (WOMAC questionnaire) and anthropometric measurement were performed.

Sample collection procedure: in the Service for Physical Medicine and Rehabilitation of the University Medical Center Kragujevac, a blood sample was taken from the peripheral vein according to the valid standards. After taking the blood, the tubes were sent to the immunological laboratory of the Faculty of Medical Sciences for analysis. The values of the following cytokines were determined from a blood sample: IL-6, TNF-α, IL-10, IL-17.

Measurement of TNF-α, IL-6, IL-17, IL-10 in serum samples. The blood samples of from all patients were collected before any therapeutic application. After the blood samples were taken, blood clot was cut and centrifuged for separating the serum. Serum samples were kept at -80 °C before testing. Serum levels of molecules of interest were measured using sensitive enzyme-linked immunosorbent assay (ELISA) kits specific for human cytokines (R&D Systems, Minneapolis, MN, for TNF-α, IL-6, IL-17, IL-10, according to the manufacturer's instructions (15). Concentrations of cytokines were determined by interpolation of a standard curve. Results were expressed as picogram per milliliterof patient serum.

The WOMAC questionnaire (Western Ontario and McMaster Universities) is a three-dimensional disease-specific instrument used in a patient with OA of the knee or hip, measuring pain, stiffness, physical function during typical daily activities. The questions refer to the patient's condition during the last 48 hours. The patient completed the WOMAC questionnaire by selecting one of the offered answers (marking with X). It consisted of three parts: the first part of the questionnaire included questions about the severity of knee pain (5 questions in total). The second part of the questionnaire included questions related to joint stiffness (2 questions). The third part of the questionnaire included questions related to the function of the patient, ie difficulties during the performance of everyday life activities (17 questions) (16).

Anthropometric evaluation included the measurement of body weight (kg) and body height (cm). Body weight was measured using a standing scale, and body height on an anthropometer. Both measures are taken to calculate the BMI (Body Mass Index) (kg / m2).

The statistical program SPSS version 20 for Windows (SPSS Inc.) was used for all statistical analyzes. Descriptive statistics methods (mean, standard deviation) were used to analyze the socio-demographic characteristics. Spearman Correlation coefficient was used to examine the association between the WOMAC, KL scale, CRP and TNF-α, IL-6, IL-17, IL-10. Level of $p>0.05$ was used to determine statistical significance.

RESULTS

Patient demographics

Our study included 60 patients with knee OA, most of whom were females 47 (78,3%). The average age of patients was 69.10±9,06 years, with average length of hardship 9.36±5.95 years. The average BMI was 29.69±4.97. Majority of patients,32 (53,3%) had 2 degree by K/L score. Table 1 shows the patient demographics.

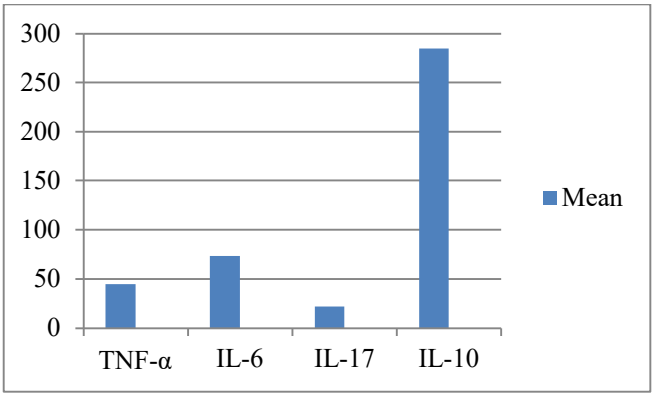
Table 1. Demographic characteristics

Demographic characteristics	
Age (years), mean±SD	69.10±9.06
Males/females (%)	13 (21.7) / 47 (78.3)
Body mass index (BMI) (kg/m^2), mean±SD	29.69 ±4.97
Length of disease, mean±SD	9.36±5.95
K/L score	
2	32 (53,3%)
3	28 (46,7%)

Blood Samples

Graph 1 shows the serum cytokine concentration (TNF-α 44,17 (±86,03), ИЛ- 6 73,51 (±63,44), ИЛ-10 284,78 (±584,11). ИЛ-17 21,74 (±47,70))

Graph 1. Serum cytokine values



Correlation Analysis

Mean WOMAC scores in the OA patients 52,47±11,51 (pain 11,14±2,50; stiffness 3,05±1,31; difficulties 38,24±8,66). Correlation analyses of variables are given in table 3. Both IL-6 and TNF-α correlated positively with WOMAC scores. WOMAC index subscales showed different association with cytokines. The most statistically significant association was between cytokines and functionality. In our studies the level of cytocines in the serum of pacients with OA knee is show not correlation with radiographic image (table 2). The CRP value was correlated with the WOMAC index, but did not show an association with cytokine values (tabel 2). The duration of the discomfort was not correlated with cytokine values (tabel 2).

Table 2. Correlation of WOMAC index and KL score, CRP, BMI

variables		TNF-α	IL-6	IL-17	IL-10
WOMAC total	Spearman*	-0,439	-0,404	0,224	-0,44
	P	0.000	0,001	0,070	0,726
WOMAC pain	Spearman*	-0,542	-0,459	0,230	-0,020
	P	0.000	0,000	0,64	0,874
WOMAC stiffnes	Spearman*	-0,235	-0,188	0,142	-0,109
	P	0,057	0,130	0,254	0,384
WOMAC difficulties	Spearman*	-0,411	-0,386	0,236	-0,031
	P	0,001	0,001	0,056	0,805
K/L score	Spearman*	-0,097	-0,030	-0,124	-0,050
	P	0,436	0,809	0,322	0,693
CRP	Spearman*	-0,099	0,000	-0,189	0,166
	P	0,430	0,999	0,128	0,184
BMI	Spearman*	-0,092	-0,042	-0,003	-0,127
	P	0,463	0,737	0,980	0,311

DISCUSSION

There are more and more studies that study the effects of cytokines on OA knee. In our study we investigated the serum concentration of proinflammatory cytokines (IL-6, IL-17, and TNF-α) and anti-inflammatory cytokines (IL-10) in patients with knee OA and associated those with the functional ability of the patient.

Numerous studies have examined the association between pain and cytokine values. There are few studies that have examined the functionality of patients with OA of the knee and the value of cytokines. By analyzing the results of our study, some differences were found between IL in terms of their correlation with the WOMAC score and its subscales. An association between WOMAC index values and TNF-α and IL-6 has been shown. Although IL-10 is an anti-inflammatory cytokine that essentially regulates the production of proinflammatory cytokines, no correlation has been found between its value and the value of the WOMAC index. Numerous studies have shown a positive association between pain, as the main symptom of knee OA, and cytokine values. One of these studies followed IL-17 level in synovial fluid was positively correlated with WOMAC pain score in OA knee patients (17). However, we also found a study showing a negative correlation between WOMAC pain and IL-17 (18). IL-17 can stimulate bone reabsorption, some studies have shown increased values of IL-17 in patients with rheumatoid arthritis. (17, 19). Also IL-17 can affect synovial cells, macrophages, osteocytes to produce TNF-α and IL-6, the major proinflammatory cytokines in OA (17). Based on previous research, IL-17 can be considered a key factor in causing inflammation in OA. The analysis of the results of our study did not find a correlation between the total WOMAC index and the value of IL-17, but showed a correlation between the WOMAC index subscale related to functionality and IL-17.

Beyond being simply a biomarker of inflammation, CRP may reflect or contribute to molecular mechanisms of disease states. Research have suggested that lower levels of CRP - as seen in chronic, subclinical inflammatory states might enhance inflammation by activating endothelium and proinflammatory cytokine synthesis. IL-6 and IL-1 are known to promote hepatocyte CRP production., studies have demonstrated that IL-6 is the chief regulator of CRP production by hepatocytes (20). Several studies have shown a correlation between synovial fluid IL-6 levels and CRP but not serum IL-6 levels (21, 22). Our results were consistent with the results of a previous study, the CRP value was not correlated with cytokine values. Babaei et al in their study showed a negative correlation between IL-10 values and CRP measured from synovial fluid (23). In our work, there was also a negative correlation between CRP and IL-10, which is explained by the fact that high values of IL-10 associated with reduced values of CRP are consistent with the anti-inflammatory and protective characteristics of IL-10.

Comparing the results of different studies, we came to the conclusion that the results differ significantly. In some studies, an association between radiographic image severity and IL-6, IL-17 values from both synovial fluid and serum was shown (10,24). However, synovial fluid (SF) levels of IL-6 have been shown not to correlate with OA knee (KL) (25). In our study, no relationship was found between interleukin values and radiographic image severity. Some studies have shown that IL-6 levels may increase in early-stage OA. As elevated IL-6 values are associated with early-stage OA of the knee, this may be useful in explaining why there is no correlation between KL scores and IL-6 values in our patients because we followed only patients who were on the KL scale at score 2 and 3. Although some studies have shown that some ILs are associated with cartilage destruction, (26) some other studies have shown that TNF-α, measured from

synovial fluid, was not correlated with the weight of the radiographic image (19).

BMI and interleukins were not correlated in our study, which was in accordance with the results of other studies.

The present study demonstrated than the concentrations of proinflammatory cytokines (TNF-α IL-6) can be correlated with WOMAC scores of knee OA patients. The results indicate that concentrations of TNF-α and IL-6 may affect knee joint function in patients with knee OA. All cytokines monitored in our study were not correlated with radiographic evaluation. Although the level of CRP may affect the synthesis of proinflammatory cytokines, our results did not show a correlation between CRP and cytokines.

The limitation of this study is that cytokine values were determined only from serum and not from synovial fluid. Subsequent studies could bridge the gap between functionality in patients with knee OA and serum cytokines and synovial fluid cytokines.

The clinical contribution of this study is that we have shown that cytokines, in addition to causing inflammation in the knee joint in OA, can also affect the functionality of patients.

ACKNOWLEDGMENT

The authors would like to express gratitude to the Faculty of Medical Science, University of Kragujevac for Grant No. JP 05/18.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. TE McAlindon, RR Bannuru, MC Sullivan, NK Arden, F Berenbaum, SM Bierma-Zeinstra, et al. OARSI guidelines for the non-surgical management of knee osteoarthritis. Osteoarthritis and Cartilage 2014; 22: 363-88.
2. Abby PE, Akhtar R, Comerford EJ, Bates KT. Tissue material properties and computational modelling of the human tibiofemoral joint: a critical review. PeerJ 2018; 6: e4298.
3. Dieppe P . Developments in osteoarthritis. *Rheumatology* 2011; 50(2): 245-7.
4. Choi YS, Park JK, Kang EH, Lee YK, Kim TK, Chung JH, et al. Cytokine signaling-1 suppressor is inducible by IL-1beta andinhibits the catabolic effects of IL-1beta in chondrocytes: itsimplication in the paradoxical joint-protective role of IL-1beta. Arthritis Res Ther 2013;15(6): R191.
5. Snelling SJB, Bas S, Puskas GJ, Dakin SG, Suva D, Finckh A, et al. Presence of IL-17 in synovial fluid identifies a potential inflammatory osteoarthritic phenotype. PLOS ONE 2017; 12(4): e0175109.
6. Sellam J, Berenbaum F. The role of synovitis in pathophysiology and clinical symptoms of osteoarthritis. Nat Rev Rheumatol 2010; 6(11): 625-35.
7. Loria MP, Dambra P, Moretti B, Patella V, Capuzzimati L, Cavallo E, et al. Tursi. Role of cytokines in gonarthrosis and knee prosthesis aseptic loosening. J Orthop Sci 2004; 9:274-9.
8. Schwarz S, Mrosewski I, Silawal S, Tanzil GS. The interrelation of osteoarthritis and diabetes mellitus: considering the potential role of interleukin-10 and in vitro models for further analysis. Inflammation Research 2018; 67(4): 285-300.
9. Schett G, Elewaut D, McInnes IB, Dayer JM, Neurath MF. How cytokine networks fuel inflammation: Toward a cytokine-based disease taxonomy. Nat Med 2013; 19(7): 822-4.
10. Wojdasiewicz P, Poniatowski LA, Szukiewicz D. The Role of Inflammatory and Anti-Inflammatory Cytokines in the Pathogenesis of Osteoarthritis. Mediators of Inflammation 2014; 1-19.
11. Haynes MK, Hume EL, Bruce Smith JB. Phenotypic Characterization of Inflammatory Cells from Osteoarthritic Synovium and Synovial Fluids. Clin Immunol 2002; 105(3): 315-25.
12. Mimpen JY, Baldwin MJ, Cribbs AP, Philpott M, Carr AJ, Dakin SG, et al. Interleukin-17A Causes Osteoarthritis-Like Transcriptional Changes in Human Osteoarthritis-Derived Chondrocytes and Synovial Fibroblasts *In Vitro.* Front Immunol 2021; 12: 676173.
13. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K et al. The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the knee. Arthritis Rheum 1986; 29: 1039-49.
14. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis 1957; 16: 494-501.
15. Jovanovic, M. Simovic Markovic B, Gajovic N, Jurisevic M, Djukic A, Jovanovic I, et al. Metabolic syndrome attenuates ulcerative colitis: Correlation with interleukin-10 and galectin-3 expression. World J Gastroenterol 2019; 25(43): 6465-82.
16. Bellamy N, Buchana WW, Goldsmith CH, Campel J, Stitt LW. Validation of WOMAC health status instrument for measuring clinically important patient relevation outcomes to antireumatic drug therapy in patients with osteoarthritis hip or knee. J Rheumatol 1988; 15: 1833-40.
17. Liu Y, Peng H, Meng Z, Wei M. Correlation of IL-17 Level in Synovia and Severity of Knee Osteoarthritis. Med Sci Monit 2015; 21: 1732-6.
18. Askari A, Naghizadeh MM, Homayounfar R, Shahi A, Afsarian MH, Paknahad A, et al. Increased serum Levels of IL-17A and IL-23 Are Associated with Decreased Vitamin D3 and Increased Pain in Osteoarthritis. PLoS One 2016; 11(11): e0164757.
19. Orita S, Koshi T, Mitsuka T, Miyagi M, Inoue G, Arai G, et al. Associations between proinflammatory cytokines in the synovial fluid and radiographic grading and pain-related scores in 47 consecutive patients with

osteoarthritis of the knee. BMC Musculoskeletal Disorders 2011; 12(1):144.
20. Stannus O, Jones G, Cicuttini F, Parameswaran V, Quinn S, Burgess J, et al. Circulating levels of IL-6 and TNF-α are associated with knee radiographic osteoarthritis and knee cartilage loss in older adults. Osteoarthritis and Cartilage 2010; 18 (11): 1441-7.
21. Smith JW, Martins TB, Gopez E, Johnson T, Hill HR, Thomas D. Rosenberg. Significance of C-reactive protein in osteoarthritis and total knee arthroplasty outcomes. Ther Adv Musculoskelet Dis 2012; 4(5): 315-25.
22. Pearle AD, Scanzello CR, George BAS, Mandl LA, DiCarlo EF, Peterson M, et al. Elevated high-sensitivity C-reactive protein levels are associated with local inflammatory findings in patients with osteoarthritis. OsteoArthritis and Cartilage 2007; 15: 516-23.
23. Babaei M, Javadian Y, Narimani H, Ranaei M, Heidari B, Basereh H, et al. Correlation between systemic markers of inflammation and local synovitis in knee osteoarthritis. Caspian J Intern Med 2019; 10(4): 383-7.
24. Chen B, Deng Y, Tan Y, Qin J, Chen LB. Association between severity of knee osteoarthritis and serum and synovial fluid interleukin 17 concentrations. J Int Med Res 2014; 42: 138-44.
25. Stannus OP, Jones G, Quinn SJ, Cicuttini FM, Dore D, Ding C. The association between leptin, interleukin-6, and hip radiographic osteoarthritis in older people: a cross-sectional study.Arthritis Res Ther 2010; 12: R95.
26. Attur M, Wang H, Kraus BV, Bukowski FJ, Aziz N, Krasnokutsky S, et al. Radiographic severity of knee osteoarthritis is conditional on interleukin-1 receptor antagonist gene variations. Ann Rheum Dis 2010; 69: 856-61.

DEVELOPMENT AND STABILITY EVALUATION OF NATURAL TOPICAL FORMULATIONS CONTAINING *PINUS SIBIRICA* ESSENTIAL OIL

Branislav Petrovic[1], Jovana Bradic[2,3], Anica Petrovic[2,3], Dragana Ivanovic[4], Marija Tabakovic[4], Svetlana Saric[4] and Vladimir Jakovljevic[3,5,6]

[1]*ADOC doo, 11 Milorada Jovanovica 11 000, Belgrade, Serbia*
[2]*Department of Pharmacy, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia*
[3]*Center of Excellence for Redox Balance Research in Cardiovascular and Metabolic Disorders, Kragujevac, Serbia*
[4]*Institute of Public Health Kragujevac, Center for Hygiene and Human Ecology, Department of Sanitary Microbiology, Serbia*
[5]*Department of Physiology, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia*
[6]*Department of Human Pathology, Sechenov First Moscow State Medical University, Moscow, Russia*

Received: 26.01.2024.
Accepted: 07.03.2024.

Corresponding author:

Associate Prof. Jovana Bradic, PhD

University of Kragujevac, Faculty of Medical Sciences, Department of Pharmacy, Kragujevac, Serbia

E-mail: jovanabradickg@gmail.com

UDK:

Eabr 2026; 27(3): 255-263
DOI: 10.2478/eabr-2024-0003

ABSTRACT

Pinus sibirica (P. sibirica) is used in folk medicine for centuries in treatment of various diseases due to its anti-inflammatory, antioxidant, and antimicrobial activity. The present study was focused on formulating semi-solid topical cream and gel preparations with P. sibirica essential oil (EOPS) and on evaluation of their stability during three months of storage. After preparation of semi-solid formulations (cream and gel), we followed parameters such as organoleptic characteristics, pH values, electrical conductivity, phase separation, washability, swelling index and microbiological contamination. Tests for physical and microbiological stability were performed considering different storage conditions at temperature (4±2° C, 25±2°C and 40±2°C) for 12 weeks. All test were performed at sampling time points of 7th, 30th, 60th, 90th day after storage. Cream with EOPS was white colour, it is homogenous with pleasant odor characteristic to used EOPS while gel with EOPS was transparent, it is homogenous with specific odor. After application to skin, EOPS cream was leaving non-greasy film while gel with EOPS was leaving sticky film and cooling effect. In all tested samples, we observed no phase separation and pH values were optimal for skin application. During three months of storage at different conditions, all tested formulations were stable and addition of EOPS did not cause any changes in followed parameters. Both cream and gel formulation remained stable since we did not notice any significant changes in all followed parameters during storage at different temperatures. Addition of 0.5% of EOPS did not change visual, sensory and microbial properties of formulations.

Keywords: *Pinus sibirica, stability, topical formulations, essential oil.*

INTRODUCTION

The continual evolution of pharmaceutical and cosmetic industry has led to the increasing number of topical products for skin diseases or cosmetic purpose (1). In fact, these products are designed to cure symptoms of various skin diseases (eczema, psoriasis, dermatitis etc) or to enhance skin beauty, slow ageing or enable skin cleaning. In recent years, companies produce numerous topical products that are natural and eco-friendly since there is increasing consumer demand for clean, sustainable, and plant-based products. Herbal products are derived from plant materials such as extracts or essential oils which has already confirmed beneficial properties for the skin (1).

Pinus sibirica (*P.sibirica*) is coniferous plant used in folk medicine worldwide for prevention and treatment of various conditions. Both leaves and seeds of *P.sibirica* are utilized to produce extracts, essential oils or oils rich with numerous active ingredients exerting different effects (lowering blood pressure, lipids and glucose as well as to inhibit allergic reactions). Different kind of extractive products (decoction and infusion) are used for curing rheumatism or kidney and liver disorders (3). In addition, wide variety of dermatological disorders (psoriasis, eczema, pimples, and scabies) and wounds are treating with topical applied *P.sibirica* essential oil (EOPS) (4-6). All abovementioned effects inspired scientists to reveal chemical composition of this coniferous plant and to determine if products of this coniferous plant exert antioxidant, antimicrobial and anti-inflammatory activity. The most abundant compounds in EOPS are α-pinene (40.19%), β-pinene (19.64%) and δ -3-carene (16.14%) (5) that are the most responsible for many pharmacological activities (7, 8).

Selection of topical semisolid dosage form (cream, gel, ointment or paste) depends on skin condition or disorder, site and way of application and solubility of ingredients. Nevertheless, designing novel quality dermatological product is demanding process that requires from manufacturer to guarantee safety and stability of final product during distribution and storage process. Instability of topical product is reflected through phase separation, creaming, aggregation or it can cause skin irritation or infections (9). On the other side, the most common process responsible for formulation instability is oxidation of lipid components that consequently lead to a loss of product efficiency (10). In that sense, numerous methods for verification of product stability during storage at different conditions are developed. All relevant information about products physical and microbiological stability should be provided before releasing product to the market. Therefore, the aim of our investigation was to formulate semi-solid topical preparations containing *P.sibirica* essential oil and to determine their stability during three months of storage.

MATERIALS AND METHODS

Preparation of semi-solid formulations (gel and cream)

One hundred percent pure *P.sibirica* essential oil was purchased from Alekpharm (Belgrade), and stearic acid, cetyl alcohol, cetearyl alcohol, polysorbate 60, sweet almond oil, glycerin, phenoxyethanol, citric acid, carbomer 940, sodium benzoate, propylene glycol and triethanolamine (TEA) were purchased from Unichempharm (Čačak).

The cream base (F1) was made by precisely measuring and mixing separately oil phase components (stearic acid, cetyl alcohol, cetearyl alcohol, polysorbate 60 and sweet almond oil) and aqueous phase (glycerin, aqua purificata, 10% TEA) heated up to 70-80° C. After heating both phases, oil phase was added to aqueous phase with continuous stirring on a laboratory stirrer RV16 basic (IKA®VERKE, Germany). Emulsion was cooled down and preservative and pH adjuster were added. To create formulation F2 EOPS was added in cooling down stage in abovementioned base by levigation method (11). On the other hand, gel base (F3) was prepared with carbomer 940 by dispersing it in water at room temperature. Produced dispersion was stirred with propylene glycol and gel was formed by adding 10% TEA solution. Gel was preserved with sodium benzoate. Formulation F4 was prepared by incorporating 0.5 g EOPS in gel base. All ingredients of F1-F4 formulations are presented in Table 1.

Physical properties of cream and gel with *Pinus sibirica* essential oil

Organoleptic test

Prepared samples were subjected to a set of organoleptic analysis estimated by visual observation and touch: color, odor, homogeneity and presence of phase separation in creams. Moreover, set of parameters were determined after application of samples to skin (type of smear, application/touch) (12).

pH values determination

The digital pH meter (Mettler Toledo, Columbus, OH, USA) was used to evaluate pH values of all formulations (F1-F4) at room temperature. The pH of each formulation was taken for at least three times, and the average was calculated (13).

Electrical conductivity determination

The conductivity meter (Eutech CON 700, Thermo Fisher Scientific, Shanghai, China) was calibrated and electrical conductivity (σ) of all samples was measured at room temperature. All experiments were done in triplicate and average value is presented (14).

Centrifugation test

Centrifugation test was directly performed after cream and gel preparation in order to evaluate changes such as

phase separation. Samples were centrifuged in laboratory centrifuge (Hettich Mikro 120, Germany) at 3000 rpm at room temperature for 30 min. Samples were considered to be stable if they remained homogenous after centrifugation (15).

Washability

In order to test the level of washing of prepared formulations (F1-F4) with distilled water, creams and gels were applied over the skin (16).

Swelling index

One gram of F1-F4 formulation was added to 10 ml distilled water in Petri dish. After that samples were taken and left in dry place for one and three hours. The mass of formulations was weighed and the degree of swelling was established from the formula (17).

$$Swelling\ Index\ (SW)\% = [(Wt - Wo)/Wo] \times 100$$

(SW) % = Equilibrium percent swelling
Wt = Weight of swollen crem/gel after time t
Wo = Original weight of cream/gel at zero time

Assessing the stability of cream and gel with P.sibirica essential oil

Stability analyses (organoleptic characteristics, pH values, electrical conductivity, and centrifugation test, microbiological test) were performed for topical formulations that are storage in incubator (ICT-R 52 FALC, Italy) at different temperatures such as 4±2° C, 25±2°C and 40±2°C. All parameters were observed at various time intervals during three months. The measurements were done after 7th days, after one month and after two and three months (18).

Both cream and gel formulations are tested according to the following standards (SRPS EN ISO 21149: 2017, SRPS EN ISO 16212: 2017, SRPS EN ISO 18416:2016, SRPS EN ISO 21150 :2016, SRPS EN ISO 22717:2016, SRPS EN ISO 22718:2016) were used to detect and identify the specified microorganisms. We identified presence of noteworthy pathogens like *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Candida albicans.* On the other hand, we have the ability to enumerate the overall aerobic mesophilic bacteria, including both the total aerobic microbial count and the total yeast and mold count (19). Analyses were performed on the day of preparation, after 7, 30, 60 and 90 days.

Statistical analysis

The results of the determinations were subjected to analysis of variance (ANOVA) ($p \leq 0.05$). The analysis was performed using the IBM SPSS 21.0 *for Windows.*

Table 1. Composition and concentration of the cream-based and gel-based formulations

Ingredients %	Cream base (F1)	EOPS cream (F2)	Ingredients%	Gel base (F3)	EOPS gel (F4)
Stearic acid	10	10	Carbomer 940	0.5	0.5
Cetyl alcohol	2	2	Propylene glycol	10	10
Cetearyl alcohol	2	2	TEA 10%	q.s	q.s
Polysorbate 60	2	2	Sodium benzoate	0.2	0.2
Sweet almond oil	4	4	Citric acid	q.s.	q.s.
Glycerin	3	3	EOPS	/	0.5
Phenoxyethanol	0.8	0.8	Aqua purificata	ad 100	ad 100
TEA 10%	q.s.	q.s.			
Citric acid	q.s.	q.s.			
EOPS	/	0.5			
Aqua purificata	ad 100	ad 100			

EOPS-*Pinus sibirica* essential oil; TEA- Triethanolamine

RESULTS

Organoleptic properties of the formulations

The results obtained for the organoleptic characteristics of both creams and gels are presented in Table 2. Cream formulation F2 has white color, it is homogenous with pleasant odor characteristic to used essential oil. After application to skin, creams spread easily, leaving non-greasy film on the skin. On the other hand, gel formulations F3 and F4 were transparent, homogenous and spread easily after application to skin with leaving sticky film and cooling effect. F4 formulation had specific odor due to addition of EOPS. Analyzing creams during three months at different temperatures, we didn't noticed changes in all observed parameters. During period of three months at different storage conditions, both gel and cream formulations were stable showing good visual and sensory properties. Addition of EOPS did not affect all abovementioned parameters.

Table 2. Organoleptic characteristics and physical appearance of gel and cream base and with EOPS after 7, 30 , 60 and 90 days of preparation and storage at different temperatures

		7th day				30th day				60th day				90th day			
Characteristics		F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4
Color	4±2°C	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	25±2°C	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	40±2°C	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Odor	4±2°C	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	25±2°C	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	40±2°C	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Homogenity	4±2°C	E	E	E	E	E	E	E	E	E	E	G	G	E	E	G	G
	25±2°C	E	E	E	E	E	E	E	E	E	E	G	G	E	E	G	G
	40±2°C	E	E	E	E	E	E	E	E	E	E	G	G	E	E	G	G
Phase separation	4±2°C	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	25±2°C	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	40±2°C	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Application/ touch	4±2°C	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	25±2°C	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	40±2°C	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Type of smear	4±2°C	E	E	E	E	E	E	E	E	E	E	G	G	E	E	G	G
	25±2°C	E	E	E	E	E	E	E	E	E	E	G	G	E	E	G	G
	40±2°C	E	E	E	E	E	E	E	E	E	E	G	G	E	E	G	G

Legend: Color, Odor: N - Normal; M – Modified; IM – Intensely Modified; Homogenity/Type of smear: G-good, E-excellent, A-average, Phase separation: No or Yes; Application /Touch: P– Pleasant touch, easy skin application (spreadability); D – Unpleasant touch, sticky, relative difficulty to apply on skin; MD – Very unpleasant touch, very sticky, compromises skin application;

pH determination

The pH values of base formulations (F1 and F3) as well as EOPS loaded formulations (F2 and F4) are shown in Figure 1A and 1B respectively. pH values of cream base were in 5.14-5.55 while that range for EOPS cream were 5.11-5.30 during 3 months of storage at different temperatures. On the other hand, pH values for gel samples (F3 and F4) were slightly higher, 6.29-6.59 for gel base and 6.49-6.68 for EOPS gel. Importantly, during 12 weeks of storage at different temperatures, pH values of the examined samples did not change remarkably. Addition of EOPS in formulations did not cause changes in pH values.

Conductivity

Figures 2A and 2B displayed the values of electrical conductivity of creams and gels after storage for up to 90 days, respectively. The values of conductivity for cream base and EOPS cream were in range from 40.1-49.1 μS and 43.6-41.2 μS respectively. Electrical conductivity values of gel base and EOPS gel were higher in comparison to cream formulations (78.5-84.0 μS/ for gel base and 72.5-80.4 μS for EOPS gel. Storage of all formulations during period of three months did not cause significant changes in electrical conductivity values although EOPS formulations (F2 and F4) had lower values relative to cream and gel bases (F1 and F3).

Centrifugation test

There were no signs of samples (F1-F4) physical instability such phase separation by conducting centrifugation test during three months of storage at different storage temperatures. Results are displayed in Table 2.

Washability

The performed washing experiments revealed that cream and gel formulations (F1-F4) can be removed easily by washing with distilled water.

Swelling index

The swelling index of all formulations is represented in Figure 3. Gel formulations (F3 and F4) with carbomer 940 showed higher swelling index in comparison to cream formulations (F1 and F2). The highest swelling index was noticed in cream with EOPS after half an hour.

Microbiological stability test

Since *E.coli, P. aeruginosa, S. aureus* and *C. albicans* are main skin pathogens we aimed to detect them as well as the numbers of aerobic mesophilic bacteria, yeast and molds (Table 3). All four formulations (F1-F4) meet all the requirements of ISO microbiological standards. There was absence of *E.coli, P. aeruginosa, S. aureus* and *C. albicans* while number of aerobic mesophilic bacteria, yeast and molds were <1 cfu/1g. Elevated value of cream aerobic mesophilic bacteria was noticed in cream base group (F1) that is stored at high temperature but still in permitted range.

Figure 1. A: pH of the cream formulations with and without *P.sibirica* essential oil during the stability testing period; B: pH of the gel formulations with and without *P.sibirica* essential oil during the stability testing period. F1-cream base; F2-EOPS cream; F3-gel base; F4-EOPS gel

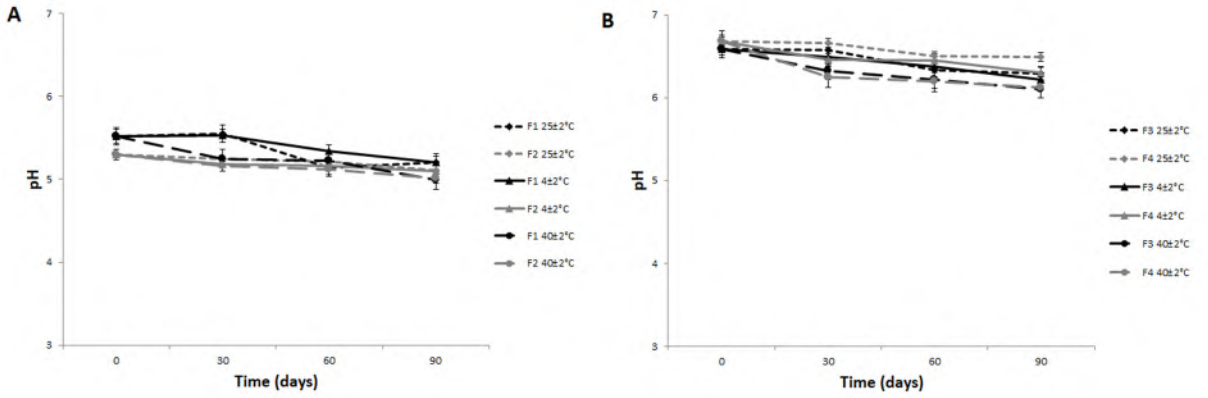


Figure 2. A: Conductivity of the cream formulations with and without *P.sibirica* essential oil during the stability testing period; B: Conductivity of the gel formulations with and without *P.sibirica* essential oil during the stability testing period. F1-cream base; F2-EOPS cream; F3-gel base; F4-EOPS gel

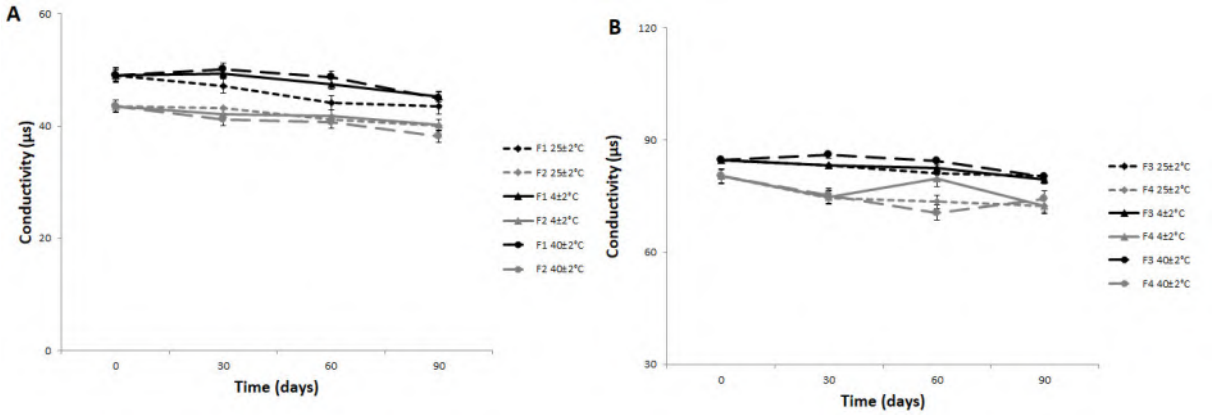


Figure 3. Swelling index of the formulations (F1-F4).
F1-cream base; F2-EOPS cream; F3-gel base; F4-EOPS gel

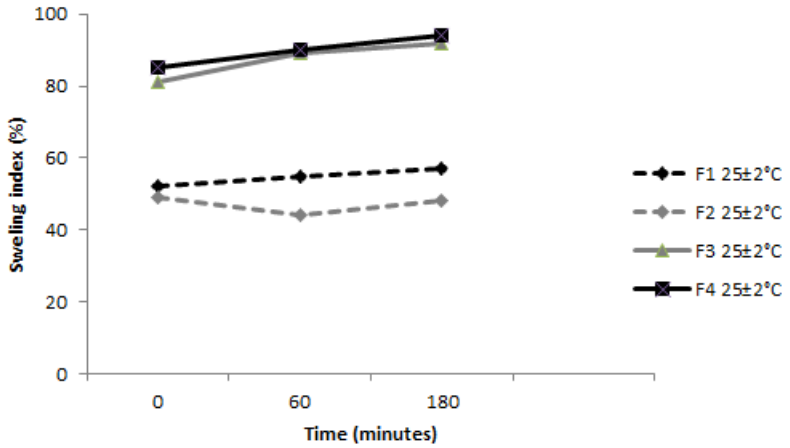


Table 3. List of tested formulations according to microbial contamination during three months of storages at different temperatures

		Day 7				Day 30				Day 60				Day 90			
Parameters		F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4
Number of AMB(cfu/g)	8±2°C	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
	25±2°C	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
	40±2°C	10	<1	<1	<1	40	<1	<1	<1	60	<1	<1	<1	80	<1	<1	<1
Number of yeast and mould (cfu/g)	8±2°C	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
	25±2°C	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
	40±2°C	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Escherichia coli (per g)	8±2°C	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	25±2°C	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	40±2°C	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Staphylococcus aureus (per g)	8±2°C	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	25±2°C	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	40±2°C	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Pseudomonas aeruginosa (per g)	8±2°C	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	25±2°C	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	40±2°C	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Candida albicans (per g)	8±2°C	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	25±2°C	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	40±2°C	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Legend: nd-not detected; AMB: aerobic mesophilic bacteria

DISCUSSION

Nowadays, herbal ingredients present very attractive components of numerous topical semi-solid formulations exerting auspicious effects on skin. Before these products find their place on market, they had to be stable during manufacturing, transport and storage period. Stability studies play a crucial role in the development and marketing of pharmaceutical and cosmetic semi-solid topical products, including creams and gels. In that sense, we aimed to formulate new topical semisolid (cream and gel) products with *Pinus sibirica* essential oil as well as to determine stability properties while storage them at different temperatures during period of three months.

In first part of our investigation, all preparations (F1-F4) showed satisfactory organoleptic properties such as color, odor and appearance. Additionally, application of all samples was pleasant and easy without leaving sticky and greasy film. As we expected, creams were emollient and cause skin hydration while gels cause cool feeling after application on skin. Moreover, results of centrifugation tests confirmed

physical stability of all examined samples, showing absence of phase separation. Importantly, addition of EOPS to both cream and gel samples, did not cause different properties except the smell (characteristic for *Pinus sibirica* essential oil). All aforementioned properties of cream and gel formulations (F1-F4) are of great importance for commercial products and may influence on consumer decision significantly (20).

After confirming similar visual and sensory features of manufactured creams and gels, we decided to determine if those formulations are physically stabile during period of three months by storage at different temperatures. Since our novel formulations, creams and gels were produced with large amount of water, measuring pH and electrical conductivity values were inevitable. There are some reasons why the pH of cream and gel formulations might decrease such as chemical interaction among acidic or alkaline substances present in the formulation, exposure to air leading to oxidation processes, microbial contamination, absorption of carbon dioxide from the air, temperature changes etc. All those factors are crucial and may lead to pH misbalance of the formulation and consequently to skin irritation, dryness, redness, inflammation and damage (21, 22). Storing of prepared formulations (F1-F4) at 4±2°C, 25±2°C and 40±2°C did not lead to significant change of pH values during 12 weeks, and pH range was from 4.99-6.58 which is suitable for topical products according to Regulations of cosmetic products in our country (23). Interestingly, we noticed linear reduction of pH values during 90 days but it was not significant change.

Another method we used to determine physical stability of creams and gels is conductrometry, which is sensitive to small changes in structure of formulations. Our results indicate that prepared cream formulations are O/W emulsions. Given the range of values observed in this investigation, the electrical conductivity of creams falls within a low to moderate range. The conductivity of a cream is influenced by its composition, encompassing components such as water, emollients, thickeners, and other ingredients (24). We did not notice significant changes of this parameter in cream formulations (F1, F2) during storage of three months. On the other side, values of electrical conductivity of prepared gels (F3-F4) was higher in comparison to cream formulations (F1-F2) due to several factors related to their composition and structure. Gels and creams are both semisolid formulations, but their specific ingredients and properties can lead to differences in electrical conductivity. Gels often have higher content of water compared to creams. Water is a polar solvent that can enhance the ionization of certain substances within the formulation, leading to increased electrical conductivity. Furthermore, presence of electrolytes or ionic species that can facilitate the flow of electrical current through the gel matrix (25, 26). Moreover, three months of storage did not have significant influence on electrical conductivity of all gel formulations. Adding the essential oil to formulations (F2, F4) electrical conductivity values remained constant, which can be explained by hydrophobic properties, low water content and nonpolar compounds, found in EOPS (6, 27). It was noticed that gel formulations showed high swelling index due to its porous nature and big surface area. Higher values of swelling index in gels relative to creams can be due to polymer nature, degree of cross-linking, ionic strength and presence of water. This effect is very important if semi-solid formulation is applied to wounds (28).

Beside physical stability, semi-solid aqueous formulations should maintain good microbiological properties during storage time. Microbes, such as bacteria, mold, and yeast, can significantly influence the stability of topical creams and gels (29). Microbial growth may cause changes in the appearance, texture, and odor of the cream, leading to spoilage of product. Main reason for spoilage of topical product by microbes is production of metabolic by-products or acids leading to pH fluctuations, aggregation or separation of phases (30, 31). Moreover, certain microbes can produce toxins that may pose health risks. Contaminated creams can become unsafe for use, leading to potential adverse effects on the skin. In that sense, topical semi-solid products should include preservatives (synthetic or natural origin), should be packed in airtight and light-resistant containers and produced according to Good Manufacturing Practices (GMP) (32, 33). Considering all this, microbial testing is necessary and crucial to detect microbiological contamination promptly. We investigated main skin pathogens and proved absence of *E.coli, P. aeruginosa, S. aureus* and *C. albicans* in all formulations. Moreover, number of aerobic mesophilic bacteria, yeast and molds also meets ISO standards for microbiological safety. We noticed slight increase of aerobic mesophilic bacteria number in cream base (F1) but still within the standard values ($<10^3$). In that sense, we believe that presence of preservatives (phenoxiethanol/sodium benzoate) as well as essential oil in our formulations led to good microbiological stability during 12 weeks. Antimicrobial effect of Siberian pine essential oil was proven earlier by Russian scientist (7).

CONCLUSION

According to the results obtained from this study, we may conclude that all cream and gel formulations showed good pH value, conductivity and physical and microbial stability during three months of storage at different temperatures. Furthermore, addition of 0.5% *P. sibirica* essential oil did not affect pH values, conductivity, sensory and stability properties of the cream and gel formulations. Further investigations of safety and efficacy of these formulations will be performed.

ACKNOWLEDGEMENTS

The authors express profound gratitude to Faculty of Medical Sciences, University of Kragujevac, Serbia for Grant No. JP 14/22.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Tapfumaneyi P, Imran M, Mohammed Y and Roberts MS. Recent advances and future prospective of topical and transdermal delivery systems. Front. Drug. Deliv. 2022; 2:957732. doi: 10.3389/fddev.2022.957732.
2. Mohd Zaid NA, Sekar M, Bonam SR, Gan SH, Lum PT, Begum MY, et al. Promising Natural Products in New Drug Design, Development, and Therapy for Skin Disorders: An Overview of Scientific Evidence and Understanding Their Mechanism of Action. Drug Des Devel Ther. 2022;16:23-66.
3. Nikolić M, Jakovljević V, Bradić J, Tomović M, Petrović B, Petrović A. Korean and Siberian Pine: review of chemical composition and pharmacological profile. Acta Poloniae Pharmaceutica – Drug Research. 2023;79 (6):785-797.
4. Lantto TA, Dorman HD, Shikov AN, Pozharitskaya ON, Makarov VG, Tikhonov V.P, et al. Chemical composition, antioxidative activity and cell viability effects of a Siberian pine (Pinus sibirica Du Tour) extract. Food Chem. 2009;112:936–943.
5. Averina ES, Seewald G, Müller RH, Radnaeva LD, Popov DV. Nanostructured lipid carriers (NLC) on the basis of Siberian pine (Pinus sibirica) seed oil. Pharmazie. 2010;65(1):25-31.
6. Nikolic M, Andjic M, Bradic J, Kocovic A, Tomovic M, Samanovic AM, et al. Topical Application of Siberian Pine Essential Oil Formulations Enhance Diabetic Wound Healing. Pharmaceutics. 2023;15(10):2437.
7. Efremov A.A., Zykova I.D., Senashova V.A., Grodnitckaya I.D., Pashenova N.V. Antimicrobial and Antiradical Activities of Individual Fractions of Pinus sibirica Du Tour and Abies sibirica Ledeb. Growing in Siberia. Russ. J. Bioorg. Chem. 2021;47:1439–1444.
8. Romanenko E.P., Domrachev D.V., Tkachev A.V. Variations in Essential oils from South Siberian conifers of the Pinaceae family: New data towards identification and quality control. Chem. Biodivers. 2022;19:e2021 00755.
9. Herbig ME.; Evers DH, Gorissen S, Köllmer M. Rational Design of Topical Semi-Solid Dosage Forms-How Far Are We? Pharmaceutics. 2023. doi:10.3390/pharmaceutics15071822.
10. Badruddoza AZM, Yeoh T, Shah JC, Walsh T. Assessing and Predicting Physical Stability of Emulsion-Based Topical Semisolid Products: A Review. J Pharm Sci. 2023;112(7):1772-1793.
11. Chen MX, Alexander KS, Baki G. Formulation and Evaluation of Antibacterial Creams and Gels Containing Metal Ions for Topical Application. J Pharm (Cairo). 2016. doi:10.1155/2016/5754349.
12. Naveed A, Shahiq-uz-zaman, Barkat AK, Haji M, Shoaib K, Mahmood A, et al. Evaluation of various functional skin parameters using a topical cream of Calendula officinalis extract. African Journal of Pharmacy and Pharmacology. 2011;5(2):199-206.
13. Ma B, Yuan J, Chen S, et al. Topical application of temperature-sensitive caerin 1.1 and 1.9 gel inhibits TC-1 tumor growth in mice. Am J Transl Res. 2020;12(1): 191-202.
14. Tadić VM, Žugić A, Martinović M, Stanković M, Maksimović S, Frank A. Enhanced Skin Performance of Emulgel vs. Cream as Systems for Topical Delivery of Herbal Actives (Immortelle Extract and Hemp Oil). Pharmaceutics. 2021;13(11):1919.
15. Stolić Jovanović A, Martinović M, Žugić A, Nešić I, Tosti T, Blagojević S. Derivatives of L-Ascorbic Acid in Emulgel: Development and Comprehensive Evaluation of the Topical Delivery System. Pharmaceutics. 2023;15(3):813.
16. Joshi P, Joshi S, Rajani U, Semwal RB, Semwal DK. Formulation and Evaluation of Polyherbal Cream and Lotion for the Treatment of Psoriasis-Induced Secondary Infections. Curr Rev Clin Exp Pharmacol. 2021;16(1):79-96.
17. Shailendra KS, Ashutosh B, Sayantan M. Development And Evaluation Of Tioconazole Loaded Emulgel. Int J App Pharm. 2017; 9(5): 83-90.
18. Arianto A, Cindy C. Preparation and Evaluation of Sunflower Oil Nanoemulsion as a Sunscreen. Open Access Maced J Med Sci. 2019;7(22):3757-3761.
19. Jairoun AA.; Al-Hemyari SS, Shahwan M, Zyoud SH. An Investigation into Incidences of Microbial Contamination in Cosmeceuticals in the UAE: Imbalances between Preservation and Microbial Contamination. Cosmetics 2020. doi:10.3390/cosmetics7040092.
20. Xu S, Kwa M, Lohman ME, Evers-Meltzer R, Silverberg JI. Consumer Preferences, Product Characteristics, and Potentially Allergenic Ingredients in Best-selling Moisturizers. JAMA Dermatol. 2017;153(11):1099-1105.
21. Lukić M, Pantelić I, Savić SD. Towards Optimal pH of the Skin and Topical Formulations: From the Current State of the Art to Tailored Products. Cosmetics. 2021; 8(3):69.
22. Buraczewska I, Lodén M. Treatment of Surfactant-Damaged Skin in Humans with Creams of Different pH Values. Pharmacology. 2005;73(1):1-7.
23. Pravilnik o kozmetickim proizvodima. Sluzbeni glasnik RS, 60, 2023.
24. Stojiljković D, Arsic I, Tasić-Kostov M. The influence of polar and non-polar emollients on the structure and skin moisturizing potential of the emulsions stabilized by mixed emulsifier. Acta Medica Medianae. 2016;55(2):25-30
25. Lupi FR, Shakeel A, Greco V, Oliviero Rossi C, Baldino N, Gabriele D. A rheological and microstructural characterisation of bigels for cosmetic and pharmaceutical uses. Mater Sci Eng C Mater Biol Appl. 2016;69:358-65.
26. Kulawik-Pióro A, Miastkowska M. Polymeric Gels and Their Application in the Treatment of Psoriasis Vulgaris: A Review. International Journal of Molecular Sciences. 2021;22(10):5124.
27. Shkreli R, Terziu R, Memushaj L, Dhamo K. Formulation and stability evaluation of a cosmetics emulsion

loaded with different concentrations of synthetic and natural preservative. JBS. 2022;5(1):38-51.
28. El-Kased, R.F., Amer, R., Attia, D. et al. Honey-based hydrogel: In vitro and comparative In vivo evaluation for burn wound healing. Sci Rep. 2017; 7: 9692.
29. Halla N, Fernandes IP, Heleno SA, et al. Cosmetics Preservation: A Review on Present Strategies. Molecules. 2018;23(7):1571.
30. Celeiro M, Varela E, Rodriguez R, Penedo M, Lores M. Tracking Bacterial Spoilage in Cosmetics by a New Bioanalytical Approach: API-SPME-GC-MS to Monitor MVOCs. Cosmetics. 2020; 7(2):38.
31. Almukainzi M, Alotaibi L, Abdulwahab A, Albukhary N, El Mahdy AM. Quality and safety investigation of commonly used topical cosmetic preparations. Sci Rep. 2022;12(1):18299.
32. Rathee P, Sehrawat R, Rathee P, Khatkar A, Akkol EK, Khatkar S, Redhu N, Türkcanoğlu G, Sobarzo-Sánchez E. Polyphenols: Natural Preservatives with Promising Applications in Food, Cosmetics and Pharma Industries; Problems and Toxicity Associated with Synthetic Preservatives; Impact of Misleading Advertisements; Recent Trends in Preservation and Legislation. *Materials*. 2023; 16(13):4793.
33. Halla N, Fernandes IP, Heleno SA, et al. Cosmetics Preservation: A Review on Present Strategies. *Molecules*. 2018;23(7):1571.

FACTORS ASSOCIATED WITH OCCURRENCE OF DEPRESSIVE SYMPTOMS AMONG PATIENTS UNDERGOING MAGNETIC RESONANCE IMAGING

Melanija Tepavcevic[1*], Valentina Opancina[2] , Ivona Marinkovic[1] , Simonida Delic[1], Nikoleta Janicijevic[1], Nikola Vunjak[2] and Jovana Milosavljević[1]

[1]*University of Kragujevac, Faculty of Medical Sciences, Department of Anatomy, Serbia*
[2]*University of Kragujevac, Faculty of Medical Sciences, Department of Radiology, Serbia*
[3]*University of Kragujevac, Faculty of Medical Sciences, Department of Hygiene and Ecology, Serbia*
[4]*University of Kragujevac, Faculty of Medical Sciences, Serbia*

Received: 23.03.2025.
Accepted: 15.12.2025.

Corresponding author:

Melanija Tepavcevic

University of Kragujevac, Faculty of Medical Sciences, Department of Anatomy, Serbia

E-mail: melanijatepavcevic@yahoo.com

UDK:

Eabr 2026; 27(3): 265-273
DOI: 10.2478/eabr-2025-0015

ABSTRACT

A lot of serious diseases can have depression as the comorbidity. The aim of this study was to inspect the socio-demographic factors and factors related to the MRI procedures, and their influence on the occurrence of depressive symptoms among patients undergoing the magnetic resonance imaging. This research was conducted as a cross-sectional study at the University Clinical Center in Kragujevac in the Department of Radiological Diagnostics. The questionnaire consists of socio-demographic characteristics, but also the questions about data relevant to the MRI. The questionnaire used to assess the presence of depressive symptoms and the degree of depression was Beck Depression Scale, which was validated and had strong internal consistency. The manifestation of symptoms of depression, looking at the sociodemographic factors, depended only of marital status. The analysis showed statistically significant differences for all variables related to radiological procedures (time running MRI scans, type of MRI scans, duration of waiting, disease type). Post-hoc analysis revealed that depressive symptoms were more common among patients undergoing MRI for the first time compared to those having it for the second time, among patients having MRI of the thorax and pelvis compared to MRI of the extremities, among oncology patients compared to orthopedic and neurological patients, and among widowed individuals compared to other marital status groups. Depression is comorbidity to a lot of diseases. This research has brought a lot of significance among the depression and examined factors, which should be acknowledged by radiologists when patient have an appointment for MRI in order to reduce the possibility of people start or continue expressing symptoms.

Keywords: *Depression, MRI, sociodemographic factors, radiology.*

INTRODUCTION

Depression is a mood disorder that causes a persistent feeling of sadness and loss of interest and can impact daily life of person (1). Physical symptoms of depression are manifested in changes in flow, sleep disturbances, fatigue and loss of energy followed with daily mood swings (1). Depression is considered as a significant public health problem and it may occur as comorbidity in a lot of difficulty diseases.

Diseases that patients suffer from, as well as diseases that are suspected but are still being diagnosed, can affect the patient's mental state and the appearance of symptoms of depression (2). Emphasis should be placed on patients who suffer from, and are in the treatment, of oncological diseases, as well as patients who are suspected of the possibility of malignant diseases. A scientific study has shown that 10% more people with malignant diseases have symptoms of depression compared to those who are healthy (2). It has also been proven that there are symptoms of depression not only in patients but also in their family members and spouses (3).

When we talk about patients diagnosed with oncological disease, attention should be drawn to the effectiveness of treatment of the first stage of the disease in which these patients are found as one of the main influences on the occurrence of depression (4). A MRI (Magnetic Resonance Imaging) scan provides them with an insight into the current state of their primary disease (regression or remission of the disease). In the case of patients who are under suspicion, magnetic resonance imaging is one of the relevant methods for diagnosing the disease (2). For this reason, the length of waiting for a MRI examination is very important, even regardless of the diagnostic suspicion. The longer the period from the establishment of suspicion and the initial medical examination to the MRI examination, patients inevitably spend this period in anticipation of receiving the diagnosis (5). In addition to depression, anxiety is also very common (6). A study conducted in Mostar proved a higher level of depression in outpatients compared to those who were examined during hospitalization (7). In addition, some studies show a close connection between the occurrence of anxiety and depression in patients waiting for a MRI scan (8). Magnetic resonance imaging (MRI) is something new and foreign to patients who encounter this type of examination for the first time (9). However, the length of the examination, the noise of the device, the small space can contribute to the feeling of discomfort and anxiety. The problem is also patients with claustrophobia in whom magnetic resonance imaging very often cannot be performed, or due to the movement results with poor-quality imaging (10).

The most common MRI scans performed are spinal imaging. Patients who suffer of pain in the cervical and lumbar part of the spine for many years are more often older patients. Magnetic resonance imaging in this case shows a better visualization of all muscle and nerve structures that are not covered by CT and radiography (11). Patients, after many years of problems and pain, most often in the cervical and lumbar part of the spine, undergo examination by magnetic resonance imaging. In a high percentage MRI scans are done when drug therapy and physical therapy no longer have an effect, and there is a progression of symptoms (12). In such patients, there is already a diagnosis formed on the basis of a clinical examination, either by a general practitioner or a neurologist. Magnetic resonance imaging of the spine can show some anatomical damage that is an indication for an operative solution to the problem (11).

A number of scientific studies have shown that there is a difference in occurrence of depression in the genders, and that women are more likely to manifest symptoms of depression. A scientific study has shown and proven a number of factors of epigenetic nature and neurotransmitters and hormones that make women more susceptible to the manifestation of symptoms of depression (13). The hormonal basis for the manifestation of depressive symptoms in women immediately after childbirth has been demonstrated, and that the percentage of depressive symptoms in postpartum women is increasing (14).

The study also proved a greater prevalence of the presence of symptoms of resemblance in women suffering from breast cancer. This is one of the indicators that patients suffering from oncological diseases are more likely to exhibit symptoms of depression. In such patients, magnetic resonance imaging is one of the possible ways to establish or confirm the diagnosis (15). There is also an inevitable connection between depression and oral health, so the impact of personal hygiene and the onset of depressive symptoms are closely related and very common among younger people (16, 17).

The aim of this study was to inspect the socio-demographic factors and factors related to the MRI procedures and their influence on the occurrence of depressive symptoms among patients undergoing the magnetic resonance imaging.

MATERIAL AND METHODS

Study Procedure and Participants

This research was conducted as a cross-sectional study in accordance with the rules of good clinical practice and the recommendations of the Declaration of Helsinki.

The research was conducted at the University Clinical Center in Kragujevac in the Department of Radiological Diagnostics. The duration was 3 months, from December 2024 to February 2025. For the conduction of this research, the Ethical approval of the University Clinical Center in Kragujevac was obtained, and registered under the number 17987/2024.

Participants were eligible for inclusion in the study if they met all of the following criteria:

1. Adults aged 18 years or older
2. Referred for magnetic resonance imaging (MRI) during the study period

3. Able to provide informed consent and participate voluntarily
4. Completed the questionnaire in full
5. Declared the presence of any known diseases as part of the questionnaire

Participants were excluded from the study based on any of the following criteria:

1. Patients who declined to participate voluntarily
2. Incomplete or partially filled-out questionnaires
3. Patients with a previously diagnosed psychiatric disorder (e.g., depression, anxiety, psychosis)
4. Patients under the age of 18

All patients participated voluntarily and anonymously, with signed consent. To conduct this research, paper questionnaires were used, which the respondents filled out in the Department for Magnetic Resonance Imaging examinations immediately before the examination. The total number of respondents is 120. Final number of participant included was 118.

Applied Questionnaires

A) Socio-Demographic Data

The questionnaire consists of socio-demographic characteristics that the respondents independently declared such as gender, age, level of education, marital status, but also the questions about the magnetic resonance like the length of waiting for the examination, the number of performed MRI in case of individual respondent, diseases they suffer from, and type of MRI referring to the body part.

The age of the patients ranged from 18 to 78 years.

Patients were divided into two groups: those who undergo a MRI scan for the first time, as well as those for whom this was at least the second time they had a MRI scan.

All patients reported the length of wait for a MRI examination, which ranged from 5 days to 16 months. The waiting time is divided into periods of 1-15 days, 15-30 days, 1-3 months, 3-6 months, 6-9 months, 9-12 months, as well as those who have waited for more than a year.

Patents were also divided accordingly to the level of their education into six groups, as follows elementary, high school, higher school, faculty, master, and PhD.

A questionnaire was also filled out about the type of examination that patients do MRI of the head, MRI of the neck, MRI of the chest, MRI of the abdomen, MRI of the small pelvis, MRI of the spinal column, MRI of the upper and lower extremities.

B) Symptoms of Depression

The questionnaire used to assess the presence of depressive symptoms and the degree of depression was validated by the Beck Depression Scale. The BDI-II scale was used to assess symptoms of depression. This scale consists of 21 questions, where each one is graded between 0 and 3 points. Intention was to record a range of depression symptoms, the person has encountered throughout the previous week. The rating scale was as follows: 0–13: no/minimal symptoms; 14–19: mild depressive symptoms; 20–28: moderate depressive symptoms; 29–63: severe depressive symptoms.

The questionnaire were validated and used in the Serbian language .Reliability and internal consistency of a scale BDI expressed by Cronbach's alpha coefficient was assessed as excellent ($\alpha = 0.91$) (18).

Statistical Analysis

Data was analysed using the Statistical Package for Social Sciences Software, version 21 (SPSS, Chicago, Illinois, version 21.0). Number of cases and frequency were generated for the descriptive data. A chi-square test was used to assess the correlation between categorical variables. Due to structure of contingency tables and presence of small expected cell counts for certain variables, the assumptions for traditional Person chi-square test were not fully met. While Fisher's Exact Test was suitable for small samples it was less practical for multi-category tables. Therefore, the Monte Carlo simulation method was selected as more appropriate alternative. We assessed impact of sociodemoraphic characteristics and factors related to the radiology diagnostic procedures with occurrence of depression symptoms. The level of significance was measured as $p < 0.05$. Post-hoc analysis – Bonferroni analysis, was performed for the categorical variables that showed significance, to determinate precise significance between investigated groups. Bonferroni use adjusted p value, where 0.05 is divided by the number of performed tests.

RESULTS

Of the 120 total participants, 2 participants were excluded from the study due to a previously known psychiatric diagnosis among men, the largest percentage did not show the presence of depressive symptoms, namely 62.3%, while in women the percentage that did not show symptoms of depression was slightly lower, and it amounted to 50.9%. Statistically, there was no significant difference between the genders in this study.

The age distribution of respondents was determined by age groups 18-24 years, 25-34 years, 35-44 years, 45-54 years, 55-64 years, and older than of 65 years. Among all age groups, the highest percentage of respondents did not show symptoms of depression. The age group 18-24 year showed the highest percentage of respondents who did not show symptoms of depression (71.5%). The largest number of subjects with mild symptoms of depression is represented among patients aged 55-64 years (35.5%) Subjects with mild depressive symptoms are most prevalent among patients over 65 years of age (6.95%), Based on the statistical data that can be seen in Table 1, it has been proven that there is no statistically significant difference between age groups and the manifest-

ation of symptoms of depression on magnetic resonance imaging.

Based on the socio-demographic questionnaire, the respondents provided information about their level of education and the most of the respondents had completed secondary school 53.4%. In all six educational groups, most respondents showed no symptoms of depression. This can be seen in Table 1. In these patients, there was no statistically significant difference in the manifestation of symptoms of depression on magnetic resonance imaging.

The most patients were those who are married, among whom was the highest percentage of respondents without symptoms of depression 61.4%, while among the respondents who are widowed, the percentage of respondents with weak symptoms of depression was 66.7%, and with weak to moderate symptoms of depression 33.3% Thus, it was established that there is a statistically significant difference according to the marital status ($p < 0.000$) (Table 1). On the basis of Bonferroni's analysis, it was found that there was a statistically significant difference in the manifestation of depressive symptoms between widowers and single people, married persons, and divorcees.. Widowers are more likely to have mild to moderate symptoms of depression than widowers who rarely have this level of depressive symptoms.

Among the subjects who underwent at least a two MRI examination, most of those showed no symptoms of depression in 69%, In patients who are examined for the first time, the percentage of people without symptoms of depression is lower and is 45%. Among them, the highest percentage of those who exhibited weak symptoms of depression was 48.3%, while respondents with weak to moderate symptoms were represented in a smaller percentage of 6.7%, while in those who came for the second time, the percentage of people with these symptoms was 1.7%. There was statistically significant difference between patients who undergo the MRI for first and at least second time ($p = 0.016$) (Table 2). Patients who come for a MRI examination for the first time are more likely to show symptoms of depression than those who come for an examination for the second or third time.

Based on the length of the wait, patients who waited 3-6 months for an examination were the most represented 28.8%. An estimated 88.9% of patients without symptoms of depression were among those who waited 9-12 months for follow-ups. Respondents with weak symptoms accounted for a maximum of 67.9% among those who waited 1-3 months for a MRI examination. There is a statistically significant difference in the length of waiting for the examination and the appearance of depressive symptoms in the examinees ($p = 0.018$) (Table 2).

We found that there is a statistically significant difference in the occurrence of symptoms of depression and illness from which patients suffer ($p = 0.006$) (Table 2). Based on the Bonferroni test, it was shown between which groups of respondents there is a statistically significant difference. Patients suffering from oncological diseases are more likely to exhibit some symptoms of depression, compared to the patients suffering from neurological and orthopedic diseases (Table 3).

The highest percentage of depression and weak to moderate symptoms were shown by patients who had a chest examination, while weak symptoms of depression were most common in patients who came for a MRI examination of the pelvis while most patients who did not show symptoms of depression were among the subjects who came for a MRI examination of the neck and extremities. There was a statistically significant difference in the manifestation of symptoms of depression and the type of MRI examination that is performed ($p = 0.002$) (Table 2).With the Bonferroni test, we showed between which groups there is a statistically significant difference. People who had a chest MRI scan are more likely to show symptoms of depression than those who came for a MRI scan of the extremities. Patients who came for a pelvic exam were also more likely to show symptoms of depression than those who come for a spine and limb scan (Table 3).

Table 1. Impact of depressive levels on Sociodemographic characteristics of study participants done by chi-square test

	Descriptive statistics		**Depressive symptoms**			**Monte Carlo Sig. 2 tailed**	
Variables	**Frequency**	**Percentage**	**No symptoms**	**Weak symptoms**	**Weak to moderate**	**Pearson X^2 value**	**Significance CI (lower and upper bound)**
Gender							
Male	61	51.7	38 62.3%	21 34.4%	2 3.3%	1.623	0.529 (0.516 – 0.542)
Female	57	48.3	29 50.9%	25 43.9%	3 5.3%		
Age groups							

	Descriptive statistics		Depressive symptoms			Monte Carlo Sig. 2 tailed	
Variables	**Frequency**	**Percentage**	**No symptoms**	**Weak symptoms**	**Weak to moderate**	**Pearson X² value**	**Significance CI (lower and upper bound)**
18-24	7	5.9	5 71.4%	1 14.3%	1 14.3%	15.140	0.124 (0.116 – 0.133)
25-34	12	10.2	7 58.3%	4 33.3%	1 8.3%		
35-44	17	14.4	12 70.6%	5 29.4%	0 0.0%		
45-54	22	18.6	15 68.2%	7 31.8%	0 0.0%		
55-64	31	26.3	19 61.3%	11 35.5%	`1 3.2%		
65+	29	24.6	9 31.0%	18 62.1%	2 6.9%		
Education							
Elementary	3	2.5	1 33.3%	1 33.3%	1 33.3%	15.368	0.122 (0.114 – 0.131)
High school	63	53.4	33 52.4%	27 42.9%	3 4.8%		
Higher school	24	20.3	14 58.3%	10 41.7%	0 0.0%		
Faculty	18	15.3	12 66.7%	6 33.3%	0 0.0%		
Master	6	5.1	4 66.7%	2 33.3%	0 0.0%		
PhD	4	3.4	3 75.0%	0 0.0%	1 25.0%		
Marital status							
Not married	20	16.9	13 65.0%	5 25.0%	2 10.0%	31.762	0.000* (0.000 – 0.000)
Married	70	59.3	43 61.4%	27 38.6%	0 0.0%		
Divorced	19	16.1	11 57.9%	8 42.1%	0 0.0%		
Widow	9	7.6	0 0.0%	6 66.7%	3 33.3%		

* Significant level, p less than 0.05

Table 2. Impact of depressive levels on factors related to the radiology diagnostic procedures of study participants done by chi-square test

	Descriptive statistics		Depressive symptoms			Monte Carlo Sig. 2 tailed	
Variables	**Frequency**	**Percentage**	**No symptoms**	**Weak symptoms**	**Weak to moderate**	**Pearson X² value**	**Significance CI (lower and upper bound)**
Time running scans							
First time	60	50.8	27 45.0%	29 48.3%	4 6.7%	7.421	0.016* (0.013 – 0.020)
Second time	58	49.2	40 69.0%	17 29.3%	1 1.7%		
Type of MRI scan							

	Descriptive statistics		Depressive symptoms			Monte Carlo Sig. 2 tailed	
Variables	**Frequency**	**Percentage**	**No symptoms**	**Weak symptoms**	**Weak to moderate**	**Pearson X^2 value**	**Significance CI (lower and upper bound)**
Head	23	19.5	15 65.2%	8 34.8%	0 0.0%		
Neck	5	4.2	4 80.0%	1 20.0%	0 0.0%		
Thorax	1	.8	0 0.0%	0 0.0%	1 100.0%		
Abdomen	12	10.2	7 58.3%	5 41.7%	0 0.0%	44.941	0.002* (0.001 – 0.003)
Pelvis	27	22.9	6 22.2%	18 66.7%	3 11.1%		
Vertebral column	31	26.3	21 67.7%	9 29.0%	1 3.2%		
Upper/lower limbs	15	12.7	12 80.0%	3 20.0%	0 0.0%		
Breast	4	3.4	2 50.0%	2 50.0%	0 0.0%		
Duration of waiting							
1-15 days	3	2.5	1 33.3%	1 33.3%	1 33.3%		
16-30 days	9	7.6	6 66.7%	3 33.3%	0 0.0%		
1-3 months	28	23.7	9 32.1%	19 67.9%	0 0.0%		
3-6 months	34	28.8	21 61.8%	11 32.4%	2 5.9%	24.992	0.018* (0.015 – 0.022)
6-9 months	28	23.7	19 67.9%	8 28.6%	1 3.6%		
9-12 months	9	7.6	8 88.9%	1 11.1%	0 0.0%		
1+ year	7	5.9	3 42.9%	3 42.9%	1 14.3%		
Disease type							
cardiovascular	2	1.7	2 100.0%	0 0.0%	0 0.0%		
endocrinological	8	6.8	3 37.5%	5 62.5%	0 0.0%		
urological	7	5.9	2 28.6%	4 57.1%	1 14.3%		
rheumatological	4	3.4	4 100.0%	0 0.0%	0 0.0%		
gastroenterological	9	7.6	4 44.4%	5 55.6%	0 0.0%	39.009	0.006* (0.004 – 0.008)
oncological	23	19.5	4 17.4%	17 73.9%	2 8.7%		
orthopedic	18	15.3	14 77.8%	3 16.7%	1 5.6%		
neurological	36	30.5	29 80.6%	7 19.4%	0 0.0%		
gynecological	11	9.3	5 45.5%	5 45.5%	1 9.1%		

* Significant level, p less than 0.05

Table 3. Post-hoc analysis of variables that showed statistical significance observing the levels of depression, Bonferroni test

Variable 1 (V1)	Variable 2 (V2)	Mean Difference (V1-V2)	Sig. (Adjusted p values)	95% CI	
				Lower bound	Upper bound
MR thorax	MR upper/lower limb	1.79	0.046*	0.02	3.55
MR pelvis	MR vertebral column	0.58	0.009*	0.10	1.06
MR pelvis	MR upper/lower limb	0.71	0.007*	0.14	1.28
Widow	Single	0.95	0.001*	0.39	1.52
Widow	Married	0.98	0.000*	0.47	1.48
Widow	Divorced	0.93	0.001*	0.36	1.50
Oncological	Orthopaedic	0.39	0.011*	0.10	1.26
Oncological	Neurological	0.47	0.002*	0.18	1.18

* Significant level, p less than 0.05

DISCUSSION

Depression is a disease that permeates all aspects of life and many factors can affect the appearance of symptoms of depression, even in people who have not had a tendency to manifest these symptoms before. Every examination of the patient and going to the doctor is a type of stress that people face, and depression and anxiety are often connected (19).However, the occurrence of depression is more common in people who have a health problem compared to people who are healthy (20). It is inevitable that people suffering from severe incurable diseases show more frequent symptoms of depression compared to patients who face health problems who have a better outcome and solution. For this reason, the onset and manifestation of symptoms of depression has been extensively investigated in many populations as well as among patients (13). Research that has been done so far has determined an increased manifestation of depression in women compared to men, as well as the reasons that can lead to it, hormonal, neurotransmitter, epigenetics, and physiological period characteristic for women (21). Within the context of our investigation, gender was not significant factor in occurrence of depressive symptoms when undergoing the MRI.

One of the most important factors in the research conducted at KBC Mostar, was the length of waiting for a MRI examination and patients who waited longer for a MRI examination were more likely to show symptoms of depression (7). More precisely, patients who had an outpatient examination had symptoms in a higher percentage compared to those who were examined in a hospital setting. Patients who were ordered to undergo an examination on an outpatient basis (and patents like this were more common) waited longer for the examination itself and waited for a longer period of time for information about the diagnosis or evaluation of the disease, as well as about the continuation of treatment and possible recurrent challenges (7). However, this was not the case in our study, and we found that waiting time for an examination was not important factor in decreasing symptoms of depression after Bonferroni analysis.

In people of different marital status, we found that there is a significant difference in the manifestation of symptoms of depression and that people who are widowed are more likely to exhibit symptoms of depression compared to single married and divorced people. In previous studies, different results have been obtained (20). People who are divorced are more likely to show symptoms of depression than people who are married. It is important to note that marital status is a major factor in the manifestation of symptoms of depression. People who are widowers have experienced the pain of losing a partner, and for this reason they are more sensitive to other factors that can lead to the manifestation of symptoms of depression. Depression is a disease that occurs both at all ages and in people of all educational levels (22). In our research, we proved that neither age nor levels of educations were significant factors influencing the manifestation of depressive symptoms among participants undergoing the MRI. A study conducted on young adolescents showed a rapid increase in the prevalence of depressive symptoms, mostly in women (23).

We must not forget that the MRI examination itself is extremely uncomfortable in terms of narrow space, duration, sound, which is a real problem, especially for patients who have claustrophobia. When we talk about patients who underwent magnetic resonance imaging for the first time, they showed symptoms of depression in this study compared to those who underwent this minimum for the second time on a MRI examination. We can relate this to the fact that these patients are encountering this type of examination for the first time and are exhibiting such symptoms, or that they are going for the first time for this type of examination that can diagnose their suspected disease (24). While people who have been repeatedly examined by magnetic resonance imaging (MRI) have significantly fewer symptoms of depression, with the assumption that they have already encountered this

type of examination, and the MRI examination was not something new to them.

Rapid, real-time magnetic resonance imaging is very important, especially for the formation of the diagnosis of the disease, monitoring the effect of therapy, and monitoring the state of the disease, i.e. their phase, regression, progression of remission. When we talk about patients, it is inevitable that the percentage of people suffering from malignant diseases is on the rise in Serbia (25). The percentage of patients suffering from oncological diseases who are treated is extremely high. Numerous studies have shown that these patients are more likely to exhibit symptoms of depression compared to patients who do not suffer from malignant diseases (26). Women with breast cancer show a higher prevalence of depressive symptoms, underscoring the increased risk of depression among oncology patients. MRI can aid in confirming such diagnoses (15). Depression is also closely linked to oral health, with poor hygiene and depressive symptoms frequently co-occurring, especially in younger populations. Oncology patients are faced with such a diagnosis, which is often life-threatening, or disrupts the quality of life to a large extent or even shortens life expectancy (16, 17). Our study showed high occurrence of depressive symptoms among oncological patients. While in patients being treated for neurological or orthopedic diseases, this was not the case. Breast cancer is leading cause of mortality among women and women with breast cancer are more likely to exhibit symptoms of depression than those who are healthy (27). Orthopedic patients are faced with diseases that can often be easily diagnosed and then cured, and do not show symptoms of depression in a high percentage as is the case with oncology patients. In this study, we proved that oncology patients are more likely to suffer from symptoms of depression compared to other patients.

When we talk about the type of examination that patients do, we have proven that patients who do a chest and small pelvic examination are more likely to show symptoms of depression compared to patients who have an examination of the upper and lower extremities and spinal column. We can assume that there is a correlation between these results and diseases that patients suffer from and for which they have undergone magnetic resonance imaging. We can assume that people suffering from prostate cancer, rectal bladders, as well as women suffering from malignant diseases of the uterus and ovaries were more likely to do magnetic resonance imaging, and the percentage of depression in these patients is higher. Or is it possible to prescribe this examination due to the evolution of the disease. So these examinations are more common in patients in whom there may be a clinical suspicion of the presence of secondary deposits in the pelvis. However, in examinations that perform imaging of the spinal column and upper and lower extremities, these are most often orthopedic conditions or possibly neurological conditions that do not greatly impact the length of life, but can certainly affect the quality of life. There is close correlation between the disease on which patient is suspected to have prior to the MRI scanned and the occurrence of depressive symptoms. In our case, oncological patients expressed more depressive symptoms in comparison to the orthopedic and neurological patients.

Limitations of our study were that we have conducted research only in one center (unicentric study), and we had a relatively small sample size. Also, we consider that our limitation may also be that we did not include the precise diagnosis of the respondents and the stage of their diseases. This can influence the expression of depressive symptoms in patients. We think that this aspect should also be included in future research.

CONCLUSION

The analysis showed statistically significant differences for all variables related to radiological procedures (time running MRI scans, type of MRI scans, duration of waiting, disease type) as well as for marital status. Post hoc analysis revealed that depressive symptoms were more common among patients undergoing MRI for the first time compared to those having it for the second time, among patients having MRI of the thorax and pelvis compared to MRI of the extremities, among oncology patients compared to orthopedic and neurological patients, and among widowed individuals compared to other marital status groups.

- This research has brought a lot of significance among the depressive and examined factors that should be considered when patient have appointment for MRI in order to reduce the possibility of people continue expressing symptoms. Radiologists and medical staff should be more patient and kind towards the individuals seeking for help and diagnostics.

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST

Authors declare no conflict of interest exists.

REFERENCES

1. Chand SP, Arif H. Depression. [Updated 2023 Jul 17]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK430847/.
2. Smith HR. Depression in cancer patients: Pathogenesis, implications and treatment (Review). Oncol Lett. 2015;9(4):1509-1514. doi: 10.3892/ol.2015.2944.
3. Lim SM, Kim HC, Lee S. Psychosocial impact of cancer patients on their family members. Cancer Res Treat. 2013;45(3):226-233. doi:10.4143/crt.2013.45.3.226.
4. Rosenstein DL. Depression and end-of-life care for patients with cancer. Dialogues Clin Neurosci. 2011;13(1):101-8. doi: 10.31887/DCNS.2011.13.1/drosenstein.

5. Nieto Alvarez I, Madl J, Becker L, Amft O. Patients' Experience to MRI Examinations-A Systematic Qualitative Review With Meta-Synthesis. J Magn Reson Imaging. 2025;61(1):480-493. doi:10.1002/jmri.29365.
6. Masalma R, Zidan T, Amasheh S, Maree M, Alhanbali M, Shawahna R. Predictors of anxiety in patients undergoing magnetic resonance imaging scans: a multicenter cross-sectional study. BMC Psychiatry. 2024;24(1):633. doi: 10.1186/s12888-024-06091-6.
7. Bagaric M, Babic D, Pavlovic M. Anksioznost I depresija kod pacijenatan kojima je ordiniran pregled magnetnom rezonancom. Zdravstveni glasnik. 2018;4(1):33-39. doi:10.47960/2303-8616.2018.7.33.
8. De La Rosa JS, Brady BR, Ibrahim MM, Herder KE, Wallace JS, Padilla AR, Vanderah TW. Co-occurrence of chronic pain and anxiety/depression symptoms in U.S. adults: prevalence, functional impacts, and opportunities. Pain. 2024;165(3):666-673. doi: 10.1097/j.pain.0000000000003056.
9. Madl JEM, Sturmbauer SC, Janka R, Bay S, Rohleder N. Preparing patients according to their individual coping style improves patient experience of magnetic resonance imaging. J Behav Med. 2022;45(6):841-854. doi: 10.1007/s10865-022-00361-y.
10. Munn Z, Moola S, Lisy K, Riitano D, Murphy F. Claustrophobia in magnetic resonance imaging: A systematic review and meta-analysis. Radiography. 2015;21(2):e59–e63. doi:10.1016/j.radi.2014.12.004.
11. Jensen MS, Olsen KR, Morsø L, Søndergaard J, Schiøttz-Christensen B. Does changed referral options affect the use of MRI for patients with low back pain? Evidence from a natural experiment using nationwide data. BMJ Open. 2019;9(6):e025921. doi:10.1136/bmjopen-2018-025921.
12. Suckling J, Nestor LJ. The neurobiology of addiction: the perspective from magnetic resonance imaging present and future. Addiction. 2017;112(2):360-369. doi: 10.1111/add.13474.
13. Albert PR. Why is depression more prevalent in women? J Psychiatry Neurosci. 2015;40(4):219-21. doi: 10.1503/jpn.150205.
14. Barnes J, Theule J. Maternal depression and infant attachment security: A meta-analysis. Infant Ment Health J. 2019;40(6):817-834. doi:10.1002/imhj.21812
15. Javan Biparva A, Raoofi S, Rafiei S, et al. Global depression in breast cancer patients: Systematic review and meta-analysis. PLoS One. 2023;18(7):e0287372. doi:10.1371/journal.pone.0287372.
16. Janicijevic N, Dimovic T, Stajic D, et al. Correlation between the Oral and Mental Health of University Students in Serbia-A Cross-Sectional Study. Healthcare (Basel). 2024;12(14):1431. doi:10.3390/healthcare12141431.
17. Thapar A, Eyre O, Patel V, Brent D. Depression in young people. Lancet. 2022;400(10352):617-631. doi:10.1016/S0140-6736(22)01012-1.
18. Beck AT, Steer RA, Brown GK. Bekov Inventar Depresije—Drugo Izdanje [Beck Depression Inventory—Second Edition]: BDI-II: Edicija Sinapse; Beograd, Serbia: 2019. 63p.
19. McGraw, Mark. Stress, Anxiety Affect MRI Patients' Experience, Imaging Workflow. Oncology Times. 2022;44(6):33. doi: 10.1097/01.COT.0000824736.71989.2e.
20. Mössinger H, Kostev K. Depression Is Associated with an Increased Risk of Subsequent Cancer Diagnosis: A Retrospective Cohort Study with 235,404 Patients. Brain Sci. 2023;13(2):302. doi:10.3390/brainsci13020302.
21. Martin LA, Neighbors HW, Griffith DM. The Experience of Symptoms of Depression in Men vs Women: Analysis of the National Comorbidity Survey Replication. JAMA Psychiatry. 2013;70(10):1100–1106. doi:10.1001/jamapsychiatry.2013.1985.
22. Pilmeyer J, Huijbers W, Lamerichs R, Jansen JFA, Breeuwer M, Zinger S. Functional MRI in major depressive disorder: A review of findings, limitations, and future prospects. J Neuroimaging. 2022;32(4):582-595. doi: 10.1111/jon.13011.
23. Keyes KM, Gary D, O'Malley PM, Hamilton A, Schulenberg J. Recent increases in depressive symptoms among US adolescents: trends from 1991 to 2018. Soc Psychiatry Psychiatr Epidemiol. 2019;54(8):987-996. doi: 10.1007/s00127-019-01697-8.
24. Zhang FF, Peng W, Sweeney JA, Jia ZY, Gong QY. Brain structure alterations in depression: Psychoradiological evidence. CNS Neurosci Ther. 2018;24(11):994-1003. doi: 10.1111/cns.12835.
25. Stepovic MM, Stajic D, Sekulic M, Rajkovic Z, Djonovic N. Analysis of Mortality from Carcinomas Primary Localized in Region of Lip, Oral Cavity and Pharynx in Central Serbia, 1999-2015. Iran J Public Health. 2020;49(2):274-282.
26. Smith HR. Depression in cancer patients: Pathogenesis, implications and treatment (Review). Oncol Lett. 2015 Apr;9(4):1509-1514. doi: 10.3892/ol.2015.
27. Rancic N, Todorovic M, Stepovic M, Vekic S, Kostic D, Ratkovic M, Radevic S, Simic R and Dragojevic Simic V (2024) Medical cost of breast cancer services in Serbia between 2010 and 2019: national data report. Front. Public Health 12:1378886. doi: 10.3389/fpubh.2024.1378886.

GAP (GLASGOW COMA SCORE - GCS, AGE, ARTERIAL PRESSURE), MGAP (MECHANISM OF INJURY, GCS, AGE, ARTERIAL PRESSURE) AND RTS (REVISED TRAUMA SCORE) AS PREDICTORS OF POOR OUTCOMES AND MORTALITY AFTER INJURY IN THE PREHOSPITAL SETTING

Ivana Stefanovic[1], Sladjana Andjelic[1], Danijele Tasic-Uros[1], Ivana Milivojcević Bevc[1] and Jelena Vuckovic Filipovic[2]

[1]*Institute for emergency medicine Belgrade, Serbia*
[2]*University of Kragujevac, Faculty of Medical Sciences, Department of Internal Medicine, Serbia*

Received: 27.09.2024.
Accepted: 16.10.2024.

Corresponding author:

Ivana Stefanovic, MD

Institute for Emergency Medicine, Bul. Franse D`Eperea 5, Belgrade, Serbia

E-mail: ivanastf@gmail.com

UDK:

Eabr 2026; 27(3): 275-280
DOI: 10.2478/sjecr-2024-0014

ABSTRACT

In out-of hospital settings (OHS), various Trauma Scores (TS) are useful as significant trauma outcome predictors. This study was to retrospectively calculate the RTS (Revised Trauma Score), MGAP (Mechanism of Injury, GAP- Glasgow Coma Score, Age, Arterial Pressure) and GAP values and then compare them as mortality predictors in OHS. A cross-sectional study was conducted on 286 trauma patients (TP) in OHS treated from 1st to 31st October 2020 and transported to the Clinical Center of Serbia (CCS). The data analysed came from medical reports filled out by the IEM Belgrade doctors and the discharge papers from the CCS. Clinical and demographic variables for all the subjects, as well as their GAP, MGAP, and RTS scores, were examined. Their AUC values were compared. While analysing the ability of the GAP, MGAP and RTS scores to predict in-hospital mortality, we found comparable values at 0.890 (GAP), 0.869 (MGAP) and 0.871 (RTS) by using the AUROC curve with high statistical significance (p< 0.001). MGAP has the highest sensitivity, and GAP has the highest specificity. No significant difference in the ability to predict mortality between these three scores. Clinical parameters and calculated TS for all TP classified according to whether the subjects survived (n=230; 80.5%) or died (n=46; 19.5%) after four weeks of treatment in the CCS were significantly statistically different in these two groups. GAP, MGAP and RTS scores are significant predictors of poor outcomes and mortality in TP. It was established that there is no difference in their predictive value.

***Keywords**: Trauma score, predictor, trauma patients, prehospital.*

INTRODUCTION

Trauma is the leading cause of death, disability and hospitalisation in the world (1). Globally, it is estimated that there are yearly approximately six million trauma-related deaths (2). About 40 million people experience permanent injuries annually, while 100 million experience temporary injuries (2,3). According to the EuReCa Serbia Trauma Register (ESTR), which collects emergency medical aid data, 45% of the population in Serbia had trauma in the last quarter of 2018 (4)]. The correct assessment of injury severity with limited resources in prehospital conditions significantly impacts triage and the outcome of trauma. This was why different scoring systems were developed based on various parameters. The first scoring systems appeared during the 1960s to aid triage in mass casualty situations. With further modifications, the scores took on a predictive role in addition to their primary role in triage. Trauma GAP (Glasgow coma score - GCS, Age, Arterial pressure) and MGAP (mechanism of injury, GCS, Age, Arterial pressure) are two predominantly physiologically based scoring systems whose value has been confirmed through research (5-7). The MGAP score includes the mechanism of injury, GCS, the patient's age, and systolic blood pressure (SBP). French research confirmed its predictive value in assessing injury severity and 30-day mortality (5). Based on this score, patients are classified into low-risk (23-29 points), moderate-risk (18-22 points) and high-risk patients (<18 points). Unlike the MGAP, the GAP score does not look into the mechanism of injury and has been clinically validated as a predictor of injury severity and mortality in Japan [6]. GAP score points range from 3 to 24, where values of 3 to 10 indicate severe, 11 to 18 moderate, and 19 to 24 slight injury. These two systems are more straightforward than the Revised Trauma Score (RTS) as they do not include breath frequency or score adjustment for head injury. Clinical research showed that the GAP score has a better predictive value for injury severity classification and outcome than RTS and MGAP scores [6].

The RTS is a trauma management scoring tool based on physiology and is currently accepted worldwide. This scoring system relies on physiological variables, systolic blood pressure and respiratory rate (FD) and, during calculation, considers the value of the Glasgow Coma Scale (GCS) variable to be more important than the other variables (7, 10-11). The maximum score is 12, while anything less than that (a score of 11 or less) indicates severe injury and the need for immediate hospitalisation.

This study aimed to retrospectively calculate the RTS, MGAP and GAP scores and compare their efficacy in predicting mortality in prehospital conditions.

MATERIALS AND METHODS

The Institute for Emergency Medicine (IEM) Belgrade provides emergency medical assistance to more than 1.6 million residents living in 11 municipalities throughout the city of Belgrade, covering an area of approximately 1500 square kilometres. The Institute provides almost one million health services annually. A cross-sectional study was conducted at the IEM from October 1st to October 31st 2020, in Belgrade. The data were collected while working in prehospital conditions. The study included 286 trauma patients, who were examined in the field and treated by the ambulance service. The data collected included demographic data (gender and age of the patient), the cause of trauma, mechanism of injury (blunt or penetrating trauma), respiratory rate, systolic blood pressure, GCS score and outcome (resuscitation, emergency intervention, admission to the intensive care unit, operative treatment), and mortality after four weeks.

Statistical analysis

The collected data were presented using tables and graphs and analysed with SPSS. The Mann–Whitney U-test was used to compare the mean scores in the classified groups. The significance of the score as a predictor was determined by calculating the area under the AUROC curve. The values for individual scoring systems were compared using the Delong test. Statistical significance was set at $p < 0.05$.

RESULTS

In October 2020, the IEM medical teams provided emergency medical assistance to 286 trauma patients. Demographic data show that the mean age of the subjects was 40.3±17.54 years and that 65.7% of the patients were male, almost twice the number of female patients (34.3%). As to the cause of injury, the majority of the patients were injured in traffic accidents (n=132, 46.2%), as a result of a fall (n=90, 31.5%), as a result of other causes (n=49, 17.1%) and as a result of violence (n=15, 5.2%) (Table 1).

The clinical data analysis shows that 191 (66.8%) patients suffered from blunt trauma, while only half of that (n=95, 33.2%) suffered from penetrating trauma. Vital signs were taken at the scene as the trauma patients were examined, and their mean values were for systolic blood pressure (SBP) 115±23.4 mmHg and for the respiratory rate (RR) 19.5±8.5 respirations per minute (Table 1).

Table 1. Clinical characteristics of the trauma patients

Clinical parameters	Statistical values
Age	40.3 ± 17.54
Gender	
M	188 (65,7%)
F	98 (34,3%)
Systolic blood pressure	115 ± 23,4
Respiratory rate	19,5 ± 8,5
Cause of injury	
Motor vehicle accidents	132 (46,2%)
Fall	90 (31,5%)
Other	49 (17,1%)
Violence	15 (5,2%)
Mechanism of injury N (%)	

Clinical parameters	Statistical values
Blunt trauma	191 (66,8%)
Penetrating trauma	95 (33,2%)
GCS score	13,54± 1,59
GAP score	21,55 ± 2,89
MGAP score	24,87± 3,22
RTS score	7,85± 2,22
Resuscitation/Emergency Intervention N (%)	
Yes	147 (51,6%)
No	139 (48,4%)
Intensive Care Unit N (%)	
Yes	79 (27,8%)
No	207 (72,2%)
Operative Treatment	
Yes	198 (69,2%)
No	89 (30,8%)
Mortality at four weeks	
Yes	56 (19,5%)
No	230 (80,5%)

The mean trauma score values calculated at the scene were for GAP 21.55±2.89, MGAP 24.87±3.22, and RTS 7.85±2.22. After processing, the Emergency Clinical Centre (ECC) treatment data showed that 51.6% of the patients included in the study had required resuscitation or emergency interventions, 69.2% had operative treatment, and 27.8% had been admitted to the intensive care unit. The total mortality rate was 19.5%. It turned out that the patients who survived the first four weeks after being injured had statistically significantly higher pre-hospital GAP scores ($\chi^2 = 125.3$, df = 2, $p < 0.01$), MGAP ($\chi^2 = 125.3$, df = 2, $p < 0.01$) and RTS ($\chi^2 = 125.3$, df = 2, $p < 0.01$) (Table 1).

There is a significant statistical difference in clinical signs and calculated trauma scores for trauma patients who were still alive after four weeks of treatment in the ECC (n=230; 80.5%) and those who died before that time (n=46; 19.5%). Patients with penetrating injuries had a fatal outcome in 84.8% of cases, while this percentage was significantly lower for blunt injuries (11.2%) (Table 2, Figure 1). All clinical signs (SBP and RR), except for the patient's age, were also significantly different in the group that survived as opposed to the group of deceased patients (p<0.001).

Table 2. Clinical signs and trauma scores in patients who survived versus those who died

Clinical signs	Survived	Deceased	p
Age	38,2 ± 17,22	41,5 ± 16.26	0,021
SBP	115,7 ± 18.28	88,4 ± 19.73	<0,001
RR	19,04 ± 3,85	14,1 ± 6,67	<0,001
Mechanism of injury N (%)			
Blunt trauma	203 (88,2%)	27 (11,2 %)	<0,001
Penetrating trauma	7 (15,2%)	39 (84,8%)	<0,001
GCS score	13,54± 1,59	7,76± 3,87	<0,001
GAP score	22,56 ± 2,93	16,21± 4,68	<0,001
MGAP score	24,98± 3,45	18,54± 5,32	<0,001
RTS score	7,95± 2,55	4,99± 2,58	<0,001

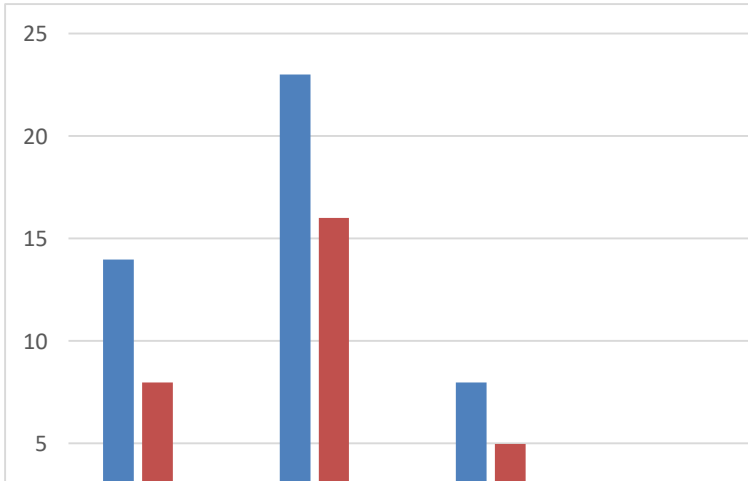


Figure 1. Trauma scores mean values for the survivors and the deceased

By using the AUROC curve to analyse the ability of GAP, MGAP and RTS scores to predict in-hospital mortality (regardless of prevalence), comparable values with high statistical significance (p<0.001) were found at 0.890 (GAP), 0.869 (MGAP) and 0.871 (RTS). MGAP had the highest sensitivity (94), and RTS had the highest specificity (100) (Table 3, Figure 2).

Table 3. Sensitivity and specificity for GAP, MGAP and RTS scores

Scores	The cut-off value for predicting mortality	Sensitivity	Specificity	AUC (95% CI)	p
GAP	<11,5	86	100	0,890 (0,822-0,934)	<0,001
MGAP	<18	94	98	0,869 (0,842-0,945)	<0,001
RTS	<7,5	83	99	0,871 (0,854-0,969)	<0,001

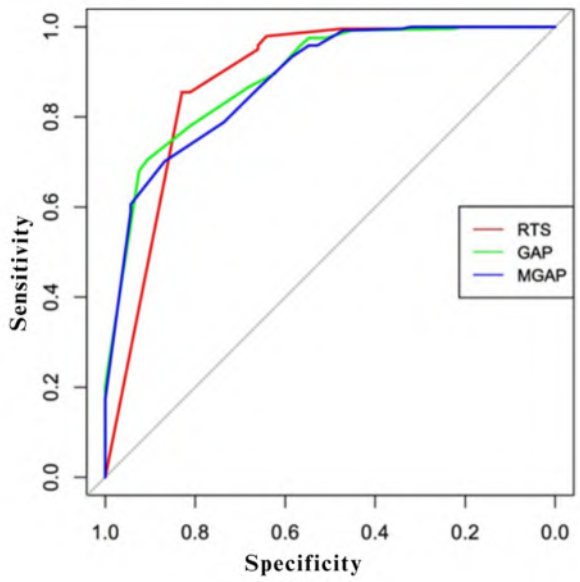


Figura 2. AUROC values, sensitivity and specificity for GAP, MGAP and RTS scores

There was no statistically significant difference in values when using the Delong test to compare the AUROC results for GAP, MGAP and RTS scores (Table 4).

Table 4. Comparing the AUROC values of GAP, MGAP and RTS scores using the Delong test

	GAP/MGAP	GAP/RTS	MGAP/RTS
p	0,28	0,54	0,89

DISCUSSION

Prehospital trauma scores are valuable tools for triage in out-of-hospital settings with limited resources. The usefulness of various trauma scores for predicting the mortality of trauma patients has also been shown through clinical research (8-9). This study aimed to analyse the predictive values of GAP, MGAP and RTS scores and compare their efficacy in prehospital settings.

The results of analysing 286 trauma patients in our study revealed that the mean age of the injured was 40.3 ± 17.54 and that injuries occurred almost twice as often in men than in women (M:F 65.7%:34.3%). Literature data on this subject are not coherent. A study by Mohammed et al. (7) conducted in Egypt showed that, on average, slightly younger people were susceptible to trauma (37.12 ± 16.43), and men were four times more likely to have suffered from injuries than women. Chou et al. (12) found in a Taiwanese study that men injured themselves 1.4 times more often than women. The most common causes of injury in our study were traffic accidents (46.2%), followed by falls (31.5%), and violence as a cause was less prevalent (5.2%). These results vary significantly from indicators at the global level, where traffic

accidents are represented as the cause of trauma in 23% of the cases and falls in 8% (1).

Regarding the trauma mechanism, blunt trauma was the most prevalent (66.8%) and twice as common as penetrating trauma. The calculated trauma scores in our sample have the following mean values: GAP 21.55 ± 2.89, MGAP 24.87 ± 3.22 and RTS 7.85 ± 2.22. Clinical outcome analysis revealed that 51.6% of the patients had resuscitation or emergency interventions, 69.2% required operative treatment, and 27.8% had to be admitted to the intensive care unit. The mortality rate as a result of trauma four weeks after the incident was 19.5%. The patients who had survived the first four weeks did have statistically significantly higher GAP, MGAP and RTS ($p<0.001$) scores initially in the pre-hospital setting. Clinical variables, such as vital signs and mechanisms of injury, were also significantly different in the survivor and deceased groups of trauma patients ($p<0.001$).

Previous studies have shown similar results regarding the role GAP, MGAP, and RTS scores have in preventing bad trauma outcomes. In our research, AUC values were for GAP 0.890 (CI 95% 0.822-0.934), MGAP 0.869 (CI 95% 0.842-0.945) and RTS 0.871 (CI 95% 0.854-0.969). The Delong test did not reveal a significant difference in the tested scores' ability to predict a lethal outcome. An Egyptian study from 2022 (7) highlighted the importance of all three examined scores as predictors of poor trauma outcomes and mortality, which is particularly important in low-income countries that need more straightforward and effective trauma scores. Cassignol et al. (13) compared the values of MGAP and RTS as predictors of hospital mortality during three years in France. The AUC value for RTS was 0.84 (0.82-0.87), and for MGAP, 0.91 (0.89-0.92), which showed the advantages of MGAP as a predictor of mortality after trauma compared to RTS. Galvagno et al. (14) conducted a study which included 43.082 patients to compare the importance of RTS and MGAP scores during triage of trauma patients in prehospital settings. MGAP showed higher sensitivity and specificity than the RTS score but did not show overall superiority compared to the RTS. In our study, MGAP had the highest sensitivity, and the GAP score had the highest specificity. In a study by Bouzat et al. (15), the prehospital MGAP score had a significantly higher AUC compared to the RTS (0.93 [CI 95% 0.91-0.95] vs 0.86 [CI 95% 0.83-0.89], $p<0.01$). The MGAP score < 23 had a sensitivity of 88% for predicting mortality. The sensitivity for RTS < 12 was 79% (15). In a study by Jokšić et al. (16), which included 307 trauma patients, the MGAP score showed the highest sensitivity and specificity (93.4). The MGAP and GAP were highly correlated and proved statistically significant predictors of treatment outcomes in trauma patients (OR 2.23; 95% CI 1.06-4.70; $p = 0.035$). It was also discovered that with an increase in the value of the MGAP score by one, the probability of survival increases 2.2 times.

Research by Tirtayas and Philippi from Indonesia showed that all three scores (GAP, MGAP and RTS) have statistically the same predictive value in trauma (17). Ahun et al. found a statistically significant difference in the mean values for all three scores between the groups that survived and did not survive four weeks after injury. The sensitivity and specificity of the MGAP and GAP were significantly higher than the RTS score for predicting the 4-week survival after injury (18).

These results indicate that the MGAP and GAP scores should be used in prehospital settings and probably have an advantage over the RTS score. Their advantage lies in their simplicity and the inclusion of anatomical and physiological factors (mechanism of injury and age). Applying simple trauma scores, such as GAP, MGAP, and RTS, can assist medical emergency teams in triaging traumatised patients and determining the degree of injury severity quickly so patients can be treated and transported promptly to the Emergency Centre. Further research should aim to determine which score is the most useful predictor of poor trauma outcomes in the prehospital setting.

CONCLUSION

Trauma scores are valuable tools for triage and mortality prediction in trauma patients. The GAP, MGAP and RTS scores are significant predictors of poor trauma outcomes and mortality. It has been established that there is no difference in their predictive value. The MGAP score has anatomic-physiological characteristics and is simple, unlike the RTS score, so future research should be focused on further comparison of these scores in prehospital settings.

CONFLICT OF INTEREST

None

FUNDING

None

ETHICAL APPROVAL

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Institute for Emergency Medicine Belgrad (No. 637/21).

REFERENCES

1. van Breugel JMM, Niemeyer MJS, Houwert RM, Groenwold RHH, Leenen LPH, van Wessem KJP. Global changes in mortality rates in polytrauma patients admitted to the ICU-a systematic review. World J Emerg Surg. 2020;15(1):55.
2. Debas HT, Donkor P, Gawande A, Jamison DT, Kruk ME, Mock CN, eds. Essential Surgery: Disease Control Priorities, Third Edition (Volume 1). Washington (DC): The International Bank for Reconstruction and Development / The World Bank; April 2, 2015.
3. Rossiter ND. Trauma-the-forgotten pandemic? Int Orthop. 2022;46(1):3-11.

4. Stojković T, Vujinović V, Fišer Z. EuReCa_Srbija Trauma registar: mesto nastanka povrede, četvoromesečna analiza. Journal Resuscitatio Balcanica. 2019;5(12):161-5.
5. Sartorius D, Le Manach Y, David JS, Rancurel E, Smail N, Thicoïpé M, et al. Mechanism, Glasgow coma scale, age, and arterial pressure (MGAP): a new simple prehospital triage score to predict mortality in trauma patients. Crit Care Med. 2010;38(3):831–7.
6. Kondo Y, Abe T, Kohshi K, Tokuda Y, Cook EF, Kukita I. Revised trauma scoring system to predict in-hospital mortality in the emergency department: Glasgow coma scale, age, and systolic blood pressure score. Crit Care. 2011;15(4):R191.
7. Mohammed Z, Saleh Y, AbdelSalam EM, Mohammed NBB, El-Bana E, Hirshon JM. Evaluation of the Revised Trauma Score, MGAP, and GAP scoring systems in predicting mortality of adult trauma patients in a low-resource setting. BMC Emerg Med. 2022;22(1):90.
8. Yadollahi M. A study of mortality risk factors among trauma referrals to trauma center, Shiraz, Iran, 2017. Chin J Traumatol. 2019;22(4):212-218.
9. Tang OY, Marqués CG, Ndebwanimana V, Uwamahoro C, Uwamahoro D, Lipsman ZW, et al. Performance of Prognostication Scores for Mortality in Injured Patients in Rwanda. West J Emerg Med. 2021;22(2):435-44.
10. Nakhjavan-Shahraki B, Yousefifard M, Hajighanbari MJ, Karimi P, Baikpour M, Razaz JM, et al. Worthing Physiological Score vs Revised Trauma Score in Outcome Prediction of Trauma patients; a Comparative Study. Emerg (Tehran). 2017;5(1):e31.
11. Javali RH, Krishnamoorthy, Patil A, Srinivasarangan M, Suraj and Sriharsha. Comparison of Injury Severity Score, New Injury Severity Score, Revised Trauma Score and Trauma and Injury Severity Score for Mortality Prediction in Elderly Trauma Patients. Indian J Crit Care Med. 2019;23(2):73-7.
12. Chou PS, Huang SH, Chung RJ, Huang YC, Chung CH, Wang BL, et al. Gender Differences in the Epidemiological Characteristics and Long-term Trends of Injuries in Taiwan from 1998 to 2015: A Cross-Sectional Study. Int J Environ Res Public Health. 2022;19(5):2531.
13. Cassignol A, Markarian T, Cotte J, Marmin J, Nguyen C, Cardinale M, et al. Evaluation and Comparison of Different Prehospital Triage Scores of Trauma Patients on In-Hospital Mortality. Prehosp Emerg Care. 2019;23(4): 543-50.
14. Galvagno SM Jr, Massey M, Bouzat P, Vesselinov R, Levy MJ, Millin MG, et al. Correlation Between the Revised Trauma Score and Injury Severity Score: Implications for Prehospital Trauma Triage. Prehosp Emerg Care. 2019;23(2):263-70.
15. Bouzat P, Legrand R, Gillois P, Ageron FX, Brun J, Savary D, et al; TRENAU Group. Prediction of intra-hospital mortality after severe trauma: which pre-hospital score is the most accurate? Injury. 2016;47(1):14-8.
16. Jokšić-Mazinjanin R, Marić N, Đuričin A, Gojković Z, Vasović V, Rakić G, et al. Prehospital Trauma Scoring Systems for Evaluation of Trauma Severity and Prediction of Outcomes. Medicina (Kaunas). 2023;59(5):952.
17. Tirtayasa P, Philippi B. Prediction of the mortality rate of trauma patients in the emergency room at Cipto Mangunkusumo Hospital by several scoring systems. Medical Journal Of Indonesia. 2013; 22(4): 227-31.
18. Ahun E, Köksal Ö, Sığırlı D, Torun G, Dönmez SS, Armağan E. Value of the Glasgow coma scale, age, and arterial blood pressure score for predicting the mortality of major trauma patients presenting to the emergency department. Ulus Travma Acil Cerrahi Derg. 2014;20(4): 241-7.

TEMPORAL KINETICS OF URINARY VERSUS SERUM AMYLASE FOR PROGNOSIS AND SEVERITY STRATIFICATION AND THEIR CORRELATION WITH CT SEVERITY INDEX AND CLINICAL OUTCOMES IN ACUTE PANCREATITIS: A PROSPECTIVE COHORT STUDY

Rekha Walwekar[1*], Harshaji Rao[2] and Arun Walwekar[3]

[1]*KLE Jagadguru Gangadhar Mahaswamigalu Moorusavirmath Medical College and Hospital, Department of Pharmacology, Hubli, India*
[2]*Oxford Medical College, Hospital & Reasearch Centre, Bangalore, India*
[3]*Karnataka Medical College and Research Institute, Department of General Surgery, Hubli, India*

Received: 02.06.2026.
Accepted: 24.07.2026.

Corresponding author:

Rekha Walwekar

E-mail: rekhaarun13@gmail.com

UDK:

Eabr 2026; 27(3): 281-296
DOI: 10.2478/eabr-2026-0031

ABSTRACT

Acute pancreatitis presents a highly unpredictable clinical course, with traditional serum amylase measurements often failing to reflect evolving tissue injury due to rapid clearance. While contrast-enhanced CT (CECT) and the Computed Tomography Severity Index (CTSI) are gold standards for assessing structural severity, they are often delayed, highlighting the need for continuous, non-invasive markers to track disease progression and predict outcomes. To compare the temporal kinetics of urinary versus serum amylase for prognosis and severity stratification, and their correlation with CT severity index and clinical outcomes in acute pancreatitis. This was a prospective observational study conducted to assess the clinical significance and correlation of these biomarkers with the severity of the disease with CTSI and clinical outcome. Participants were observed over time from the moment of admission to the hospital, with data collected at predefined intervals to monitor the progression of the disease and its response to prognostic markers. A strong positive correlation between high-tier urinary amylase levels (≥1000 U/L) and greater radiological severity via the CT Severity Index (CTSI) (rs) = 0.81, $p < 0.001$)). 66.7% of patients exhibiting normal levels of serum amylase at admission, with despite CT-proven acute pancreatitis. Serum amylase peaks at 24 hours ($r_s = 0.58$, $p < 0.001$) but rapidly clears within 72 hours, demonstrating that longitudinal urinary amylase tracking provides a broader prognostic window for assessing disease severity. The data confirms a strong positive correlation between high-tier urinary amylase levels and severe radiological involvement on the CT Severity Index (CTSI), utilizing urinary amylase as a sequential, non-invasive marker provides excellent prognostic value.

Keywords: *Urinary amylase, serum amylase, acute pancreatitis.*

INTRODUCTION

Acute pancreatitis is a serious condition characterized by inflammation of the pancreas. [1] It can lead to varying degrees of severity, ranging from mild, self-limiting disease to severe, life-threatening conditions with multi-organ involvement. [2] This disease often presents with abdominal pain, nausea, vomiting, and elevated serum enzyme levels, making early diagnosis essential to initiating appropriate management and preventing complications. [3] Diagnosis of acute pancreatitis typically involves clinical assessment, imaging studies, and the measurement of biomarkers like amylase and lipase. [4]

Acute pancreatitis presents a highly unpredictable clinical course, with traditional serum amylase measurements often failing to reflect evolving tissue injury due to rapid clearance. [5] While contrast-enhanced CT (CECT) and the Computed Tomography Severity Index (CTSI) are gold standards for assessing structural severity, they are often delayed, highlighting the need for continuous, non-invasive markers to track disease progression and predict outcomes. [6] This study investigates the temporal kinetics of urinary and serum amylase in correlation with CTSI scores to establish a dynamic, cost-effective method for monitoring disease severity. [7] Urinary amylase is an enzyme excreted by the kidneys, and its levels can reflect pancreatic function. [8] In the setting of acute pancreatitis, amylase is filtered by the kidneys and excreted in the urine, sometimes at levels higher than in the serum. [9]

Urinary amylase provides insights into the severity of acute pancreatitis. [10] Studies have indicated that patients with severe acute pancreatitis have markedly elevated urinary amylase levels compared to those with milder forms of the disease. [11] As the kidneys attempt to clear the excessive amylase released by the inflamed pancreas, urinary amylase levels can become disproportionately elevated. [12] This relationship may offer an additional avenue for determining the severity of pancreatitis, thus aiding clinical decision-making. [13]

While cross-sectional imaging via Contrast-Enhanced Computed Tomography (CECT) is the gold standard for grading pancreatic necrosis using the CT Severity Index (CTSI), routine or repetitive scanning is limited by high costs, radiation exposure, and lack of real-time bedside utility.[14] Therefore, clinicians rely heavily on biochemical markers to track disease progression.[15] Traditionally, serum amylase has been the most widely used biomarker. However, it possesses severe structural limitations: its levels peak rapidly and often drop back to normal baseline ranges within 48 to 72 hours of symptom onset. [16] This rapid clearance creates a massive diagnostic and monitoring blind spot for the substantial percentage of patients who present late to the hospital. [17] Urinary amylase presents a highly promising but under-utilized alternative. Due to altered glomerular permeability during acute pancreatic injury, the kidneys clear massive amounts of the enzyme, allowing urinary amylase levels to remain significantly elevated for 7 to 10 days. [18] Despite this physiological advantage, sequential tracking of urine enzyme levels over critical clinical intervals remains poorly researched. Furthermore, there is a lack of robust data correlating these urinary clearance curves directly with definitive radiological damage (CTSI) and clinical outcomes. [19]

This prospective cohort study advances acute pancreatitis management by mapping the synchronized temporal kinetics of urinary versus serum amylase against CT Severity Index (CTSI). By directly correlating the prolonged kinetic tail of urinary amylase with 72-96 hour CTSI scores, this research bridges the clinical time-lag gap that often causes rapid-clearing serum markers to miss structural pancreatic damage.

OBJECTIVES OF THE STUDY

This study is done to compare the temporal kinetics of urinary versus serum amylase for prognosis and severity stratification, and their correlation with CT severity index and clinical outcomes in acute pancreatitis.

METHODOLOGY

Study Design

This was a prospective observational study conducted to evaluate the diagnostic value of serum amylase and urinary amylase levels in suspected cases of acute pancreatitis. The study aimed to assess the clinical significance, sensitivity, and correlation of these biomarkers with the severity of the disease. As a prospective study, participants were observed over time from the moment of admission to the hospital, with data collected at predefined intervals (initial, 24 hours, and 72 hours post-admission). This observational approach allowed the research team to monitor the progression of the disease and its response to diagnostic markers. The study also incorporated diagnostic imaging techniques, including ultrasonography and CT scan, in patients with severe pancreatitis. This design allowed for a comprehensive evaluation of biomarkers in relation to clinical outcomes, thus offering insights into the role of biochemical markers in the early diagnosis and management of acute pancreatitis.

Study Setting

The study was conducted at the Department of General Surgery, Karnataka Medical College and Research Institute (KMC-RI) Hubballi, India. This hospital is a tertiary care institution that provides a wide range of diagnostic and therapeutic services to patients from urban and rural areas. The department of general surgery at KMC-RI is well-equipped to handle cases of acute pancreatitis, with access to modern

diagnostic tools such as ultrasound, CT scans, and laboratory investigations. The hospital's multidisciplinary team, consisting of surgeons, physicians, and radiologists, is well-suited to evaluate the severity of acute pancreatitis and monitor its progression. As a major medical center in the region, KMC-RI provides an ideal setting for studying the clinical utility of biomarkers in diagnosing and managing acute pancreatitis in a real-world healthcare environment.

Study Duration

The study was conducted over a period of two years, from September 2023 to September 2025. This duration allowed sufficient time to enroll a representative sample of patients, track their progress, and collect comprehensive data on the role of serum amylase and urinary amylase in diagnosing acute pancreatitis and assessing the effectiveness of biomarkers in predicting the severity of the disease. The two-year period was necessary to accommodate the number of patients needed for statistical analysis, especially considering the inclusion and exclusion criteria.

Participants - Inclusion and Exclusion Criteria

Inclusion Criteria:

- All suspected cases of acute pancreatitis admitted to the Department of General Surgery at KMC-RI, Hubballi.
- Patients who gave verbal or written consent to participate in the study.

Exclusion Criteria:

- Immuno-compromised patients (e.g., those with HIV/AIDS, patients on immunosuppressive drugs).
- Patients diagnosed with chronic liver disease (CLD), chronic kidney disease (CKD), or other severe systemic illnesses.
- Patients who were not mentally capable of giving consent due to mental illness or other medical conditions.
- Oncological patients who had received or would receive chemotherapy, radiotherapy, or biological therapy.

The inclusion and exclusion criteria were defined to ensure that the study focused on patients with acute pancreatitis and minimized confounding factors, such as the effects of other comorbidities or treatments on the biomarkers being studied.

Study Sampling

The study employed a non-random sampling method, selecting all suspected cases of acute pancreatitis who were admitted to the Department of General Surgery at Kmcri during the study period. The sampling approach aimed to include every patient who met the inclusion criteria and consented to participate in the study. As a prospective observational study, participants were monitored from the time of admission. This approach ensured that the study could accurately reflect the progression of acute pancreatitis, the changes in biomarker levels, and the relationship between the biomarkers and clinical outcomes. By selecting a diverse sample of patients, the study aimed to provide a broad understanding of how serum amylase and urinary amylase relate to the severity of acute pancreatitis across different patient demographics.

Study Sample Size

Sample size calculated using roc curve:

Area under curve 0.878

Severe pancreatitis prevalence 16.36 (reference- Comparative Analysis of Urinary Amylase and Serum Amylase in the Diagnosis and Prognosis of Acute Pancreatitis-South Eastern European Journal of Public Health (SEEJPH) - ISSN: 2197-5248) [20]

Type 1 error 0.001(alpha error)

Power 95 percent (1-beta)

Formula

$$n = (Z_{\omega/2} + Z_{\beta})^2 \times (0.0099 + 0.41 \times AUC\,(1 - AUC))$$

$$(AUC - 0.5)^2$$

Sample size =114

Study Groups

This study did not include specific experimental or control groups. Instead, it observed all suspected cases of acute pancreatitis admitted to the hospital during the study period.

The patients were categorized according to BISAP score and CT severity index. This stratification allowed for a better understanding of how biomarkers correlate with different levels of disease severity and provided insights into their potential clinical use in stratifying patients based on prognosis.

Study Parameters

The primary study parameters included:

- Serum amylase and urinary amylase levels at admission. Clinical severity of acute pancreatitis assessed using the BISAP score and CTSI.
- All the cases were CT proven pancreatitis

Secondary parameters involved patient demographics (age, gender, comorbidities) and the presence of complications, including pancreatic necrosis, pseudocysts, or abscesses. These parameters were measured to correlate with the levels of biomarkers and to assess their sensitivity and specificity in diagnosing acute pancreatitis and predicting its severity.

Study Procedure

Upon admission to the hospital, patients were assessed for suspected acute pancreatitis based on clinical symptoms,

such as abdominal pain, nausea, and vomiting. Routine investigations, including complete blood count (CBC), renal function tests (RFT), and abdominal ultrasound, were performed to confirm the diagnosis. Serum amylase and urinary amylase levels were measured at the time of admission and followed up at 24 hours 72 hours.

Study Data Collection

Data was collected through a combination of laboratory tests, clinical evaluations, and imaging studies. Laboratory data, including serum amylase and urinary amylase levels, were collected from the hospital's central laboratory. Clinical assessments, including patient history, symptoms, and the severity of pancreatitis, were recorded by the attending medical staff. Imaging data, including ultrasound and CT scan results, were collected from the hospital's radiology department. Data was compiled in a secure database, with each patient's unique identifier ensuring confidentiality. The collected data was then analyzed to assess the correlation between biomarkers and disease severity, as well as the overall diagnostic value of the biomarkers in acute pancreatitis.

Data Analysis

The collected data was analyzed using standard statistical methods. Descriptive statistics were used to summarize patient demographics, clinical findings, and biomarker levels at each time point. The sensitivity, specificity, and predictive values of serum amylase and urinary amylase in diagnosing acute pancreatitis were calculated using standard formulas. Statistical tests, including the chi-square test and t-tests, were used to compare biomarker levels between patients with mild and severe pancreatitis. A statistical analysis Spearman's rank correlation coefficient and Kruskal-Wallis tests demonstrate urinary amylase levels and serum amylase correlation with the CT Severity Index (CTSI) to differentiate mild from severe acute pancreatitis to evaluate the prognostic accuracy of the biomarkers in relation to disease severity. A p-value of <0.05 was considered statistically significant.

Ethical Considerations

The study was conducted in accordance with ethical guidelines and was approved by the Institutional Ethics Committee of KMC-RI, Hubballi (Reg. No- ECR/486/Inst/KA/2013/RR-16, No: KIMS: ETHICS COMM: 864: 2022-23). All patients were informed about the nature of the study and gave written or verbal consent to participate. The study ensured the confidentiality and privacy of patient data, with only authorized personnel having access to the data. Participation in the study was voluntary, and patients were free to withdraw from the study at any time without any adverse effects on their treatment. The study adhered to the principles of beneficence, non-maleficence, and respect for autonomy, ensuring that the rights and well-being of the participants were prioritized throughout the research process.

RESULTS

Demographic profile

In this study total of 138 patients was taken and sample size is 114. The highest incidence of acute pancreatitis in this study was observed among individuals aged 40-49 years (24.6%), followed closely by those aged 30-39 years (23.9%) and 20-29 years (20.3%). This distribution indicates that acute pancreatitis is most common among adults in their third to fifth decades of life. The relatively low occurrence in the very young (14-19 years, 4.3%) and the elderly (70+ years, 1.4%) suggests a possible association between lifestyle, environmental exposures, or cumulative health risks and disease onset in middle age. Detailed data are available in supplementary table 1and supplementary figure 1.

Males accounted for a significantly higher proportion of the study population, with 85.5% male and only 14.5% female patients. This male predominance may reflect gender-related risk factors, particularly higher alcohol consumption rates among men, which is a major contributor to acute pancreatitis in this cohort. Detailed data are available in supplementary table 2 and supplementary figure 2.

Etiology

A substantial majority (77.5%) of patients reported a history of alcohol consumption, reinforcing alcohol as a leading etiological factor in acute pancreatitis. This finding supports the notion that alcohol-induced pancreatitis remains a critical public health concern, especially in populations with high alcohol use. Detailed data are available in supplementary table 3 and supplementary figure 3.

Gallstones were identified as the causative factor in 18.1% of cases, making it the second most common etiology after alcohol. While less prevalent, gallstone-related pancreatitis remains clinically significant and requires a different diagnostic and management approach compared to alcohol-induced cases. Detailed data are available in supplementary table 4 and supplementary figure 4.

Only 5.8% of the cases were classified as idiopathic, indicating that in the vast majority of patients (94.2%), an underlying cause—such as alcohol or gallstones—could be clearly identified. This reflects good diagnostic clarity and comprehensive workup in the study population. Detailed data are available in supplementary table 5 and supplementary figure 5.

Days of admission

Most patients (42.8%) were hospitalized for 6-10 days, with an additional 22.5% requiring 11-15 days. Only one patient had a prolonged stay of 47 days, likely due to severe complications. These figures highlight the variable clinical course of acute pancreatitis, where severity and complications often dictate the duration of hospitalization. Detailed

data are available in supplementary table 6 and supplementary figure 6.

Serum amylase

Serum amylase levels were ≤100 U/L in 68.2% of patients, which is below or within the normal diagnostic threshold. Only 8% had levels above 500 U/L. This observation raises questions about the diagnostic reliability of serum amylase alone, particularly in delayed presentations or mild cases. Detailed data are available in supplementary table 7 and supplementary figure 7.

Serum amylase at 24h and 72h

At 24 hours, a majority of patients (51) had serum amylase between 101-500 U/L, indicating ongoing enzymatic activity. Around 22 patients still had levels >1000 U/L, suggesting persistent inflammation. By 72 hours, more patients (49) had levels drop to ≤100 U/L, showing biochemical recovery in many cases. The declining trend across categories demonstrates that serum amylase tends to normalize within 72 hours in most patients. Detailed data are available in supplementary table 8 and supplementary figure 8.

Urinary amylase

Urinary amylase levels were more widely distributed, with 40.6% of patients having levels 100-499 U/L and 32.6% of patients having levels 500-999 U/L. Notably, higher values were observed in a subset of patients, and suggesting urinary amylase may remain elevated longer and could better reflect ongoing pancreatic enzyme activity, especially in the moderate-to-severe spectrum. Detailed data are available in supplementary table 9 and supplementary figure 9.

Urinary amylase at 24h and 72h

Urinary amylase peaked around 24h in many patients, with 44 having levels above 1000 U/L. By 72h, a shift toward lower levels was evident, with 62 patients having levels in the 100-499 U/L range. This trend suggests that urinary amylase remains elevated longer than serum amylase and may be useful in ongoing monitoring of enzymatic activity. Detailed data are available in supplementary table 10 and supplementary figure 10.

Amylase at admission

Approximately 22.5% of patients had elevated urinary amylase at admission despite normal serum amylase levels. This highlights the potential diagnostic advantage of urinary amylase in detecting early or atypical presentations of acute pancreatitis. It reinforces the need to consider urinary testing when serum markers are inconclusive. Detailed data are available in supplementary table 11.

BISAP (Bedside Index for Severity in Acute Pancreatitis) Score

The majority of patients (55%) had a BISAP score between 0-2, indicating a lower risk of severe outcomes. However, 29 patients (21%) had scores ≥3, signaling potential for higher morbidity or ICU need. BISAP score distribution suggests a predominance of mild to moderate pancreatitis in the study cohort. Detailed data are available in Table 1 and supplementary figure 11.

Table 1. BISAP Score

BISAP Score	Number of patients	Percentage (%)
0	20	14.5%
1	36	26.1%
2	40	29.0%
3	25	18.1%
4	12	8.7%
5	5	3.6%

CT Severity Index (CTSI)

CTSI scoring revealed that 42.8% of patients had moderate severity, while 19.6% had severe pancreatitis based on imaging. Mild disease was seen in 37.7% of cases. These findings indicate that a considerable proportion of patients had moderate-to-severe disease on imaging, supporting the utility of CT in severity stratification. Detailed data are available in supplementary table 12 and supplementary figure 12.

Severity of acute pancreatitis

Using the Modified Atlanta Classification, 39.9% of cases were classified as mild, 37.7% as moderate, and 22.5% as severe pancreatitis. This distribution highlights that nearly two-thirds of the patients had moderate-to-severe disease, which corresponds with longer hospital stays and potential complications, emphasizing the need for early risk stratification. Detailed data are available in supplementary table 13and supplementary figure 13.

Treatment

All patients in the study were managed conservatively, reflecting current trends in supportive care for acute pancreatitis, unless complications dictate surgical or interventional procedures. This also suggests a uniform clinical approach was used throughout the study period. Detailed data are available in supplementary table 14 and supplementary figure 14.

Outcome of the study

Among 138 patients treated with conservative methods, 26.1% developed complications, while the overall mortality rate was 7.2%. The majority of patients (73.9%) did not experience complications, with 92.8% surviving. Detailed data are available in table 2.

Table 2. Outcome of the study

Outcome Parameter	Category	Number of Patients (n = 138)	Percentage (%)
Complications	No Complications	102	73.9%
	Developed Complications	36	26.1%
Survival Status	Survived (Discharged)	128	92.8%
	Deceased (Mortality)	10	7.2%

Complications

Complications were observed in 30 (26.1%) patients, indicating that approximately one in four patients developed adverse clinical events during hospitalization. These could include necrosis, infection, or organ dysfunction, and they contribute significantly to morbidity and resource utilization. Acute fluid collection was observed in 18 patients (50.0%) of complicated cases and accounts for 13.0% of total cohort, pancreatic necrosis was observed in 10 patients (27.8%) of complicated cases and 7.2% of total cohort), ARDS /Organ failure was observed 6 patients (16.7%) of complicated cases (4.3% of total cohort), pancreatic pseudo cyst was observed in 2 patients (5.6%) of complicated cases (1.4% of total cohort). Detailed data are available in table 3.

Table 3. Breakdown of Specific Complications

Clinical Complication	Patient Count	Percentage within Complications Group *(n = 36)*	Percentage of Total Study Cohort *(n = 138)*
Acute Fluid Collection (APFC)	18	50.0%	13.0%
Pancreatic Necrosis	10	27.8%	7.2%
ARDS / Organ Failure	6	16.7%	4.3%
Pancreatic Pseudocyst	2	5.6%	1.4%
Total Patients with Complications	36	100.0%	26.1%

Mortality Rate

The mortality rate in this cohort was 7.2%, reflecting the serious and potentially fatal nature of severe acute pancreatitis. While most patients recovered, this data reinforces the importance of early detection, prognostic assessment, and intensive care in severe cases. Detailed data are available in supplementary table 15 and supplementary figure 15.

6 \10 =cause of death was ARDS (Acute Respiratory Distress Syndrome)

4\10 =cause of death was sepsis

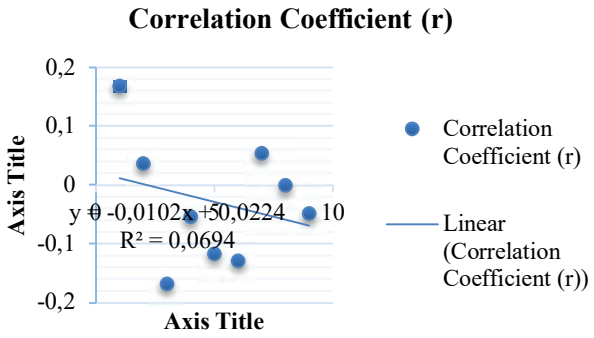
Analysis parameters

The correlation analysis reveals several relationships between the variables studied. Age shows a weak positive correlation with serum amylase levels ($r = 0.169$, $p = 0.05$), suggesting a slight tendency for serum amylase to increase with age, though its correlation with urinary amylase is negligible and not significant ($r = 0.037$, $p = 0.666$). Serum amylase and urinary amylase themselves have a weak negative correlation ($r = -0.168$, $p = 0.051$), indicating they do not strongly rise or fall together. Neither BMI nor WBC show significant correlation with the severity of pancreatitis ($r = -0.054$ and -0.117, $p > 0.1$), implying that may not be reliable indicators of disease severity in this data. Similarly, alcohol use and gall stone presence have no significant correlation with severity ($r = -0.129$, $p = 0.13$; $r = 0.054$, $p = 0.533$, respectively). Notably, complications show a strong and statistically significant positive correlation with severity of pancreatitis ($r = 0.494$, $p < 0.01$), indicating that as severity increases, the likelihood or extent of complications also rises markedly. Days of admission are not significantly correlated with severity ($r = -0.048$, $p = 0.579$). Data on death in relation to severity was not available for analysis. Overall, the data highlights complications as a key factor closely linked with severity, while other variables, including amylase levels and alcohol, appear less directly associated in this sample. Detailed data are available in Table 4 and Figure1.

Table 4. Correlation coefficients between variables

Variable 1	Variable 2	Correlation Coefficient (r)	Significance (p-value)
Age	Serum Amylase	0.169	0.05
Age	Urinary Amylase	0.037	0.666
Serum Amylase	Urinary Amylase	-0.168	0.051
BMI	Severity of Pancreatitis	-0.054	0.533
WBC	Severity of Pancreatitis	-0.117	0.172
Alcohol	Severity of Pancreatitis	-0.129	0.13
Gall Stone	Severity of Pancreatitis	0.054	0.533
Complication	Severity of Pancreatitis	0.494**	0
Days of Admission	Severity of Pancreatitis	-0.048	0.579
Death	Severity of Pancreatitis	(data not available)	(data not available)

Figure 1. Correlation Coefficients between variables



Longitudinal Urinary Amylase Excretion and CTSI Severity

Analysis of the CTSI data and urinary amylase levels shows a significant positive correlation between high-tier urinary amylase levels (≥1000 U/L) and greater radiological severity. A Kruskal-Wallis test confirms that as CTSI scores increase, there is a corresponding significant rise in urinary enzyme excretion, particularly during the 24-to-72-hour window. This demonstrates that tracking longitudinal urinary amylase kinetics can accurately reflect and categorize the severity of acute pancreatitis. Detailed data are available in Table 5.

Table 5. Longitudinal Urinary Amylase Excretions by CTSI Severity

Clinical Time Point & Enzyme Range	**Mild CTSI (0-3) (N = 52)**	**Moderate CTSI (4-6) (N = 55)**	**Severe CTSI (7-10) (N = 31)**	**Total Patients (N = 138)**	**Statistical Significance**
I. AT ADMISSION					Kruskal-Wallis (H)-test:
• 100 - 499 U/L	42	14	0	56 (40.6%)	χ^2= 84.32
• 500 - 999 U/L	10	33	2	45 (32.6%)	p < 0.001 (Highly Sig.)
• 1000 - 1499 U/L	0	7	4	11 (8.0%)	
• 1500 - 1999 U/L	0	1	0	1 (0.7%)	Spearman's Rank (rs):
• Normal / Sub-clinical (<100 U/L)	0	0	25	25 (18.1%)	rs = 0.74, p < 0.001
II. AT 24 HOURS					Kruskal-Wallis (H)-test:
• 100 - 499 U/L	35	8	0	43 (31.2%)	χ^2= 91.15
• 500 - 999 U/L	17	30	4	51 (37.0%)	p < 0.001 (Highly Sig.)
• 1000 - 1499 U/L	0	16	13	29 (21.0%)	
• +1500 U/L	0	1	14	15 (10.9%)	Spearman's Rank (rs)
					(rs)= 0.81, p < 0.001

Clinical Time Point & Enzyme Range	Mild CTSI (0-3) (N = 52)	Moderate CTSI (4-6) (N = 55)	Severe CTSI (7-10) (N = 31)	Total Patients (N = 138)	Statistical Significance
III. AT 72 HOURS					Kruskal-Wallis (H)-test:
• 100 - 499 U/L	48	14	0	62 (44.9%)	χ^2= 78.46
• 500 - 999 U/L	4	36	8	48 (34.8%)	p < 0.001(Highly Sig.)
• 1000 - 1499 U/L	0	5	15	20 (14.5%)	
• +1500 U/L	0	0	8	8 (5.8%)	Spearman's Rank (rs):
					(rs)= 0.78, p < 0.001

Longitudinal Serum Amylase and CTSI Severity

Longitudinal analysis of 138 patients reveals that 66.7% present with normal serum amylase (1-100 U/L) at admission, indicating a significant, early-stage diagnostic blindspot for severe acute pancreatitis, with peak diagnostic correlation occurring at 24 hours ($\mathbf{r_s}$= 0.58). Serum levels show a rapid decline by 72 hours, particularly in mild cases (73% returning to normal), validating that delayed, serial testing is more indicative of CTSI severity than single-point admission tests. Detailed data are available in Table 6.

Table 6. Longitudinal Serum Amylase Excretions by CTSI Severity

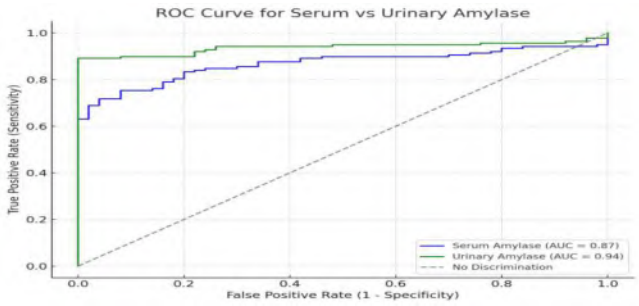
Clinical Time Point & Serum Amylase Range	Mild CTSI (0-3) (n = 52)	Moderate CTSI (4-6) (n = 59)	Severe CTSI (7-10) (n = 27)	Total Patients (N = 138)	Statistical Significance
I. AT ADMISSION					Kruskal-Wallis H-test:
• 1 - 100 U/L (Normal Range)	48	32	12	92 (66.7%)	χ^2 = 18.42
• 101 - 500 U/L	4	22	9	35 (25.4%)	p = 0.001 (Significant)
• 501 - 1000 U/L	0	4	2	6 (4.3%)	
• >1000 U/L	0	1	4	5 (3.6%)	Spearman's Rank (rs):
					rs = 0.38, p = 0.001
II. AT 24 HOURS					Kruskal-Wallis H-test:
• 1 - 100 U/L (Normal Range)	22	6	0	28 (20.3%)	χ^2 = 46.12
• 101 - 500 U/L	26	21	4	51 (37.0%)	p < 0.001 (Highly Sig.)
• 501 - 1000 U/L	4	24	9	37 (26.8%)	
• >1000 U/L	0	8	14	22 (15.9%)	Spearman's Rank (rs):
					rs= 0.58, p < 0.001

Clinical Time Point & Serum Amylase Range	Mild CTSI (0-3) (n = 52)	Moderate CTSI (4-6) (n = 59)	Severe CTSI (7-10) (n = 27)	Total Patients (N = 138)	Statistical Significance
III. AT 72 HOURS					Kruskal-Wallis H-test:
• 1 - 100 U/L (Normal Range)	38	11	0	49 (35.5%)	$\chi^2 = 39.85$
• 101 - 500 U/L	14	33	7	54 (39.1%)	$p < 0.001$ (Highly Sig.)
• 501 - 1000 U/L	0	13	12	25 (18.1%)	
• >1000 U/L	0	2	8	10 (7.3%)	Spearman's Rank (rs):
					rs= 0.51, $p < 0.001$

Prognostic ROC curve

Urinary Amylase (green curve) shows a higher AUC, indicating superior discriminative ability. Serum Amylase (blue curve) has a lower AUC, reflecting less effective performance, especially in later presentations. Both markers perform better than chance (gray diagonal line). Detailed data are available in Figure 2.

Figure 2. ROC Curve for serum vs. urinary amylase



DISCUSSION

This research examined the prognostic significance of urinary amylase in patients who were diagnosed with acute pancreatitis, with a comparative assessment in relation to the usually utilized serum markers—amylase. This prospective cohort study advances acute pancreatitis management by mapping the synchronized temporal kinetics of urinary versus serum amylase against delayed radiological evolution (CTSI). By directly correlating the prolonged kinetic tail of urinary amylase with 72-96 hour CTSI scores, this research bridges the clinical time-lag gap that often causes rapid-clearing serum markers to miss structural pancreatic damage.

The results obtained from this research provide a new perspective into the role of biochemical parameters in acute pancreatitis, highlighting the potential value of urinary amylase as a prognostic marker, especially in delayed presentations or when serum markers produce inconclusive results.

The population profile seen in this study follows trends well established in current literature, wherein acute pancreatitis shows greater prevalence in males with a high concentration in patients between the age groups 30-49. [21] This population trend follows the most frequent risk factor exposure to lifestyle-related factors like alcohol abuse, which was the most common etiological factor encountered in our study. The significance of alcohol-related pancreatitis is in line with epidemiological observations in various areas, especially South Asia and parts of Europe. [22] Gallstones accounted for the second most frequent cause, reinforcing the global perception that these two pathologies predominate the clinical profile of AP. [23] That the comparatively low figure of idiopathic cases can be accounted for by the stringent diagnostic workups done in the course of the study, involving imaging and biochemical studies, so that not all uncommon or secondary etiologies of pancreatitis were omitted, supports the need for extensive diagnostic workups to diminish the ratio of unclassified or idiopathic cases, thus facilitating more targeted treatment protocols. [24]

Urinary amylase had a much broader range of elevation than serum amylase and was elevated for a longer period. This is especially important in patients who come late in the course of the disease. In these patients, serum amylase can already have returned to normal or near-normal levels, underestimation of the severity of the disease. [25] The duration of increased urinary amylase in these patients provided a biochemical correlate for persistent pancreatic inflammation. In addition, the investigation demonstrated statistically significant correlations between the levels of urinary amylase and disease severity markers such as the development of local and systemic complications, the requirement for intensive care, the length of stay in the hospital, and death. These correlations suggest a prognostic value for urinary amylase, an area not as widely investigated as serum markers but with potential to inform clinical decisions and predict complications [26].

The results of our study are supported by previous work, which also reported the maintained elevation of urinary amylase in acute pancreatitis and its value in prognostic situations where serum amylase levels have ceased to be raised. [27] For example, earlier clinical studies have shown that urinary amylase may be elevated for as long as 7 days after the onset of symptoms, while serum amylase usually returns to normal within 3 to 5 days. This physiological phenomenon is due to the renal clearance and excretion mechanisms of amylase, where ongoing renal processing of the enzyme leads to its extended detectability in the urine. [28] This property makes urinary amylase especially valuable in evaluating cases with delayed medical presentation—a typical situation in rural or resource-poor environments where prompt access to healthcare is usually not available [29].

The idea of combining urinary amylase with serum markers and CTSI to create a prognostic panel is a central contribution of this study. Such a combination might provide a richer and more detailed evaluation of the patient's clinical status; the finding of significantly increased urinary amylase may be further supportive of pancreatitis and may signal an extended or complicated disease process. [30] Decreasing levels of urinary amylase in response to treatment can be a surrogate marker of clinical improvement. [31]

Clinically, the addition of urinary amylase testing can increase prognostic accuracy and patient stratification, particularly in situations where access to immediate imaging modalities like contrast-enhanced CT is not available. This becomes extremely pertinent in primary care centers and emergency departments, where rapid decisions have to be made with limited capabilities. [32] Ease and non-invasiveness of urine collection also account for the convenience of this test. In contrast to serum sampling, which is done by skilled staff and involves negligible but definite risks of infection or hematoma, urine sampling is usually easier and may be repeated several times without much discomfort to the patient [33].

Secondly, the value of urinary amylase in resource-poor settings cannot be emphasized enough. Most low- and middle-income nations have systemic diagnostic infrastructure challenges such as access to advanced imaging. [13] In such situations, urinary amylase—especially when measured with low-cost colorimetric assays—may serve as a feasible biochemical option for the initial diagnosis and follow-up of acute pancreatitis. This would allow earlier commencement of supportive treatment and prompt referral to tertiary facilities, hence enhancing overall patient outcomes [34].

A further possible avenue of investigation is the creation of urinary amylase ratios or indices that would control for urinary creatinine excretion or urine volume, thus normalizing amylase levels and enhancing interpretability. A number of studies in nephrology have employed enzyme-to-creatinine ratios to improve diagnostic specificity in urine tests. [35] Analogous strategies could be applied to urinary amylase in the diagnosis of pancreatitis, with potentially more stable markers of disease status and progression. Additionally, the temporal dynamics of urinary amylase—its increase, peak, and decline—might be modelled to create time-dependent prediction tools to supplement current scoring systems like the Ranson, APACHE II, or BISAP scores. [36]

This work also paves the way for reassessing current diagnostic algorithms and revising clinical practice guidelines to include urinary amylase as an optional or conditional test in the AP diagnostic algorithm, use of urinary amylase must not be overlooked. It might be especially beneficial in triaging patients in large-volume centers or in epidemic bursts of pancreatitis, when unavailability of imaging and delayed reporting of laboratory values can hinder on-time treatment. [37]

This study successfully demonstrated a strong positive correlation between high-tier urinary amylase levels (≥1000 U/L) and greater radiological severity via the CT Severity Index (CTSI) ((r_s) = 0.81, $p < 0.001$)). According to study done by Samal SS et al, serum amylase maintain only a moderate correlation with acute pancreatitis severity indexes ($r \approx 0.43 - 0.46$). By tracking urinary amylase longitudinally, our protocol achieves superior prognostic stratification compared to single-point serum tests. [38]

Data from 138 patients indicates a significant diagnostic limitation for serum amylase at admission, with 66.7% of patients exhibiting normal levels despite CT-proven acute pancreatitis. Serum amylase peaks at 24 hours ($r_s = 0.58$, $p < 0.001$) but rapidly clears within 72 hours, demonstrating that longitudinal urinary amylase tracking provides a broader prognostic window for assessing disease severity. For clinical application, consider prioritizing 24-hour serum amylase testing to better correlate with CTSI-assessed severity, as admission levels may be unreliable for severity stratification. According to study done by, Akula V et al. Measuring urine amylase allowed superior monitoring of patients, because this measure remained elevated for longer than the serum markers. Acute pancreatitis is a sensitive, specific, and prolonged diagnostic marker in the form of urinary amylase, which surpasses serum markers for early diagnosis and disease monitoring. Routine urinary amylase testing is recommended to improve clinical management. [39]

The prospect of urinary amylase as a point-of-care test (POCT) holds significant promise, especially in clinical settings where timely diagnostic information is critical. The potential for integrating advanced dipstick technology and portable point-of-care analyzers into routine practice can lead to faster, more efficient patient care. These devices could enable bedside rapid estimation of urinary amylase levels, providing immediate diagnostic insights that accelerate clinical decision-making, particularly in acute conditions such as pancreatitis. [40] This would be invaluable in environments where rapid intervention is required, such as rural clinics, ambulances, or disaster-stricken areas, where laboratory access is limited or unavailable. Moreover, POCTs for urinary amylase could enhance triage capabilities and inform immediate

therapeutic strategies, potentially improving patient outcomes. However, the successful implementation of POCTs in diverse healthcare settings would require extensive validation. Pilot studies focused on assessing the accuracy, cost-effectiveness, and overall feasibility of urinary amylase POCTs are essential to establish their clinical utility. Research in this area would not only inform best practices but also guide policymakers in determining the practical applications of these devices within healthcare systems, particularly in low-resource environments. The outcomes of such studies could pave the way for a broader integration of POCTs in prognostic protocols, improving healthcare accessibility and efficiency globally. [41]

Strengths of the study

The study yields strong evidence to establish the prognostic value of urinary amylase in acute pancreatitis, especially where serum markers yield restricted data are not available. The evidence indicates that urinary amylase, is distinctively advantageous in late-presenting cases and limited-resource settings. Its continued elevation, concordance with the severity of the disease, and simplicity in obtaining make it an important addendum to the clinician's toolset. Although larger, multicenter studies are required to substantiate and extrapolate these findings, the data of this study support an expanded re-evaluation of urinary amylase within the prognostic pathway of acute pancreatitis. In leveraging the entire arsenal of biochemical understanding—serum and urinary—physicians can gain a more detailed picture of disease processes and thereby achieve better outcomes through earlier and more focused intervention.

Future directions

Future research could explore larger, multicenter trials to validate the findings of this study and confirm the diagnostic and prognostic thresholds of urinary amylase. Studies should also examine the establishment of standardized urinary amylase indices, including the amylase-to-creatinine ratio, to standardize results in different patient populations and enhance their clinical applicability. It would also be useful to study the behavior of urinary amylase in various patient populations, such as with underlying renal dysfunction or with comorbidities. The use of urinary amylase with current severity scoring systems (such as APACHE II, Ranson, or BISAP scores) may improve the prognostication of patient outcomes. Additionally, the creation of point-of-care testing (POCT) devices for urinary amylase may simplify prognosis in emergency or rural conditions, enabling faster clinical decisions and enhanced patient care in resource-constrained situations.

Limitations of the study

Limitation of this investigation is that it was a single-center study with a relatively modest sample size, and results may be limited by generalizability. Larger multicenter trials are needed to validate the findings and to assess the generalizability of urinary amylase in a more varied patient population. The investigation also failed to control for possible confounding variables of renal function variability, hydration state, and simultaneous urinary tract disorders that can affect amylase levels in the urine. Standardized pre-analytical baseline parameters—such as timed 24-hour urine collections, precise fluid balance tracking, or concurrent urinary creatinine monitoring to calculate an amylase-creatinine clearance ratio (ACCR)—were not systematically gathered across all 138 patients at the time of admission. We acknowledge this as a significant limitation of our diagnostic evaluation. Though patients with underlying renal impairment were excluded to minimize bias, clinical practice in the real world with patients having these comorbidities means that more research needs to be conducted on how these variables might affect urinary amylase measurements. Also, as an observational study, causality was not possible to establish, and future prospective studies would need to determine the actual clinical benefits of adding urinary amylase to the diagnostic and prognostic pathways for acute pancreatitis.

CONCLUSION

This prospective cohort study confirms that longitudinal urinary amylase tracking is a robust, accessible, and clinically valuable tool for managing acute pancreatitis in a tertiary healthcare setting. By choosing an entirely CT-proven cohort, this study shifts focus away from basic emergency room screening. Instead, it highlights how tracking renal enzyme clearance over a 72-hour period can effectively mirror the internal progression of pancreatic tissue damage. The data confirms a strong positive correlation between high-tier urinary amylase levels and severe radiological involvement on the CT Severity Index (CTSI). Ultimately, utilizing urinary amylase as a sequential, non-invasive marker provides excellent prognostic value.

REFERENCES

1. Yadav D, Lowenfels AB. The epidemiology of pancreatitis and pancreatic cancer. Gastroenterology. 2013;144(6): 1252-1261.
2. Banks PA, Freeman ML. Practice guidelines in acute pancreatitis. Am J Gastroenterol. 2006;101(10):2379-2400.
3. Tenner S, Baillie J, Dewitt J, Vege SS. American College of Gastroenterology guideline: management of acute pancreatitis. Am J Gastroenterol. 2013;108(9):1400-1415
4. Rompianesi G, Hann A, Komolafe O, Pereira SP, Davidson BR, Gurusamy KS et al. Serum amylase and lipase and urinary trypsinogen and amylase for diagnosis of acute pancreatitis. Cochrane Database of Systematic Reviews. 2017(4).
5. Yadav D, Agarwal N, Pitchumoni CS. A critical evaluation of laboratory tests in acute pancreatitis. Official journal of the American College of Gastroenterology| ACG. 2002 Jun 1;97(6):1309-18.
6. Gupta J, Gupta A, Pao K. Comparative study on sensitivity of serum and urinary amylase in the diagnosis of acute pancreatitis. J Med Sci Clin Res. 2017;5(3):10.JMSCR.

7. Shahid N, Khurram Y, Shahid G. Urinary amylase as the first line diagnostic tool for acute pancreatitis. Pak J Med Dent. 2021;11(2):9.ojs.zu.edu.pk.
8. Ismail OZ, Bhayana V. Lipase or amylase for the diagnosis of acute pancreatitis? Clin Biochem. 2017 Dec;50(18):1275-1280.
9. Saad SE, Mohamed AB, Elsayed MM. The predictive value of urinary trypsinogen dipstick versus amylase and lipase in early diagnosis of acute pancreatitis. Benha Medical Journal. 2025 Feb 27.
10. Xiao H, Huang JH, Zhang XW, Ahmed R, Xie QL, Li B et al. Identification of potential diagnostic biomarkers of acute pancreatitis by serum metabolomic profiles. Pancreatology. 2017 Jul 1;17(4):543-9.
11. Rompianesi G, Hann A, Komolafe O, Pereira SP, Davidson BR, Gurusamy KS. Serum amylase and lipase and urinary trypsinogen and amylase for diagnosis of acute pancreatitis. Cochrane Database Syst Rev. 2017 Apr 21;4(4):CD012010. doi: 10.1002/14651858.
12. Wani MD, Chalkoo M, Ahmed Z, Yousuf AM, Arafat Y. Clinical significance of urinary amylase in acute pancreatitis. Arch Surg Clin Res. 2017; 1: 021-031.
13. Judal H, Ganatra V, Choudhary PR. Urinary amylase levels in the diagnosis of acute pancreatitis: a prospective case control study. Int Surg J 2022;9:432-7.
14. Terui K, Hishiki T, Saito T, Mitsunaga T, Nakata M. Urinary amylase/urinary creatinine ratio (uAm/uCr)-a less-invasive parameter for management of hyperamylasemia. BMC Pediatrics. 2013;13: 205.
15. Shahid N, Baqai KB, Murtaza YM, Bilal SB, Ishtiaq GI, Bhatti MA et al. Urinary amylase as the first line diagnostic tool for acute pancreatitis. Pakistan Journal of Medicine and Dentistry. 2022;11(2):50-6.
16. Jamry A. The prognostic value of serum and urine amylase levels and blood count parameters in assessing the risk of post-endoscopic pancreatitis development. Gastroenterology Review/Przegląd Gastroenterologiczny. 2021 Jun 4;16(2):132-5.
17. Liu X, Meng W. Effects of individualized comprehensive nutritional support on inflammatory markers, serum amylase (AMS), prealbumin (PA), albumin (ALB), calcium ion (Ca2+) in patients with severe pancreatitis. Journal of Medical Biochemistry. 2024 Nov 16;43(6):843.
18. Pitchumoni CS, Agarwal N, Jain NK. Systemic complications of acute pancreatitis. American Journal of Gastroenterology (Springer Nature). 1988 Jun 1;83(6).
19. Walkowska J, Zielinska N, Tubbs RS, Podgórski M, Dłubek RJ, Olewnik Ł et al. Diagnosis and treatment of acute pancreatitis. Diagnostics. 2022 Aug 15;12(8):1974.
20. Akula V, Mathias N, Gudipati A. Comparative analysis of urinary amylase and serum amylase in the diagnosis and prognosis of acute pancreatitis: A cross-sectional study. South East Eur J Public Health. 2025;26(S1):1557-1568.seejph.com.
21. Nagar AB, Gorelick FS. Epidemiology and pathophysiology of acute pancreatitis. Pancreatitis and its complications. Totowa, NJ: Humana Press; 2005:3-15.
22. Cappell MS. Acute pancreatitis: etiology, clinical presentation, diagnosis, and therapy. Medical Clinics of North America. 2008 Jul 1;92(4):889-923.
23. Staubli SM, Oertli D, Nebiker CA. Laboratory markers predicting severity of acute pancreatitis. Critical reviews in clinical laboratory sciences. 2015 Nov 2;52(6):273-83.
24. Hung WY, Lanfranco OA. Contemporary review of drug-induced pancreatitis: a different perspective. World journal of gastrointestinal pathophysiology. 2014 Nov 15;5(4):405.
25. Liu P, Xia L, Zhang WL, Ke HJ, Su T, Deng LB et al. Identification of serum microRNAs as diagnostic and prognostic biomarkers for acute pancreatitis. Pancreatology. 2014 May 1;14(3):159-66.
26. Kiriyama S, Gabata T, Takada T, Hirata K, Yoshida M, Mayumi T et al. New diagnostic criteria of acute pancreatitis. Journal of hepato-biliary-pancreatic sciences. 2010 Jan;17:24-36.
27. Werner J, Hartwig W, Uhl W, Müller C, Büchler MW. Useful markers for predicting severity and monitoring progression of acute pancreatitis. Pancreatology. 2003 May 12;3(2):115-27.
28. Keogh JB, McGeeney KF, Drury MI, Counihan TB, Donnell OD. Renal clearance of pancreatic and salivary amylase relative to creatinine in patients with chronic renal insufficiency. Gut. 1978 Dec 1;19(12):1125-30.
29. Ambromovage AM, Howard JM, Pairent FW. The twenty-four hour excretion of amylase and lipase in the urine: correlation with the functional state and operative injury of the pancreas. Annals of Surgery. 1968 Apr;167(4):539.
30. Smith RC, Keely SJ, Chesher D. Should serum pancreatic lipase replaces serum amylase as a biomarker of acute pancreatitis? ANZ journal of surgery. 2005 Jun;75(6):399-404.
31. Wani M, Chalkoo M, Ahmed Z, Yousuf AM, Arafat Y, Arsalan SS et al. Clinical significance of urinary amylase in acute pancreatitis. Hellenic J Surg Res. 2017;1(1):21-31.
32. Rocha AP, Schawkat K, Mortele KJ. Imaging guidelines for acute pancreatitis: when and when not to image. Abdominal Radiology. 2020 May;45:1338-49.
33. Gupta J. Comparative study on sensitivity of serum and urinary amylase in the diagnosis of acute pancreatitis. J Med Sci Clin Res. 2017;5(3):18323-30.
34. Bharwani N, Patel S, Prabhudesai S, Fotheringham T, Power N. Acute pancreatitis: the role of imaging in diagnosis and management. Clinical radiology. 2011 Feb 1;66(2):164-75.
35. Hong W, Geng W, Chen B, Basharat Z, Wu Q, Zimmer V et al. Predictors of acute pancreatitis with low elevation of serum amylase. Ther Clin Risk Manag. 2017;13:1577-84.
36. Judal H, Ganatra V, Choudhary P. Urinary amylase levels in the diagnosis of acute pancreatitis: a prospective case control study. Int Surg J. 2022;10(2):67-75.
37. Inatomi O, Bamba S, Nakai Y, Kusumoto K, Kawakami T, Suzuki T et al. Diagnostic value of Serum amylase levels indicating computed tomography-defined post-endoscopic retrograde cholangiopancreatography pancreatitis:

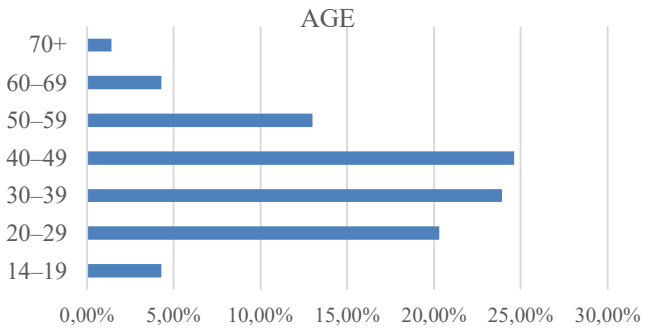
A prospective multicenter observational study. Pancreas. 2020 Aug 1;49(7):955-9.
38. Samal SS, et al Comparative Study between Serum Amylase and Lipase, BISAP Score and CT Severity Index in Assessing the Severity of Acute Pancreatitis. International Journal of Pharmacy Research & Technology. 2026: 16 (1);2761-2767.
39. Akula V, Mathias N, Gudipati N. Comparative Analysis of Urinary Amylase and Serum Amylase in the Diagnosis and Prognosis of Acute Pancreatitis: A Cross-Sectional Study SEEJPH. XXVI(S1); 2025: 1557-1568.
40. Mogekar S, Jayakar S, Sampath KS, Badangi V. A study on urinary amylase and serum amylase in diagnosing acute pancreatitis. Cureus. 2024;16(3):324-31.
41. Simha A, Saroch A, Pannu AK, Dhibar DP, Sharma N, Singh H et al . Utility of point-of-care urine trypsinogen dipstick test for diagnosing acute pancreatitis in an emergency unit. Biomarkers in Medicine. 2021; 15(14), 1271-1276.

SUPPLEMENTARY MATERIAL

Supplementary Table 1. Age group

Age Group (Years)

Age Group (Years)	Number of patients	Percent
14-19	6	4.30%
20-29	28	20.30%
30-39	33	23.90%
40-49	34	24.60%
50-59	18	13.00%
60-69	6	4.30%
70+	2	1.40%

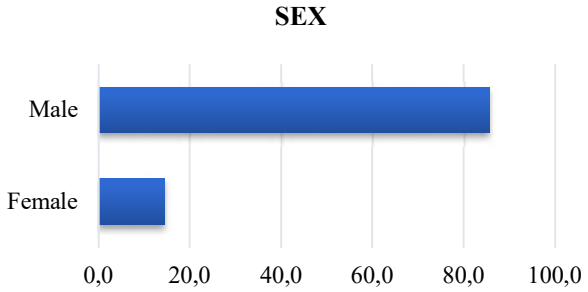


Supplementary Figure 1. Age Group

Supplementary Table 2. Gender distribution

SEX

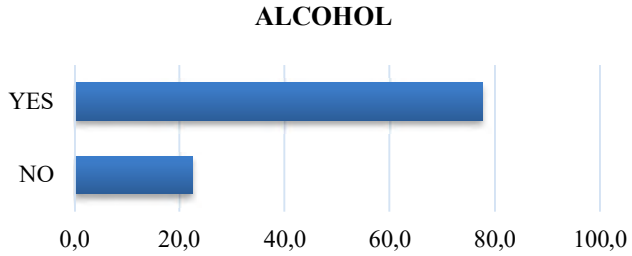
Sex	Number of patients	Percent
Female	20	14.5
Male	118	85.5



Supplementary Figure 2. Gender distribution

Supplementary Table 3. Alchol as etiology

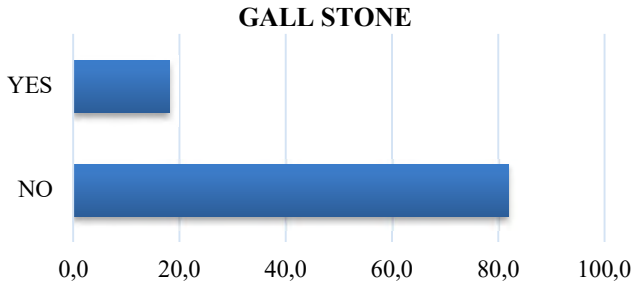
	Number of patients	Percent
NO	31	22.5
YES	107	77.5



Supplementary Figure 3. Alchol as etiology

Supplementary Table 4. Gall Stone

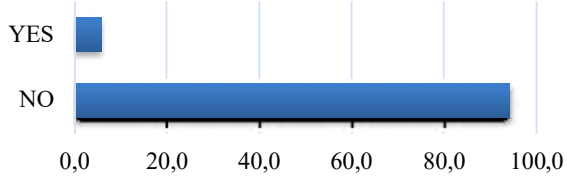
	Number of patients	Percent
NO	113	81.9
YES	25	18.1



Supplementary Figure 4. Gall Stone

Supplementary Table 5. Idiopathic

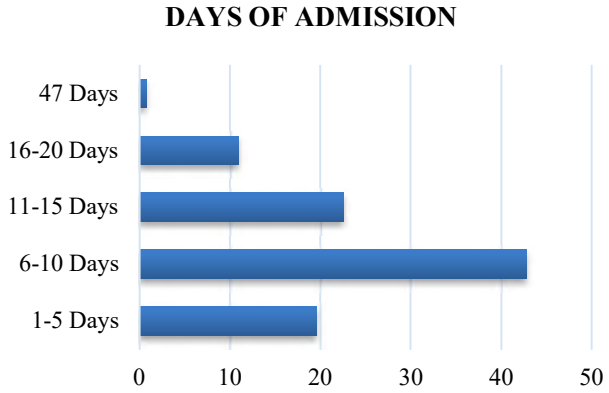
IDIOPATHIC		
	Number of patients	Percent
NO	130	94.2
YES	8	5.8



Supplementary Figure 5. Idiopathic

Supplementary Table 6. Days of Admission

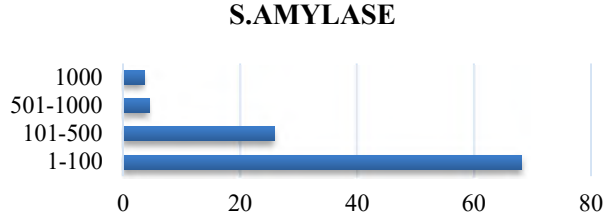
Category	Number of patients	Percent (%)
<1 week	27	19.6
2-3 weeks	59	42.8
3-4 weeks	31	22.5
4-5 weeks	15	10.9
>6 weeks	1	0.7



Supplementary Figure 6. Days of Admission

Supplementary Table 7. S.Amylase

S.AMYLASE		
Category	**Number of patients**	**Percent (%)**
1-100	92	68.2
101-500	35	25.9
501-1000	6	4.4
1000	5	3.6

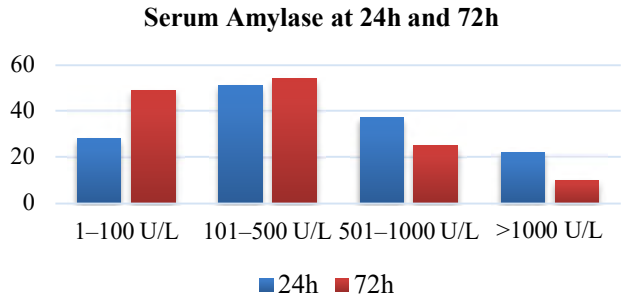


Supplementary Figure 7. S.Amylase

Supplementary Table 8.

Serum Amylase at 24h and 72h

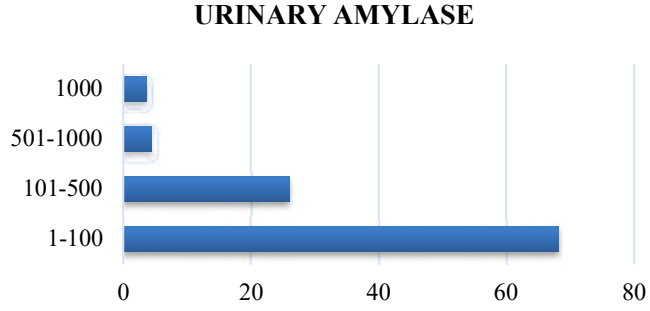
Time Point	1–100 U/L	101–500 U/L	501–1000 U/L	>1000 U/L
24h	28	51	37	22
72h	49	54	25	10



Supplementary Figure 8. Serum Amylase at 24h and 72h

Supplementary Table 9. Urinary Amylase

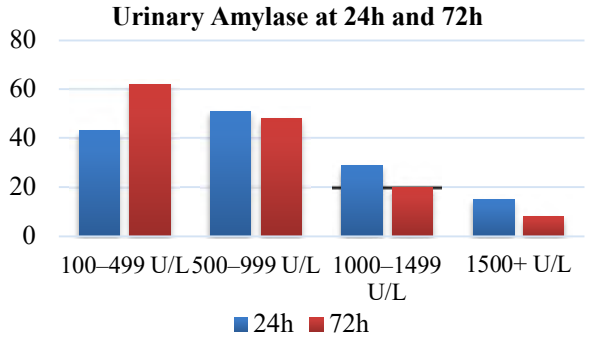
Value Range	Number of patients	Percent
100 - 499	56	40.6
500 - 999	45	32.6
1000 - 1499	11	8
1500 - 1999	1	0.7



Supplementary Figure 9. Urinary Amylase

Supplementary Table 10. Urinary Amylase at 24h and 72h

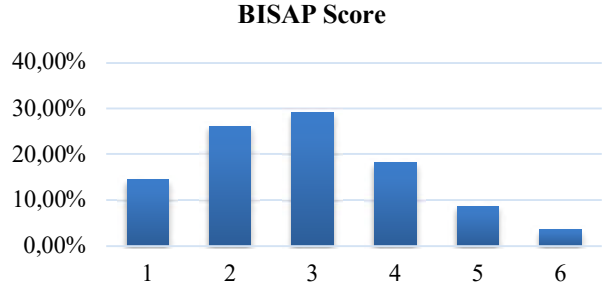
Time Point	100–499 U/L	500–999 U/L	1000–1499 U/L	1500+ U/L
24h	43	51	29	15
72h	62	48	20	8



Supplementary Figure 10.
Urinary Amylase at 24h and 72h

Supplementary Table 11. Amylase at Admission

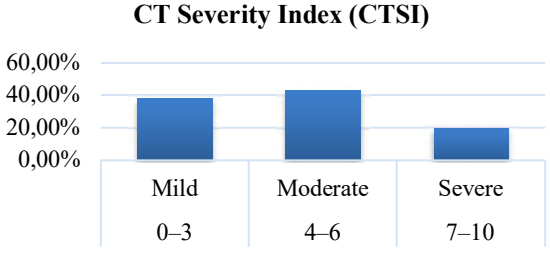
Category	Number of patients	Percent (%)
↑ Urinary Amylase & Normal Serum Amylase	31	22.5%



Supplementary Figure 11. BISAP Score

Supplementary Table 12. CT Severity Index (CTSI)

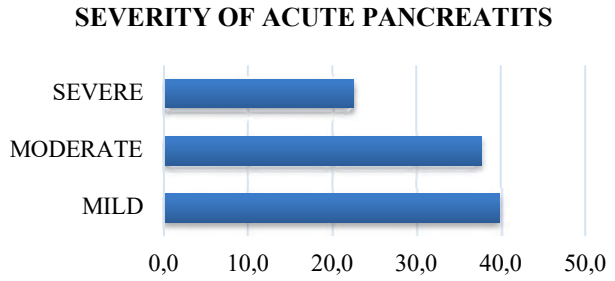
CTSI Score Range	Severity	Number of patients	Percent (%)
0–3	Mild	52	37.7%
4–6	Moderate	59	42.8%
7–10	Severe	27	19.6%



Supplementary Figure 12. CT Severity Index (CTSI)

Supplementary Table 13. Severity of acute pancreatitis using the Modified Atlanta Classification.

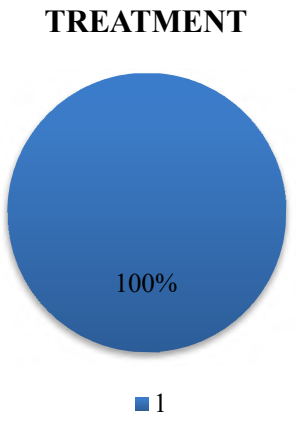
	Number of patients	Percent
MILD	55	39.9
MODERATE	52	37.7
SEVERE	31	22.5



Supplementary Figure 13. Severity of acute Pancreatitis

Supplementary Table 14. Treatment

	Number of patients	Percent
CONSERVATIVE	138	100.0



Supplementary Figure 14. Treatment

Supplementary Table 15. Death

	Number of patients	Percent
NO	128	92.8
YES	10	7.2



Supplementary figure 15. Death

DEVELOPMENT OF SIMULATION METHODS IN BIOMEDICAL SCIENCES

Rasa Mladenovic, Dragana Dakovic and Kristina Mladenovic

[1] *University of Pristina, Faculty of Medicine, Serbia*
[2] *University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia*
[3] *University of Kragujevac, Faculty of Medical Sciences, Department of Pathophysiology, Kragujevac, Serbia*

Received: 03.05.2020.
Accepted: 26.08.2020.

Corresponding author:

Rasa Mladenovic

University of Pristina, Faculty of Medicine, Serbia

E-mail: rasa.mladenovic@med.pr.ac.rs

ABSTRACT

Simulation is an imitation of the operation of a real process or system over time that is applied for a variety of purposes, including entertainment, education, training, system evaluation, and research. Medical simulation is an artificial presentation of real clinical situations, which is applied in education. Medical simulation allows the acquisition of clinical skills without the risk of harming the patient. Medical simulations have been developed and refined over the years- simulation models, cadavers, actors and robots have found wide application in medical training. Of more sophisticated simulation technologies, Virtual and Augmented Realities are used. The presence of science in the digital world is necessary in order to market the proven knowledge acquired in an adequate manner. The traditional teaching process, despite serious and thorough research, seems non-inspirational, and it is important that educators and teachers keep up with the times and provide students with the latest teaching and work methods.

Keywords: *Simulation, medicine, dentistry.*

UDK:

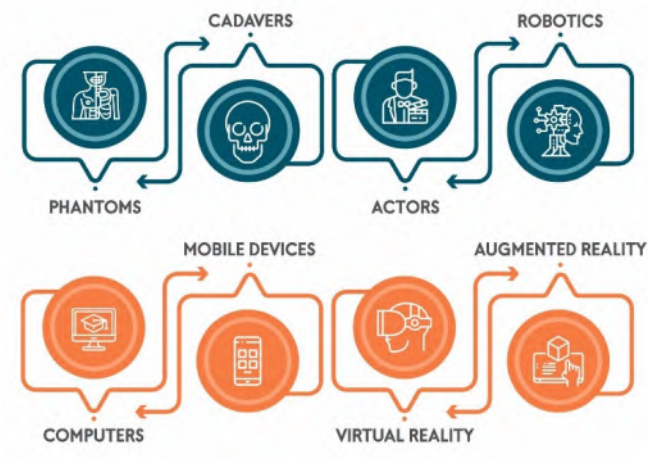
Eabr 2026; 27(3): 297-303
DOI: 10.2478/sjecr-2020-0051

INTRODUCTION

Simulation is an imitation of the operation of a real process or system over time. Today, simulation is used for a variety of purposes, including entertainment, education, training, system evaluation and research. It is commonly used in areas where people need to manipulate or control complex systems, including aviation, healthcare, power plant operations, and the like. The effective use of simulators in aerospace industry provided the basis for their application in other industries (1).

Medical simulation is an artificial presentation of real clinical situations. Medical simulation allows the acquisition of clinical skills without the risk of harming the patient. It can be used both for individual learning and practice as well as for *multidisciplinary team working* (2). The goal of simulation-based medical training is to provide an optimal relationship between knowledge acquisition and patient safety (3). In addition, medical simulation has the potential to simulate a scenario that happens less often in practice, such as urgent, critical, and life-threatening conditions. Medical simulation can be applied both in graduate and postgraduate studies, as well as in continuing medical education to reconstruct critical situations and emergencies that occur in natural disasters and war (Figure 1).

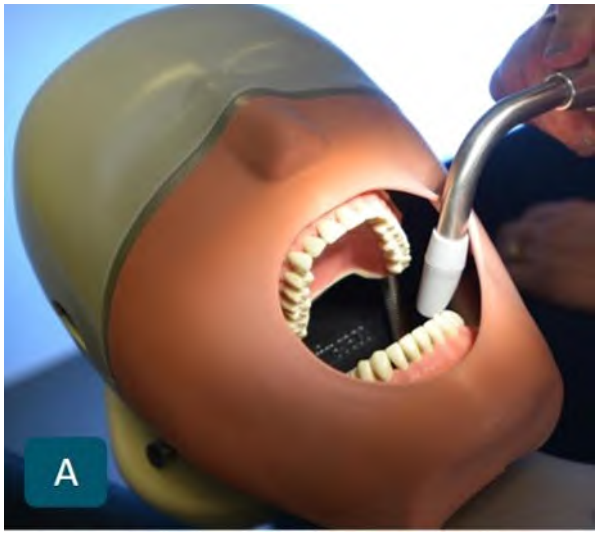
Figure 1. Development of simulation methods: Phantoms, cadavers, actors, robotics, computers and mobile devices, Virtual Reality and Augmented Reality



Simulation models

Phantom / manikins based simulators represent a patient with different levels of physiological and anatomical detail. They may represent age groups (eg newborn, baby, child and adult) or organ parts (4). Today, manikins are used in almost all aspects of medical education, from the anatomy of the human body to surgical simulation scenarios. Manikins help students to involve in assessing the patient's condition, by being able to reproduce vital signs, and thus to develop better clinical skills, cognitive thinking, and communication in a professional healthy environment. In order to create a more realistic image of the patient, certain props are often added to them, such as wigs or clothes, and the environment in which the doll is used is of exceptional importance for the entire simulation course. (5). In dental practice, Oswald Fergus introduced the first 'Phantom head' in 1894, thus laying the cornerstone of dental education (6). This type of simulation is still prevalent in everyday preclinical dental practice (Figure 2A) (7). The main limitation of the preclinical model for exercise is the poor presentation of soft tissues, thus making the learning of more complex realistic clinical procedures difficult. Moreover, students cannot practice without limitations, among other things due to material costs.

Figure 2. A) Dental phantom for exercise in restorative dentistry (6); B) Use of cadaver to learn the anatomy of the head (7)





Cadavers

The tradition of using cadavers originated in ancient Greece in the third century B.C. Through the centuries, it has

become an important method that has given students a 3D view of human anatomy. In addition to formalin embalming, Thiel's (Walter Thiel) embalming method (8) has been successfully used to demonstrate a number of oral surgery techniques (9) (Figure 2B). Performing these procedures on a cadaver may enhance students' confidence prior to operating on their first patients and reduce the incidence of complications (10). Cadavers allow surgeons to practice procedures in a realistic environment, where they can make mistakes without endangering the patient's life (11). Today, due to traditional customs and culture, cadavers for medical education are rarely donated.

Actors

To make the simulation flow as realistic as possible, 'simulated patients' have also been employed in medical education. These are trained performers - actors, who simulate different clinical conditions and diseases and interact with students relying on medical history and specific segments of physical examination (12). Simulated patients can also be instructors and thus can give feedback to students on communication, technical and non-technical skills (5).

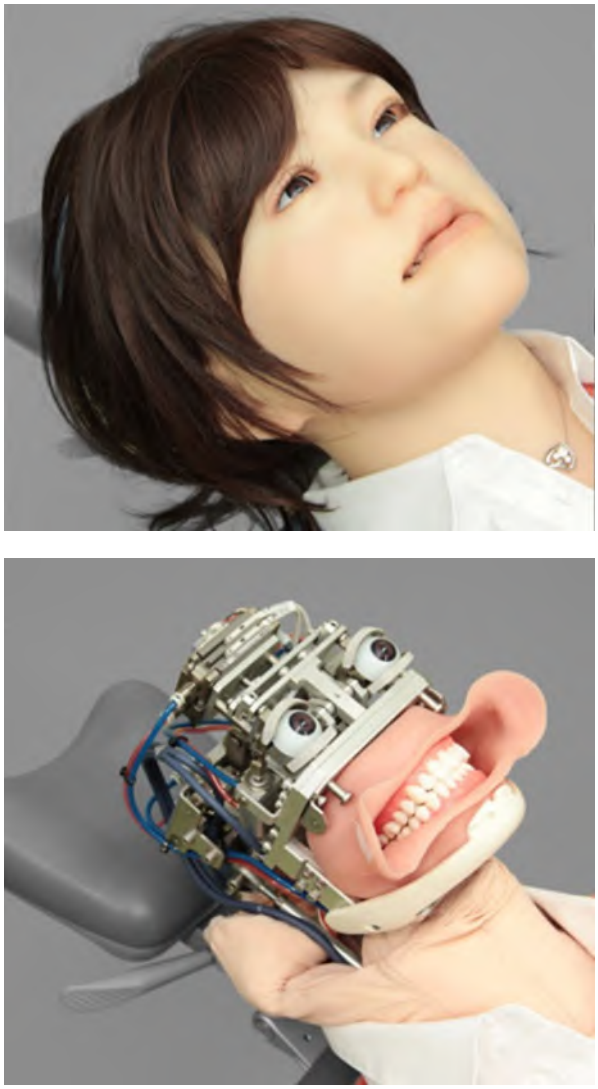
Robotics

This scientific branch is focused on the development of robots for automation of operations, and one of the main areas of robotic systems and artificial intelligence applications in the modern medical field. Tmsuk R&D Inc. and Tottori University Hospital of Yonago have jointly developed a Mikoto robot for medical training. This robot is specifically designed for endotracheal intubation, endoscopy and aspiration (13). In dental education, a Hanako robot (Hanako - Dentaroid, Nissin, Netherland) is being applied today, designed to simulate a patient with anatomical and physiological characteristics that the instructor can control at a touch of the panel interface on the controller (14) (Figure 3). The application of robots in medical branches gives high accuracy and can reduce the probability of making a mistake during the operation. Also, it can affect better diagnosis and thus better treatment of the disease. So, the main purpose of the development of medical robotics is to increase the efficiency of treatment, to reduce the risk and harm to human health, but from the financial aspect, it is still not widely applied in everyday education (15).

Computer-Aided Learning / mLearning

By introducing computers in the curriculum of health sciences (Computer-Aided Learning), the acceptance has been achieved, both by teachers and students. Computers effectively complement conventional learning and help students to acquire specific knowledge and to develop problem-solving skills. They can facilitate problem-based learning, by using realistic, risk-free clinical simulations and providing student-centered instructions under the control of the educator. This can result in a more enjoyable learning experience that encourages users to try to understand better and to use learning resources more often (16). With the development of mobile devices, smartphones have been increasingly used in the educational process (Mobile-Aided Learning, mLearning).

Figure 3. Robotics in dentistry
(Hanako - Dentaroid, Nissin, Netherland)



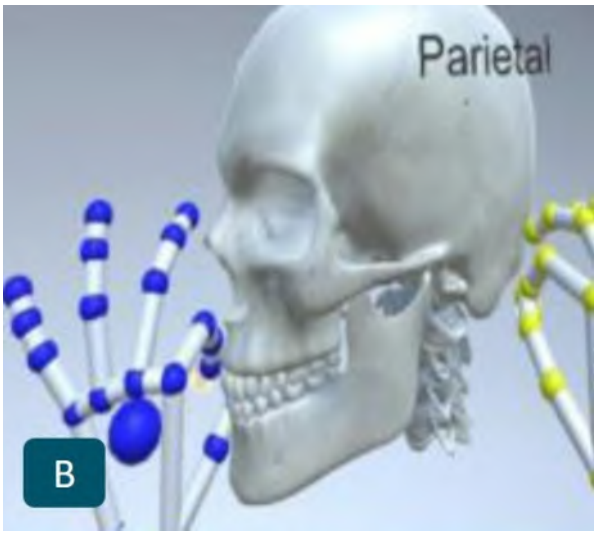
Undoubtedly, the use of mobile devices pervades the field of biomedical education in the form of simulation models, complementing conventional teaching. Numerous mobile applications can transform the medical environment and positively impact student education while also allowing students to learn at their own pace (17-20). The usage of computers and mobile learning devices usually requires students to follow the content without direct or indirect supervision of teachers, and occupies a special place in distance learning, both in the transfer of knowledge in remote areas and during emergencies and pandemics (21). Obstacles to success are mainly technical and practical and can be overcome through cooperation between institutions (22).

Virtual Reality and Augmented Reality

Virtual Reality (VR) is a computer-generated simulation made up of a set of images and sounds that represent a real place or situation, with which the user integrates with special equipment (the basis is VR glasses) (23, 24). Virtual Reality creates a world that man can experience and explore virtually, through his senses. The first application of Virtual Reality in medicine began in the early 1990s due to the need for medical staff to visualize complex medical procedures (25). Many VR systems are available today that allow a more detailed measurement of student performance by looking through precision, accuracy, speed, and error rate. The use of VR in medicine today is an important tool for improving surgical skills and has a positive effect on diagnosis and surgery planning (26) (Figure 4) (27, 28). In addition to surgery, VR has found application in endoscopy (29), neurology (30) and anesthesiology (31). Technological advances have made it possible to incorporate Virtual Reality simulation technology in dental education, and above all in the preclinical restorative dentistry (32, 33). Virtual Reality simulators provide the ability to integrate clinical scenarios into the operating environment and facilitate tactile skills using haptic technology (34). Haptic technology provides tactile feedback in the form of vibrations, movements, and the feeling of touch when applying force. This is very useful in a number of situations, including the ones where surgeons use minimally invasive techniques and manipulate long wires or instruments (35). The main advantage of applying these technologies is that the system regularly and routinely records data on all aspects of operator's performance. These data are not limited to criteria such as "completion time", but they include parameters such as the intensity of the tremor, the force that the instrument exerts on the tissues, the number of movements (5).

Figure 4. Virtual Reality simulation in Maxilofacial Surgery - A) environment sees; B) operator sees over VR glasses (26) (Images reproduced under creative commons)





Augmented Reality (AR) is a simulation of a three-dimensional environment created using hardware and software that provides the user with realistic experiences and ability to interact. Augmented Reality offers an interactive experience and aims to enhance the existing environment by adding virtual elements, rather than replacing it with a completely new environment. The key to using Augmented Reality was the development of personal computer devices, and the advancement of technology has brought augmented reality concepts to mobile devices. The concept of AR has found wide application in the arts, gaming industry, architecture, daily life, industrial design, navigation, auto industry, archeology. With increased need for catheterization and endoscopic interventions, AR has found an important role in many medical disciplines (36) (Figure 5) (37-39). In dental education, it is increasingly used in everyday clinical practice and education. It is most widely used in maxillofacial surgery due to complex clinical procedures, and is increasingly used in implantology, oral surgery and anaesthesia, aesthetics and morphology (Figure 6) (40-46). Augmented Reality shows great potential in facilitating learning, whether it helps in learning anatomy by providing interactive 3D models, improving fine motor skills, or helping students to see interactions from a patient's point of view. This technology provides students with new ways to better understand the complex structures described in textbooks and to apply them in real-world situations (47).

The biggest disadvantage of these sophisticated learning methods is the need for a good knowledge of technology and the concept of work. Contact with these technologies is largely unknown, so training with an educator and practicing is required. As physical contact with virtual objects is not achieved through Augmented Reality and Virtual Reality, it is necessary to work on better user integration in combination with haptic simulators and robotics (48).

Most researchers agree on the significant educational and motivational potential of virtual technologies. These new technologies are constantly advancing, becoming more

affordable, and users also have a positive attitude in implementing them (49).

Figure 5. Usage Augmented reality in operative surgery (36)

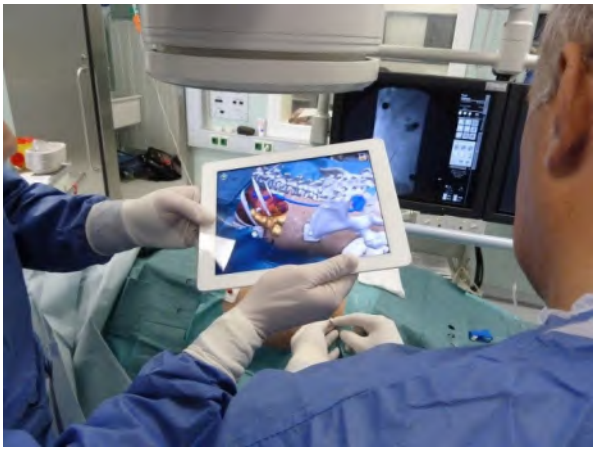


Figure 6. Learning local anaesthesia with Augmented Reality mobile simulator (Image by the author)



Martín-Gutiérrez et al (50) identified *four main aspects* regarding the *advantages* of using *virtual* technologies:

- increase student motivation and engagement, and 3D models enhance the learning experience;
- provide better access to learning - students interact with virtual objects, allowing them to explore, experiment and receive feedback;
- available through mobile devices (devices already owned and used by students) and
- Enable greater interaction than traditional teaching aids.

CONCLUSION

The development of clinical competence requires the assimilation of large amounts of knowledge combined with the acquisition of clinical skills and clinical problem-solving ability. The student should understand the concept of the procedure and develop fine motor skills to perform it. The acquisition of technical skills and the transfer of these skills to clinical practice is of paramount importance. The technological age we live in is greatly shaped by our understanding of space and time. The social circumstances created by the advancement of technology raised the question of adapting to new ways of knowledge transfer (42). The presence of science in the digital world is necessary in order to disseminate acquired knowledge in an adequate manner. In the battle for attention, science has competitors embodied in the entertainment and leisure industries. Standard textbooks, scientific monographs, and the conventional teaching process, despite serious and thorough research, seem uninspiring. The solution to the successful transfer of knowledge is to use new technologies. It is very important that educators and teachers always keep up with the times and provide students with the latest methods of learning and training. In doing so, they acknowledge students' interests and facilitate the learning process.

CONFLICT OF INTEREST

The authors declare no financial or commercial conflict of interest.

REFERENCES

1. Seymour NE, Gallagher AG, Roman SA, O'Brien MK, Bansal VK, Andersen DK, Satava RM. Virtual reality training improves operating room performance. Annals; 2002.
2. Datta R, Upadhyay K, Jaideep C. Simulation and its role in medical education. Medical Journal Armed Forces India. 2012;68(2):167-172.
3. Hssain I, Alinier G, Souaiby N. In-situ simulation: A different approach to patient safety through immersive training. Mediterranean JEmerg Med 2013;15:17-28.
4. Lippert A, Dieckmann P, Oestergaard D. Simulation in medicine. Notfall+Rettungsmedizin.2009;12(S2):49-52.
5. Forrest K, McKimm J. Healthcare simulation at a glance. Wiley-Blackwell; 2019.
6. Yekta SS, Lampert F, Kazemi S, Kazemi R, Brand HS, Baart JA, et al. Evaluationof new injection and cavity preparation model in local anesthesia teaching. J Dent Educ. 2013;77(1):51-7.
7. Dental Phantom. Do You Know What It Is? [Internet]. ARTEDENTAL. 2019 [cited 12 August 2019]. Available from: https://www.artedentalclinic.com/en/dental-phantom-do-you-know-what-it-is/.
8. Thiel W. Die Konservierung ganzer Leichen in natürlichen Farben. Ann Anat.1992;174:185-95.
9. Functional Cadaver Laboratory | L1-B | PTIFA [Internet]. Ptifa.com. 2019 [cited 12 August 2019]. Available from: https://ptifa.com/level-1/level1b/.

10. Eisma R, Lamb C, Soames R. From formalin to Thiel embalming: what changes? One anatomy department's experiences. Clin Anat 2013;26:564-71.
11. Salameh F, O'Reilly B, Balta J. The cadaver is not dead. Anatomical Science International. 2020;. https://doi.org/10.1007/s12565-020-00534-2.
12. Ikeyama T, Shimizu N, Ohta K: Low-cost and ready-to-go remote-facilitated simulation-based learning. Simul-Healthc 2012;7(1):35-9.
13. Otake T. Tottori venture touts simulated patient for budding medical professionals | The Japan Times [Internet]. The Japan Times. 2020 [cited 2 April 2020]. Available from: https://www.japantimes.co.jp/news/2017/04/12/national/science-health/tottori-venture-touts-simulated-patient-budding-medical-professionals/#.XoWq1YgzaUk.
14. Tanzawa T, Futaki K, Tani C, Hasegawa T, Yamamoto M, Miyazaki T et al. Introduction of a robot patient into dental education. European Journal of Dental Education. 2011;16(1):e195-e199.
15. Myronenko O. Robotics in medical education. Academic Notes Series Pedagogical Science 2019;1(183); 126-129 doi: 10.36550/2415-7988-2019-1-183-126-129
16. Lambert TJ, Kirkby KC, Dunn JC. Clinical education and the computer: A proposed model for computer-aided learning in the medical curriculum. Australas Psychiatr 1997;5:19-22.
17. Mohapatra D, Mohapatra M, Chittoria R, Friji M, Kumar S. The scope of mobile devices in health care and medical education. Int J Adv Med Health Res. 2015;2(1):3.
18. Muttappallymyalil J, Mendis S, John LJ, Shanthakumari N, Sreedharan J, Shaikh RB. Evolution of technology in teaching: blackboard and beyond in medical education. Nepal J Epidemiol. 2016;6(3):588-92.
19. Mladenović R, Pereira L, Đorđević F, Vlahović Z, Mladenović K, Cvetković A, Martinović B, Mladenović J, Popovski J. The use of mobile-aided learning in education of local anesthesia for the inferior alveolar nerve block. Vojnosanitetski pregled, 2018 OnLine-First (00):154-154 doi: 10.2298/VSP180622154M.
20. Mladenovic R, Cvetkovic A, Martinovic B, Mladenovic K, Zivkovic M, Arsic Z, Mladenovic S, Zecevic Lukovic T, Dakovic D. Efficiency of chewable toothbrush in reduction of dental plaque in students. BMC Oral Health. 2019;19:58 doi: 10.1186/s12903-019-0748-y.
21. Mladenovic R, Bukumiric Z, Mladenovic K. The Influence of a Dedicated Mobile Application on Studying Traumatic Dental Injuries During Student Isolation. J Dent Educ. 2020;1-3. https://doi.org/10.1002/jdd.12250
22. Dev P, Schleyer T.K.L. Computers in Health Care Education. In: Shortliffe E., Cimino J. (eds) Biomedical Informatics. Springer, London. 2014 https://doi.org/10.1007/978-1-4471-4474-8_23.
23. Park M, Kim D, Lee U, Na E, Jeon H. A Literature Overview of Virtual Reality (VR) in Treatment of Psychiatric Disorders: Recent Advances and Limitations. Frontiers in Psychiatry. 2019;10.
24. Li L, Yu F, Shi D, Shi J, Tian Z, Yang J, et al. Application of virtual reality technology in clinical medicine. Am J Transl Res 2017;9:3867-80. 10.4247/AM.2017.ABH187.
25. Pensieri C, Pennacchini M. Virtual Reality in Medicine. Progress in IS. 2016;:353-401.
26. Ayoub A, Pulijala Y. The application of virtual reality and augmented reality in Oral & Maxillofacial Surgery. BMC Oral Health 2019:19;238 https://doi.org/10.1186/s12903-019-0937-8.
27. Govea-Valladares E, Medellin-Castillo H, Ballesteros J, Rodriguez-Florido M. On the Development of Virtual Reality Scenarios for Computer-Assisted Biomedical Applications. Journal of Healthcare Engineering. 2018;2018:1-13.
28. Medellín-Castillo H. I., Govea-Valladares E. H., Pérez-Guerrero C. N., Gil-Valladares J., Lim T., Ritchie James M. The evaluation of a novel haptic-enabled virtual reality approach for computer-aided cephalometry. Computer Methods and Programs in Biomedicine. 2016;130:46-53. doi: 10.1016/j.cmpb.2016.03.014.
29. Lohre R, Wang J, Lewandrowski K, Goel D. Virtual reality in spinal endoscopy: a paradigm shift in education to support spine surgeons. Journal of Spine Surgery. 2020;6(S1):S208-S223.
30. Matamala-Gomez M, Donegan T, Bottiroli S, Sandrini G, Sanchez-Vives M, Tassorelli C. Immersive Virtual Reality and Virtual Embodiment for Pain Relief. Frontiers in Human Neuroscience. 2019;13.
31. Alaterre C, Duceau B, Sung Tsai E, Zriouel S, Bonnet F, Lescot T et al. Virtual Reality for PEripheral Regional Anesthesia (VR-PERLA Study). Journal of Clinical Medicine. 2020;9(1):215.
32. LeBlanc VR, Urbankova A, Hadavi F, Lichtenthal RM. A preliminary study in using virtual reality to train dental students. J Dent Educ 2004;68(3):378-83.
33. Buchanan JA. Use of simulation technology in dental education. J Dent Educ 2001;65:1225-1231.
34. Dutã M, Amariei C, Bogdan C, Popovici D, Ionescu N, Nuca C. An overview of virtual and augmented reality in dental education. Oral Health and Dental Management 2011;10(1):42-49.
35. Forrest K, McKimm J, Edgar S. Essential simulation in clinical education. Chichester, West Sussex: John Wiley & Sons; 2013.
36. Augmented reality and medicine, is the Middle East ready? - Wamda [Internet]. Wamda.com. 2019 [cited 12 August 2019]. Available from: https://www.wamda.com/memakersge/2015/08/augmented-reality-medicine-middle-east-ready.
37. Suenaga H, Tran H, Liao H, Masamune K, Dohi T, Hoshi K et al. Vision-based markerless registration using stereo vision and an augmented reality surgical navigation system: a pilot study. BMC Medical Imaging. 2015;15:51.
38. Pokhrel S, Alsadoon A, Prasad P, Paul M. A novel augmented reality (AR) scheme for knee replacement surgery by considering cutting error accuracy. The International Journal of Medical Robotics and Computer Assisted Surgery. 2018;15(1):e1958.

39. Budhathoki S, Alsadoon A, Prasad P, Haddad S, Maag A. Augmented reality for narrow area navigation in jaw surgery: Modified tracking by detection volume subtraction algorithm. The International Journal of Medical Robotics and Computer Assisted Surgery. 2020;. https://doi.org/10.1002/rcs.2097.
40. Mladenovic R, Pereira L, Mladenovic K, Videnovic N, Bukumiric Z, Mladenovic J. Effectiveness of Augmented Reality Mobile Simulator in Teaching Local Anesthesia of Inferior Alveolar Nerve Block. Journal of Dental Education 2019;83(4):423-428 doi: 10.21815/JDE.019.050.
41. Mladenovic R, Pereira L, Mladenovic K. The Impact of an Augmented Reality Application on Perception and Anxiety Level in Students during Administration of First Local Anesthetic Injection. BMC Oral Health. 2019;19:257:P42 doi:10.1186/s12903-019-0954-7.
42. Mladenovic R, Dakovic D, Pereira L, Matvijenko V, Mladenovic K. Effect of augmented reality simulation on administration of local anaesthesia in paediatric patients. Eur J Dent Educ. 2020;24:507-512 https://doi.org/10.1111/eje.12529.
43. Touati R, Richert R, Millet C et al. Comparison of Two Innovative Strategies Using Augmented Reality for Communication in Aesthetic Dentistry: A Pilot Study. Journal of Healthcare Engineering 2019:7019046.
44. Juan M-C, Alexandrescu L, Folguera F et al. A Mobile Augmented Reality system for the learning of dental morphology. Digital Education Review 2016;30:234-247.
45. Llena C, Folguera S, Forner L et al. Implementation of augmented reality in operative dentistry learning. Eur J Dent Educ 2018;22(1):e122-30.
46. Pellegrino G, Mangano C, Mangano R et al. Augmented reality for dental implantology: a pilot clinical report of two cases. BMC Oral Health 2019;19:158.
47. Campisi CA, Li EH, Jimenez DE, Milanaik RL. Augmented Reality in Medical Education and Training: From Physicians to Patients. In: Geroimenko V. (eds) Augmented Reality in Education. Springer Series on Cultural Computing. Springer, Cham; 2020.
48. Gandaglia G, Schatteman P, De Naeyer G, et al. Novel technologies in urologic surgery: a rapidly changing scenario. Curr Urol Rep.2016;17:19.
49. Liu D, Bhagat K, Gao Y, Chang T, Huang R. The Potentials and Trends of Virtual Reality in Education. Smart Computing and Intelligence. 2017;105-130.
50. Martín-Gutiérrez J. Virtual Technologies Trends in Education. EURASIA Journal of Mathematics, Science and Technology Education. 2016;13(1).

A CASE OF BIPHASIC TICK-BORNE ENCEPHALITIS VIRUS MENINGITIS IN A PRESUMED LOW-RISK REGION OF CENTRAL SERBIA

Sara M. Petrovic[1,2*], Ivana Rakovic[1,2], Nemanja Djordjevic[1,2], Sofija Sekulic Markovic[1,2], Ivana Lesnjak[1,2], Andrijana Krstic[1,2], Pavle Banovic[3,4] and Biljana Popovska Jovicic[1,2]

[1]*University of Kragujevac, Faculty of Medical Sciences, Department of Infectious Diseases, Serbia*
[2]*University Clinical Center Kragujevac, Clinic for Infectious Diseases, Serbia*
[3]*University of Novi Sad, Faculty of Medicine, Department of Microbiology with Parasitology and Immunology, Serbia*
[4]*Pasteur Institute Novi Sad, Department of Prevention of Rabies and Other Infectious Diseases, Clinic for Lyme Borreliosis and Other Tick-Borne Diseases, Serbia*

Received: 01.05.2026.
Accepted: 17.06.2026.

Corresponding author:

Sara M. Petrovic

E-mail: sara.nikolic.kv@gmail.com

ABSTRACT

Tick-borne encephalitis (TBE) is an emerging viral infection in many European regions. It typically presents with a biphasic illness characterized by an initial nonspecific febrile syndrome followed by neurological involvement. We report a case of an adult patient from Central Serbia admitted with persistent headache, recurrent fever and vomiting. The patient had a history of multiple tick bites in the months prior to illness onset. Laboratory findings revealed lymphocytic pleocytosis, elevated cerebrospinal fluid (CSF) protein, and positive TBEV-specific and IgG in both serum and CSF. Other viral serologies and PCR analyses were negative. This case highlights the importance of considering TBE virus as a potential cause of fever of unknown origin or central nervous system infection, even in regions not traditionally regarded as endemic. The occurrence of TBE suggests a potential shift in the epidemiological profile of Sumadija region, which has historically been considered a low-incidence or peripheral zone for TBE transmission. This case underscores the need for increased clinical vigilance and strengthened epidemiological surveillance in regions not previously considered at risk.

Keywords: *Tick-borne encephalitis virus, prolonged fever, meningitis, region of Sumadia, Serbia.*

UDK:

Eabr 2026; 27(3): 305-309
DOI: 10.2478/eabr-2026-0026

INTRODUCTION

Tick-borne encephalitis (TBE) is a zoonotic disease caused by the tick-borne encephalitis virus (*Orthoflavivirus encephalitidis*; TBEV), which belongs to the genus Orthof*lavivirus*. It is a significant cause of central nervous system (CNS) infections across Europe and parts of Asia. The disease is endemic in Central, Eastern, and Northern Europe. In several countries, the incidence of tick-borne encephalitis (TBE) fluctuates, reflecting the influence of climatic changes and the geographic spread of tick vectors and their animal hosts. Human exposure to TBE virus (TBEV) is accidental and is associated with entering or residing in TBEV-endemic foci, as well as consuming contaminated milk or dairy products (1). Most cases occur during seasons of increased tick activity, typically between April and November. The illness often demonstrates a biphasic pattern, beginning with a nonspecific febrile phase and, in a proportion of patients, progressing to CNS involvement. The clinical spectrum is broad, ranging from isolated meningitis to severe forms such as meningoencephalitis or myelitis, which can complicate early recognition of the disease due to its heterogeneous presentation (2).

In Serbia, TBE is rarely considered in the routine diagnostic evaluation of CNS infections and consequently remains substantially underrecognized. Nevertheless, seroepidemiological data indicate prior human exposure to TBEV, while molecular studies of Ixodes ricinus ticks have revealed the presence of viral genetic material, suggesting the existence of local natural transmission foci (3). Although Serbia has not been regarded as a country with high endemicity for TBE, available clinical reports suggest that it occupies an intermediate epidemiological position within the European landscape of TBE risk (4, 5).

Within this context, we present a case of tick-borne encephalitis meningitis in a patient from Lapovo, in the Sumadija region. Beyond its clinical relevance, this case draws attention to a geographic area in which the risk of TBE may be underestimated and underscores the need for increased clinical awareness and epidemiological surveillance. Our findings add to the growing evidence of TBE occurrence in regions previously considered to be at low risk.

CASE REPORT

A 54-year-old male was admitted to the Clinic for Infectious Diseases on June 9, 2025, due to prolonged fever and headache. The headache had been present since May 22, 2025. In the week preceding symptom onset, the patient reported daily prolonged sun exposure while performing agricultural work. During the initial days of illness, he experienced low-grade fever, with temperatures up to 37.5°C, followed by a short transient afebrile interval. Subsequently, fever recurred with higher peaks, reaching up to 39°C. Four days prior to hospitalization, the patient developed nausea and vomiting, occurring three to four times daily and exacerbated by head movement. He denied other accompanying symptoms. Fever responded transiently to treatment with a nonsteroidal anti-inflammatory drug (ibuprofen), while the headache persisted. No clinically significant abnormalities in laboratory parameters were observed at admission or during treatment (Table 1). The patient was initially evaluated by a neurologist. Neurological assessment, supplemented by brain MSCT and MSCT cerebral angiography, demonstrated no pathological findings. Epidemiological history revealed multiple tick bites in the months preceding illness onset, as well as mosquito bites.

Upon admission, the patient underwent thorough laboratory testing, including complete blood count, biochemical parameters, and relevant serological tests (Table 1). Brain imaging with multislice computed tomography (MSCT) showed no abnormalities.

Table 1. Laboratory test results at admission and before discharge in patient with Tick-borne encephalitis

Parameters	Day of illness		Reference range
SERUM	**17**	**24**	
WBC (10^9 /L)	7.4	4.1	3.70 – 10.00
Lymphocytes (10^9 /L)	1.3	1.0	1.20 – 3.40
PLT (10^9 /L)	319	364	135 – 450
C-reactive protein (mg/L)	5.15	0.48	< 5.00
AST (U/L)	15	19	0 – 40
ALT (U/L)	15	21	0 – 40
Glucose (mmol/L)	5.0	4.8	3.80 – 6.10
Na^+ (mmol/L)	133	139	137 – 147
K^+ (mmol/L)	3.6	3.5	3.50 – 5.30
CSF			
Cytology	89 Lymphocytes	21 Lymphocytes	0 – 5 Lymphocytes
Protein (g/L)	1.1	0.7	< 0.4
Glucose (mmol/L)	3.0 (out of 5)	2.8 (out of 4.8)	≈ 50–66% of serum glucose

Cerebrospinal fluid (CSF) obtained was clear and under elevated pressure, prompting further cytological and biochemical analyses. Initial cytological analysis demonstrated 89 lymphocytes, which decreased to 21 lymphocytes on follow-up examination. Biochemical analysis revealed elevated CSF protein levels (1.1 g/L initially, decreasing to 0.7 g/L), while CSF glucose values remained within the normal range.

In accordance with socio-epidemiological data indicating exposure to vector bites, additional diagnostic investigations were performed. A multiplex meningitis/encephalitis (ME) PCR test of cerebrospinal fluid (CSF), targeting the most common causes of meningoencephalitis in this geographic area, yielded negative results. Serological testing for West Nile virus infection was also negative. Given the clinical course of the disease and the history of tick exposure, further analyses were expanded to include a broader panel of arboviral pathogens (Table 2). First-tier serological testing for TBEV-reactive antibodies was performed at the national reference laboratory, the Institute of Virology, Vaccines and Sera "Torlak". To avoid ELISA-associated cross-reactivity with antibodies against other orthoflaviviruses, a neutralization assay was subsequently conducted at the Pasteur Institute Novi Sad. The TBEV NT100 titer was determined to be 1:80 in serum sample and 1:20 in CSF, indicating strong neutralizing activity.

Table 2. Combined serological and molecular analysis of cerebrospinal fluid and serum

Pathogen	Sample		Reference range
	serum	CSF	
multiplex meningitis/encephalitis PCR assay of CSF	Ø	negative	negative
Enterovirus PCR	negative	negative	negative
West Nile IgM/IgG PCR	negative	negative	negative: < 0.80 positive: > 1.10 PCR negative
Denga IgM/IgG PCR	negative	negative	negative: < 0.80 positive: > 1.10 PCR negative
Zika IgM/IgG PCR	negative	negative	negative: < 0.80 positive: > 1.10 PCR negative
TBE IgM/IgG NT100	IgM positive (1.43) IgG positive (1.64) 1:80	IgM positive (3.14) 1:20	negative: < 0.80 positive: > 1.10 negative: <1:10
Borelia burgdorferi IgM/IgG	negative	negative	negative
Toxoplasma gondii IgM/IgG	negative	Ø	negative

The patient was treated with anti-edematous (mannitol), vitamin supplementation including benfotiamine, and supportive symptomatic treatment, with resolution of fever and gradual withdrawal of symptoms.

DISCUSSION

Although TBE is primarily endemic in parts of Central and Eastern Europe, its occurrence in Serbia has historically been low and sporadic, with limited clinical recognition and surveillance. Serological and molecular studies have documented the presence of TBEV in *I. ricinus* ticks and low human seroprevalence in potential microfoci such as Fruska Gora and rural areas around Belgrade, indicating that TBEV exposure does occur within Serbian territories, but may be underrecognized due to the lack of comprehensive surveillance programs (6, 7). Serbia has also reported imported arboviral infections, including a fatal case of TBE acquired in Switzerland, as well as cases of imported Dengue virus infection (5, 8, 9). These observations illustrate a shifting epidemiological pattern of arboviral infections affecting CNS and febrile illness diagnostics in Serbia, underscoring the need for heightened clinical awareness and strengthened surveillance.

Recent predictive models integrating ecological and exposure determinants demonstrate that TBE risk in Europe is dynamic, with shifting spatio-temporal patterns extending into areas previously considered low risk (10). Such evidence supports the potential for autochthonous cases in Central Serbia and highlights the importance of strengthened epidemiological monitoring and heightened clinical awareness.

TBE remains a clinically challenging diagnosis due to its variable presentation and subtle early neurological symptoms. In endemic regions, a high index of suspicion is crucial, particularly in patients presenting with febrile illness following tick exposure or a biphasic course progressing to neurological involvement (11). West Nile virus (*Orthoflavivirus nilense*; WNV) is well established in many parts of Europe, with numerous locally acquired cases, and is often considered in the differential diagnosis of viral meningoencephalitis (12, 13). In our patient, common causes of CNS febrile illness, including WNV, were initially excluded, followed by an expanded evaluation for other arboviral pathogens.

Around 80% of European TBE cases follow a biphasic course, with initial nonspecific systemic symptoms such as fever, headache, myalgia, and malaise, followed by

neurological manifestations indicative of CNS involvement (11, 14). According to European Centre for Disease Prevention and Control (ECDC) criteria, a confirmed case requires clinical evidence of CNS inflammation, such as meningitis or encephalitis, together with laboratory confirmation of TBEV infection. Laboratory confirmation may be achieved through detection of TBEV-specific IgM and IgG in serum, IgM in CSF, seroconversion or a four-fold rise in paired sera, viral RNA detection, or virus isolation; a single positive IgM in serum is sufficient for a probable case (15).

The clinical spectrum of TBE is wide, ranging from asymptomatic infection and mild febrile illness to severe forms such as meningitis, encephalitis, or meningoencephalomyelitis. Disease severity varies, and although most patients recover, a proportion may develop long-term neurological sequelae, while case-fatality rates in Europe are generally low but not negligible (11, 16). In the presented case, the disease manifested as a milder clinical form with predominant meningeal involvement, a favorable clinical course, and complete recovery following supportive therapy.

Laboratory testing remains essential for diagnosis. Detection of TBEV-specific IgM in serum or CSF is considered definitive for acute infection, while seroconversion to IgG provides additional support. PCR testing may be informative during the early viremic phase but is often negative once neurological symptoms appear (14, 16). CSF analysis typically shows mild lymphocytic pleocytosis and elevated protein, while glucose levels usually remain normal. Neuroimaging can aid in differential diagnosis and exclusion of alternative causes but is not required for confirmation.

These findings are consistent with our case, in which diagnosis was established based on epidemiological exposure, neurological symptoms, and detection of TBEV-neutralizing antibodies in both serum and CSF. This case underscores the importance of integrating epidemiological, clinical, and laboratory data to achieve timely diagnosis and initiate appropriate management, particularly in areas with low or sporadic TBE incidence.

CONCLUSION

This case suggests that tick-borne encephalitis can occur autochthonously in regions historically considered at low risk, potentially highlighting the evolving epidemiological landscape in Serbia. It also underscores the critical importance of maintaining clinical suspicion for TBEV infection and the need for enhanced epidemiological surveillance to detect and manage emerging cases.

ACKNOWLEDGEMENT

None.

CONFLICT OF INTEREST

The authors declare no financial or commercial conflict of interest.

REFERENCES

1. European Centre for Disease Prevention and Control (ECDC). Tick-borne encephalitis – Annual Epidemiological Report for 2022. Stockholm: ECDC; 2024. Available from: https://www.ecdc.europa.eu/en/publications-data/tick-borne-encephalitis-annual-epidemiological-report-2022 (accessed 22 March 2026).
2. Bogovic P, Strle F. Tick-borne encephalitis: A review of epidemiology, clinical characteristics, and management. World J Clin Cases 2015; 3(5): 430-441.
3. Banovic P, Díaz-Sánchez AA, Galon C, Simin V, Mijatovic D, Obregón D, et al. Humans infested with Ixodes ricinus are exposed to a diverse array of tick-borne pathogens in Serbia. Ticks Tick Borne Dis. 2021;12(2): 101609.
4. Poluga J, Barac A, Katanic N, Rubino S, Milosevic B, Urosevic A, et al. Tick-borne encephalitis in Serbia: A case series. J Infect Dev Ctries. 2019;13(6):510-515.
5. Popovic Dragonjic L, Vrbic M, Tasic A, Simin V, Bogdan I, Mijatovic D, et al. Fatal Case of Imported Tick-Borne Encephalitis in South Serbia. Trop Med Infect Dis. 2022;7(12):434.
6. Potkonjak A, Petrovic T, Ristanovic E, Lalic I, Vracar V, Savic S, et al. Molecular Detection and Serological Evidence of Tick-Borne Encephalitis Virus in Serbia. Vector Borne Zoonotic Dis. 2017;17(12):813-820.
7. Banovic P, Obregón D, Mijatovic D, Simin V, Stankov S, Budakov-Obradovic Z, et al. Tick-Borne Encephalitis Virus Seropositivity among Tick Infested Individuals in Serbia. Pathogens. 2021;10(3):301.
8. Petrovic V, Turkulov V, Ilic S, Milosevic V, Petrovic M, Petric D, et al. First report of imported case of dengue fever in Republic of Serbia. Travel Med Infect Dis. 2016;14(1):60-61.
9. Rakovic I, Petrovic S, Protic J, Lesnjak I, Djordjevic N, Pete M, et al. Case report: Clinical presentation of imported cases of dengue: a case series. Front. Trop. Dis. 2026; 7:1771412. doi: 10.3389/fitd.2026.1771412
10. Dagostin F, Erazo D, Marini G, Da Re D, Tagliapietra V, Avdicova M, et al. Predicting the spatio-temporal risk of human tick-borne encephalitis (TBE) in Europe by combining hazard and exposure drivers. One Health. 2026;22:101331.
11. Halsby K, Gildea L, Madhava H, Angulo FJ, Pilz A, Erber W, et al. Clinical manifestations and outcomes of Tick-borne encephalitis: A systematic literature review. Ticks Tick Borne Dis. 2024;15(6):102407.
12. European Centre for Disease Prevention and Control (ECDC). Historical data on local transmission in Europe for West Nile virus; 2024. Available from: https://www.ecdc.europa.eu/en/west-nile-fever/surveillance-and-disease-data/historical
13. Petrovic T, Sekler M, Petric D, Vidanovic D, Debeljak Z, Lazic G, et al. Intensive West Nile Virus Circulation in Serbia in 2018-Results of Integrated Surveillance Program. Pathogens. 2021;10(10):1294.
14. Dumpis U, Crook D, Oksi J. Tick-borne encephalitis. Clin Infect Dis. 1999;28(4):882-90.

15. European Centre for Disease Prevention and Control (ECDC). 2023. Fact sheet about TBE. https://www.ecdc.europa.eu/en/tick-borne-encephalitis/ facts/factsheet (accessed 07 June 2023).
16. Ruzek D, Avsic Zupanc T, Borde J, Chrdle A, Eyer L, Karganova G, et al. Tick-borne encephalitis in Europe and Russia: Review of pathogenesis, clinical features, therapy, and vaccines. Antiviral Res. 2019;164:23-51.

CHALLENGES IN REMOVING A BROKEN ABUTMENT SCREW: A CASE REPORT

Sonja Milosavljevic[1], Milica Jovanovic[1*], Vladan Radisavljevic[1] and Marko Milosavljevic[1]

[1]*University of Kragujevac, Faculty of Medical Sciences, Department of Dentistry, Serbia*

Received: 26.03.2026.
Accepted: 08.07.2026.

Corresponding author:

Milica Jovanovic

Phone: +381 34 342 944

E-mail: micamonro@gmail.com

ABSTRACT

One of the common complications in implant-supported prostheses is fracture or loosening of the abutment screw, which can lead to disruption of the harmony between the implant-supported prosthesis and the implant, and subsequently compromise the entire restoration. This case report demonstrates the protocol for removing a fractured abutment screw, as well as the continuation of prosthetic rehabilitation. A 51-year-old patient presented to the clinic due to looseness of a maxillary implant-supported denture. After clinical and radiographic examination, a fracture of the abutment screw within the implant at position 22 was diagnosed. A commercially available system (Rhein'83 Broken Screw Extractor Kit, Bologna, Italy) was used for the removal of the fractured screw. Adherence to the recommended protocol and use of the extraction kit enabled removal of the fractured screw without damaging the implant threads and without removing the implant. Preservation of the implant allowed placement of a new OT Equator abutment and re-establishment of the retention and stabilization function of the prosthesis. This management approach proved to be an effective and safe method for preventing implant damage and ensuring adequate removal of the fractured screw.

Keywords: *Dental implants, Removal kit, OT Equator abutment, Screw fracture.*

UDK:

Eabr 2026; 27(3): 311-316
DOI: 10.2478/eabr-2026-0027

INTRODUCTION

From the beginning of human history to the present day, various attempts at replacing missing teeth have been recorded (1). The first steps in dental tooth replacement involved the use of shells and ivory, eventually leading to modern implantology based on titanium dental implants (2,3). According to their high success rate and long-term predictability, dental implants are today considered the gold standard for the replacement of missing teeth (4).

Intending to achieve adequate functional and esthetic rehabilitation of edentulous patients, implant-supported dentures have undergone significant advancement, along with their attachment systems. Currently, low-profile attachment systems based on the Locator and OT Equator principles are widely used (5). One of the main advantages of the OT Equator system (Rhein'83, Bologna, Italy) over other attachment systems is its reduced size and high retention for implant-supported dentures. In addition, favorable force distribution and the possibility of use in cases of reduced prosthetic space make this system unique (5,6).

With the development of dental implants, their precise design, and the use of biocompatible materials, treatment success has increased. However, the growing use of implants in tooth replacement has also led to an increased risk of complications and implant failure (7). Implant failure may be influenced by poor general health and the presence of autoimmune diseases such as diabetes mellitus, rheumatoid arthritis, multiple sclerosis, and Sjögren's syndrome (8).

In contrast to these general factors, improper surgical technique, inadequate prosthetic planning, and unfavorable loading of the implant-supported restoration are also factors that can lead to complications and implant failure (7,9).

According to the above, mechanical complications that may occur include fracture of the crown or implant, ceramic chipping or cracking, and loosening or fracture of the abutment screw. These complications are most often caused by excessive occlusal forces, improper screw design, and material fatigue, while screw loosening and fracture are most commonly the result of inadequate tightening technique or insufficient torque (10,11).

Abutment screw fracture, which may occur at the level of the screw head, shank, or threads, although relatively rare, requires removal of the fractured component (12). However, there are cases in which the position of the fractured fragment necessitates leaving it in place. Conventional methods, such as ultrasonic devices and explorers, have shown a success rate of approximately 73% in removing fractured fragments, while significantly higher success has been reported with mechanical techniques involving precise drilling and extraction of the fragment (11).

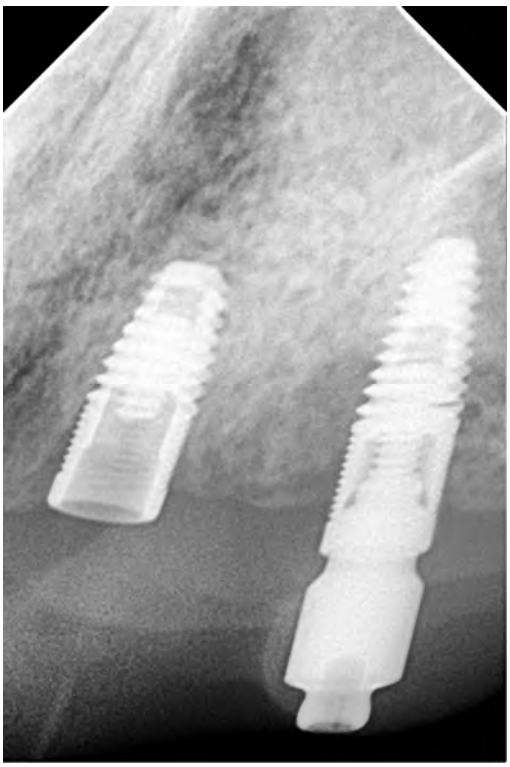
These fragment removal techniques may generate heat, which can be transmitted through the metal to the bone–implant interface, potentially compromising osseointegration (11). Nevertheless, extraction of the fractured fragment is generally recommended, as its presence may allow bacterial infiltration, which, together with mechanical complications, can impair the osseointegration process, lead to bone loss, and ultimately result in implant fracture and failure (13).

This case report aimed to demonstrate the clinical protocol for managing a fracture of an OT Equator abutment using the Rhein'83 Broken Screw Extractor Kit**,** emphasizing the safe and effective removal of the fractured screw**,** preservation of the implant thread integrity, and the subsequent prosthetic rehabilitation**.**

CASE REPORT

A 51-year-old male patient presented to the Department of Dentistry, Faculty of Medical Sciences, University of Kragujevac, complaining of reduced retention of his maxillary overdenture. In the patient's history, the mobility of the prosthesis occurred after mechanical trauma. Review of the dental records revealed that four implants (B&B Dental, Bologna, Italy) had been placed two years earlier in positions 14 (3.5 × 10 mm), 12 (4.0 × 8 mm), 22 (4.0 × 8 mm), and 24 (3.5 × 10 mm). After the osseointegration period, a maxillary implant-supported overdenture retained by OT Equator abutments (Rhein 83, Bologna, Italy) was fabricated. At the time of presentation, the prosthesis had been in function for approximately 18 months. During this period, the patient attended regular six-month follow-up visits, during which retentive caps were routinely replaced. In the mandible, the patient had a semicircular metal-ceramic bridge. Medical history revealed arterial hypertension under therapy, and the patient was an active smoker. Clinical examination, prompted by the patient's complaint of prosthesis mobility, revealed fracture of the Equator abutment at implant position 22. Radiographic assessment, including retroalveolar radiographs, confirmed the presence of a fractured abutment screw within the implant at position 22, as well as a fracture line affecting the implant at position 24 (Figure 1).

Figure 1. Retroalveolar radiograph showing fractured abutment screw (22) and implant fracture (24)



Following comprehensive clinical and radiographic evaluation, the OT Equator abutment at position 24 was removed. During this procedure, the fractured coronal portion of the implant was retrieved together with the abutment (Figure 2). The remaining implants were clinically stable and osseointegrated.

Figure 2. Retrieved fractured implant fragment (24) and abutment (22)



Considering the preservation of osseointegration and the absence of inflammatory changes, a conservative treatment approach was planned. The objective was to retrieve the fractured screw fragment at position 22 and replace it with a new abutment, while subsequently evaluating the possibility of placing an additional implant in the region of the fractured implant or distal to it in order to achieve more favorable load distribution and increased prosthetic support. After discussion of the proposed treatment plan and obtaining written informed consent, the corrective procedure was scheduled.

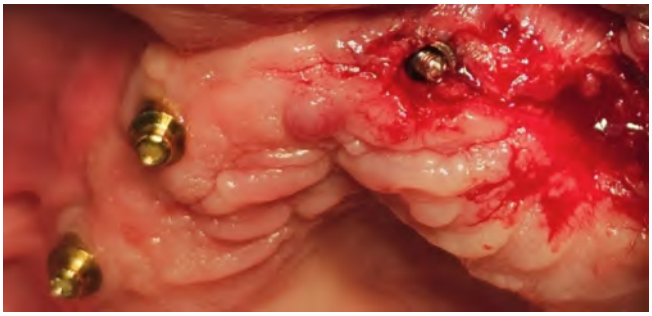
For removal of the fractured abutment screw at implant position 22, a dedicated retrieval kit (Rhein 83 Broken Screw Extractor Kit, Bologna, Italy) was utilized. A surgical flap was elevated in the affected region to ensure adequate access and visibility. The fractured fragment was retrieved using the Claw Reamer bur mounted on a contra-angle handpiece in combination with a manual centering device, according to the manufacturer's protocol.

The centering device was first positioned within the implant to ensure precise coaxial alignment and to protect the internal thread configuration. The Claw Reamer bur was then introduced through the guide and initially operated at a low rotational speed of 30 rpm under continuous irrigation (Figure 3). As the fractured screw fragment could not be mobilized at this speed, the rotational velocity was carefully increased to 60 rpm to enhance mechanical engagement while maintaining thermal safety. Controlled engagement of the coronal aspect of the fractured fragment was subsequently achieved using gentle apical pressure. Once stable interlocking was established, counterclockwise rotation enabled gradual mobilization and atraumatic retrieval of the retained screw segment (Figure 4).

Figure 3. Placement of extractor kit elements – centering device and Claw Reamer bur



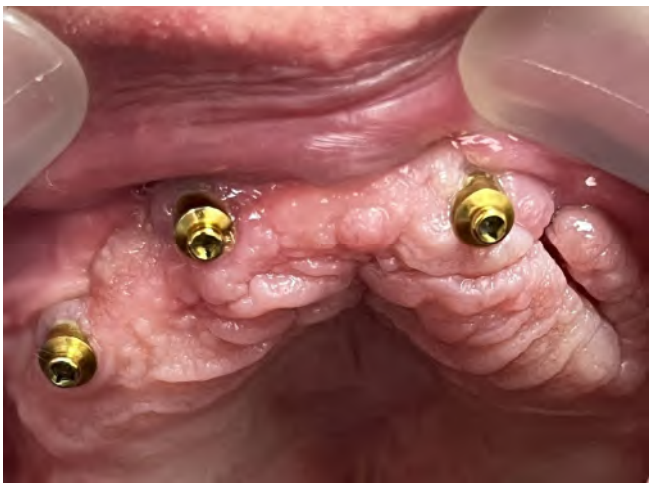
Figure 4. Removed fractured abutment screw segment



Following extraction, the implant chamber was thoroughly irrigated and carefully inspected to confirm preservation of the internal threads and complete removal of metallic debris. The integrity of the implant–abutment interface was verified, allowing continuation of prosthetic rehabilitation without the need for implant removal. The surgical site was sutured, and the patient was scheduled for follow-up. Sutures were removed seven days postoperatively, with uneventful soft tissue healing observed.

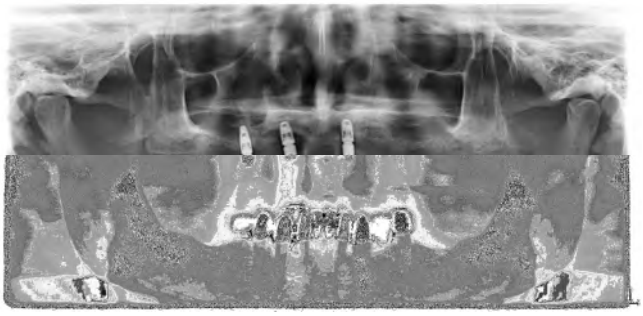
Based on the previously documented transmucosal height of the OT Equator abutment (5 mm), a new abutment of identical height (Rhein 83) was placed at implant position 22 (Figure 5). The retentive caps within the overdenture were replaced, resulting in re-establishment of satisfactory prosthesis stability and retention. After replacing the OT Equator abutment and retention caps, re-occlusion was performed with articulating paper to eliminate premature and strong contacts. In order to ensure adequate distribution of masticatory forces on the protective work, correction of occlusal contacts was performed in places where these premature and strong contacts would lead to the creation of strong forces, in an unfavorable direction. At the control visit, the patient reported significant improvement in function and comfort, without pain or mobility.

Figure 5. Newly placed OT Equator abutment at implant position 22



Regarding implant position 24, due to the confirmed implant fracture, the remaining implant portion was atraumatically removed. The extraction site was thoroughly debrided and filled with a bone substitute material to preserve ridge volume and support future implant placement (Figure 6).

Figure 6. Control orthopantomography after removing fractured implant and bone substitution



After an appropriate healing period, clinical and radiographic evaluation will be performed to assess bone remodeling and potential resorption at the grafted site. Based on the findings, the optimal position and timing for placement of a new implant will be determined in order to ensure favorable load distribution and long-term prosthetic stability.

DISCUSSION

This case presented the clinical management of mechanical complications associated with an implant-retained prosthesis retained by the OT Equator system, including abutment screw fracture and implant fracture in the maxilla. The results indicate that accurate diagnosis and the application of a structured extraction protocol may allow for the preservation of osseointegration and the continuation of prosthetic rehabilitation through a minimally invasive therapeutic approach.

The use of dental implants represents a standard protocol for replacing missing teeth; however, complications such as abutment screw fracture remain a clinical challenge (10). The most common causes of screw loosening and fracture include improper implant–abutment fit, excessive occlusal forces, and material deformation (11). When the preload force is insufficient to counteract separating forces, screw loosening occurs, potentially leading to fracture and peri-implant complications. Reported rates of implant damage (9.8%) and internal thread damage (13.33%) during fragment retrieval highlight the limitations of currently available techniques (12). Fractures most frequently occur at the junction between the screw head and shank or between the shank and threaded portion. Various removal methods have been described. Raju et al. recommend explorers or ultrasonic scalers depending on fragment position (13), while Gooty et al. suggest creating an indentation to facilitate ultrasonic vibration–assisted loosening (14). Nayana et al. introduced a modified 557 bur operating in counterclockwise rotation (15). Despite these

approaches, no standardized protocol has been established, and the risk of internal thread damage remains significant (12).

In the present case, removal was performed using a dedicated extraction kit (Rhein 83 USA Broken Screw Extractor Kit), differing from the noninvasive methods proposed by Raju and Gooty. Senet-Vicente et al. reported that the Rhein 83 drilling technique achieved superior outcomes, with an 84.4% success rate and shorter retrieval time compared to Phibo and Sanhigia techniques (16). Prolonged drilling time, as observed with Phibo and Sanhigia systems, may increase metal temperature and complicate removal (17). Similarly, Agustin-Padanero et al. found ultrasonic techniques to be more complex and time-consuming than dedicated removal kits (18). Although Martinez-Alcaraz et al. did not observe significant differences in implant wear between conventional and drilling techniques, most studies indicate that drilling kits such as Rhein 83 reduce the risk of internal thread damage (12,13,15,19).

A thorough understanding of abutment mechanics is essential to prevent such complications. Schwarz et al. reported that screws are frequently tightened 30–50% below recommended torque values, contributing to loosening and fracture (20). Retightening after 10 minutes is recommended to compensate for settling and maintain preload stability (21,22). Material selection also plays a role: titanium abutments generally demonstrate superior mechanical resistance compared to PEEK and zirconia, although Kohal et al. reported favorable performance of alumina-reinforced zirconia and carbon fiber–reinforced PEEK under artificial loading (24,25).

In this case report, the etiological factors that can lead to fracture of the abutment and implant include chronic mechanical trauma, the presence of a metal-ceramic bridge in the mandible, and potential interferential occlusal contacts. The continuous action of the primary etiological factors can lead to material fatigue, structural weakness and ultimately its fracture, which in this particular case manifested. In addition to the above factors, smoking should be seen as an important predisposing factor, whose cumulative effect with the previously mentioned primary factors could contribute to the occurrence of this complication. In the current literature, active smoking can indirectly lead to a weakening of the osseointegration bond, a decrease in the quality of the peri-implant bone and a decrease in the biomechanical stability of the implant and prosthetic work. Taking all of the above into account, the most likely reason for the occurrence of this complication is the continuous cumulative effect of primary and secondary predisposing factors (26).

Although the Rhein'83 Extractor Kit has proven to be a reliable tool for the removal of fractured abutment screws, certain limitations should be considered. The system is primarily used in conjunction with OT Equator abutment screws within the Rhein83 implant-prosthetic portfolio, and its applicability to other implant systems may depend on screw design and manufacturer specifications. The success of the procedure largely depends on the clinician's experience and technical skill, particularly in cases involving deeply positioned or difficult-to-access screw fragments. In addition, mechanical manipulation within the implant channel may potentially generate heat and metallic debris, especially if excessive pressure is applied. Therefore, adequate irrigation and careful instrumentation are essential to prevent thermal damage and to preserve the integrity of the implant–abutment interface. Within these limitations, the Rhein'83 Extractor Kit represents a clinically viable and conservative approach for managing fractured abutment screws, with a low risk of complications when used according to recommended clinical protocols.

CONCLUSION

The present case supports the clinical feasibility and reliability of a controlled drilling protocol for the retrieval of fractured OT Equator abutment screws using the Rhein 83 USA Broken Screw Extractor Kit, while preserving implant integrity and maintaining the continuity of implant–prosthetic rehabilitation. Given the inherent risk of thread damage during fragment removal, such procedures remain clinically demanding and technique-sensitive. The applied protocol demonstrated that a carefully controlled drilling technique can ensure predictable fragment retrieval while preserving the integrity of the implant threads and preventing excessive heat generation. By maintaining osseointegration and enabling continuation of prosthetic rehabilitation, this approach may represent a reliable and clinically valuable strategy in the management of similar mechanical complications, contributing to long-term implant-prosthetic success.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

INFORMED CONSENT STATEMENT

Informed consent was obtained from the participant or their representatives.

FUNDING

None.

REFERENCES

1. Abraham CM. A brief historical perspective on dental implants, their surface coatings and treatments. Open Dent J. 2014;8:50–55.
2. Fareed WM, Hossam M, Mossa E, Ekrami M, Abouseif M, Almutairi M, et al. Evolution of dental implant and implant surface treatments: a narrative review. Bull Stomatol Maxillofac Surg. 2025.
3. Jain S, Hemavardhini A, Ranjan M, Pasricha N, Thakar S, Soni K, et al. Evaluation of survival rates of dental implants and the risk factors: a retrospective follow-up study. Cureus. 2024;16:e55360.

4. Sharma M, Solanki S, Razdan RA, Bhargava A, Srivastava A, Menon I. Linking prosthodontics and periodontics: an implant success rate. Bioinformation. 2025;21(3):447–451.
5. Tallarico M, Fiorillo L, Montanari M, Scrascia R, Cristache CM, Ferrari E, et al. One-year multicenter randomised controlled trial comparing OT Equator® and Locator attachments to retain an early loaded implant overdenture on two implants. Int J Biomater. 2023;2023:2745262.
6. Khalifa AK, Alokda MM, Said MM. Using different low-profile abutments for assisting mandibular implant overdenture: a split-mouth study. Int J Dent. 2022; 2022:8738220.
7. Mahendra J, Subbiah U, GopalaKrishnan P, Karunanidhi K, Rajendran S, Mohan S. Decoding success: a five-year retrospective study of dental implant survival. J Oral Biol Craniofac Res. 2025;15(5):948–954.
8. Chitumalla RK, Gopinathan PA, Jamjoom FZ, Munaga S, Alaifi A, Siddeeqh S. Success rate of dental implants in patients with autoimmune disorders: a systematic review and meta-analysis. J Int Soc Prev Community Dent. 2025;15(5):402–414.
9. Chen ST, Buser D, Sculean A, Belser UC. Complications and treatment errors in implant positioning in the aesthetic zone: diagnosis and possible solutions. Periodontol 2000. 2023;92(1):220–234.
10. Arshad M, Mohammadi M, Habibzadeh S, Peláez J, Joda T, Mosaddad S, et al. The efficacy of techniques for managing fractured dental implant abutment screws: a systematic review and meta-analysis. J Evid Based Dent Pract. 2026;102226.
11. Chowdhary R, Sonnahalli N, Gala J. Implant abutment screw fracture and techniques of retrieval: a literature review based on a novel abutment screw fracture classification. J Osseointegr. 2023;15(1):32–39.
12. Martínez-Alcaraz P, Flores-Fraile J, Agustín-Panadero R, Senent G, Gómez-Polo C, Cruz-Jiménez S, et al. Retrievability of fractured abutment screws and damage to implant–abutment connections: an in vitro comparative analysis of conventional vs. drilling techniques. Appl Sci. 2024;14:11846.
13. Raju S, Nair VV, Karunakaran HK, Manjuran NG. Management of perishing implants with abutment screw fracture: a systematic review. J Indian Prosthodont Soc. 2021;21(3):229–239.
14. Gooty JR, Palakuru SK, Guntakalla VR, Nera M. Noninvasive method for retrieval of broken dental implant abutment screw. Contemp Clin Dent. 2014;5:264–267.
15. Nayana P, Nayak SS, Chatterjee A, Sivaraman K, Srikanth G, Singh C. Retrieval of fractured implant abutment screws: a narrative review. J Int Soc Prev Community Dent. 2022;12(3):287–294.
16. Senent-Vicente G, Baixauli-López M, González-Angulo E, Fernández-Bravo L, Zubizarreta-Macho Á, Gómez-Polo M, et al. In vitro analysis of the removability of fractured prosthetic screws within endosseous implants using conventional and mechanical techniques. Materials (Basel). 2023;16(23):7317.
17. Brisman DL. The effect of speed, pressure, and time on bone temperature during the drilling of implant sites. Int J Oral Maxillofac Implants. 1996;11(1):35–37.
18. Agustín-Panadero R, Baixauli-López M, Gómez-Polo M, Cabanes-Gumbau G, Senent-Vicente G, Roig-Vanaclocha A. In vitro comparison of the efficacy of two fractured implant-prosthesis screw extraction methods: conventional versus mechanical. J Prosthet Dent. 2020;124(6):720–726.
19. Moorthy A, Aljudaibi S, Donnelly-Swift E, Polyzois I, Grufferty B. An in vitro evaluation of 2 methods for retrieving fractured abutment screw fragments from the intaglio of 4 different implant systems. J Prosthet Dent. 2024;131(2):282–290.
20. Schwarz MS. Mechanical complications of dental implants. Clin Oral Implants Res. 2000;11 Suppl 1:156–158.
21. Alevizakos V, Bergmann AL, von See C. Removal of broken abutment screws using ultrasonic tip - a heat development in-vitro study. BMC Oral Health. 2024; 24(1):2.
22. Soleimani M, Żmudzki J, Pakieła W, Jaśkowska A, Krasny K. Dental implant abutment screw loss: presentation of 10 cases. J Funct Biomater. 2024;15(4):96.
23. Sagheb K, Görgen CI, Döll S, Schmidtmann I, Wentaschek S. Preload and friction in an implant-abutment-screw complex including a carbon-coated titanium alloy abutment screw: an in vitro study. Int J Implant Dent. 2023;9(1):8.
24. Salman I. The effect of implant abutment material of titanium, zirconia, and polyether ether ketone on prosthetic screw fracture resistance. Front Biomed Technol. 2025;12(4):764–770.
25. Kohal RJ, Schikofski T, Adolfsson E, Vach K, Patzelt SBM, Nold J, et al. Fracture resistance of a two-piece zirconia implant system after artificial loading and/or hydrothermal aging—an in vitro investigation. J Funct Biomater. 2023;14(12):567.
26. Chrcanovic BR, Albrektsson T, Wennerberg A. Smoking and dental implants: A systematic review and meta-analysis. J Dent. 2015 May;43(5):487-98.

INFORMATION FOR AUTHORS

AIMS AND SCOPE

Experimental and Applied Biomedical Research (*EABR)* former *Serbian Journal of Experimental and Clinical Research* is a peer-reviewed, open access journal which publishes original research articles, reviews, case reports and letters to the editor in all areas of the biomedical sciences that have not been published previously. The journal comprises both basic and clinical research in the field of biomedicine. Current acceptance rate is 60%. *EABR* was founded in 2000 under the name *Medicus* and over more than two decades has grown into one of the leading national journals in the field of biomedical sciences. *Experimental and Applied Biomedical Research* is owned and published by Faculty of Medical Sciences University of Kragujevac. The journal adheres to the policies of the International Committee of Medical Journal Editors (ICMJE) and publishing ethics guidelines provided by the Committee on Publication Ethics (COPE).

TYPES OF MANUSCRIPTS

- *Original research articles*: *EABR* considers all original research manuscripts which present the results of an original research study (experimental or clinical). These manuscripts must contain sufficient information on all relevant research methods, as well as a detailed analysis of the results obtained.

- *Reviews*: *EABR* considers literature reviews, systematic reviews and meta analyses addressed to a particular subject area, with special reference to new knowledge and facts. Manuscripts in this category must not be shorter than 6000 words, the text must cite more than 70 references of which 50% have been published in the previous 5 years. Systematic reviews should follow the PRISMA guidelines.

- *Case reports*: *EABR* considers case reports presenting detailed information on the symptoms, signs, diagnosis, treatment (including all types of interventions), and outcomes of an individual patient. Case reports should usually describe new or uncommon conditions that serve to enhance medical care or highlight diagnostic approaches. Case reports should follow the CARE guidelines.

- *Letters to the editor: EABR* considers letters to the editor related to different clinico-laboratory observations. They should be titled, not exceed 500 words, and have a maximum of 5 references. Up to 1 table or figure may be submitted, but will be published at the discretion of the Editor. No more than 3 authors should appear.

MANUSCRIPT SUBMISSION

Manuscripts submitted to *Experimental and Applied Biomedical Research* must neither be published previously nor be under consideration for publication in another journal. Manuscripts are acompanied with a suitable *cover letter* stating that: the manuscript is not

submitted for publication elsewhere; all authors have agreed to submission; the study is carried out in accordance with relevant ethical international guidelines.

EABR considers only manuscripts written in English using *Microsoft Office Word* format and uploaded online at https://www.editorialmanager.com/sjecr/.

Plagiarism, data fabrication and image manipulation are not tolerated. Plagiarism includes copying text, ideas, images, or data from another source, even from authors own publications, without providing any reference to the original source. If a study's design or the manuscript's structure or language has been inspired by previous works, these papers must be explicitly cited. All manuscripts submitted to *Experimental and Applied Biomedical Research* are checked for plagiarism using the academic standard software prior to the first step of the editorial process.

MANUSCRIPT PREPARATION AND ORGANISATION

Title Page

The Title Page should contain the following informations:

- Manuscript title
- Full author(s) names
- The affiliation(s) of the author(s)
- A clear indication and an active e-mail address of the corresponding author

Manuscript title should be concise and informative.

It is necessary to state the full names and surnames (middle letter or name is optional) of all authors and the exact affiliations of all authors - institution, (department), city, (state), country. *Experimental and Applied Biomedical Research* remains neutral with regard to jurisdictional claims in institutional affiliations. Responsibility for affiliations ultimately rests with the author.

Abstract

Provide an abstract of 150 to 250 words. Abstract should be structured (Background, Methods, Results, Conclusion), citation-free, without abbreviations if possible.

Keywords

Three to five relevant keywords need to be added after the abstract. Keywords should be specific to the manuscript, yet reasonably common within the subject discipline.

Text Formatting

Manuscripts should be submitted in *Microsoft Office Word*. The authors should use normal, plain *Times New Roman* font (12pt) for text. Pages should be numbered automatically. Italics may be used for emphasis. Abbreviations should be defined at the first mentioning in the text and used consistently thereafter (do not use a separate subtitle for abbreviations only). Please use no more than three levels of displayed headings. International System (SI) of Units should be used (imperial, US customary and other units should be converted to SI units).

Original research articles should contain following sections: Introduction, Materials and Methods, Results, Discussion, Conclusions, Acknowledgments, Conflict of Interest, and References. *Reviews* may require different formats, while *Case reports* manuscripts should follow the CARE guidelines.

Introduction. This section should contain context or background for the study, rationale, clear aim of research or tested hypothesis.

Materials and Methods. This section should provide sufficient detail for replication of the study. If more than one method is used in the research, use subsections with appropriate subheadings. The *Materials and Methods* section should also contain following statements:

a) Informed Consent Statement. In cases where the identification of personal information is necessary for scientific reasons, authors should obtain informed consent from all individuals included in the study
b) Human Right Statement. Manuscripts containing information related to human should clearly state that the research has complied with all relevant international and national regulations and institutional policies and has been approved by the authors' institutional Ethics committee.
c) Animal Right Statement. Manuscripts containing information related to animals should clearly state that the research has complied with all relevant international and national regulations and institutional policies and has been approved by the authors' institutional Ethics committee.

For details and examples of statements please see part 'Research and publication ethics'.

Results. The results should be presented in logical sequence in the manuscript. Do not repeat all the data in the tables or figures in the text.

Conclusions. Within the *Conclusions* section the authors should clearly explain the main conclusions of the article, highlighting its importance and relevance.

Acknowledgments. Acknowledgments of people, grants, funds, etc. should be placed in a separate section after the Conclusions section. The names of funding organizations should be written in full. This may include administrative and technical support, or donations in kind (e.g., materials used for experiments).

Conflict of Interest. Authors must declare all relevant interests that could be perceived as conflicting. If there is no conflicts exist, the authors should state this. Submitting authors are responsible for coauthors declaring their interests.

References. *References* must be numbered in order of appearance in the text (including table captions and figure legends) and listed individually at the end of the manuscript. In the text, reference numbers should be placed in round brackets ( ), and placed before the punctuation – e.g. (1), (1–3) or (1, 3). The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text.

The reference list should include contain surnames and the first letter of the author's name, full title, abbreviated title of the journal, year of publication, volume, number and pagination (Vancouver style guide). In case where the list of authors are more than six, please use et al. after the sixth author.

The examples of correct referencing:

For journal papers:
Shoji F, Haro A, Yoshida T, Ito K, Morodomi Y, Yano T, et al. Prognostic significance of intratumoral blood vessel in- vasion in pathologic stage IA non-small cell lung cancer. Ann Thorac Surg. 2010;89(3):864-9.

For journal papers by DOI:
Ewy MW, Patel A, Abdelmagid MG, Mohamed Elfadil O, Bonnes SL, Salonen BR, et al. Plant-Based Diet: Is It as Good as an Animal-Based Diet When It Comes to Protein? Curr Nutr Rep. 2022. doi: 10.1007/s13668-022-00401-8.

For books:
Kleiner FS, Mamiya CJ, Tansey RG. 2001. Gardner's art through the ages (11th ed.). Fort Worth, USA: Harcourt College Publishers.

For chapter in an edited book:
Mettam GR, Adams LB. How to prepare an electronic version of your article. In: Jones BS, Smith RZ, editors. Introduction to the electronic age, New York: E-Publishing Inc; 2009, p. 281–304.

Tables, figures and images

Tables

Tables should always be cited in text in consecutive numerical order. For each table, please supply a table caption (title) explaining the components of the table. Identify any previously published material by giving the original source in the form of a reference at the end of the table caption. Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

Figures

Please submit each figure as an individual file separate from the manuscript text. All figures are to be numbered using Arabic numerals. Figures should always be cited in text in consecutive numerical order. Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.

For vector graphics, the preferred format is EPS, for halftones, please use TIFF format. *Microsoft Office* files are also acceptable. Vector graphics containing fonts must have the fonts embedded in the files.

Line art:

- Definition: Black and white graphic with no shading.
- Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.
- All lines should be at least 0.1 mm (0.3 pt) wide.
- Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.
- Vector graphics containing fonts must have the fonts embedded in the files.

Halftone art:

- Definition: Photographs, drawings, or paintings with fine shading, etc.
- If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.
- Halftones should have a minimum resolution of 300 dpi.

Images

Supply vector-based files such as those produced by *CorelDraw*, *Adobe Illustrator* or similar software. Vector files give us maximum flexibility for sizing your figures properly. Do not rasterize line art or text. Photographic images should have a minimum resolution of 300 dpi at final print size. Embedded images within a vector file should also have a minimum resolution of 300 dpi. Up sampling artwork (artificially increasing file size or resolution) will not improve quality and causes production problems. At final print size, line weights can be no thinner than 0.28 pt.

PEER REVIEW PROCESS

All submitted manuscripts received by the Editorial Office will be evaluated by a professional *Editorial board* to determine whether they posses sufficient quality, are they properly prepared and follow the ethical policies of *Experimental and Applied Biomedical Research*. Manuscripts that do not fit with the quality and ethical standards of *EABR* will be rejected before peer-review. Manuscripts that are not properly prepared according to the Instruction for authors will be returned to the authors for revision and resubmission.

Once a manuscript passes the initial evaluation, it will be assigned to at least two independent experts for single-blind peer-review process. If the outcomes of the performed reviews are opposite, the third review is required. The peer-review outcomes are one of following:

- *Accept (without any changes)* - the journal will publish the paper in its original form. This type of decision outcome is rare.
- *Minor revision* - the manuscript have high chance to be accepted after fulfillment of minor corrections. Authors will be asked to resubmit the revised manuscript within a suitable time frame, and the revised version will be returned to the reviewer for further comments.
- *Reconsider after Major Revision* - the acceptance of the manuscript would depend on the revisions. The authors are required to perform extensive and significant

improvements in their manuscript. Authors will be asked to resubmit the revised manuscript within a suitable time frame, and the revised version will be returned to the reviewer for further comments.

- *Reject* - the manuscript is rejected for two reasons: 1. it has serious flaws, and/or makes no original significant contribution; 2. corrections and improvements during the (major) revision were not sufficient and satisfactory. No offer of resubmission to the journal is provided.

All reviewer comments should be responded point-by-point in a separate document entitled 'Answers to reviewers comments'. Corrections should be marked within the text in a red colour or as a track changes. During the submission process, author should suggest two potential reviewers with the appropriate expertise to review the manuscript. Proposed reviewers should be from different institutions than the authors.

Upon editor's approval, after received positive manuscript reviews, the manuscript is accepted in the system, and the corresponding author receives information about the manuscript accepted for publication to the email address. Once accepted, the manuscript will undergo professional copy-editing, English editing, proofreading by the authors, pagination and publication. The Editorial board reserves the right to correct the English language after proofreading by the authors.

DOI number is assigned to the paper and, after proofreading and text break according to the Journal instructions, the paper is published as *Ahead of Print* first on *Sciendo* platform (https://sciendo.com/journal/sjecr) and then in one of the next issues of the Journal.

RESEARCH AND PUBLICATION ETHICS

Research Involving Human Subjects

When reporting on research that involves human subjects, human material, human tissues, or human data, authors must declare that the investigation was carried out following the rules of the Declaration of Helsinki of 1975 (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/), revised in 2013. As a minimum, a statement including number of approval and name of the ethics committee must be stated in Section 'Statement of Human Rights' of the article. In addition, the protection of privacy is a legal right that must not be breached without individual informed consent. In cases where the identification of personal information is necessary for scientific reasons, authors should obtain full documentation of informed consent, including written permission from the patient prior to inclusion in the study.

Example of Statement of Human Rights: "The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of *Name of the Institution* (No. *number of approval*)."

Example of Statement of Informed Consent: "All subjects gave their informed consent for inclusion before they participated in the study".

For non-interventional studies (e.g. surveys, questionnaires, social media research), all participants must be fully informed if the anonymity is assured, why the research is being conducted, how their data will be used and if there are any risks associated. As with all

research involving humans, ethical approval from an appropriate ethics committee must be obtained prior to conducting the study.

Research involving Animals

When reporting on research that involves animal subjects, animal material or animal tissues, authors must declare that the investigation was carried out following the rules of the European Directive for the welfare of laboratory animals (No. 2010/63/EU) and national and institutional regulations. As a minimum, a statement including number of approval and name of the ethics committee must be stated in Section 'Statement of Animal Rights' of the article. Statements on animal welfare should confirm that the study complied with all relevant legislation. Also, authors must include details on housing, husbandry and pain management in their manuscript (section Materials and methods).

Example of Statement of Animal Rights: "All research procedures were carried out in strict accordance with the European Union Directive for the welfare of laboratory animals (No. 2010/63/EU) and approved by the Ethics Committee of *Name of the Institution* (No. *number of approval*)."

EABR

