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ORIGINAL ARTICLE

Endoscopic retrograde cholangiopancreatography and endoscopic sphincterotomy for sphincter of Oddi dysfunction: Short-term results

Ivan Mamontov, Tamara Tamm, Kostiantyn Kramarenko, Dmitro Riabushchenko, Erika Bilousova
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ABSTRACT

Aim: To analyze the short-term results of ERCP and endoscopic sphincterotomy (ES) in patients with sphincter of Oddi dysfunction (SOD).

Materials and Methods: We retrospectively analyzed the medical data of 120 cases of SOD with undergoing ERCP between January 2013 and December 2020. Mean values of factors with standard deviation were used. To compare mean values, Student's t-test was used. To compare non-parametric factors, the Chi-square test ($\chi 2$) was used. The value $p<0.05$ was considered statistically significant. All calculation were performed with SPSS® version 19 (IBM, USA).

Results: Biliary SOD was in 99 (82.5%) patients, mixed – bilio-pancreatic – in 21 (17.5%). Jaundice was in 59 (49.2%); dilatation of the common bile duct (CBD) was in 117 (97.5%); 18 (15%) patients had acute pancreatitis; 2 (1.7%) – cholangitis. All 117 (97.5%) patients with successful ERCP underwent ESP and/or Precut. Disappearance/reduction of abdominal pain was in 19 (90.5%) of 21 patients with pancreatic SOD, and in 60 (60.6%) of 99 patients with biliary SOD ($\chi 2=6.872$, $p=0.009$). Within 24-48 hours after ERCP, CBD size decreased from 10.9±2.3 to 8.2±1.7 mm ($p<0.001$). After 72-96 hours total bilirubin level decreased from 43.8±36.2 to 23.4±14.3 μmol/l ($p<0.001$). Adverse events associated with ERCP were pancreatitis (14.2%), bleeding (1.7%) and cholecystitis (0.8%). Among the 21 patients with bilio-pancreatic SOD, no worsening of pancreatitis symptoms or onset of acute pancreatitis was observed. All cases of post-ERCP pancreatitis occurred exclusively in patients with biliary SOD ($\chi 2=4.201$, $p=0.041$).

Conclusions: In SOD with signs of cholestasis, ES leads to a decrease of CBD size and normalization of bilirubinemia. Elimination of abdominal pain immediately after ES is more typical for bilio-pancreatic SOD compared to biliary SOD. The most common adverse event after ERCP and ES in patients with SOD is acute pancreatitis – specific for biliary SOD and not typical for bilio-pancreatic SOD.

KEYWORDS: ERCP, Sphincter of Oddi dysfunction, endoscopic sphincterotomy, cholestasis

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INTRODUCTION

Sphincter of Oddi dysfunction (SOD) is a benign disorder leading to a delay in the bile and/or pancreatic outflow due to dyskinesia or anatomical stenosis [1-3]. The diagnoses of stenosis of the major duodenal papilla (MDP), papillostenosis, and chronic papillitis used by surgeons and endoscopists essentially correspond to the diagnosis of SOD.

Sphincter of Oddi dysfunction, due to its anatomy, can lead to both biliary and pancreatic disorders. It can occur in patients with an intact biliary system, but it is more commonly observed in patients who have undergone cholecystectomy [4].

According to clinical presentation, laboratory and visualisation findings, biliary SOD classified into three types [4]:

Type I SOD is defined as biliary-type pain with both elevated liver enzymes (ALT, AST) and a dilated bile duct. Type II SOD presents with biliary-type pain with either elevated liver enzymes or a dilated bile duct. Type III SOD patients have only biliary-type pain without biochemical or imaging abnormalities.

Endoscopic retrograde cholangiopancreatography (ERCP) in SOD is aimed to determining the delay of contrast outflow, excluding choledocholithiasis and other disorders leading to biliary obstruction [5]. And endoscopic treatment of SOD is focused on reducing the sphincter of Oddi tonus or curing the stenosis. Endoscopic sphincterotomy (ES) is an effective method for management the biliary SOD types I and II, what was confirmed by retrospective and prospective randomized trials [4-8]. However, in SOD type III, ES has no advantage over placebo [8]. In pancreatic SOD, ES prevents recurrent attacks of acute pancreatitis [8, 9].

AIM

To analyze the short-term results of ERCP and endoscopic sphincterotomy (ES) in patients with sphincter of Oddi dysfunction (SOD).

MATERIALS AND METHODS

We retrospectively analyzed the medical data of 120 cases of SOD with undergoing ERCP between January 2013 and December 2020 at Municipal non-profit enterprise city clinical hospital №2 named after prof. O.O. Shalimov of Kharkiv City Council. We included only patients with naive papilla. Due to incomplete medical data, we have not included patients who were treated in other hospitals and were referred to our institution only for ERCP procedure.

Indications for ERCP were determined on the basis of clinical and visualisation finding. Exclusion criteria were: cases with history of Billroth II gastrectomy, ERCP in history and incomplete medical data.

ENDOSCOPIC RETROGRADE CHOLANGIOPANCREATOGRAPHY

ERCP was performed by two experienced operators. Before the procedure all patients received diclofenac (100 mg) per rectum for post-exposure prevention (PEP). Premedication with an injection of scopolamine butylbromide (10-20 mg) and local anesthesia of the pharynx with 8 % lidocaine were done.

Procedure usually was started with a guided sphincterotomy or cannulation. Priority was given to obtain selective biliary cannulation which is defined as deep canulation of common bile duct (CBD) through naïve papilla followed by cholangiography without pancreatic duct canulation/opacification. Needle-knife precut sphincterotomy (Precut) was used to achieve biliary access in case of failure of selective biliary cannulation after 5-10 attempts or approximately 5 min of trying.

For the SOD treatment ES was applied. In cases when precut was performed and there was no delay of contrast outflow after cholangiography, ES was not used; on the contrary, when after precut and cholangiography the contrast did not freely outflew to the duodenum, ES was performed. In order to reliably exclude CBD stones, especially small stones and biliary sludge, after ERCP and ES, revision of CBD by a basket was done.

We never used transpancreatic pre-incision manometry, sphincter manometry, or balloon dilation. And we have not used prophylactic pancreatic stents placement in patients of this series.

After the procedure, the patient fasted until the next morning, received an intravenous infusion and ceftriaxone (2 g). Blood tests – hemoglobin, bilirubin and amylase levels were measured at baseline, 4-8 hours after the procedure, and next morning.

The effectiveness of endoscopic procedures in SOD was assessed by clinical presentation (pain), laboratory data (blood bilirubin, AST, AST, amylase) and the size of the common bile duct (CBD) – according to abdominal ultrasound. ERCP-related adverse events according European Society of Gastrointestinal Endoscopy (ESGE) guideline were recorded [10].

STATISTICAL ANALYSIS

Mean values of factors with standard deviation were used. To compare mean values, Student's t-test was used. To compare non-parametric factors, the Chi-square test (χ^2) was used. The value $p<0.05$ was considered statistically significant. All calculation were performed with SPSS® version 19 (IBM, USA).

ETHICS

This work complies with the principles of the Declaration of Helsinki.

Table 1. Characteristics of patients with SOD (n=120)

Characteristics	Value
Male/Female	21(17.5%)/99(82.5%)
Age, years	63.5±13.3 (21-88)
History of cholecystectomy	16 (13.3%)
Total bilirubin, μmol/l	43.8±36.2 (10-174)
Common bile duct, mm	10.9±2.3 (7-20)
Acute pancreatitis	18 (15%)
Cholangitis	2 (1.7%)
Parapapillary diverticulum	19 (15.8%)
Success of ERCP	117 (97.5%)
Selective biliary cannulation	69 (57.5%)
Pancreatic cannulation/injection	31 (25.8%)
Precut	46 (38.3%)
EST	98 (81.7%)
Adverse events associated with ERCP	20 (16.7%)
Acute pancreatitis	17(14.2%)
Bleeding	2 (1.7%)
Perforation	0 (0%)
Cholangitis	0 (0%)
Cholecystitis	1 (0.8%)

Source: compiled by the authors of this study

AI STATEMENT

The author states that no AI tools were used in writing this article.

RESULTS

Biliary SOD was in 99 (82.5%) patients, mixed – biliary and pancreatic – in 21 (17.5%) patients. Among the studied patients there were no cases of pancreatic SOD. All patients with biliary SOD were type I or II; there were no cases of type III.

The basic characteristics of patients with SOD are presented in Table 1.

The main complaint of all patients with SOD was upper abdominal pain. Jaundice was present in 59 (49.2%) patients. Dilatation of the CBD (> 8 mm) was in 117 (97.5%) patients.

Elevated serum amylase level was observed in 21 (17.5%) patients, in 18 (15%) of whom the amylase level was more than 3 times the upper limit of normal. So, these 18 (15%) patients had acute pancreatitis, the course of which in all these cases was mild.

Moreover, there were 2 (1.7%) cases of mild cholangitis, confirmed by the presence of pus in the bile during ERCP. Both of these cases also had the criteria for acute pancreatitis.

In total, only in 3 (2.5%) cases ERCP was not successful (Table 1). Despite Precut, only pancreatic duct opacification was achieved in these patients, and selective biliary cannulation was not achieved. Due to the absence of jaundice in these patients, the decision was not to perform further attempts of cholangiography. Later to exclude CBD stones these patients were referred to MRI.

All 117 (97.5%) patients with successful ERCP underwent ESP and/or precut. Only precut was performed in 10 (8.3%) patients; EST alone – in 74 (61.7%) patients; and a combination of precut and EST was done in 33 (27.5%) patients. In order to exclude small bile stones and sludge, all 117 patients underwent revision of the CBD by basket.

Next day after endoscopic interventions, 79 (65.8%) patients noted improvement. Disappearance or reduction of abdominal pain was in 19 (90.5%) of 21 patients with pancreatic SOD, and in 60 (60.6%) of 99 patients with biliary SOD ($\chi 2=6.872$, $p=0.009$). In all 18 patients with acute pancreatitis next day after EST, abdominal pain syndrome was absent, including two patients with cholangitis. The latter also had no clinical manifestations of cholangitis – hyperthermia, chills.

Transabdominal ultrasound performed within 24-48 hours after ERCP, showed the CBD size decrease from 10.9±2.3 to 8.2±1.7 mm ($p<0.001$); aerobilia was noted in 47 (39.2%) patients. The total bilirubin level at the same time decreased from 43.8±36.2 to 36.1±24.2 μmol/l ($p>0.05$). After 72-96 hours, the CBD size did not significantly differ from its previous value and was 7.8±1.8 mm ($p>0.05$). The total bilirubin level at the same time significantly decreased compared to its initial value and was 23.4±14.3 μmol/l ($p<0.001$). In patients with hyperbilirubinemia (n=64), normalization of total bilirubin level occurred on average on day 5 (from day 1 to day 8).

Among adverse events associated with ERCP (table 1), the most common was acute pancreatitis – 17 (14.2%). One case had moderate severity, the other 16 – mild pancreatitis. In 2 (1.7%) cases bleeding occurred after precut and/or EST, which did not require any intervention. One (0.8%) patient had acute cholecystitis and an urgent cholecystectomy was performed.

There were no cases of perforation and cholangitis after ERCP and endoscopic interventions in the studied group of patients. There were also no cases of mortality.

DISCUSSION

Sphincter of Oddi dysfunction includes a variety of clinical conditions associated with its malfunctioning, resulting in transient or permanent bile and/or pancreatic obstruction. Biliary obstruction leads to jaundice with specific laboratory marker (elevated serum bilirubin, AST, AST); involvement of the pancreatic duct can lead to pancreatitis [1, 3, 9].

Most of the patients with suspected sphincter of Oddi dysfunction are women aged 30–50 years who have undergone cholecystectomy. The reasons for the higher incidence of female are unclear, but this may simply reflect the higher incidence of gallstone and the higher rate of cholecystectomy in women [1, 3, 11]. It is known that there are patients with SOD without a history of cholecystectomy [3]. In our study, women did predominate, there were 82.5% of them, but only 13.3% of patients had undergone cholecystectomy (Table 1). The majority of patients had biliary SOD (82.5%), and 17.5% had mixed – biliopancreatic SOD, which in 15% manifested as acute pancreatitis. It is noteworthy that among all the studied patients with SOD, cholangitis occurred in 2 (1.7%) cases, which in both cases was accompanied with acute pancreatitis.

With implementing of CT, MRI, and endoscopic ultrasound the diagnostic role of ERCP has almost gone. And nowadays ERCP is used mainly as therapeutic procedure in malignant or benign biliary obstruction, CBD stones and other bilio-panreatic pathology. Suspected cases of SOD requiring ERCP are gradually decreasing [12]. Nonetheless ERCP with EST is an effective treatment modality in biliary SOD types I and II while having no advantage in SOD type III [2, 9, 13, 14]. In this study, we analyzed cases of ERCP usage in biliary SOD types I and II and pancreatic SOD and focused on the short-termed results of endoscopic interventions.

Since the main manifestation of SOD was signs of biliary obstruction, the main criteria for assessing the effectiveness of endoscopic interventions were the CBD size according to ultrasound and the level of total bilirubin. In addition, we focused on abdominal pain.

As it was told above, EST led to a decrease or disappearance of abdominal pain in 65.8% of patients: in 90.5% with bilio-pancreatic SOD and in 60.6% with biliary SOD ($\chi 2=6.872$, $p=0.009$). So, in bilio-pancreatic SOD, EST is significantly more effective in relieving abdominal pain syndrome compared to biliary SOD.

Our data also show that in the studied patients, EST leads to the elimination of cholestasis, which is manifested by a

significant ($p<0.001$) decrease in the CBD size already 24-48 hours after the procedure and a significant ($p<0.001$) decrease in the level of total bilirubin after 72-96 hours.

The most common adverse event was acute pancreatitis (14.2%). Sphincter of Oddi dysfunction is a known risk factor for post-ERCP pancreatitis [15, 16]. In this regard, the risk of pancreatitis calls into question the appropriateness of using ERCP in all patients with biliary SOD [11]. In pancreatic SOD, the effect of ERCP and EST on the development pancreatitis as an adverse event after these endoscopic interventions is unclear [9]. Among the 21 patients with bilio-pancreatic SOD, no worsening of pancreatitis symptoms or onset of acute pancreatitis was observed. On the contrary, clinical manifestations of acute pancreatitis disappeared after EST. So all cases of post-ERCP pancreatitis occurred exclusively in patients with biliary SOD – in 17 (17.2%) out of 99 ($\chi 2=4.201$, $p=0.041$).

The incidence post-EST bleeding varies from 1 to 48% [17]. In our study, bleeding occurred in 2 (1.7%) patients, but both of these patients did not require any intervention.

Acute cholecystitis, as an adverse event after ERCP develops in 0.5-5.2% [10]. In our series, acute cholecystitis developed in one patient (0.8%), which was an indication for emergency cholecystectomy.

CONCLUSIONS

In summary, this study suggests that in SOD with signs of cholestasis, EST leads to a decrease of CBD size and normalization of bilirubinemia. Elimination of abdominal pain immediately after EPST is more typical for biliopancreatic SOD compared to biliary SOD. The most common adverse event after ERCP and EST in patients with SOD is acute pancreatitis, the development of which is specific for biliary SOD and not typical for bilio-pancreatic SOD.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Psychoeducation as a mental health strategy in wartime: A meta-analysis of its effectiveness

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ABSTRACT

Aim: To synthesize current evidence on the effectiveness of psychoeducational interventions through a systematic review and meta-analysis.

Materials and Methods: A systematic search of peer-reviewed literature was conducted to identify studies evaluating psychoeducational interventions for individuals with post-traumatic stress and anxiety disorders. Eight studies, including randomized controlled trials (RCTs) and quasi-experimental designs, with a total of 993 participants, met the inclusion criteria for the review. Methodological quality was assessed using predefined criteria, and studies with a high risk of bias were excluded from the primary meta-analysis. The meta-analysis included six RCTs comprising 872 participants. Pooled effect sizes were calculated using a random-effects model, and standardized mean differences (SMD) with 95% confidence intervals (CI) were reported.

Results: The aggregated findings demonstrated a statistically significant reduction in psychological symptoms, with a pooled effect size of SMD = 0.34 (95% CI [0.13, 0.54]), reflecting a moderate therapeutic effect. Interventions delivered in group settings and those integrated with pharmacological treatment yielded comparatively stronger outcomes. Digital delivery formats showed practical value in settings with restricted access to in-person mental health services, including conflict-affected and displaced communities.

Conclusions: Psychoeducation constitutes a scalable, adaptable, and empirically supported element of contemporary mental health systems, especially relevant under wartime conditions in Ukraine.

KEY WORDS: Psychoeducation, Post-Traumatic Stress Disorder, anxiety disorders, meta-analysis, mental health, war, war-related trauma, digital interventions

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INTRODUCTION

Psychoeducation is an important component of psychological and psychiatric care aimed at increasing patients' and family members' awareness of mental health conditions, their course, self-help strategies, and relapse prevention. The growing prevalence of mental disorders, including depression, post-traumatic stress disorder (PTSD), anxiety disorders, and schizophrenia, underscores the need for effective interventions that not only provide treatment but also promote informed symptom management and long-term stabilization. Traumatic events are closely associated with the development of mental disorders and involve complex neurobiological mechanisms, including alterations in neurotransmitters, metalloenzymes, and hormonal regulation [1]. Recent evidence has also demonstrated associations between combat-related traumatic injuries, pain outcomes, and mental health disturbances, emphasizing the multidimensional impact of trauma on psychological functioning [2]. Mental disorders remain among the leading causes of disability worldwide and are associated with substantial social and economic burden. Despite advances in pharmacological and psychotherapeutic approaches, relapse rates remain high, and treatment adherence is often insufficient, highlighting the need for scalable, evidence-based supportive interventions.

Psychoeducation developed in the second half of the twentieth century as a structured response to the need for scientifically grounded and humane mental health care. The term was introduced in the 1960s in the context of schizophrenia treatment to describe interventions aimed at teaching patients and their families skills for coping with symptoms and interacting effectively with the health-care system [3]. Contemporary definitions of psychoeducation emphasize the provision of evidence-based information alongside the development of coping and self-regulation skills, as described in foundational work by McFarlane [4]. As an interdisciplinary intervention, psychoeducation integrates educational, therapeutic, and preventive components and is grounded primarily in cognitive-behavioral principles that emphasize patient insight and active participation in treatment [5]. Its structured and ethically oriented format aims to reduce stigma, strengthen coping strategies, and support long-term stabilization.

Psychoeducation is widely applied across psychiatric conditions, including schizophrenia, bipolar disorder, depression, PTSD, and anxiety disorders, and its effectiveness

has also been demonstrated in the management of chronic pain, cardiovascular diseases, and diabetes [6, 7]. It is now widely recognized that individuals with mental disorders can actively participate in their own treatment and act as key agents of change for themselves, while illness management skills -including understanding of the disorder and its treatment, development of coping strategies, and relapse prevention - play a crucial role in the recovery process. Owing to its flexibility and adaptability, psychoeducation is increasingly integrated into multilevel health, educational, and social systems, including large-scale initiatives implemented in cooperation with organizations such as the World Health Organization and UNICEF. In this context, the integration of Internet of Things (IoT) technologies has been identified as a promising approach to enhance health system responsiveness by enabling real-time monitoring, data-driven decision-making, and improved healthcare delivery. The concept of Healthcare 4.0 further underscores the importance of advanced digital solutions, such as artificial intelligence and big data analytics, in strengthening healthcare systems and enabling effective responses to global challenges [8, 9].

However, despite the widespread implementation of psychoeducational programs, the available evidence remains methodologically heterogeneous. Studies vary considerably in diagnostic focus, intervention format (individual vs. group, face-to-face vs. online), duration, and outcome indicators. In particular, the comparative effectiveness of psychoeducation in reducing symptoms of post-traumatic stress disorder and anxiety disorders has not been systematically quantified using meta-analytic methods. This limits the development of evidence-based clinical guidelines.

A comprehensive synthesis of current evidence is essential for determining the clinical value of psychoeducation, optimizing intervention models, and informing mental health policy. Clarifying its effectiveness across diagnostic categories is particularly relevant in contexts characterized by increased psychological burden and limited access to specialized care, such as populations affected by war and large-scale humanitarian crises [10].

Clarifying its effectiveness across diagnostic categories is particularly relevant in contexts characterized by increased psychological burden and limited access to specialized care, including war-affected populations. Recent studies have highlighted the profound impact of direct hostilities on self-systems in military personnel and raised concerns about the long-term psychological and social consequences of trauma for affected civilians [11, 12]. Furthermore, the systematic provision of mental health care has been shown to predict better subjective well-being among Ukrainians during the second year of the Russian invasion, and wartime psychoanalytic contributions continue to shape the evolving landscape of mental health support in Ukraine [13].

AIM

The aim of this study was to analyze psychoeducation as a therapeutic method by examining its historical foundations, theoretical framework, and clinical effectiveness, and to evaluate its potential for further development in medical and psychotherapeutic contexts, particularly among war-affected populations. Additionally, a systematic review and meta-analysis were conducted to assess the effectiveness of psychoeducational interventions in reducing symptoms of post-traumatic stress disorder and anxiety, to identify the most effective program characteristics, and to compare different delivery formats (individual, group-based, and passive) as well as their combinations with other treatment approaches. Special attention was given to the application of psychoeducation in the context of the war in Ukraine and its implications for the development of interventions targeting war-related psychosomatic conditions.

This article also lays the foundation for further research in the field of psychoeducation, the development of psychoeducational programs for patients with mental and psychosomatic disorders caused by the war.

MATERIALS AND METHODS

A comprehensive search strategy was developed after defining the research question and eligibility criteria. The literature search was conducted across multiple databases, including the Cochrane Central Register of Controlled Trials (CENTRAL), PsycINFO, PubMed, and Scopus. Each database was searched using a tailored approach to account for differences in indexing systems and search functionalities. The search was conducted from database inception to September 2025.

The search strategy was based on key concepts derived from the research question and structured according to the PICO framework, with primary emphasis on population (individuals exposed to trauma, including war-related trauma) and intervention (psychoeducation). Additional concepts included outcomes (PTSD and anxiety) and study design (clinical trials), in line with the eligibility criteria.

Relevant keywords and their synonyms were identified for each concept. Terms within the same concept were combined using the Boolean operator "OR" (e.g., "PTSD" OR "post-traumatic stress disorder"; "anxiety" OR "anxiety disorders"; "war trauma" OR "combat exposure"), while different concepts were combined using "AND" (e.g., "psychoeducation" AND "PTSD" AND "anxiety"). Where applicable, controlled vocabulary (e.g., MeSH terms in PubMed) was used alongside free-text keywords to enhance search sensitivity. Truncation and alternative spellings were applied where appropriate to capture variations of key terms.

The search strategy was iteratively refined to achieve an optimal balance between sensitivity and specificity. Limits were applied to include only peer-reviewed articles published in English.

INCLUSION/ EXCLUSION CRITERIA

Inclusion criteria:

Randomized controlled trials (RCTs) assessing the effectiveness of psychoeducation.

Participants were described as suffering from PTSD, anxiety disorders, or having elevated scores on depression, anxiety, or psychological distress scales.

Studies using a control group (no intervention, placebo, or waitlist).

Exclusion criteria:

Review articles or other meta-analyses.

Studies where psychoeducation was combined with other psychotherapy methods, in order to isolate the specific effect of psychoeducation.

Interventions targeting specific groups, such as parents or caregivers of patients.

Data were extracted independently by two reviewers using a standardized form. Disagreements were resolved through discussion or consultation with a third reviewer. Extracted data included study characteristics, sample size, intervention details, and outcome measures.

Data synthesis and meta-analysis were performed using a random-effects model. Effect sizes were calculated as standardized mean differences (SMDs) with 95% confidence intervals (CIs). Statistical heterogeneity was assessed using the I^2 statistic. Publication bias was evaluated using funnel plots and Egger's regression test. The risk of bias was assessed using the Cochrane Risk of Bias 2 tool. A p-value < 0.05 was considered statistically significant. Due to the small number of included studies, the assessment of publication bias should be interpreted with caution.

The study was conducted in accordance with the "Rules of Ethical Principles of Human Research" and the Helsinki Declaration (1964-2013). The study was approved by the bioethics committee at the Ivano-Frankivsk National Medical University (Expert Opinion No. 153/25 dated September 17, 2025).

FRAMEWORK

The work is a fragment of the research project "Research into mental disorders among civilians, military personnel, and other population groups caused by the impact of hostilities in Ukraine" (№ state registration 0126U002072) on the Department of Psychiatry, Narcology and Medical Psychology, Ivano Frankivsk National Medical University.

AI STATEMENT

The author states that no AI tools were used in writing this article.

RESULTS

PRESENTATION OF THE MAIN MATERIAL OF THE SYSTEMATIC REVIEW

Numerous empirical studies have demonstrated the effectiveness of psychoeducation in improving clinical outcomes, enhancing treatment adherence, reducing stigma, and promoting quality of life. The integration of psychoeducational programs into comprehensive care for affective and psychotic disorders is supported by evidence showing their capacity to reinforce pharmacological and psychotherapeutic interventions. Meta-analyses indicate that psychoeducation significantly reduces relapse rates in schizophrenia and bipolar disorder. For instance, Pitschel-Walz et al. reported improved adherence among patients receiving psychoeducation compared with those receiving pharmacotherapy alone, while Bauml et al. found a 40% reduction in rehospitalization rates, attributable to increased awareness and self-management skills [14, 6, 15].

An important factor in the effectiveness of psychoeducation is its impact on overcoming social stigmatization. Mental disorders have traditionally been perceived as phenomena accompanied by high levels of social isolation and discrimination, which negatively affect the rehabilitation process. The study by Corrigan et al. confirmed that psychoeducation for patients and their families contributes to reducing negative stereotypes and improving the social functioning of people with mental disorders [16].

In addition to its impact on reducing relapses and stigmatization, psychoeducation significantly increases adherence to pharmacological therapy. The study by Anderson showed that patients who participated in psychoeducational programs were significantly more likely to adhere to medical recommendations and had a lower risk of premature treatment discontinuation, which contributed to better symptom control [3].

Psychoeducation is associated with statistically significant improvements in patients' emotional state, particularly with reductions in anxiety and depression, which are especially pronounced in individuals with chronic mental disorders [4].

The results of the meta-analysis by Donker et al. also indicate a positive effect of psychoeducation on the duration of remission in depressive and anxiety disorders. Patients who underwent psychoeducational programs demonstrated improved stress resilience and the ability to cope more effectively with emotional difficulties compared to those who received standard treatment without psychoeducational support [17].

Group-based psychoeducation. Group psychoeducation is one of the main formats of implementing psychoeducational programs, which provides not only the transfer of knowledge about mental disorders but also creates a space for support, social learning, and exchange of experience among participants. The main focus of group psychoeducation, as in the individual format, is to teach the mechanisms of the disorder, explain its symptoms, course, treatment methods, and adaptation strategies. An additional important factor is the interaction between participants, which contributes to the formation of a supportive community [18].

The structure of group psychoeducation includes several stages: familiarization with information, skills training, role-playing, discussion of real situations, and consolidation of knowledge and support for the application of skills in everyday life. An important component is family involvement, which creates more favorable conditions for rehabilitation [3].

The meta-analysis by Pitschel-Walz et al. demonstrated that participation in group programs reduces the risk of schizophrenia relapse by 45%. McFarlane et al. confirmed that group psychoeducation has a preventive effect, helping patients recognize signs of exacerbation [4, 6].

Social interaction contributes to improved interpersonal relationships and reduced isolation. Participants in group programs demonstrated better communication skills and lower levels of social alienation, while group participation was also associated with significantly higher adherence to pharmacotherapy recommendations

The inclusion of family members in psychoeducational interventions has been shown to reduce caregiver stress, improve communication, and enhance quality of life. Thus, group psychoeducation represents an effective approach associated with reduced relapse rates, improved psycho-emotional state, increased treatment adherence, and better social functioning. Involving the family in structured psychoeducation, which, in addition to imparting knowledge, also teaches skills such as stress management and problem-solving training, promotes empowerment, resilience and the sense of coherence and, consequently, the adherence and social competence of families; ultimately, in cases of severe mental illness, this leads to a significant reduction in relapse rates and an improvement in quality of life [19, 20]. In terms of effect size regarding relapse rates, multi-family intervention is clearly superior to all other psychosocial interventions [21, 22].

Digital psychoeducation. Digital psychoeducation is one of the directions actively developing due to the integration of modern information technologies into the field of mental health. It covers various formats, including online platforms, mobile applications, telemedicine services, chatbots, and the integration of psychoeducation into social networks and messengers. This approach makes educational materials accessible to a wide audience, particularly to people with limited abilities, residents of remote regions, and those who cannot participate in traditional psychoeducational programs and those who cannot participate in traditional psychoeducational programs [23, 24].

One key component of digital psychoeducation is the use of websites and online platforms that offer educational courses, interactive exercises, mental state self-assessment tests, and self-help recommendations [24, 25]. Social networks and messengers play a significant role in spreading psychoeducational content. Thanks to their accessibility, specialists can effectively communicate with the audience, distribute materials, conduct educational webinars, and create supportive online communities.

Chatbots and artificial intelligence algorithms are gradually being integrated into psychoeducational programs. They respond to questions, provide modules, recommend materials, and monitor changes in users' conditions. An example is Woebot, which is successfully applied to support individuals with depressive and anxiety disorders [25]. Mobile applications such as PTSD Coach and MoodGYM offer flexibility in learning, allow keeping mood diaries, and provide reminders and recommendations, which may be particularly beneficial for war-affected populations and military personnel exposed to high stress [11, 12, 23]. The effectiveness of online programs depends on personalization: language adaptation, cultural tailoring, and alignment with the target group's needs [24].

The study by Linardon et al. showed that online programs for patients with depression and anxiety reduced symptoms and increased resilience to stress reduced symptoms and increased resilience to stress, which is particularly relevant given the neurobiological mechanisms implicated in trauma-related disorders [26]. A systematic review by Ebert et al. confirmed that digital psychoeducation improves treatment adherence through platform interactivity [23].

The meta-analysis by Firth et al. proved the effectiveness of mobile applications in early intervention for mental disorders among adolescents and young people. In addition to clinical benefits, digital psychoeducation is economically feasible, as confirmed by the study of Donker et al., which showed a reduction in the need for face-to-face consultations [25, 27].

Thus, digital psychoeducation is an evidence-based and effective approach to increasing awareness, reducing stigma, improving adherence to treatment, and enhancing quality of life. It has broad prospects for use in health-care and social-support systems.

Adaptability. A key factor in the effectiveness of psychoeducation is its adaptability, which enables its use across diverse social, clinical, and cultural contexts. The flexibility of psychoeducation allows it to be tailored to the type of disorder, individual patient characteristics, and the format of material delivery.

Cultural adaptation includes language adaptation, localization of materials, and consideration of beliefs about mental health. Chowdhary et al. proved that adapted programs were significantly more effective among patients with depression in low- and middle-income countries [28].

Adaptability also manifests itself in the choice of format: individual or group sessions, in-person or online. Studies show that remote formats can be as effective as traditional meetings [23, 27]. During the COVID-19 pandemic, psychoeducational programs were successfully delivered digitally [24].

Personalization is another aspect of adaptability. AI algorithms enable the adaptation of psychoeducational programs to individual users. For example, applications can analyze behavioral patterns and recommend materials based on individual needs [27].

Psychoeducation is integrated into health-care systems, education, corporate environments, prevention, and crisis interventions. In schools, it helps prevent various forms of deviant behavior; in companies and work teams, it reduces emotional burnout; and during emergencies, it stabilizes the psycho-emotional state.

Thus, the adaptability of psychoeducation extends its reach and enables its use across the most diverse contexts, thereby increasing the effectiveness of support at both the individual and societal levels.

Psychoeducation in somatic medicine. Psychoeducation is an effective method not only in the field of mental health but also in somatic medicine. It plays an important role in improving patients' understanding of their illnesses, increasing adherence to treatment, reducing anxiety before medical procedures, and improving overall prognosis in chronic diseases. Adopting psychoeducational strategies for somatic illnesses helps foster an active patient role in their treatment, a critical factor for achieving successful outcomes. One of the key directions of psychoeducation is working with patients who have chronic diseases such as diabetes, cardiovascular pathologies, and autoimmune disorders. Psychoeducational strategies are also relevant for patients with connective tissue

disorders and related somatic comorbidities, where awareness of disease mechanisms and metabolic features can improve self-management and treatment adherence. For example, studies show that patients with diabetes who participated in psychoeducational programs had better glycaemic control, attributed to increased awareness of disease mechanisms and the importance of regular blood sugar monitoring. Furthermore, psychoeducational interventions such as Blood Glucose Awareness The Hypoglycemia Awareness Restoration Program (HARPdoc), alongside Blood Glucose Awareness Training (BGAT), has been shown to reduce the incidence of severe hypoglycemia by over 50% in individuals with type 1 diabetes using advanced glucose-monitoring technologies, while also improving hypoglycemia awareness and alleviating diabetes-related distress and anxiety symptoms. Another important aspect is the use of psychoeducation in oncology. Patients with oncological diagnoses often face high levels of anxiety, fear, and depressive symptoms, which can negatively affect their adherence to treatment and overall health. Psychoeducation helps patients understand the specifics of treatment, possible side effects, and ways to reduce their impact, as well as teaches strategies of psychological adaptation to the disease. Somatic medicine also widely uses psychoeducational programs for patients undergoing surgical treatment. It has been proven that patients who receive psychoeducation before operations demonstrate lower anxiety levels, which contributes to better recovery and fewer complications after surgery.

Psychoeducation also plays an important role in the treatment of chronic pain. Patients who receive psychoeducational interventions regarding pain mechanisms and methods of managing it achieve better results than those who receive only pharmacological therapy. This is explained by the fact that understanding the physiological processes underlying pain helps patients change their behavioral responses and reduce pain catastrophizing [7].

The integration of psychoeducation into somatic medicine is a promising approach that can improve patients' quality of life, reduce complication rates, and increase treatment effectiveness. Educational programs for patients can be implemented in both traditional formats (individual consultations, group sessions) and through digital technologies (online courses, mobile applications, interactive platforms), ensuring wide coverage and personalized approaches. The effectiveness of such programs may depend on motivational factors and learning challenges, as highlighted in studies examining medical students' engagement with educational materials [29].

Thus, psychoeducation is an important tool in general medicine, helping patients better understand their illnesses, increasing adherence to treatment, and contributing to improved therapy outcomes. Its use among patients with chronic, oncological, and surgical diseases has an evidence base and is an effective strategy for supporting their physical and psychological well-being.

The full-scale war in Ukraine has caused unprecedented strain on the health-care system, especially in the field of mental health. Prolonged traumatic events associated with hostilities have led to a massive increase in psycho-emotional disorders among all segments of the population, including military personnel, internally displaced persons, civilians in frontline regions, as well as people living outside active combat zones. The consequences of these experiences - physical losses, injuries, destruction, the death of loved ones, and forced displacement - have led to a significant increase in the prevalence of PTSD, anxiety, and depressive disorders.

A separate systemic problem is the critical shortage of specialists in the field of mental health: psychiatrists, clinical psychologists, and psychotherapists. Medical institutions, especially in rural and remote regions, often lack qualified specialists to provide psychological assistance. According to WHO estimates, the level of psychiatric service provision in Ukraine remains one of the lowest among European countries.

Another barrier to the formation of an effective mental-health system is the high stigmatization of mental disorders. A large part of the population avoids seeking psychological and especially psychiatric help because of fears of judgment, discrimination, or loss of social status. This situation reduces the effectiveness of early diagnosis, timely intervention, and prevention of symptom chronicity, which is particularly dangerous in wartime conditions.

The functioning of the health-care system is also complicated by the physical destruction of infrastructure: some institutions were destroyed or damaged due to hostilities, and medical staff work under resource shortages, high workload, and increased personal risk. In regions where a significant number of internally displaced persons have relocated, existing medical institutions are overloaded and lack sufficient staff, places, or technical support.

At the same time, the current situation has spurred the introduction of innovative solutions. In particular, digital tools for psychological support have been actively developed. Online platforms such as "You Are Not Alone" and "Diia. Mental Health" provide basic psychoeducation, self-help methods, consultations with specialists, and interactive educational programs. Research data show that the use of such platforms contributes to reduced anxiety, depressive symptoms, and improved psycho-emotional condition among users, particularly among internally displaced persons.

In this context, psychoeducation appears as an extremely effective tool for responding to the crisis in the field of mental health. Its universality, accessibility, evidence-based effectiveness, and flexibility in adapting to different target audiences make psychoeducation a strategically important part of the systemic response. Programs can be oriented to different groups, such as military personnel, displaced persons, children, people who have experienced loss, medical workers, etc.

We decided to conduct our own study of the effectiveness of psychoeducation in reducing PTSD and anxiety symptoms in the format of a meta-analysis of studies by other authors.

Given these challenges, we conducted a meta-analysis to evaluate the effectiveness of psychoeducation. The study was guided by the following hypotheses:

1. Psychoeducation has a positive effect on symptoms of PTSD and anxiety.
2. Combined interventions (psychoeducation plus pharmacotherapy) yield greater effects than psychoeducation alone.
3. Intervention format (individual, group, or passive delivery) influences effect size.

In this meta-analysis, psychoeducation is defined as an intervention involving the provision of information, educational materials, or feedback/recommendations. Passive psychoeducation may include handouts, posters, audiovisual guides, lectures, internet materials, or software. Such interventions are aimed at educating about the nature and treatment of depressive/anxiety disorders or psychological distress. Psychoeducation can be delivered in primary or secondary care settings, universities, community centers, or other public institutions. Its delivery may occur via mail, email, face-to-face lectures, or web resources.

Passive psychoeducation was defined as not requiring homework or relaxation exercises and not providing active treatment. Programs incorporating principles of active psychotherapies (e.g., cognitive-behavioral therapy (CBT) or interpersonal therapy (IPT) were excluded from the analysis. In addition, interventions combining psychoeducation with other components, such as CBT or multi-factor interventions, were also excluded.

RESULTS OF META-ANALYSIS

At the selection stage, 154 articles were identified. After removing 54 duplicates, 100 articles remained. Following a relevance review, 62 articles were excluded for irrelevant outcomes, leaving 38 for full-text assessment. Ultimately, 8 studies met the predefined inclusion criteria and were included. In total, 146 articles were excluded at various stages.

STATISTICAL ANALYSIS OF DATA

A total of eight studies were included in the review, comprising 993 participants. Among these, six were randomized controlled trials (RCTs), including 872 participants, and two employed quasi-experimental designs, comprising 121 participants. The key characteristics of all included studies, including sample sizes, interventions, and outcomes, are summarized in Fig. 1 and Table 1.

A pooled analysis of the six RCTs using a random-effects model showed a small but positive effect of psychoeducational interventions, with a standardized mean difference (SMD) of 0.21 (95% CI: 0.01–0.41). Heterogeneity among these RCTs was high ($Q = 34.9$, $df = 7$, $I^2 \approx 72\%$), indicating substantial variability in study designs, interventions, and outcome measures. The two quasi-experimental studies were not included in the pooled analysis due to the absence of randomization but are described narratively to provide additional context.

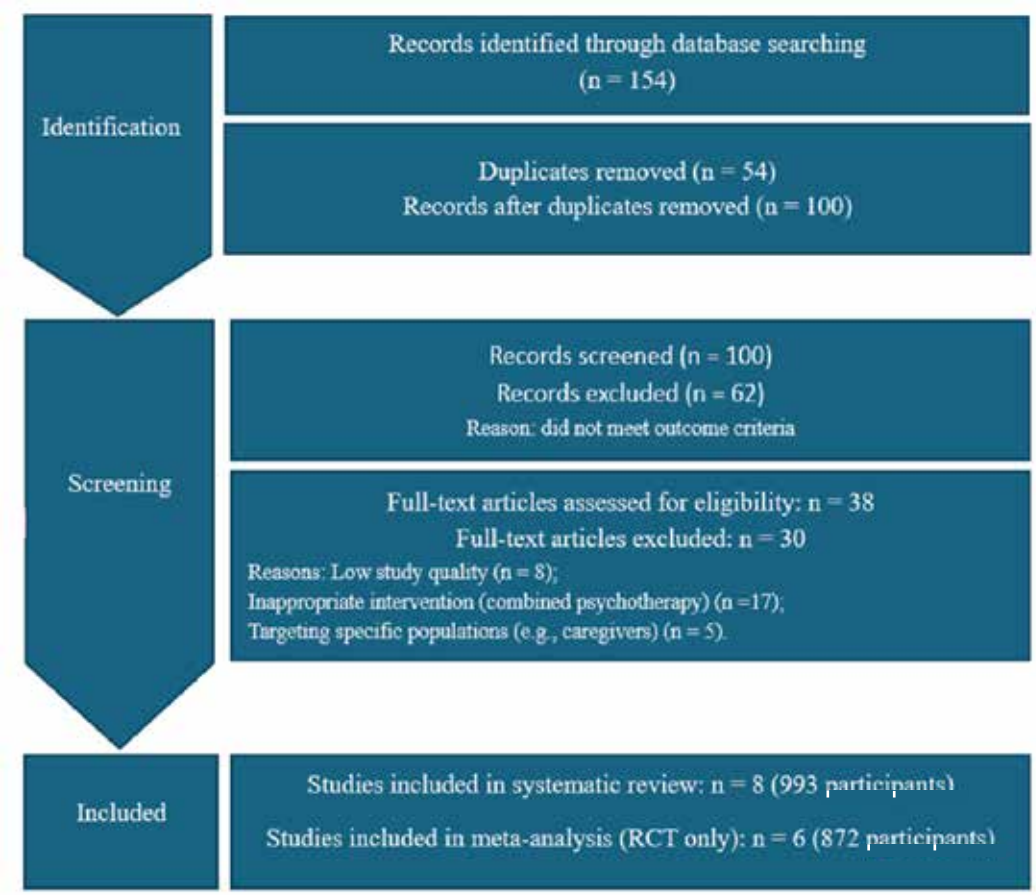

Fig. 1. PRISMA 2020 flow diagram of study selection process

Picture taken by the authors

Table 1. Characteristics of included studies (n = 8, total sample N = 993)

Study	Country	Sample	Intervention vs control	Outcomes	Effect	95% CI / p
Turpin et al., 2005	United Kingdom	n=142	Self-help booklet vs control	PTSD, anxiety, depression	d≈0.27, ns	p≈0.06
Yeomans et al., 2010	Sub-Saharan Africa, Burundi	n=120	Reconciliation training ± psychoeducation vs waitlist	PTSD	No additional effect	–
Pratt et al., 2005	USA	n=70	Inpatient psychoeducation *vs* no intervention	PTSD knowledge	↑ knowledge; no clinical effect detected	–
Oflaz et al., 2008	Turkey	n=51	Psychoeducation + medication vs medication	PTSD, coping	SMD≈0.69	Reported
Wong et al., 2013	USA	n=99	Psychoeducational video *vs* usual care	PTSD	SMD≈0.29	–
Wong et al., 2016	Hong Kong	n=182	Psychoeducation vs MBCT *vs* usual care	GAD	SMD≈0.40	p<0.05 (vs UC)
Morland et al., 2022	USA	n=274	CBCT vs psychoeducation	PTSD, relationships	d=0.59–0.76	95% CI [0.17-1.19]
Possemato et al., 2022	USA	n=55	Mobile psychoeducation *vs* mindfulness	PTSD, emotional regulation	d=0.71-0.99	Pilot

Source: compiled by the authors of this study

QUALITY OF STUDIES AND RISK OF BIAS

Risk of bias was assessed using the Cochrane Risk of Bias 2.0 (RoB 2.0) tool. Two independent reviewers evaluated each included randomized controlled trial across five domains: (1) the randomization process, (2) deviations from intended interventions, (3) missing outcome data, (4) measurement of outcomes, and (5) selection of the reported result. Each domain was rated as "low risk", "some concerns", or "high risk". The overall risk of bias judgment for each study was determined by combining assessments across all domains for the primary outcome. Disagreements were resolved through discussion and consensus.

Among the eight included studies, four were classified as low risk of bias, two as some concerns, and two as high risk of bias [7, 26, 30-35]. This indicates that the meta-analysis is primarily based on studies of low to moderate risk of bias, although some studies have methodological limitations that should be considered when interpreting the results. Accordingly, we conducted the statistical analysis excluding studies assessed as high risk of bias.

Statistical analysis excluding studies assessed as high risk of bias yielded the following results: the pooled standardized mean difference (SMD) was 0.34 (95% confidence interval: 0.13-0.54), indicating a moderate effect. Heterogeneity among studies was moderate to substantial, with Q = 14.67 (df = 5), I^2 = 65.9%, and τ^2 = 0.041.

DISCUSSION

The present meta-analysis provides comprehensive evidence supporting the effectiveness of psychoeducation as an adjunctive intervention for the management of post-traumatic stress disorder (PTSD), anxiety, and depression. A total of eight studies were included in the review, comprising 994 participants. Among these, six were randomized controlled trials (RCTs) and two employed quasi-experimental designs. The meta-analysis, focusing on the six RCTs (873 participants), demonstrated a statistically significant, small-to-moderate reduction in clinical symptoms (pooled SMD = 0.34, 95% CI [0.13, 0.54]) [7, 17, 33-36]. This effect remained robust even after excluding studies assessed as high risk of bias, indicating that the observed benefit is not driven by methodological limitations.

The results underscore the multifaceted value of psychoeducation. By enhancing patients' understanding of their condition, promoting adherence to treatment, and strengthening coping mechanisms, psychoeducation addresses both cognitive and behavioral aspects of mental health management [5, 6, 15, 17]. These effects may extend beyond symptom reduction and include improvements in psychosocial functioning, perceived quality of care, and adaptation to chronic illness, especially in patients with schizophrenia and concomitant somatic disorders [37]. The biopsychosocial interaction between physical trauma, chronic pain, emotional distress, and mental well-being further supports the importance of integrated psychoeducational interventions in rehabilitation and long-term recovery [41]. While the overall effect size is small to moderate, it carries considerable clinical significance, particularly in contexts where pharmacological or intensive psychotherapeutic interventions are less accessible, more costly, or associated with greater risk [30, 33, 34]. The low cost and scalability of psychoeducation further support its integration into broader healthcare strategies, including primary care and public health programs [9, 38].

Heterogeneity among studies was substantial (I^2 = 65-72%), reflecting variability in intervention formats, population characteristics, and outcome measurement tools. Interventions differed in delivery (individual versus group), mode (offline versus online), and duration (short-

term versus long-term), while participant populations ranged from veterans to civilians and displaced individuals [10, 21, 23, 25]. Outcome assessments also varied, contributing to differences in effect estimates. Nevertheless, sensitivity analyses confirmed the stability of findings, suggesting that psychoeducation retains meaningful effects across diverse contexts and populations [17, 22, 35].

Digital psychoeducational programs emerged as a particularly promising approach. Online or video-based interventions demonstrated efficacy comparable to traditional formats and offer practical advantages in settings with limited access to in-person therapy, including conflict-affected communities and displaced populations [24-26, 36]. Such digital formats provide opportunities to expand the reach of mental health support, maintain continuity of care, and enhance patient engagement. In parallel, recent psycholinguistic approaches aimed at overcoming anxiety states may complement psychoeducational interventions by improving emotional processing and adaptive communication strategies [40] (Table 2).

Despite these results, several methodological considerations warrant attention. The variability in study design, intervention delivery, and outcome assessment may influence effect estimates and limit the generalizability of findings [23, 24, 28]. Moreover, the relatively short follow-up periods in most studies constrain conclusions regarding long-term efficacy. Future research should focus on high-quality, longitudinal studies to better understand sustained effects, optimize intervention models, and explore the synergistic impact of psychoeducation combined with pharmacotherapy or other psychosocial treatments [4, 22, 37]. Additional investigation into the relative effectiveness of different delivery modes, particularly digital interventions, and their application across diverse populations, including vulnerable and displaced groups, will further inform evidence-based implementation strategies [10, 24, 39].

In summary, this meta-analysis demonstrates that psychoeducation is an effective, low-risk, and accessible adjunctive intervention for reducing symptoms of PTSD, anxiety, and depression. Its applicability across diverse populations, combined with its potential for digital delivery, underscores its value as a scalable component of contemporary mental health systems. By supporting symptom reduction, enhancing treatment adherence, and contributing to relapse prevention, psychoeducation represents a meaningful addition to mental health care, particularly in contexts affected by conflict or limited access to specialized services [6, 10, 15, 17, 38].

LIMITATIONS

Despite the positive findings, this meta-analysis has several methodological limitations. Substantial heterogeneity was observed across the included studies ($I^2 = 65\text{-}72\%$), reflecting differences in intervention formats (individual vs. group, offline vs. online, short-term vs. long-term), participant populations (veterans, civilians, refugees), and outcome measurement tools.

Some studies were deemed high risk, and most had relatively short follow-up periods, limiting conclusions about long-term effectiveness. Variation in the delivery and content of psychoeducational programs, as well as differences in symptom assessment methods, may also affect the generalizability of the results.

FUTURE RESEARCH DIRECTIONS

Further high-quality, longitudinal studies are needed to clarify the sustained effects of psychoeducational interventions and to optimize their implementation in clinical and public health settings.

Future research should compare the effectiveness of different intervention formats, including online versus offline delivery, group versus individual settings, and short-term versus long-term programs. It is also important to evaluate outcomes across diverse populations, particularly vulnerable and displaced groups, and to investigate the combined effects of psychoeducation with pharmacological or other psychosocial treatments.

Additionally, the role of digital and video-based programs in expanding access, maintaining continuity of care, and enhancing patient engagement within public health systems warrants thorough exploration. Addressing these areas will provide critical evidence to refine psychoeducational programs and strengthen their integration into comprehensive mental health care strategies.

Table 2. Risk of Bias Assessment (Cochrane RoB 2.0)

Study	Randomization	Deviations	Missing data	Measurement	Selective reporting	Overall risk
Turpin et al., 2005	Low	Some concerns	Low	Low	Low	Low
Yeomans et al., 2010	Some concerns	High	Some concerns	Low	Some concerns	Some concerns
Pratt et. al., 2005	Some concerns	Some concerns	High	Low	Some concerns	High
Oflaz et al., 2008	Low	Some concerns	Low	Low	Some concerns	Some concerns
Wong et al., 2013	Low	Low	Some concerns	Low	Low	Low
Wong et al., 2016	Low	Low	Low	Low	Low	Low
Morland et al., 2022	Low	Some concerns	Low	Low	Low	Low
Possemato et. al., 2022	Some concerns	Some concerns	High	Some concerns	Some concerns	High

Source: compiled by the authors based on [24-26, 36, 40]

CONCLUSIONS

The meta-analysis demonstrates that psychoeducation is an effective adjunctive intervention for reducing symptoms of PTSD, anxiety, and depression. A total of eight studies, comprising 993 participants, were included in the review, of which six were randomized controlled trials (RCTs) and two employed quasi-experimental designs. The quantitative synthesis, based on the six RCTs (872 participants), showed a statistically significant, small-to-moderate reduction in clinical symptoms (pooled SMD = 0.34, 95% CI [0.13, 0.54]). Excluding studies assessed as high risk of bias did not alter this effect, confirming the robustness of the findings. Psychoeducation enhances patients' awareness of their condition, strengthens coping strategies, improves adherence to treatment, and supports relapse prevention. Its accessibility, low cost, and minimal risks make it a practical and scalable component of contemporary mental health systems, particularly in contexts with limited access to specialized care.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Determinants of biochemical factors and microalbuminuria among type 2 Diabetes mellitus patients

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ABSTRACT

Aim: Progression of type 2 diabetes produces capillary and macrovascular problems, including microalbuminuria, an early renal impairment marker. This study examines biochemical factors associated to microalbuminuria in T2DM patients of varying durations.

Materials and Methods: The study included 90 healthy controls, patients with type 2 diabetes mellitus (T2DM) for less than five years, and those with the illness for more than five years. Fasting blood glucose, HbA1c, lipid profile (HDL, LDL, vLDL, TC), serum albumin, globulins, creatinine, and urine microalbumin were tested. To compare groups, ANOVA and independent t-tests were utilized.

Results: Patients with long-term illness showed higher fasting glucose (210.17±5.98 mg/dL), HbA1c (8.94±0.51%), serum creatinine (122.01±0.28 µmol/L), and reduced albumin (33.42±3.61 g/L Diabetics often had greater LDL, vLDL, and TC levels than controls ($p<0.001$). Microalbuminuria is connected to renal and metabolic dysfunction.

Conclusions: These findings suggest biochemical markers and microalbuminuria may predict T2DM. Early detection of these indications may help doctors prevent chronic kidney disease and cardiovascular issues.

KEYWORDS: albumin, creatinine, diabetes mellitus, microalbuminuria, renal function, type 2 diabetes

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INTRODUCTION

Microalbumin, a tiny protein, in type I diabetics' urine predicts nephropathy and end-stage renal disease [1]. High urine albumin suggests kidney disease, which may lead to cardiovascular, diabetes, and hypertension [2]. First, diabetic nephropathy lowers glomerular filtration rate, then microalbuminuria [3]. Many diabetics develop diabetic nephropathy when microalbuminuria reaches 20% yearly [4].

Diabetics need HbA1c and lipid profile components involving HDL, LDL, vLDL, and total cholesterol to evaluate glycemic control and metabolic health [5]. According to related research, microalbuminuria is a comprehensive measure for renal and heart risk reduction in type 2 diabetics (T2DM) and accelerates diabetic nephropathy with high blood pressure. Microalbuminuria is connected to diabetes time frame, heart rate, FBS, HbA1c, serum insulin, dyslipidaemia, smoking, and Overweight [6].

Diabetes is a group of metabolic illnesses that cause hyperglycemia owing to insulin production or action problems. In its early stages, type 2 diabetes, the most prevalent variety, may go unnoticed for years. Chronic hyperglycemia in diabetes damages the eyes, kidneys, nerves, heart, and blood cells over time. Uncontrolled type 2 diabetes increases stroke, heart, and peripheral vascular disease risk. They acquire obesity, dyslipidemia, and hypertension. Diabetes screening may be helpful if early detection and treatment reduce its symptoms. This policy statement promotes diabetes testing in physicians' offices and other healthcare settings [7]. Diabetes increases thirst, urine, hunger, and weight loss. Non-specific symptoms include fatigue, impaired vision, sweet-smelling urine or semen, and Candida-induced genital pruritus.

At least half of individuals afflicted exhibit no symptoms [8-11].

To solve a critical gap, this research analyzes biochemical and renal function markers in non-diabetic controls, the T2DM patients with less than five years of illness, and T2DM patients with more than five years. This strategy seeks a statistically significant relationship between illness

duration and biochemical and renal decline. In traditional clinical screening, microalbuminuria may predict diabetes complications.

The study questions addressed are as follows: firstly, how do key biochemical markers such as FBG, HbA1c, serum creatinine, albumin, in addition to lipid profile differ between diabetic patients and healthy individuals, secondly, to what extent does the duration of diabetes impact the severity of these biochemical changes.

AIM

This research aimed to investigate biochemical markers and microalbuminuria in type 2 diabetic patients with different disease durations.

MATERIALS AND METHODS

STUDY DESIGN

This cross-sectional study examines type 2 diabetic microalbuminuria scientific variables. From July 19 to September 1, 2025, a Baghdad diabetic treatment hospital performed the research. The college's assessment body provided ethical and informed approval to all participants.

STUDY POPULATION AND GROUPING

A total 90 persons were enlisted and categorized into three groups, including Group 1 (Control Group): 30 healthy persons without diabetes, matched by age and sex. Group 2 (= or ≤ 5 Years DM): 30 individuals diagnosed with T2DM for five years or less. Group 3 (>5 Years DM): 30 patients diagnosed with T2DM for over five years. The inclusion criteria for the diabetic cohorts were people aged under 5 and between 5 to 70 years, a verified diagnosis of T2DM, and the absence of other chronic conditions. The exclusion criteria were those with type 2 diabetes, established renal or hepatic illness, urinary tract infections, or those on nephroprotective medicines.

DATA COLLECTION AND SAMPLE HANDLING

Clinical examinations included demographics, medical information, and anthropometric measures (age, weight, height, and BMI) for every patient. After overnight fasting, 12 mL venous blood was aseptically distributed, as estimated HbA1c in 2 mL EDTA tubes, using fluoride oxalate tubes to measure 2 mL of fasting plasma glucose (FPG), with serum analysis, coagulate 8 mL in simple tubes and centrifuge, and finally collected overnight midstream urine samples for microalbumin and creatinine in sterile containers.

BIOCHEMICAL ANALYSIS

Most biochemical variables were tested using standardised and confirmed techniques per supplier protocols, such as: Fasting Plasma Glucose (FPG): Measured using the glucose oxidase enzymatic method, glycated Hemoglobin (HbA1c): Determined by the cation exchange resin method, serum Creatinine: Quantified using the Jaffe kinetic method, serum Urea: Measured enzymatically using the urease method, with lipid Profile (HDL, LDL, vLDL, and TC): Assessed using the CHOD-POD enzymatic colorimetric method. Urinary Microalbumin, as determined by immunoturbidimetric agglutination method: Measured using the kinetic alkaline picrate method. Finally, albumin/Creatinine Ratio (ACR): Calculated to assess microalbuminuria.

STATISTICAL ANALYSIS

Data were provided and analysed using SPSS 25.0, and data were presented as mean±SD for initial parameters. Post hoc Tukey's test investigated intergroup diversity after one-way ANOVA showed significant differences, and control and diabetes group pairwise comparisons using independent t-tests, with a p-value under 0.05 was significant. Box plots and bar charts showed biochemical indicator variations across groups.

ETHICAL APPROVAL

The Declaration of Helsinki-compliant research was authorised by the University of Baghdad College of Science Ethics Committee. All subjects provided written permission before the study, and all data were secure and used just for this study. The Declaration of Helsinki-compliant research was authorised by the University of Baghdad College of Science Ethics Committee, and subjects provided written permission before the study. All data was secure and used just for this study.

RESULTS

The research included 90 individuals, evenly divided into three groups: 30 controls, 30 patients with T2DM under 5 years old, and 30 patients with T2DM beyond 5 years old. Tables 1 and 2 provide outcomes, and as shown in Table 1, the control group had a mean age of 31.86±6.69 years compared to the <5 years diabetic group (3.6± 5.24 years) and the >5 years diabetic group (34.64±4.38 years). The mean body weight was slightly higher in the control group (78.56±5.20 kg) compared to patients with <5 years (29.46±6.45 kg) and >5 years diabetes (70.7±4.79 kg). Height was relatively comparable across the groups, ranging from 88.73±6.60 cm in the <5 years group to 168.76±4.53 cm in the >5 years group. A BMI estimation showed large group differences. The diabetic group aged <5 years had the greatest BMI (37.42 kg/m^2), which may be related to metabolic imbalance or stages of growth. In the control group, the BMI was high (27.58 kg/m^2), suggesting near-overweight. After 5 years of diabetes, the group with the lowest BMI (24.82 kg/m^2) was within normal range, perhaps because of improved weight control or disease adaptation.

The control group had a greater mean age (31.86±11.6 years) compared to the <5 years diabetes group (3.6±0.71 years) and >5 years diabetic group (34.64±12.36 years) (Figure 1 and Table 1). The mean body weight was somewhat greater in the control group (78.56±5.20 kg) compared to patients with <5 years (29.46 kg) and >5 years diabetes (70.7±4.79 kg). Height was similar across groups, with the control group measuring 168.73±6.60 cm and the <5 years group measuring 88.73±4.53 cm. Interestingly, the group with diabetes for less than 5 years had the highest BMI (37.42±1.96 kg/m^2), suggesting a trend towards higher BMI with longer disease duration.

Table 1. Anthropometric characteristics of control and type 2 diabetic patients according to disease duration

Measured Variables	Control (n=30)	Less than 5 years DM (n=30)	>5 years DM (n=30)
Age (years)	31.86	3.6	34.64
Weight (kg)	78.56	29.46	70.7
Height (cm)	168.73	88.73	163.76
BMI (kg/m^2)	27.58	37.42	24.82

Source: Compiled by the authors of this study

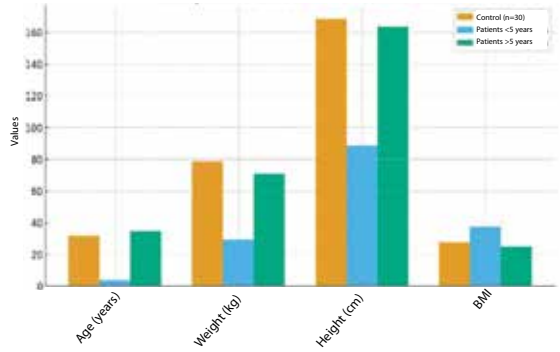

Fig. 1. Anthropometric characteristics of measured variables

Source: Own materials

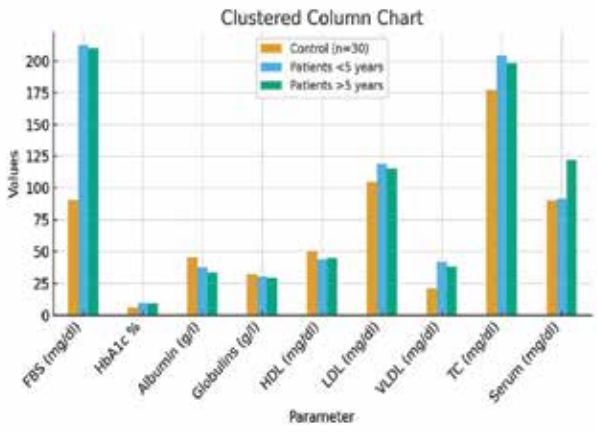

Fig. 2. Clustered column chart of biochemical parameters

Source: Own materials

The diabetes substantially increased fasting blood glucose (FBS), with the highest mean in the >5 years group (210.17 ±5.98 mg/dl), followed by the <5 years group (212.12±5.78 mg/dl) and the control group (90.20±3.90 mg/dl) (p<0.001), as shown in Figure 2 and Table 2. While, HbA1c values were also significantly higher in both diabetes groups. The <5 years group had the highest mean (9.66±0.39%), followed by the >5 years group (8.94±0.51%) and the control group (5.82±0.57%) (p<0.001).

Serum albumin levels declined progressively with disease duration: 45.21±3.17 g/l in controls, 37.42±3.17 g/l in <5 years diabetics, and 33.42±3.61 g/l in >5 years diabetics. Globulin levels also showed a decreasing trend: 31.90±1.79 g/l in controls, 30.52±2.14 g/l in <5 years, and 29.21±2.05 g/l in >5 years (p<0.001 for both markers).

The HDL cholesterol parameter was less significantly in T2DM (43.61±3.48 mg/dl and 44.96±3.99 mg/dl) compared to controls (50.04±3.00 mg/dl), while LDL levels were elevated in both diabetic groups: 118.66±4.68 mg/dl in <5 years and 114.96±3.71 mg/dl in >5 years versus 104.89 mg/dl in controls. TC levels increased from 177.13±4.80 mg/dl in controls to 204.15±6.36 mg/dl and 198.16±5.22 mg/dl in diabetic categories (p<0.05 for all lipid parameters); vLDL levels similarly showed identical patterns.

In relation to kidney function, creatinine in the serum was significantly substantially higher in the >5 years group (122.01±0.28 µmol/L) compared to the control (89.92±2.81 µmol/L) and <5 years diabetes groups (91.44±2.22 µmol/L). Similarly, urinary creatinine levels followed the same trend, increasing from 80.76±5.80 µmol/L in controls to 105.90±7.39 µmol/L and 118.85±23.11 µmol/L in the less and more 5 years with T2DM, respectively (p<0.001).

These findings support the progression of metabolic and vascular problems in diabetes by showing that T2DM patients' biochemical changes and renal function impairment increase with illness duration. CALP and XOD values in DN, diabetic, and control groups are shown in Table 3. The diabetic group with CALP levels below 5 had the greatest mean value (61.75 ng/ml), followed by the group over 5 years and control as (76.3±8.27 ng/ml) and (40.59 ng/ml), respectively. The control group (0.95 ng/ml), diabetes group under 5 years (1.11 ng/ml), and diabetic group over 5 years (1.52 ng/ml) exhibited no significant XOD variation. Both groups had considerably higher levels than the control group (0.95±0.12 ng/ml).

Table 3. Analysis values of CALP and XOD in different groups.

DISCUSSION

A prevalent metabolic illness T2DM increases global morbidity and death. Microalbuminuria in diabetic nephropathy indicates early renal decline. Oxidative stress, endothelial damage, and glomerular hyperfiltration increase renal impairment with poor glycemic control. HbA1c test glycemic control and predict microvascular problems. Microalbuminuria identification early may decrease disease development. Knowing how glycemic control affects

Table 2. Comparative analysis of biochemical parameters and renal markers in control and type 2 diabetic groups

Parameter	Control (n=30)	Patients less than 5 years	Patients above 5 years	P-value
FBS (mg/dl)	90.20±3.90	212.12±5.78	210.17±5.98	p<0.001
HbA1c %	5.82±0.57	9.66±0.39	8.94 ±0.51	p<0.001
Albumin (g/l)	45.21±3.17	37.42±3.17	33.42±3.61	p<0.001
Globulins (g/l)	31.90±1.79	30.52±2.14	29.21±2.05	p<0.001
HDL (mg/dl)	50.04±3.00	43.61±3.48	44.96±3.99	p<0.05
LDL (mg/dl)	104.89±	118.66±4.68	114.96±3.71	p<0.05
vLDL (mg/dl)	21.18±1.67	41.87±2.74	38.23±2.05	p<0.05
TC (mg/dl)	177.13±4.80	204.15±6.36	198.16±5.22	p<0.05
Serum Creatinine (µmol/l)	89.92±2.81	91.44±2.22	122.01±.28	p<0.001
Urea mg/dl	38.65±1.26	40.88±1.91	53.92±3.00	p<0.05
Cholesterol mg/dl	108.36±4.13	143.65±2.86	231.04±7.14	p<0.05

p-value ≤0.05

Table 3. Analysis values of CALP and XOD in different groups

Parameter	Control (n=30)	Patients less than 5 years	Patients above 5 years	P-value
CALP ng/ml	40.59±5.76	61.75±5.80	76.3±7.37	p<0.05
XOD ng/ml	0.95±0.12	1.11±0.19	1.52±0.24	p<0.05

microalbuminuria helps diabetes treatment. Our results complement earlier studies relating type 2 diabetics' poor glycemic control to microalbuminuria.

Both diabetic groups showed greater FBS and HbA1c than healthy controls, with the greatest HbA1c in less than 5 years of diabetes. This suggests that T2DM produces poor glycemic control, which worsens in the initial stages after diagnosis due to inadequate lifestyle adjustments and treatment.

Serum albumin and globulin dropped dramatically in long-term diabetes, Lamsal et al. (2025) found that microalbuminuria is the first symptom of diabetic nephropathy, and a decrease in serum albumin indicates renal impairment [12].

Moreover, Claudel and Verma (2025) found that diabetics with low blood albumin levels had higher cardiovascular disease risk and urine albumin excretion [13]. According to Chadha and Morris (2015), diabetic individuals have changed protein fractions and lower globulin levels, which may indicate chronic systemic inflammation or altered protein synthesis [14]. These findings support previous research and demonstrate serum proteins' ability to predict early diabetic kidney injury.

In both diabetes groups, HDL was considerably lower while LDL, vLDL, and TC were higher. Hirano (2018) and Huhtala et al. (2023) found that insulin resistance in T2DM inhibits lipoprotein metabolism, resulting in atherogenic lipids [15, 16].

Mooradian (2009) also found that poor glycaemic management and increased adipose tissue free fatty acid flow increases LDL and vLDL in diabetics. This study indicates that dyslipidaemia is a well-established and expected biochemical abnormality in T2DM and a substantial cardiovascular risk factor [17].

Diabetics, particularly those with a disease duration over five years, have higher serum creatinine levels, suggesting renal impairment. Rivetti et al. (2023) found that chronic microalbuminuria causes late creatinine elevation [18]. In addition, Jung and Yoo (2022) found that uncontrolled diabetes increases blood creatinine levels and decreases GFR [19]. In diabetic groups, urinary creatinine was likewise increased, suggesting compensatory hyperfiltration in early nephropathy. These results are congruent with Qiu et al. (2022), who found comparable urine creatinine changes with diabetes duration [20].

HbA1c values were greater in both diabetic groups, however the group with less than 5 years of diabetes had a slightly higher mean value. These findings contradict the projected glycemic control deterioration. Although Le et al. (2022) reported a linear drop in glycemic control, this may suggest greater treatment adherence in long-term diabetes. The development in HbA1c readings over 5 years likely have been due to more reliable treatment regimens or vigorous clinical care [21].

This finding contradicts the literature and are possible due to cross-sectional design or unmeasured factors such

medication compliance, lifestyle choices, or comorbidities. Thus, the literature is against this finding, so proceed with care. Mahmudul et al. (2022) found that renal disease patients had substantially higher XOD values than diabetic and healthy patients (2.04±0.38, 1.52±0.24, and 0.95±0.12) [22]. High insulin levels in diabetes increase XOD activity in renal glomerular endothelial cells, leading to reactive oxygen species (H_2O_2 and O_2^-) and oxidative stress, eventually damaging the endothelium [23].

Many studies confirm that patient serum CALP values increased. Calprotectin, an S100 protein, is essential for proinflammatory signaling [24]. Calprotectin complexes may indicate inflammation and track disease development. CALP activates TLR4 and AGE receptors, causing micro- and macrovascular problems in diabetes [25]. These processes may cause diabetic nephropathy. Table 3 suggests that CALP may be a sensitive and specific biomarker for early identification and diagnosis of diabetes complications, notably nephropathy [26]. High-risk screening and illness progression monitoring are possible due to the high AUC values and strong diagnostics. Figure 1 shows that CALP is a very effective diagnostic marker with an AUC value of 0.844. Its ability to distinguish diabetic and renal patients from healthy persons makes it useful for diabetes diagnosis and encourages treatment research [27].

CONCLUSIONS

This study shows that biochemical markers are essential for type 2 diabetes renal and metabolic impairment monitoring. Metabolic illnesses like microalbuminuria are linked to the duration of diabetes and its management. Patients with more than five years of illness had greater HbA1c, fasting glucose, creatinine, and cholesterol amounts, but albumin and globulin levels decreased. The data imply that biochemical degradation is gradual and may be identified by standardized testing. HbA1c, albumin, and microalbuminuria testing may prevent complications. The microalbuminuria indicates nephropathy and cardiovascular risk, making it crucial. Regular biochemical monitoring is needed with diabetes. We'll finish the other applications by expanding our research with bigger samples and longitudinal follow-up. Future study should examine the molecular basis of these alterations and how therapeutic treatments reverse early kidney impairment. Genetic vulnerability to microalbuminuria may also inform diabetes personalised medicine.

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CONFLICT OF INTEREST
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ORIGINAL ARTICLE

Laser technique for enhancement the bacterial strain of yogurt production

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ABSTRACT

Aim: The present study aims to study the biological effect of low intensity semiconductor laser 630 nm at 12 mw laser power on three bacterial strain used as starter culture in many yogurt production, such as Streptococcus thermophilus, Lactobacillus delbrueckii sub-bulgaricus, and Bifidobacterium.

Materials and Methods: Low intensity semiconductor laser 630 nm at 12 mw laser power on three bacterial strain used. The bacterial strain exposed to different periods 3, 5 and 7 min 12 mw laser power.

Results: All results reported and compared with control group, in the present work we measured account of starter culture, ability of antioxidant, β-galactosidase enzyme activity and also we measure changes in total acidity and pH of the yogurt production. Results showed that laser irradiation significantly enhanced cell growth and metabolic activity, with the 5-minute treatment exhibiting the best results across all studied variables. The Viable Cell Count (for Streptococcus thermophiles, Lactobacillus, and Bifidobacterium bacterial strains) was 8.9, 8.7, and 9.2 ($\times 10^8$) respectively, and the Antioxidant Capacity (for the selected strains) was 48%, 45%, and 55%. Highest levels of β-galactosidase enzyme activity were also recorded for aforementioned strains, at 0.45, 0.39, and 0.52 units/ml respectively.

Conclusions: Acidity values of product during three irradiation periods with the control treatment showed an increase in total acidity at an exposure time of 5(93) and pH 5 compared to the control treatment (which opens new horizons for improving the quality and efficiency of fermented dairy product production) and The use of lasers has led to an improvement in the flavor of dairy products.

KEYWORDS: low intensity semiconductors, bacteria, laser light, yogurt

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INTRODUCTION

A wide range of fermented dairy products can be produced by adding starter cultures, which change the lactose in milk into lactic acid. Small amounts of curdled milk were employed as starter cultures to inoculate fresh milk in the past, when bacteriology was unknown [1-2]. People have chosen to eat a variety of fermented milk products in many parts of the world, particularly during hot weather, because their strong acidity has kept people safe by getting rid of dangerous germs [3-4]. People who lived in warmer climates were particularly affected by this offering natural nutrients, probiotic yogurt products greatly improve human health delivering natural nutrients and adding probiotic strains and other lactic acid bacteria (LAB) to the gut microbiome. The foundation of fermented dairy products, such as yogurt, is lactic acid bacteria (LAB) helping to create the finished product's flavor, texture, and health advantages. The quality of yogurt product depends on activity of the bacterial starter which is influent manly on the viability of bacterial and also enhance the flavor and advantage [5]. The probiotic which laying in the GIT system keep healthy function if we intake in a sufficient amount [6]. The probiotic microorganisms in yogurt and which can be classified into two groups: standard cultured yogurt and probiotic-cultured yogurt [7], therefore the culture of probiotics that used in yogurt production continues viable during the path from production until consumption. The origin of word "probiotic 'comes from Greek words „pro" and „bios," meaning „for all life," i.e., any substance or organism that prolongs life [8].

PHOTO BIO-MODULATION (PBM)

This is the method of using the light in general and the laser as specific tool at certain wavelength to stimulate

cellular process. In recent research many topics talk about using certain wavelength in visible region from blue, green and red as stimulated device for growth a microorganisms. In practical case the range of wavelengths from yellow to green 500-600 nm will contribute in increase the growth rate of bacterial strain. The treatment by low intensity laser will produce the phenomena called photobimodulation its new technique in cellular effects. In the mitochondria, leading to increased energy production (ATP) and activation of various metabolic pathways. Hamblin (2017) [9] indicated that photobiomodulation (PBM) is the transmission of photon energy to the cell by non-ionizing visible and infrared light sources by giving molecules energy at particular light wavelengths, PBM modifies some of their inherent characteristics. As life has evolved, Sunlight was used to energize semiconductor metals. These molecules developed into photoreceptors. The authors in [10] were expressed in cell membranes and prokaryotic cells. The physiology of eukaryotic cells was subsequently impacted by the elements of the electron transport chain in mitochondria [11]. As a result, even if many species did not use light as a source of energy, many molecules involved in their physiological processes were nevertheless able to receive light in a primitive way [12]. The authors in [13] indicated that low-level lasers can positively affect microorganisms, including enhancing their growth and metabolic activity. Studies have shown that laser irradiation can increase antioxidant capacity and enzyme activity in some strains of lactic acid bacteria [14]. Accordingly, this study hypothesizes that the application of a 630 nm wavelength laser could be an effective tool for improving the functional properties of yogurt starters, potentially shortening the fermentation period and improving the sensory and health properties of the product.

AIM

The present study aims to study the biological effect of low intensity semiconductor laser 630 nm at 12 mw laser power on three bacterial strain used as starter culture in many yogurt production, such as Streptococcus thermophilus, Lactobacillus delbrueckii sub-bulgaricus, and Bifidobacterium.

MATERIALS AND METHODS

BACTERIAL STRAINS

Three pure strains were used:

1. (ST) Streptococcus thermophilus
2. (LB) Lactobacillus delbrueckii subsp. Bulgaricus
3. (B) Bifidobacterium animalis

They were obtained from an approved bacterial culture bank and preserved in MRS Broth medium with glycerol at -80°C. The used bacterial strain activated by during the experiment, the bacterial strains were treated by activating them through two consecutive transfers to their respective growth media such as (MRS for LB and BA, and M17 for ST). They were incubated under anaerobic conditions at the ideal temperature for each strain [15].

LASER TREATMENTS

The sample was treatment by using semiconductor laser CW have a power 12 mw which influence on the strain of bacterial by adjustment the power density across the cultures. All the cultures was divided into four groups such as:

1. Control group where this group not suffered to laser source
2. Treatment of group one T1 this group exposed to 3 min of time exposer
3. Treatment of group two T 2 the time of exposer a rounded 5 min

Treatment of group three T 3 the exposer time 7 min.

After the laser treatment for specific time for all the samples the count was achieved for live colonies measured and incubation using plate method. The antioxidant potency .Antioxidant properties: These were assessed using a free radical scavenging assay (2, 2-diphenyl-1-picrylhydrazyl). The DPPH inhibition ratio was measured as a clear indicator of antioxidant activity (Mohammad et al. 2019). Beta-galactosidase enzyme activity was measured: measurements were performed under ideal conditions and enzyme activity was measured using an ONPG substrate (o-nitrophenyl-β-D-galactopranoside), where the amount of emitted o-nitrophenol was determined and measured spectroscopically at a wavelength of 420 nm [16]. The PH values level measured by using PH-330i meter (WTW GmbH Germany) 0.5 N NaOH with 1% phenolphthalein as an indicator was used for calibration.

YOGURT MANUFACTURING

By adding 30 grams of skim milk powder and dividing it into four equal batches, the milk's total solids content was standardized to 12%.All batches were heated to After 15 minutes at 80°C and cooling to 42±1°C, yogurt starting culture was added to each batch. ABT-2 probiotic starter culture (0.05% wight/wight) and (0.02% w/w). The mixture was placed in 200 ml plastic cups and incubated at 42±1°C until coagulation was complete. The yogurt treatments were then transferred to a cold storage area at 4±1°C.

ANALYSIS OF STATISTICS

The means±standard deviation were used to express all of the data. One-way analysis of variance (ANOVA) and Duncan's multiscale test were used for statistical analysis; a p-value of ≥0.05 was deemed statistically significant (using Genstat software, 2010).

RESULTS

Figure 1 shows the pH and total acidity % levels of four different yogurt samples, while the yogurt curdles by reducing the pH to promote meselle bonding, the casein in the milk starts to precipitate when the pH hits 4.6 [16]. The control sample TC's pH on the first day of storage was 4.3, and the samples exposed to irradiation were in T1 4.5, while T2 was 5, and T3 was 4.8. There are significant differences in pH across the four groups (TC, T2, T1, T3) (Fig. 1A). Figure 1B shows the total acidity, which was (0.80%, 0.86%, 0.93%, and 0.89%), respectively. A decrease the yogurt samples showed an increase in total acidity % and pH values. The difference between the treatments is attributed to the effect of light exposure on the metabolic activity of the starter

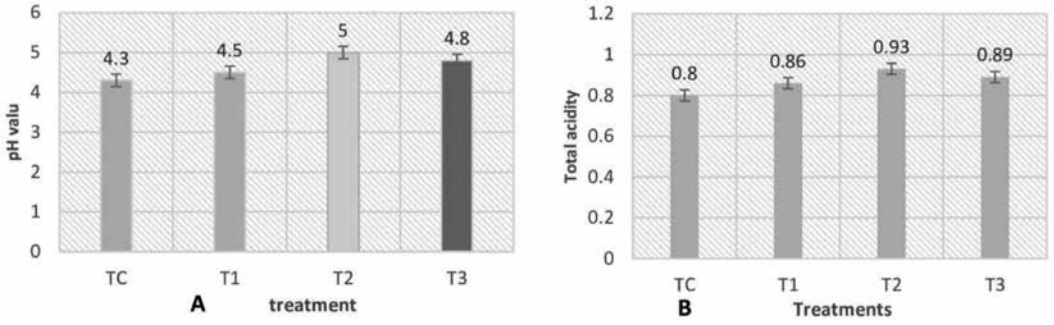

Fig. 1. A-B. The impact of 630 nm laser radiation on the pH and overall acidity of yogurt samples following a 24-hour period of refrigeration

Source: Own material

bacteria. The bacteria's ability to convert lactose to lactic acid increased, leading to improved texture and consistency in the product. The activity of living cells increases when exposed to light at low energies and for a specific duration due to the transfer of energy from the photon to the cell, which increases their ability to reproduce and improves cellular metabolism.

LIVE CELL COUNT

Table 1 presents the effect of 630 nm laser irradiation at 12 mW on the viable cell count of starter bacteria after 24 hours. For S. thermophilus, the control group (TC) had 8.2 $\times 10^8$ CFU/mL, while treated groups T1, T2, and T3 showed 8.6, 8.9, and 8.7 $\times 10^8$ CFU/mL, respectively. L. bulgaricus viable cell counts were 8.0 $\times 10^8$ CFU/mL (TC), and 8.4, 8.7, 8.5 $\times 10^8$ CFU/mL for T1, T2, and T3, respectively. For B. animalis, the counts were 8.5 $\times 10^8$ CFU/mL (TC), and 8.9, 9.2, 9.0 $\times 10^8$ CFU/mL for T1, T2, and T3, respectively. A clear difference in viable cell counts was observed, with the highest counts generally noted at 5 minutes of exposure (T2).

EFFECT OF LASER IRRADIATION ON ANTIOXIDANT CAPACITY (INHIBITION RATIO)

Table 2 details the antioxidant capacity (DPPH inhibition percentage) of the bacterial strains. For S. thermophilus, inhibition percentages were 25% (TC), 35% (T1), 48% (T2), and 42% (T3). L. bulgaricus showed inhibition rates of 22% (TC), 31% (T1), 45% (T2), and 38% (T3). B. animalis exhibited inhibition percentages of 30% (TC), 42% (T1), 55% (T2), and 51% (T3). A significant increase in antioxidant capacity was observed across all treated groups, with the 5-minute exposure time (T2) yielding the highest inhibition rates

Table 3 illustrates the effect of laser irradiation on the activity of the β-galactosidase enzyme. For S. thermophilus,

Table 1. Effect of laser irradiation on the live cell count (CFU/mL)×10^8 after 24 hours

Bacterial strain	Control group	3 minutes (T1)	5 minutes (T2)	7 minutes (T3)
S. thermophilus	8.2	8.6	8.9	8.7
L. bulgaricus	8.0	8.4	8.7	8.5
B. animalis	8.5	8.9	9.2	9.0

Source: Compiled by the authors of this study

Table 2. Effect of laser irradiation on antioxidant capacity (DPPH inhibition %)

Bacterial strain	Control group	3 minutes (T1)	5 minutes (T2)	7 minutes (T3)
S. thermophilus	25%	35%	48%	42%
L. bulgaricus	22%	31%	45%	38%
B. animalis	30%	42%	55%	51%

Source: Compiled by the authors of this study

Table 3. Effect of laser irradiation on enzyme activity (β-galactosidase unit/ml)

Bacterial strain	Control group	3 minutes (T1)	5 minutes (T2)	7 minutes (T3)
S. thermophilus	0.30	0.38	0.45	0.41
L. bulgaricus	0.25	0.32	0.39	0.35
B. animalis	0.35	0.44	0.52	0.48

Source: Compiled by the authors of this study

enzyme activity levels were 0.30 units/mL (TC), 0.38 units/mL (T1), 0.45 units/mL (T2), and 0.41 units/mL (T3). L. bulgaricus showed activities of 0.25 units/mL (TC), 0.32 units/mL (T1), 0.39 units/mL (T2), and 0.35 units/mL (T3). B. animalis exhibited enzyme activities of 0.35 units/mL (TC), 0.44 units/mL (T1), 0.52 units/mL (T2), and 0.48 units/mL (T3). These results indicate a significant increase in β-galactosidase enzyme activity in laser-treated samples, with the 5-minute exposure time (T2) again demonstrating the highest activity.

DISCUSSION

The application of low-intensity laser irradiation at 630 nm significantly influenced the metabolic activity and functional properties of starter bacteria used in yogurt production, as evidenced by changes in pH, total acidity, viable cell counts, antioxidant capacity, and β-galactosidase enzyme activity. The most pronounced enhancements were consistently observed at a 5-minute exposure time, suggesting an optimal photobiomodulation window for the bacterial strains studied. The observed decrease in pH and increase in total acidity in laser-treated yogurt samples, particularly at 5 minutes of exposure, indicate an accelerated fermentation process. This is attributed to the enhanced metabolic activity of the starter bacteria, leading to a more efficient conversion of lactose into lactic acid. The curdling process in yogurt is directly linked to pH reduction, where casein precipitation occurs around pH 4.6 [16]. The improved metabolic activity, as a result of light exposure, facilitates this process, potentially leading to improved texture and consistency of the final product. The transfer of energy from photons to bacterial cells at low energies and specific durations is known to stimulate cellular reproduction and metabolism, aligning with our findings regarding increased lactic acid production. The significant increase in viable cell counts across all three bacterial strains (S. thermophilus, L. bulgaricus, and B. animalis) following laser irradiation further supports the stimulatory effect of photobiomodulation. The 5-minute exposure time consistently yielded the highest cell counts, while a 7-minute exposure showed a slight decrease, suggesting that prolonged exposure might exceed the optimal threshold and induce cellular stress. This stimulatory effect is likely due to the absorption of light energy, which enhances ATP production, providing additional energy for cell growth and division [17]. This mechanism is consistent with the principles of photobiological alteration, where non-ionizing visible and infrared light sources impact inherent molecular characteristics by supplying energy at specific wavelengths [10-12]. The enhanced antioxidant capacity, measured by DPPH inhibition percentage, in laser-treated bacterial strains is a crucial finding. The 5-minute exposure time again resulted in the highest antioxidant activity. This can be explained by the activation of metabolic pathways that produce antioxidant compounds or enhance the activity of defensive enzymes. This property is vital for protecting the bacteria from oxidative stress during manufacturing and storage and for improving the health value of the final product for consumers [18]. The stimulation of superoxide dismutase (SOD), a potent antioxidant enzyme, by increased cellular metabolism, as suggested by [19], could be a contributing factor to the observed increase in antioxidant capacity. Finally, the significant increase in β-galactosidase enzyme activity in laser-irradiated samples is particularly noteworthy. This enzyme is critical for lactose breakdown during fermentation, and its enhanced activity implies faster fermentation and a quicker pH decrease. This could lead to reduced production time and improved product texture. The elevated enzyme levels in treated samples are likely a consequence of increased cellular metabolism and bacterial cell quantity, consistent with previous research [11]. The size of the inoculation and the activity of the culture are known to play a key role in β-galactosidase production by lactic acid bacteria. This finding also has implications for individuals with lactose intolerance, as increased β-galactosidase activity can aid in the conversion of lactose to lactic acid, making yogurt more digestible.

PBM IN FOOD BIOTECHNOLOGY AND INDUSTRIAL APPLICATIONS

Beyond the laboratory, the application of photon energy, including laser technology, is gaining traction in the food industry for various purposes [20-21]. These applications extend beyond microbial stimulation to encompass critical aspects of food processing, safety, and quality control:

ENHANCED FERMENTATION PROCESSES

Similar to our findings, PBM can be utilized to optimize the growth and metabolic activity of starter cultures in large-scale industrial fermenters. This can lead to reduced fermentation times, increased production efficiency, and lower energy consumption, directly impacting the economic viability of dairy production [22].

FOOD SAFETY AND STERILIZATION

High-intensity pulsed light and UV radiation, forms of photon energy, are effectively used for surface decontamination of food products and packaging materials. This non-thermal sterilization method helps extend shelf-life and reduce the reliance on chemical preservatives, aligning with consumer demand for cleaner labels [23].

QUALITY CONTROL AND AUTHENTICATION

Spectroscopic techniques employing lasers are increasingly used for real-time, non-destructive analysis of food composition, ripeness, and the detection of contaminants or adulterants. This ensures consistent product quality and enhances food safety throughout the supply chain [24].

NUTRITIONAL FORTIFICATION

Photostimulation, including specific light wavelengths, has been shown to increase the concentration of bioactive compounds, such as vitamins, antioxidants, and polyphenols, in various food products, including sprouts and fermented foods. This aligns with the observed increase in antioxidant capacity in our laser-treated yogurt, suggesting a pathway for developing nutritionally enhanced products [25-26]

CLINICAL AND PRODUCTION IMPLICATIONS

This has several significant implications.

PRODUCTION EFFICIENCY

The accelerated fermentation process, evidenced by increased acidity and viable cell counts, translates directly into reduced production cycles for dairy manufacturers. This can lead to significant cost savings in terms of energy and labor, and an increase in overall plant throughput.

IMPROVED PRODUCT QUALITY AND FUNCTIONAL VALUE

The enhanced antioxidant capacity and β-galactosidase activity contribute to a more functional and healthier yogurt product. The increased antioxidant properties can protect the product from oxidative degradation during storage and offer enhanced health benefits to consumers. The elevated β-galactosidase activity is particularly beneficial for lactose-intolerant individuals, making the yogurt more digestible and expanding its market appeal.

SENSORY ENHANCEMENT

The improved metabolic activity of the starter cultures can lead to a more desirable flavor profile and texture in the final product. Optimized fermentation contributes to the balanced production of flavor compounds, resulting in a smoother, more consistent, and palatable yogurt [27].

CONCLUSIONS

In this research paper confirms that low-intensity laser irradiation at 630 nm significantly enhances the viability and metabolic functionality of S. thermophilus, L. bulgaricus, and B. animalis. The optimal exposure time of 5 minutes leads to accelerated fermentation, increased antioxidant capacity, and elevated β-galactosidase activity. These findings have profound implications for the dairy industry and consumer health Industrial Efficiency. PBM can serve as a novel biostimulant in industrial yogurt production, leading to reduced fermentation times, lower energy consumption, and increased throughput, thereby enhancing overall production efficiency. The laser process enhance the bacterial growth and amin functions such as antioxidant and also fermentation efficiency. The main and important results was obtained at 5 min exposer time with 15 mw laser power at uniform density area of samples. The use of lasers has led to an improvement in the flavor of dairy products by increasing flavor compounds resulting from increased cellular metabolism.

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ORIGINAL ARTICLE

War, crisis and adaptation: Resilience self-assessment in Ukrainian medical and psychological education

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ABSTRACT

Aim: To assess self-reported resilience among Ukrainian medical students, physicians, teachers, and psychologists under conditions of full-scale war and to identify the main factors and strategies supporting adaptation.

Materials and Methods: An original cross-sectional mixed-methods study was conducted in 2026. The questionnaire was developed following a targeted review of publications on resilience, education, and healthcare in wartime Ukraine. The anonymous Google Forms survey was distributed through communication channels to approximately 2500 potential respondents, of whom 295 responses were received (response rate: 11.8%). Quantitative data were analysed using descriptive statistics. Open-ended responses were analysed thematically.

Results: The sample was predominantly female (216; 73.2%) and consisted mainly of students or PhD students (214; 72.5%). Most respondents assessed their resilience as moderate or high: 101 (34.2%) reported 51-75%, and 99 (33.6%) reported >75%. The most frequent resilience resources included family relationships, social support, responsibility for others, future goals, professional activity, civic engagement, faith, routine, and self-care. The main coping strategies were cognitive reframing, acceptance, short-term planning, maintaining daily structure, social connectedness, restorative activities, and reflective practices. A smaller group reported exhaustion, lack of support, avoidance behaviours, alcohol or sedative use, and depressive symptoms.

Conclusions: Resilience among teachers, medical students, physicians, and psychologists is an important area of study. Self-reported data indicated relatively high levels of resilience in the fifth year of full-scale war. However, the study revealed a concerning trend of depressive symptoms and emotional burnout. Strengthening resilience requires institutional support, psychoeducational interventions, and professional development activities.

KEYWORDS: war, Ukraine, resilience, physicians, teachers, psychologists

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INTRODUCTION

The full-scale Russian invasion of Ukraine has created a prolonged social, educational and healthcare crisis. Medical teachers, physicians, psychologists and students have had to continue professional or academic activity while facing air attacks, displacement, loss, uncertainty and overload. In such circumstances, resilience represents a process of maintaining functioning, restoring meaning and adapting to changing threats [1-4].

Previous studies conducted during the war have shown high psychological pressure on Ukrainian educators, including stress, burnout, rapid transition to online or hybrid teaching, and the need to support students experiencing their own trauma [5-11]. Medical students and PhD students also faced anxiety, interrupted education and the need to preserve professional development under crisis conditions [12-17]. Healthcare workers experienced secondary traumatic stress, lack of resources, professional overload and threats to personal safety [18-22]. Psychologists and psychosocial support workers reported emotional exhaustion, professional quality-of-life risks and the need for mutual support and supervision [23-27].

Although these findings describe separate professional groups, less is known about how representatives of medical and psychological education communities in Ukraine assess their own resilience and which factors they name as helpful. This article therefore presents an original survey-based study focused on self-assessment and thematic interpretation of resilience under wartime conditions.

AIM

To assess self-reported resilience among Ukrainian medical students, physicians, teachers, and psychologists under conditions of full-scale war and to identify the main factors and strategies supporting adaptation.

MATERIALS AND METHODS

This was an original cross-sectional mixed-methods study. A targeted literature review was used solely to formulate the questionnaire and interpret the findings; the empirical component of the study is based on an anonymous survey conducted by the authors.

The survey was conducted in March-April 2026 via Google Forms and disseminated through educational, professional, and social media channels of Bogomolets

National Medical University and Lviv Polytechnic National University. The invitation was sent to approximately 2500 potential respondents. Participation was voluntary and anonymous.

The target groups included medical and psychological students, PhD students, practising physicians, psychologists, and teachers. Inclusion criteria were affiliation with one of these groups, age ≥18 years, and voluntary completion of the questionnaire. The anonymous Google Forms survey was distributed through communication channels to approximately 2500 potential respondents, of whom 295 responses were received (response rate: 11.8%). Quantitative data were analysed using descriptive statistics.

The questionnaire contained closed-ended items on gender, age group, professional background, self-assessed resilience since the beginning of the full-scale invasion, temporary departure from Ukraine, and intention to continue studying or working in Ukraine. It also included two open-ended questions: what supports resilience, and which resilience strategies respondents had developed for themselves.

Closed-ended responses were analysed using descriptive statistics and presented as frequencies and percentages. Open-ended responses were analysed thematically: recurring meanings were identified, grouped into broader categories, and compared with quantitative findings.

ETHICS

The survey was anonymous, voluntary and did not collect patient data or personal identifiers. Respondents were informed about the aim of the study before completing the form. The authors followed the principles of the Declaration of Helsinki and ICMJE recommendations. The study was approved by the Bogomolets National Medical University Research Department Commission. No part of the manuscript contains plagiarism, fabrication or falsification. All cited sources are presented in the reference list.

AI STATEMENT

AI GPT CHAT 5.2 was used for formatting of the bibliography as well as language proofreading and correction, both under the authors' further revision. The scientific statements, conclusions, and results are the authors' own intellectual contribution.

FRAMEWORK

The study was conducted within the research project of Bogomolets National Medical University: 0124U001087 "Effective teaching techniques in postgraduate medical education", 01.2024-12.2026. Bogomolets National Medical University issued an Expert Certificate dated 22.03.2026 approving open publication of the study results.

RESULTS

The characteristics and resilience self-assessment of respondents are represented in Table 1. A total of 295 respondents completed the questionnaire, with a response rate of 11.8%. The sample was predominantly female (216; 73.2%). The largest age category was 20+ (138; 46.8%), followed by respondents under 20 (62; 21.0%). Students and PhD students constituted most of the sample (214; 72.5%), while teachers represented 47 respondents (16.0%), physicians 26 (8.8%) and psychologists 8 (2.7%).

Most respondents assessed their resilience as 51-75% or more than 75%, which together accounted for 200 respondents (67.8%). At the same time, 95 respondents (32.2%) placed their resilience below 51%, showing that perceived adaptation coexisted with vulnerability. Most participants had not temporarily left Ukraine since the full-scale invasion (229; 77.6%). A majority expressed an intention to continue studying or working in Ukraine: 210 respondents (71.2%) chose "definitely yes" or "more yes than no". However, 65 participants (22.0%) found it difficult to answer, which indicates uncertainty about future trajectories.

The thematic analysis of open-ended responses showed that resilience was supported by several interconnected groups of factors and strategies (Table 2).

The most stable resilience resource was social support, especially family, close relationships and friends. Respondents also connected resilience with responsibility for others, future goals, education, work, volunteering and contribution to Ukraine. These answers show that resilience was often understood as the continuation of meaningful activity despite threat.

The most frequent strategies were cognitive reframing, acceptance, focus on controllable tasks, short-term planning and preservation of routine. Respondents mentioned work, study, discipline, information hygiene, sleep, physical activity, hobbies, solitude, communication and psychological reflection. Some referred to psychotherapy, counselling, antidepressants or psychopharmacotherapy as forms of support. A smaller group reported maladaptive or risky strategies, including alcohol, sedatives, overeating, avoidance, withdrawal and absence of any strategy (Table 2).

DISCUSSION

The findings demonstrate the complex and substantial nature of wartime resilience in Ukraine. Most respondents reported moderate or high levels of resilience, which corresponds with studies describing adaptive capacity among Ukrainian educators, healthcare workers, and students during the war [9-11, 21-22]. However, open-ended responses indicate that resilience does not equate to the absence of distress. Rather, it appears as a dynamic process sustained by relationships, purpose, routine, and adaptive thinking, coexisting with fatigue, uncertainty, and emotional depletion.

The sample structure is important for interpretation. The predominance of young respondents and students explains the importance of education, professional formation, future goals, and the intention to remain in Ukraine. For this group, resilience was closely linked to the preservation of developmental and professional trajectories. Similar patterns have been described in studies of medical and psychology students, where continuing education, volunteering, and professional identity supported adaptation under martial law [12-17].

Table 1. Characteristics and resilience self-assessment of respondents

Indicator	Category	n (%)
Gender	Female	216 (73.2)
	Male	79 (26.8)
Age	Under 20	62 (21.0)
	20+	138 (46.8)
	30+	23 (7.8)
	40+	33 (11.2)
	50+	39 (13.2)
Profession	Students / PhD students	214 (72.5)
	Teachers	47 (16.0)
	Physicians	26 (8.8)
	Psychologists	8 (2.7)
Self-assessed resilience	Less than 25%	22 (7.4)
	25-50%	73 (24.8)
	51-75%	101 (34.2)
	More than 75%	99 (33.6)
Temporarily left Ukraine	Yes	66 (22.4)
	No	229 (77.6)
Intention to continue study/work in Ukraine	Definitely yes	115 (39.0)
	More yes than no	95 (32.2)
	Difficult to answer	65 (22.0)
	More no than yes	15 (5.1)
	Definitely no	5 (1.7)

Source: compiled by the authors of this study

Table 2. Main thematic clusters in open-ended responses

Cluster	Main content	Examples
Social ties and responsibility	Family, partners, children, friends, colleagues, mutual support and responsibility for others.	"My family"; "Do not stay alone"; "Responsibility for loved ones"
Meaning, work and civic identity	Future goals, study, professional activity, volunteering, helping the army, patriotism and belief in Ukraine.	"Plans for the future"; "Study"; "Doing useful things for Ukraine"
Cognitive regulation	Reframing, acceptance of reality, focus on what can be controlled, short-term planning.	"Acceptance"; "If I cannot influence it, there is no sense in worrying"
Routine and self-care	Daily structure, discipline, sleep, sport, walks, hobbies, information hygiene and restorative practices.	"Daily routine"; "Sport and study"; "Less news, more sleep"
Psychological and spiritual coping	Psychotherapy, counselling, diary writing, reflection, prayer, faith and meaning-making.	"Psychotherapy"; "Prayer"; "Keeping a diary"
Risk patterns	Lack of support, hopelessness, alcohol or sedative use, emotional withdrawal, exhaustion and depressive experiences.	"Nothing"; "Alcohol"; "I have had depression"

Source: compiled by the authors of this study

Social connection emerged as the strongest recurring theme. Family, friends, colleagues, and responsibility for loved ones represented key sources of meaning. This confirms that wartime resilience is relational and socially embedded. Respondents also relied on civic identity, faith, volunteering, and belief in victory, suggesting that individual coping is closely connected with collective endurance.

Cognitive and behavioural self-regulation constituted the second major mechanism. Reframing, acceptance, short-term planning, and maintaining daily routines helped respondents restore predictability in an unpredictable environment. Such strategies correspond to research on coping among teachers, healthcare professionals, and mental health workers in Ukraine [5, 9, 22-24]. The results also underline the importance of everyday practices such as sleep, exercise, hobbies, information hygiene, and regular work or study activities.

At the same time, the study revealed a vulnerable group whose answers included hopelessness, emotional exhaustion, withdrawal, alcohol, sedatives, overeating or absence of coping strategies. These responses are important because they show that self-reported resilience may mask chronic overload and survival-oriented functioning. Therefore, resilience-building should not be reduced to motivational messages. Educational and healthcare institutions need practical measures: peer support, psychoeducation, access to counselling, supervision, flexible schedules, restorative spaces and early recognition of burnout and maladaptive coping.

The study has limitations. The sample was not representative of all Ukrainian professional groups and was dominated by students and women. Resilience was self-assessed in percentage form rather than measured by a validated psychometric scale. The survey was distributed online, which may have limited participation by those with severe overload or poor access to digital communication. Nevertheless, the combination of closed-ended and open-ended data allowed identification of the main perceived resilience resources and risk patterns in medical and psychological education communities.

CONCLUSIONS

Most respondents assessed their resilience as moderate or high five years after the beginning of the full-scale war, although almost one-third reported resilience below 51%. The main resilience resources included family and close relationships, social support, responsibility for others, future goals, work and study, professional identity, volunteering, civic engagement, faith, routine, and self-care. The most frequent strategies were reframing, acceptance, focusing on controllable tasks, short-term planning, maintaining daily structure, information hygiene, communication, and restorative practices. A smaller but important group reported emotional exhaustion, hopelessness, avoidance behaviours, alcohol or sedative use, and a lack of coping strategies, which indicates the need for institutional support. Resilience programmes in medical and psychological education should combine psychoeducation, peer support, access to counselling, flexible academic or work arrangements, and early prevention of burnout.

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ORIGINAL ARTICLE

Influence of IFNG rs2069709 on the severity of varicella in children

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ABSTRACT

Aim: To assess the association between IFNG rs2069709 genotypes (CC, AC), as well as demographic factors such as sex and age, with the risk of complicated varicella in children.

Materials and Methods: The study included 132 children with confirmed varicella. Genotyping was performed using PCR with the TaqMan Genotyping Assay. Statistical analysis comprised the χ^2 test, allele frequency evaluation, and logistic regression to determine the effects of genotype, sex, and age on disease severity.

Results: The AC genotype was detected in 67.2% of children, while the CC genotype accounted for 20.3%; samples lacking genotype data (12.5%) were excluded from analysis. The C allele predominated in the uncomplicated group (64.2%), whereas the A allele was more frequent among children with complicated varicella (41.3%). Logistic regression demonstrated a tendency toward increased risk of complicated disease in AC carriers (OR=2.05; p=0.10). Sex and age showed no significant association with disease severity (p>0.05). Although statistical significance was not reached, the findings indicate that variability in the IFNG locus may influence individual immune responses to varicella-zoster virus.

Conclusions: The IFNG rs2069709 C allele may act as a protective factor against severe varicella, while the A allele may increase susceptibility to complications. The observed trend for the AC genotype highlights the potential role of genetic markers in predicting disease course. Larger studies are required to confirm these associations and clarify their underlying mechanisms.

KEYWORDS: children, allele, complicated course, logistic regression

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INTRODUCTION

Varicella (chickenpox) is an infectious disease caused by the varicella-zoster virus (VZV). It is widespread globally and represents a significant public health burden. According to the World Health Organization, the annual global burden of varicella is estimated at approximately 140 million cases, including 4.2 million severe complications requiring hospitalization and 4,200 deaths.

Despite substantial progress in understanding the immunological mechanisms underlying the host response to varicella-zoster virus, the factors determining individual variability in disease severity remain insufficiently studied [1].

Varicella is a highly contagious disease transmitted primarily via airborne droplets, with particularly high transmission rates in temperate climates. Although it has traditionally been assumed that the virus spreads predominantly through the respiratory tract, supporting evidence remains limited. In fact, viral entry is thought to occur mainly through the skin, where VZV accumulates in vesicular lesions. Skin cells and cell-free virus are actively shed and likely represent the principal source of airborne transmission, whereas individuals without skin lesions are not considered contagious [2].

Following primary infection, VZV can persist in a latent state and may reactivate years or decades later, leading to herpes zoster (shingles). Varicella and its complications are more severe in immunocompromised individuals. One of the most common and serious complications of VZV reactivation is postherpetic neuralgia (PHN) [3]. In addition, reactivation may result in a wide range of neurological disorders, including VZV vasculopathy, myelitis, focal motor weakness, zoster sine herpete, Ramsay Hunt syndrome, and meningoencephalitis [4].

In recent years, increasing attention has been given to single nucleotide polymorphisms (SNPs) that influence both innate and adaptive immune responses. In the present study, we focused on the IFNG rs2069709 polymorphism, located in the promoter region of the IFNG gene. IFNG encodes interferon-γ, a cytokine that plays an important role in antiviral immune responses. The IFN-γ signaling pathway plays a key role in controlling herpesvirus infections, including VZV; however, the contribution of specific polymorphisms such as rs2069709 to disease severity remains poorly understood.

Studies on inborn errors of immunity have demonstrated that defects in interferon signaling pathways (e.g., POLR3A, POLR3C) may predispose individuals to severe VZV infections [5, 6]. Moreover, it has been shown that interindividual variability in immune responses to VZV vaccination is largely determined by genetic factors, including HLA and cytokine-

related pathways [7, 8]. These findings suggest that genetic variability may influence both the natural course of infection and the effectiveness of preventive strategies.

Interest in this topic is driven not only by the need to better understand virus-host interactions but also by its clinical relevance. Identification of genetic predictors may facilitate early risk stratification and timely identification of children at increased risk of a complicated disease course. Therefore, investigating the relationship between genotype and the severity of varicella in children represents a promising research direction with both scientific and practical significance.

In this study, we analyzed genotype (CC, AC), sex, and age data in a cohort of 132 individuals to identify potential associations between genetic variants and the clinical form of varicella ("O" – complicated and "N" – uncomplicated).

AIM

To assess the association between IFNG rs2069709 genotypes (CC, AC), as well as demographic factors such as sex and age, with the risk of complicated varicella in children.

MATERIALS AND METHODS

Data collection was conducted at the inpatient department of the Infectious Diseases Center of the Regional Clinical Hospital (Karaganda, Kazakhstan) from March 2024 to May 2025 and included children with a confirmed diagnosis of varicella.

The study was approved by the Local Bioethics Committee of the Non-profit Joint Stock Company "Karaganda Medical University" (protocol No. 3, dated February 27, 2024). Informed consent was obtained from the parents or legal guardians of all participants.

Genotyping was performed using polymerase chain reaction (PCR) with the TaqMan Genotyping Assay (Thermo Fisher Scientific, USA), specific for the IFNG rs2069709 (A/C) polymorphism. Primers and probes recommended by the manufacturer were used. Genotypes were classified as CC or AC; homozygous AA variants were not detected in the study sample.

PCR-based genotyping was carried out in 132 children diagnosed with varicella. Total DNA extraction was performed under sterile conditions using the Qiagen Investigator Kit in accordance with the manufacturer's protocol. For preparation of the PCR mixture, the Genotyping Master Mix (Thermo Fisher Scientific, USA) and TaqMan Genotyping Assay (40X) were used. Amplification was performed according to the manufacturer's instructions.

The dataset was obtained from the file "Genotypes_22052025.xlsx" and included data from 132 individuals, comprising subject identifier, genotype (CC, AC), disease form (O – complicated or N – uncomplicated), sex (M or F), and age (Fig.1).

GENOTYPE ANALYSIS

Frequencies of the CC and AC genotypes and their distribution according to disease form and sex were calculated. The chi-square (χ^2) test was used to assess the association between genotype and disease form.

ALLELE ANALYSIS

Allele frequencies for C and A were calculated based on genotype distribution (CC=2C; AC=1A and 1C). The frequencies of alleles were compared between the groups with complicated (O) and uncomplicated (N) forms of varicella.

LOGISTIC REGRESSION

Logistic regression analysis was performed to evaluate the effects of genotype (CC as the reference category, AC as the predictor), sex (M=0, F=1), and age (years) on the probability of having an uncomplicated disease course (N status).

The model was specified as follows:

$\text{logit}(P(N)) = \beta_0 + \beta_1 \cdot AC + \beta_2 \cdot Sex + \beta_3 \cdot Age.$

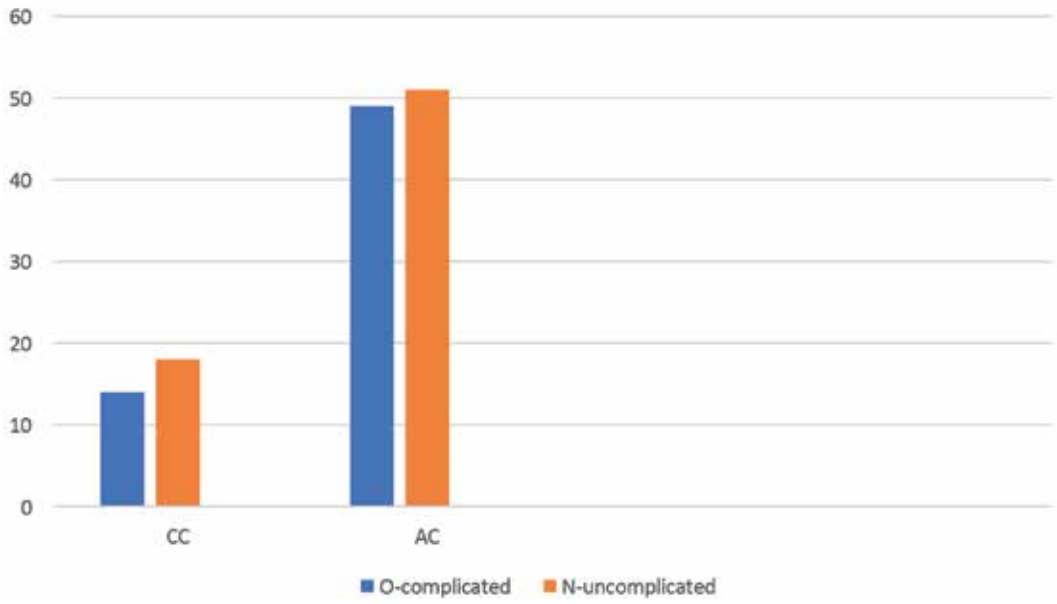

Fig. 1. Distribution of genotypes by disease form

Picture taken by the authors

Parameter estimation was performed using the maximum likelihood method. Model fit was assessed using McFadden's pseudo-R^2.

ETHICS

This work complies with the principles of the Declaration of Helsinki.

AI STATEMENT

The author states that no AI tools were used in writing this article.

RESULTS

A total of 132 children aged 0 to 18 years were included in the study and divided into two groups: 63 children with a complicated form of varicella and 69 children with an uncomplicated course. The study population comprised 44 girls and 88 boys.

Among all patients, central nervous system complications accounted for 24%, respiratory complications for 33%, generalized systemic inflammation (sepsis) for 5%, and skin-related complications for 38%.

Respiratory complications included viral-bacterial pneumonia associated with *Staphylococcus aureus* (10^5), *Staphylococcus hominis* (10^6), as well as acute simple and obstructive bronchitis associated with *Enterobacter aerogenes* (10^6), *Staphylococcus aureus* (10^6), *Staphylococcus haemolyticus* (10^6), *Streptococcus pneumoniae* (10^6), *Candida albicans* (10^4), and *Klebsiella pneumoniae* (10^6).

Inflammatory diseases of the skin and subcutaneous tissue were represented by pyoderma (streptoderma) and phlegmon.

Central nervous system involvement included meningococcal meningitis and hepatic encephalopathy.

GENOTYPE ANALYSIS

A total of 132 observations were analyzed, of which 32 (24.2%) corresponded to the CC genotype and 100 (75.8%) to the AC genotype. The distribution of genotypes according to disease form and sex is presented in Table 1 and Table 2.

The chi-square test showed no statistically significant association between genotype and disease form (χ^2=1.89, p=0.39).

ALLELE ANALYSIS

Allele analysis was performed in 132 patients, including 32 with the CC genotype and 100 with the AC genotype. A total of 264 alleles were analyzed, of which 164 (62.1%) corresponded to the C allele and 100 (37.9%) to the A allele.

Allele frequencies according to disease form are presented in Table 3.

The frequency of the C allele was higher among children with an uncomplicated form of varicella (63.0%) compared with those with a complicated course (61.1%). Conversely, the A allele was more frequently observed in patients with complicated disease (38.9% vs. 37.0% in the uncomplicated group).

LOGISTIC REGRESSION

A logistic regression model was constructed to predict disease form (complicated vs. uncomplicated) based on data from 132 patients. The results of the analysis are presented in Table 4.

The AC genotype was associated with a 2.05-fold increase in the odds of having an uncomplicated disease course (N status) compared with the CC genotype; however, this effect was only marginally significant (p≈0.10). Sex and age did not demonstrate statistically significant effects (p>0.05). The model's pseudo-R^2 was 0.06, indicating low explanatory power.

ASSOCIATION WITH DISEASE STATUS

After adjustment for sex and age, the IFNG rs2069709 A/C genotype was associated with an odds ratio (OR) of 1.49 (95% CI: 0.70-3.16; p=0.29). No statistically significant association with complicated varicella was identified.

INTERACTION WITH SEX

The interaction between IFNG rs2069709 genotype and sex was not statistically significant (p=0.29). However, among females carrying the A/C genotype, a trend toward

Table 1. Distribution of genotypes according to disease form

Disease form	AC, n (%)	CC, n (%)	Total
Uncomplicated (N)	51 (73.9%)	18 (26.1%)	69
Complicated (O)	49 (77.8%)	14 (22.2%)	63
Total	100 (75.8%)	32 (24.2%)	132

Source: compiled by the authors of this study

Table 2. Distribution of genotypes according to sex

Sex	AC, n (%)	CC, n (%)	Total
Male (M)	63 (71.6%)	25 (28.4%)	88
Female (F)	37 (84.1%)	7 (15.9%)	44
Total	100 (75.8%)	32 (24.2%)	132

Source: compiled by the authors of this study

Table 3. Allele frequencies by disease form

Group	A, n (%)	C, n (%)	Total
Form N (uncomplicated)	51 (37.0%)	87 (63.0%)	138
Form O (complicated)	49 (38.9%)	77 (61.1%)	126
Total	100 (37.9%)	164 (62.1%)	264

Source: compiled by the authors of this study

Table 4. Results of logistic regression

Predictor	Coefficient (β)	Standard Error	OR (exp(β))	p-value
Genotype AC	0.72	0.44	2.05	0.10
Sex	0.38	0.36	1.46	0.29
Age	0.02	0.02	1.02	0.32

Source: compiled by the authors of this study

an increased risk was observed (OR=2.86), although the confidence intervals were wide.

DISCUSSION

In the present study, we evaluated the association between the IFNG rs2069709 polymorphism and the severity of varicella in hospitalized children. Although no statistically significant differences were observed, carriers of the AC genotype demonstrated a trend toward an increased risk of a complicated disease course, suggesting potential clinical relevance of this genetic variant.

Interferon-γ-mediated immune responses play a crucial role in host defense against herpesvirus infections, including varicella-zoster virus (VZV) infection [6, 9, 10]. The IFNG gene encodes a key component of this signaling pathway and is essential for the activation of both innate and adaptive immune mechanisms. Disruptions in interferon signaling, including those associated with inborn errors of immunity, have previously been described in patients with severe herpesvirus infections, including complicated varicella [11, 12].

Our findings are consistent with current evidence highlighting the role of host genetic factors in determining the clinical heterogeneity of varicella. Several studies have demonstrated that polymorphisms in genes involved in immune regulation may be associated with susceptibility to complications and adverse outcomes in viral infections. For instance, polymorphisms in the IL-10 gene have been linked to severe VZV infection [13, 14]. Furthermore, genetic epidemiology and Mendelian randomization studies support the contribution of host immune variability to diverse clinical phenotypes of VZV infection [1, 15, 16].

The absence of a statistically significant association between the IFNG rs2069709 polymorphism and disease severity in our study is likely attributable to the relatively small sample size and the hospital-based design, which may have limited the statistical power of the analysis. Importantly, the observed odds ratio exceeding 2.0 suggests a potentially meaningful effect size despite the lack of statistical significance. Such findings should be interpreted as indicative of a possible association rather than evidence of no effect, and they may reach statistical significance in studies with larger sample sizes. Similar borderline results have been reported in studies investigating IFNG polymorphisms in other infectious and inflammatory conditions [8].

The application of Mendelian randomization approaches has further advanced the understanding of the relationship between immune responses and VZV-related clinical phenotypes. Yu et al. demonstrated that genetically determined anti-VZV IgG levels are associated with a broad range of phenotypes; however, these findings are largely influenced by genetic instruments located within the major histocompatibility complex (MHC) region [16].

Comparable observations have been reported for polymorphisms within the interferon-γ signaling pathway. A meta-analysis by Cheng et al. showed that variations in IFNGR1 rs2234711 may be associated with susceptibility to infectious diseases, particularly tuberculosis, with effects varying across different ethnic groups [9]. More recently, Girault et al. demonstrated that IFNG is a critical factor in limiting VZV replication, further supporting its biological relevance in infection control [11].

Negative or borderline findings, such as those observed in the present study, are also reflected in the literature. For example, Kaymaz et al., analyzing the IFNGR1 rs2234711 polymorphism in patients with sarcoidosis, did not identify significant associations [17]. This underscores that the effects of individual single nucleotide polymorphisms (SNPs) may be modest or masked by other factors, including immune status, comorbidities, age, and population-specific genetic characteristics.

It should also be noted that a standardized treatment approach was applied in this study in accordance with current clinical guidelines, including antiviral and supportive therapy. This reduces the likelihood that differences in treatment influenced the observed associations between genotype and disease severity.

Both cellular and humoral immune responses are known to play a key role in antiviral defense and in determining

the clinical course of varicella [18-21]. Genetic variability in components of the interferon signaling pathway may modulate the effectiveness of the immune response, making such polymorphisms potential markers of susceptibility to complicated disease. Of particular interest is the comparison of our findings with studies focusing on immunological predictors of severe and complicated varicella in children, which emphasize the contribution of genetically determined immune response variability to disease heterogeneity. These observations further support the hypothesis that the IFNG rs2069709 polymorphism may contribute to the risk of complicated VZV infection [22].

The limitations of this study include the lack of functional data to assess the impact of the rs2069709 polymorphism on IFNG expression or activity, as well as the inability to generalize the findings to patients with mild, outpatient forms of varicella. Nevertheless, this study represents one of the first investigations of the IFNG rs2069709 polymorphism in a pediatric population and contributes to a better understanding of genetic factors underlying individual variability in the clinical course of varicella.

CONCLUSIONS

The IFNG rs2069709 AC genotype was the most frequently observed; however, its association with a complicated course of varicella demonstrated only a suggestive trend.

The IFNG rs2069709 C allele may represent a potential protective factor, as it was more frequently observed in children with an uncomplicated disease course.

Conversely, the IFNG rs2069709 A allele may be associated with an increased risk of a complicated course of varicella.

Sex and age did not show a statistically significant effect on the likelihood of complications.

FUTURE RESEARCH

To confirm these findings, studies with larger sample sizes and additional investigation of the functional role of IFNG rs2069709 are required.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

In silico study of thiazole-based amino coumarin derivatives with promising carbonic anhydrase inhibitory activity

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ABSTRACT

Aim: In this study, a series of novel aminocoumarin derivatives incorporating an amino-thiazole moiety were designed to evaluate their inhibitory activity against the carbonic anhydrase IX enzyme (PDB ID: 6fe0) and to explore their potential anti-neoplastic properties through molecular docking analysis.

Materials and Methods: Molecular docking was performed using the Molecular Operating Environment (MOE) software, version 2015.10. The Standard Score (S-score) and Root Mean Square Deviation (RMSD) parameters were used to evaluate the binding affinities of the ligands toward the target enzyme.

Results: The four synthesized ligands (IIIa, IIIb, IIIc, and IIId) demonstrated stronger binding affinities to carbonic anhydrase IX compared with the reference ligand, acetazolamide. Among them, compound IIId showed the most favorable binding profile, having an S-score of -10.6.

Conclusions: These findings suggest that the produced 6-aminocoumarin derivatives, specifically compound IIId, could serve as promising, effective inhibitors of carbonic anhydrase with potential anti-cancer activity targeting the carbonic anhydrase IX enzyme.

KEYWORDS: molecular docking study, 6-aminocoumarin derivatives, thiazol moiety, carbonic anhydrase, anti-cancer

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INTRODUCTION

Cancer is a complex and diverse disease characterized by a series of genetic and molecular abnormalities that result in uncontrolled cell growth and proliferation, thereby rapidly increasing tissue mass in affected regions of the body. Typically, cells are signaled to undergo apoptosis and be replaced by healthier cells [1-2]. On the other hand, cancer occurs when these damaged cells multiply and spread instead of dying, a process called apoptosis [3]. Without proper management, cancer has the potential to metastasize, or spread to other organs and tissues, interfering with the regular functioning of healthy cells and potentially leading to death. Both internal and external factors can contribute to cancer formation. These include cigarette smoke, chemicals, radiation, and infectious agent exposure; genetic mutations; hormonal imbalances; immune system disorders; and random genetic abnormalities. Many complex factors are involved in cancer development, and our understanding of these causes is limited. Certain dietary variables, certain diseases, insufficient physical activity, obesity, and environmental pollutants can increase the risk of cancer [4]. There are millions of new cases of cancer every year, making it a huge public health concern worldwide. There is a strong correlation between it and death rates worldwide [5]. Many physiological and molecular processes contribute to anticancer drug resistance, including conserved but enhanced drug efflux, genetic changes, epigenetic modifications, and others [6]. A lack of selectivity, drug resistance, excessive toxicity, and adverse effects limit the current chemotherapeutic medications. Consequently, the development of novel drug candidates is necessary to solve these issues [7]. Tumor microenvironmental factors, such as hypoxia and acidity, influence cancer biology. Acidosis and hypoxia typically make tumor treatment much more challenging by increasing cancer cell aggression, emigration, and proliferation; hence, treatment methods that block these processes are favored [8]. Consequently, numerous studies on cancer have focused on the carbonic anhydrase enzyme family, which controls the pH within cells [9]. Carbonic anhydrases (CAs), zinc-containing metalloenzymes, are crucial in the conversion of carbon dioxide (CO2) to bicarbonate (HCO3), as well as in gas exchange reactions, respiration, and carbon dioxide transport [10, 11]. In biology, CAs are classified into distinct families based on their genetic composition. The α-, β-, γ-, δ-, and ζ-CAs are included in this group [12-14]. In mammals, distinct alpha carbonic anhydrase (α-CA) isoforms are responsible for carrying out critical physiological functions [15]. Humans have 15 isoforms of carbonic anhydrase enzymes, which differ in their catalytic activity and localization. CA I, CA III, CA VII, and CA XIII are cytosolic carbonic anhydrases. CA VA and CA VB are mitochondrial carbonic anhydrases. CA VI is a secretory carbonic anhydrase that is present in saliva and colostrum. CA IV, CA IX, CA XII, CA XIV, and CA XV are membrane-bound carbonic anhydrases [16]. The transmembrane CAIX consists of a single transmembrane domain, an N-terminal

proteoglycan-like (PG) domain, an extracellular facing catalytic domain, and a short intracellular C-terminal tail [17]. CAIX, considered the most effective CA for the CO_2 hydrating process, significantly influences tumor acid-base balance. Its high catalytic activity may be attributed to its N-terminal proteoglycan-like (PG) domain, which is distinctive to CAIX within the CA family. The enzyme's ability to function efficiently at acidic pH levels is facilitated by the PG-like domain, which is situated adjacent to the catalytic domain. Additionally, it has been suggested that this PG domain is implicated in tumor invasion and cell adhesion [18]. Overexpression in a wide variety of tumor types is one of the distinctive features of CAIX. Additionally, this isoform exhibits negligible expression in healthy tissues. Consequently, its inhibition could lead to a considerably lower number of side effects than conventional anticancer agents, rendering it a promising target for therapeutic intervention [19], which has the potential to affect the radiosensitivity, migration, apoptosis, and clonogenic survival of cancer cells [20]. Coumarins (2-H-chromen-2-one) are a diverse family of heterocyclic compounds that contain a benzopyrone moiety. The fusion of a-pyrone ring with a benzene ring forms this moiety. Coumarins can be derived from both natural and synthetic sources [21]. Coumarin and its derivatives display a fascinating activity of pharmacological properties such as anticancer [22] antifungal, [23-24] antibacterial [25], and antimalarial [26-27]. The antineoplastic activity of coumarins is due to the ability of these substances to inhibit DNA repair enzymes, induce cell cycle inhibition, induce cell death, and reduce angiogenesis [28-29]. The biological activities of five-membered heterocyclic compounds, particularly those that contain multiple heteroatoms, are diverse in the field of medicinal chemistry [30]. Thiazole is a heterocyclic molecule with the chemical formula C_3H_3NS, comprising both nitrogen and sulfur. Thiazole rings are aromatic and planar [31-32]. Its high reactivity, caused by the acidic proton at C-2, makes it a desirable precursor for creating a huge number of novel chemicals. Thiazole derivatives' chemical, physical, and pharmacological features have long fascinated synthetic and biological scientists. Numerous novel compounds with distinct therapeutic potentials have been generated by modifying the thiazole ring at various places, including antioxidants, antineoplastics, antibiotics, diuretics, and anti-inflammatory medications [33]. Because of their diverse pharmacological potential, coumarin–thiazole analogues exhibit a wide range of biological activities, including anti-inflammatory [34], Anticancer [35], Antioxidant [36], and carbonic anhydrase inhibitors [37]. Consequently, the objective of this study is to introduce thiazole moieties into the design of hybrid pharmacophores that contain 6-aminocoumarin as a novel inhibitor for the carbonic anhydrase enzyme. Table 1 contains a sequence of target chemicals that are designated according to their compound codes (IIIa to IIId) and their corresponding substitute molecules (R groups). The chemical and physical properties of the compound can be substantially influenced by the specific chemical moiety represented by each R-group, which is connected to the basic structure of the compound. A molecular docking investigation was carried out to determine the compound's binding affinity to the carbonic anhydrase enzyme. The most powerful molecules with strong enzymatic actions will be chosen for further chemical production.

Table 1. Target compounds. The IIIa, IIIb, IIIc, and IIId are target compounds corresponding to Br, Cl, OH, and OCH3, respectively

Compound name	R group
IIIa	Br
IIIb	Cl
IIIc	OH
IIId	OCH3

Source: Compiled by the authors of this study

AIM

In this study, a series of novel aminocoumarin derivatives incorporating an amino-thiazole moiety were designed to evaluate their inhibitory activity against the carbonic anhydrase IX enzyme (PDB ID: 6fe0) and to explore their potential anti-neoplastic properties through molecular docking analysis.

MATERIALS AND METHODS

SYSTEM AND SOFTWARE CONFIGURATION

All computational studies were carried out on an MSI system equipped with an Intel Core i3-2330M processor (2.20 GHz) and 4.00 GB of RAM. ChemDraw Ultra 12.0 was used for drawing chemical structures, while MOE 2015 was employed for molecular modeling and related calculations.

Ligand and receptor preparation using the molecular docking technique

In this work, the molecular docking operation encompasses two steps.

STEP 1.

Ligand preparation: The ligands' molecular structures were created using ChemDraw Professional 12.0 which were subsequently converted into 3D conformations. Due to the well-documented mechanism of coumarin-based carbonic anhydrase inhibitors, which entails the hydrolysis of the parent coumarin scaffold within the enzyme's active site to generate the corresponding 2-hydroxycinnamic acid derivative [38-40]. In order to more accurately mimic the actual binding conformation, the hydrolyzed form, which is the genuine inhibitory species, was generated in silico and utilized for docking. Following this, the hydrolyzed ligand was protonated at physiological pH, and partial atomic charges were assigned. After that, energy minimization was implemented. In order to facilitate docking, the conformer that was minimized was finally stored in PDBQT format (Fig. 1).

STEP 2.

Protein organization: Carbonic anhydrase IX's crystalline framework (PDB code: 6fe0) is obtained from the PDB website

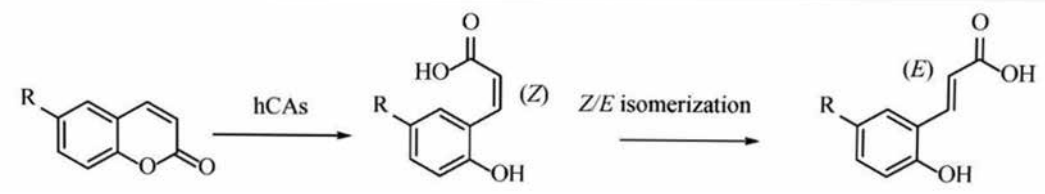

Fig. 1. Hydrolysis of coumarin scaffold and Z|E geometrical isomerization [40], R=NH2

Source: Own materials

and imported into the Molecular Operational Environment (MOE 2015) for protein production. The protein of interest was constructed in the following order: eliminating chains that were not involved in the protein activity, prioritizing the construction of the active site, and modifying the potential of the protein atoms, introducing hydrogen to obscure bonds, deleting small compounds, and removing water molecules. Once the MOE consumed the ligand that had been previously generated from the saved data, the docking procedure was initiated.

RESULTS AND DISCUSSION

Molecular docking is used to verify the principal interaction of specific ligands with the target. The MOE was implemented because of its thorough graphical description of ligand locations and receptor residue binding, which enables the visualization, definition, and estimation of protein-ligand interactions. In the molecular operating environment, the compounds that are presumed to be present establish a distinctive binding to the enzyme CA IX. Table 2 contains the RMSD and S. score values of the final compounds IIIa, IIIb, IIIc, and IIId, as well as the identification of substantial amino acids that are involved in these interactions. These characteristics correspond to the binding energy and structural alignment of the target proteins and the designed compounds. The S. score and the RMSD values, which demonstrate the mean interaction distance between the target point and the ligand atoms for the anticancer drug, are indicative of this approach. Results with a low RMSD and a high S score indicate that compounds are in their ideal posture. The S. score of the entire target products (IIIa-IIId) is higher than that of the control (acetazolamide). Therefore, they are more effective as carbonic anhydrase inhibitors than other agents. Moreover, their S. score is -9.98, -9.95, -10.12, and -10.6, and RMSD values are 1.8, 1.8, 1.5, and 1.7, respectively, as mentioned in Table 2.

Coumarins are not direct inhibitors of CA IX. Instead, they function as prodrugs. The esterase activity of the enzyme hydrolyzes the lactone ring of the coumarin upon its entry into the active site of CA IX. This hydrolysis transforms the coumarin into its active form, 2-hydroxycinnamic acid (or its derivatives). This transformation is essential because the open-ring form is the actual species that binds and inhibits the enzyme. Consequently, this mechanism is frequently referred to as „suicidal inhibition" due to the fact that the enzyme catalyzes the conversion of the prodrug into the active molecule that inhibits it. The hydrolyzed 6-aminocoumarin derivatives interact with numerous amino acid residues in the active site of CA IX. These residues are essential for the stabilization of the inhibitor through hydrogen bonding, van der Waals forces, and hydrophobic interactions on the basis of molecular docking and structural investigations. This binding will interrupt the proton shuttle mechanism, which is essential for the catalytic cycle, and prevent substrates (CO_2 and H_2O) from binding with the catalytic site. Coumarins are intriguing candidates for targeted cancer therapy due to their selectivity for CA IX over other isoforms. Further, it exhibits minimal inhibition to the isoforms (CA I and II), thereby minimizing the risk of adverse effects, as shown in Table 2. Acetazolamide is a carbonic anhydrase inhibitor that is both well-known and extensively investigated, making it a priceless reference. The MOE visualization of acetazolamide with hCA IX (PDB code: 6fe0) is depicted in Figure 2, which shows the three-dimensional and two-dimensional structures of the ligand-protein interaction, respectively. Acetazolamide specifically binds to the amino acid threonine 199 in the active site of hCA IX and the zinc ion, as illustrated in Figure 1. The docking visualization of compounds (IIIa-IIId) with hCA IX is depicted in Figures 3-6 using the PDB code 6fe0.

Table 2. Molecular docking of the test ligands (IIIa-IIId) in comparison to acetazolamide displays the RMDS, S-score, and primary amino acids that are included in the ultimate ligand interactions

Compound name	R Group	S. Score	RMDS	No. of binding interactions	Binding amino acids
Acetazolamide	***	-5.18	1	2	Zn301, ThrA199
IIIa	Br	-9.98	1.8	5	Zn301, Thr A199, His A96, His A119, Asn A62
IIIb	Cl	-9.95	1.8	5	Zn301, Thr A199, His A96, His A119, Asn A62
IIIc	OH	-10.12	1.5	5	Zn301, Thr A199, His A96, His A119, Asn A62
IIId	OCH3	-10.6	1.7	6	Zn301, Thr A199(2), His A96, His A119, His A94

Source: Compiled by the authors of this study

Each figure illustrates the 3D and 2D structures of the ligand-protein interactions, respectively. Zinc and the essential amino acid Threonine199 are identified by each of the tested compounds through a hydrogen bond. The compounds IIIa, IIIb, and IIIc exhibit additional binding interactions with key amino acids, notably His96, His94, and His119; furthermore, binding of the Thiazol moiety with the amino acid Asn64, within the hCA IX. The extra binding interactions refers to enhancing the significance of a thiazole ring, which provides greater flexibility to the structure and enhances the probability of interaction with the receptor. Figures 3-5 show these interactions in both 2D and 3D forms, which illustrate the MOE visualization of compounds IIIa, IIIb, and IIIc with hCA IX. With the highest

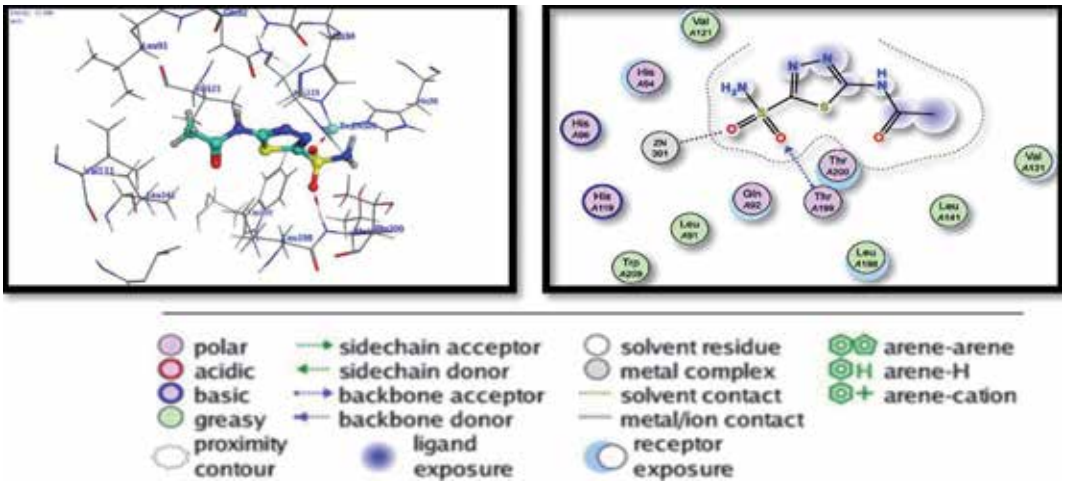

Fig. 2. Docking simulation of the reference ligand (acetazolamide) with hCA IX (PDB code: 6fe0) (above) is the 3D structure, (below) is the 2D structure

Source: Own materials

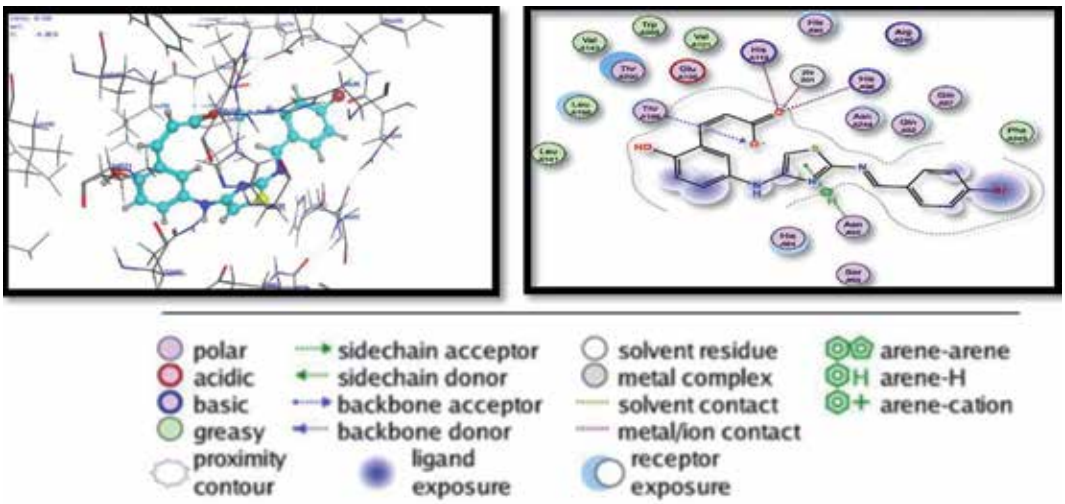

Fig. 3. Docking simulation of compound IIIa with hCA IX (PDB code: 6fe0) (above) is the 3D structure, (below) is the 2D structure

Source: Own materials

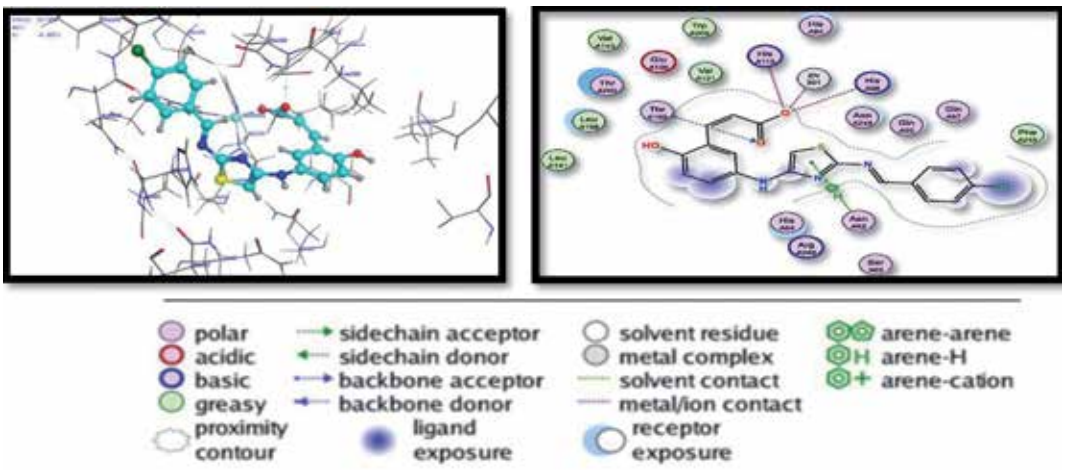

Fig. 4. Docking simulation of compound IIIb with hCA IX (PDB code: 6fe0) (above) is the 3D structure, (below) is the 2D structure

Source: Own materials

S. score of -10.6, compound IIId exhibits a considerable binding affinity. This high S. score could be the consequence of more Asn64 hydrogen bonding, which is crucial for catalytic activity in CAIX. The fact that the interaction levels of benzaldehyde derivatives differ depending on which groups are substituted at the fourth position of the benzene ring emphasizes their significance. The docking visualization of compound IIId is represented in Figure 6.

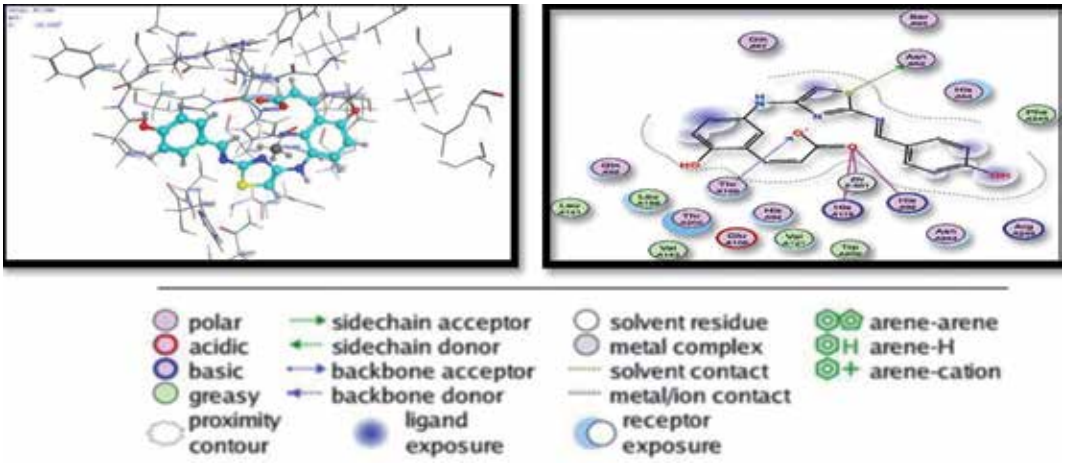

Fig. 5. Docking simulation of compound IIIc with hCA IX (PDB code: 6fe0) (above) is the 3D structure, (below) is the 2D structure

Source: Own materials

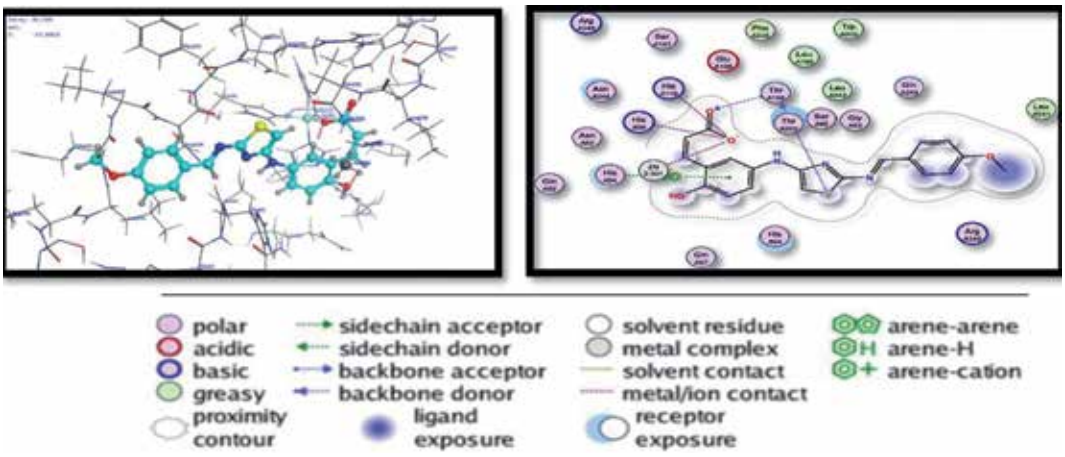

Fig. 6. Docking simulation of compound IIId with hCA IX (PDB code: 6fe0) (above) is the 3D structure, (below) is the 2D structure

Source: Own materials

CONCLUSIONS

The four aminocoumarin derivatives with thiazole moiety (IIIa-IIId) have been shown to have anti-cancer properties and a viable carbonic anhydrase inhibition effect in the ongoing investigation. Most compounds demonstrated a greater affinity for the target protein in comparison to acetazolamide. The most promising compound is IIId, which has an S. score of -10.6 and an OCH3 substituent on the benzene ring.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Efficiency of mucogingival osteoplasty in stage II-III generalized periodontitis

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ABSTRACT

Aim: To study the efficacy of mucogingival osteoplasty techniques combined with pharmacological therapy in the treatment of stage II–III generalized periodontitis

Materials and Methods: Seventy individuals (aged 35-60 years, both sexes) were examined, including 10 systemically and dentally healthy participants and 60 patients with stage II-III chronic generalized periodontitis. The 60 patients were divided equally into four treatment groups (n=15 per group): Control group: mucogingival osteoplasty and standard therapy; Group 2: mucogingival osteoplasty with multiple periosteal penetrations and standard therapy; Group 3: mucogingival osteoplasty with multiple periosteal penetrations, standard therapy, and local treatment with Miramistin oral rinses; Group 4: mucogingival osteoplasty with multiple periosteal penetrations, standard therapy, local treatment with Miramistin oral rinses, and oral administration of arginine glutamate. Dental status and salivary and serum biochemical markers were assessed preoperatively and 1, 6, and 12 months postoperatively. Tissue samples collected during surgery were also subjected to ultrastructural analysis.

Results: Comprehensive patient assessment before surgery and during recovery demonstrated the efficacy of mucogingival osteoplasty, further enhanced by additional periosteal penetrations and a pharmacological regimen supported by a mechanistic rationale. The proposed salivary biomarkers may serve as diagnostic tools for assessing periodontal status, with serum transferase levels associated with periodontitis providing complementary information.

Conclusions: All treatment approaches for patients with stage II–III generalized periodontitis effectively improved periodontal indices, the salivary biochemical profile, and transaminase levels; however, the greatest effect was observed in Group 4, likely due to the use of agents with antiseptic, antioxidant, antihypoxic, and membrane-stabilizing properties.

KEYWORDS: generalized periodontitis, mucogingival osteoplasty, periosteal penetrations, biochemical markers, pharmacological treatment.

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INTRODUCTION

Environmental factors and lifestyle influence the course of periodontal inflammation by altering the immune response to infection and initiating metabolic changes that affect the periodontal apparatus [1, 2]. Periodontitis results from the activity of periodontopathogenic bacteria, leading to tissue degradation and alveolar bone resorption [3]. Candida species form persistent biofilms on oral cavity surfaces and, as part of these complex and heterogeneous infections, contribute to chronic inflammatory processes. Moreover, their strains exhibit high resistance to conventional antifungal agents [4].

The modern concept of periodontitis treatment involves a combination of non-surgical and surgical approaches, with surgical therapy including both resective and regenerative procedures [5, 6]. Inadequate dental care leads to impaired organ and system function, as well as aesthetic defects, resulting in social and psychological consequences and an increasing number of forensic medical examinations [7]. Given its associations with cardiovascular diseases and metabolic disorders, periodontitis should be managed as part of the patient's overall medical care rather than as a purely local dental condition [8-10]. Arginine glutamate, due to its antioxidant, endothelium-protective, and membrane-stabilizing properties, acts as a non-specific metabolic regulator and exerts a beneficial effect on periodontal tissues [11, 12]. Miramistin is a potent antiseptic with low toxicity and a wide spectrum of potential clinical applications [13]. Analysis of salivary and serum biomarkers enables non-invasive, systemic assessment of inflammation and treatment efficacy in periodontitis [14]. Superoxide dismutase (SOD) is associated with oxidative stress and is implicated in the pathogenesis of periodontitis [15]. Inflammation in periodontitis may lead to elevated ceruloplasmin levels [16]. The established causal relationship between periodontitis and systemic inflammation supports the routine measurement of C-reactive protein (CRP) [17]. Periodontitis has also been associated with increased levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [18].

AIM

To study the efficacy of mucogingival osteoplasty techniques combined with pharmacological therapy in the treatment of stage II-III generalized periodontitis.

MATERIALS AND METHODS

Seventy individuals (aged 35-60 years, both sexes) underwent a comprehensive examination, comprising 10 systemically and dentally healthy participants and 60 patients with stage II-III chronic generalized periodontitis. The 60 patients were divided equally into four treatment groups (n=15 per group): Control group: mucogingival osteoplasty combined with standard therapy (lincomycin 500,000 IU intramuscularly twice daily for 5 days, loratadine 1 tablet orally twice daily for 5 days, and four oral rinses with 0.05% chlorhexidine); Group 2: mucogingival osteoplasty with multiple periosteal penetrations in combination with standard therapy; Group 3: mucogingival osteoplasty with multiple periosteal penetrations in combination with standard therapy and local treatment with 0.01% Miramistin oral rinses four times daily for 15 days; Group 4: mucogingival osteoplasty with multiple periosteal penetrations combined with standard therapy, local treatment with 0.01% Miramistin oral rinses, and oral administration of arginine glutamate (0.75 g) three times daily for 15 days. All patients underwent mucogingival osteoplasty under combined anesthesia, including conduction anesthesia (bilateral torus, bilateral tuberal, bilateral palatal, and incisive nerve blocks) and infiltration anesthesia with Ultracain D-S Forte (1.6 mL per injection; 5 cartridges administered sequentially). The multiple periosteal penetration technique, applied to patients in Groups 2, 3, and 4, was developed to promote bone regeneration. The procedure involved creating depressions in the periosteum to a depth of half its thickness (0.6-0.75 mm) using a #2 round bur, with 2-3 mm spacing between perforations. This procedure was performed after elevating a mucoperiosteal flap using a physiodispenser. Simultaneously, the tissues were irrigated with physiological saline and suctioned to maintain a clear surgical field [19]. During surgery, altered tissue was excised by necrotomy, and the excised granulation tissue was processed into blocks for ultrastructural analysis. Subgingival calcified deposits were also removed. Follow-up examinations were conducted 1, 6, and 12 months postoperatively. Salivary (SOD, ceruloplasmin, and CRP activity) and serum (ALT and AST activity) biochemical parameters were assessed preoperatively and 1, 6, and 12 months postoperatively.

Data analysis was performed with STATISTIC (StatSoF, Inc., 2011) and Microsoft Excel 2010, using parametric methods for descriptive statistics. Differences were considered statistically significant at a p-value ≤ 0.05.

RESULTS

Clinical examination revealed that the inflammatory component manifested as catarrhal gingivitis in 58 patients (96.7%) and as edematous hypertrophic gingivitis in 2 patients (3.3%). The mean periodontal pocket depth (PPD) was 6.5 mm. Other clinical indices were as follows: the oral hygiene index (OHI) 2.49±0.10, papillary-marginal-alveolar (PMA) index 66.35±0.72%, iodine number 7.45±0.08, bleeding on probing (POB) index 1.98±0.08, and periodontal index 6.32±0.14. These findings indicated poor oral hygiene and severe inflammatory and degenerative changes in the periodontium.

Biochemical analysis revealed that patients with stage II-III generalized periodontitis exhibited a significant increase in salivary ceruloplasmin activity (1.61-fold) and a decrease in SOD activity (1.23-fold) ($p<0.05$), indicating enhanced protein peroxidation and reduced antioxidant defense (Fig. 1, 2). Concurrently, CRP levels increased 26.8-fold ($p<0.05$), indicating a pronounced inflammatory response (Fig. 3). Additionally, serum ALT and AST activities were elevated 1.85- and 1.84-fold, respectively, compared to healthy controls ($p<0.05$), indicating hepatic stress (Fig. 4, 5).

Ultrastructural examination of the periodontium in patients with stage II-III generalized periodontitis revealed

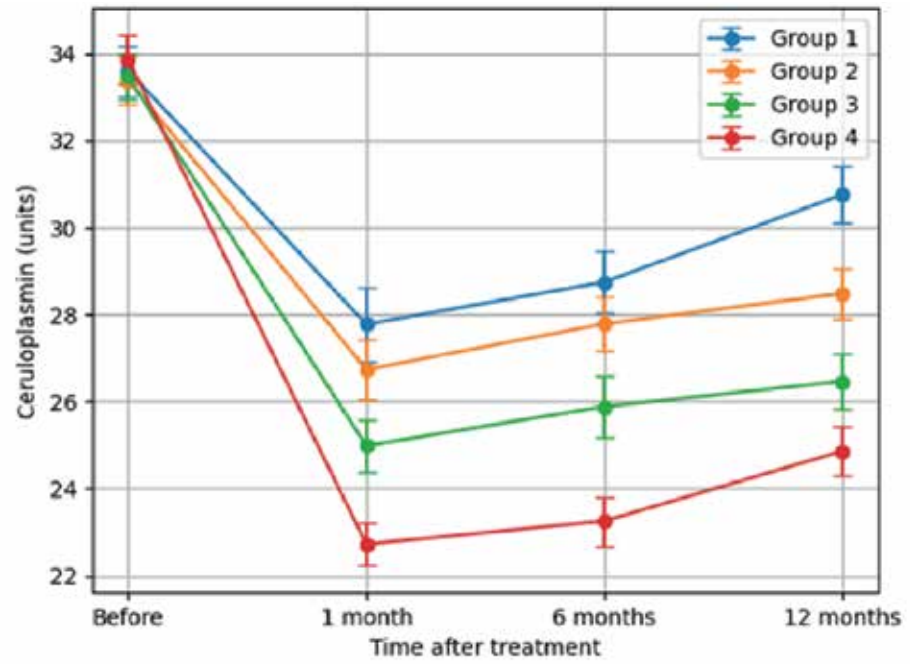

Fig. 1. Changes in salivary ceruloplasmin levels in patients with stage II-III generalized periodontitis before treatment and 1, 6, and 12 months after therapy. Data are presented as mean±SEM. The dashed horizontal line represents the mean value in healthy controls. $p<0.05$ *versus* healthy controls.

Source: Own materials

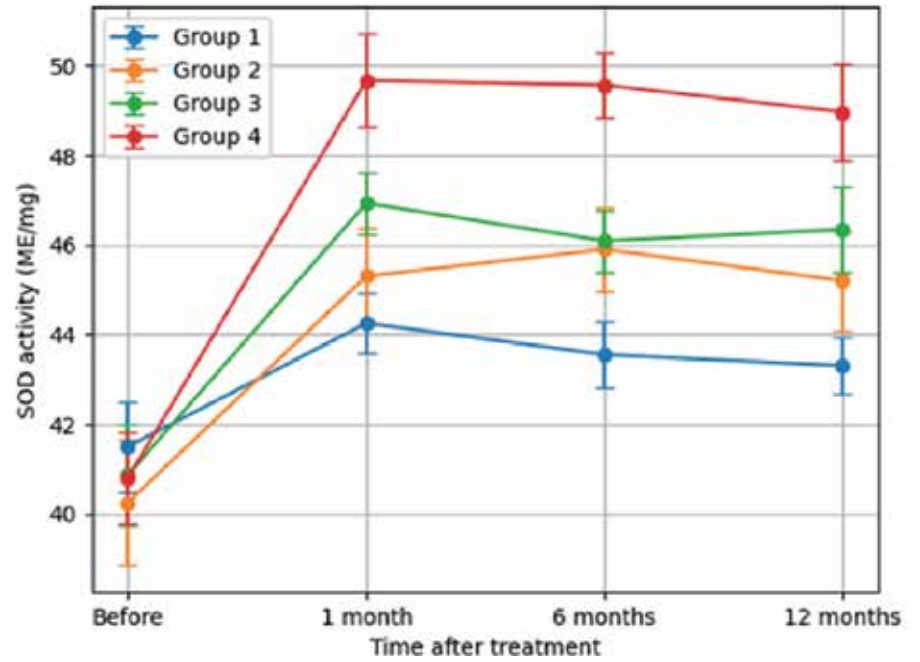

Fig. 2. Changes in salivary superoxide dismutase activity in patients with stage II-III generalized periodontitis before treatment and 1, 6, and 12 months after therapy. Data are presented as mean±SEM. The dashed horizontal line represents the mean value in healthy controls. $p<0.05$ *versus* healthy control.

Source: Own materials

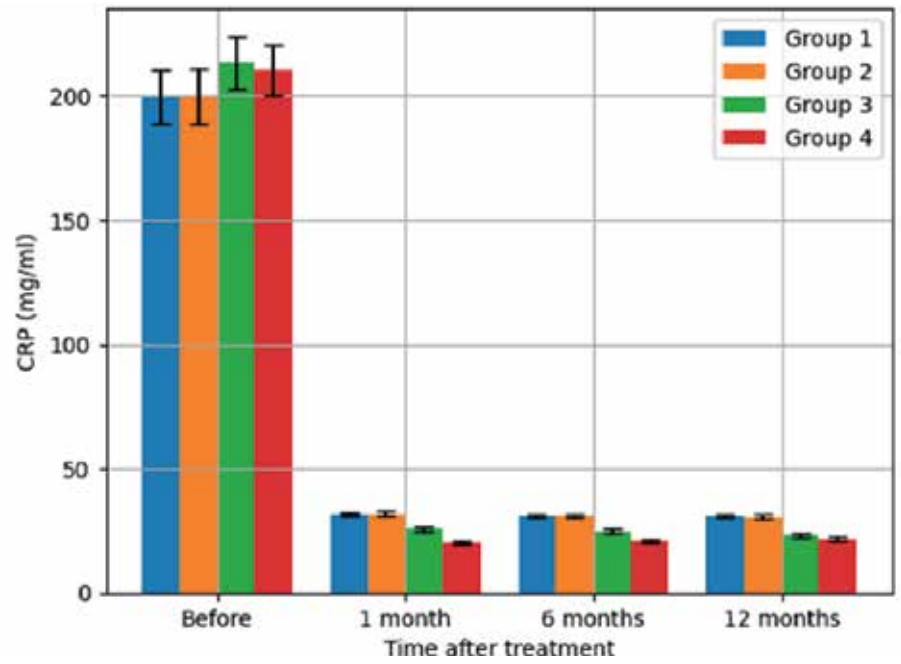

Fig. 3. Changes in salivary C-reactive protein levels in patients with stage II-III generalized periodontitis before treatment and 1, 6, and 12 months after therapy. Data are presented as mean±SEM. The dashed horizontal line represents the mean value in healthy controls. $p<0.05$ *versus* healthy control.

Source: Own materials

deformation of the arteriolar lumen, with protrusion of endothelial cell nuclei and blebbing of the luminal plasma membrane. The basement membrane appeared electron-lucent, fragmented, and tortuous. Smooth muscle cells were surrounded by edematous connective tissue components of the vascular wall, while the adventitial layer was poorly defined and indistinguishable from the surrounding electron-lucent extracellular matrix, which contained swollen, disorganized collagen fibers (Fig. 6A). Edema of the connective tissue components of the periodontium predominated in all fields of view. Fibroblasts exhibited numerous elongated processes, with sparse secretory granules of moderate electron density in the cytoplasm. The amorphous extracellular matrix (ground substance) appeared electron-lucent (Fig. 6B, 6C).

One month after mucogingival osteoplasty with periosteal penetrations combined with pharmacological therapy, the OHI, PMA index, iodine number, POB index, and periodontal index were reduced significantly 2.14-, 4.10-, 5.59-, 2.54-, and 2.23-fold, respectively ($p_1<0.05$), and exhibited minimal further changes 6 and 12 months postoperatively ($p_{2/3}<0.05$). This intervention accelerated postoperative wound healing,

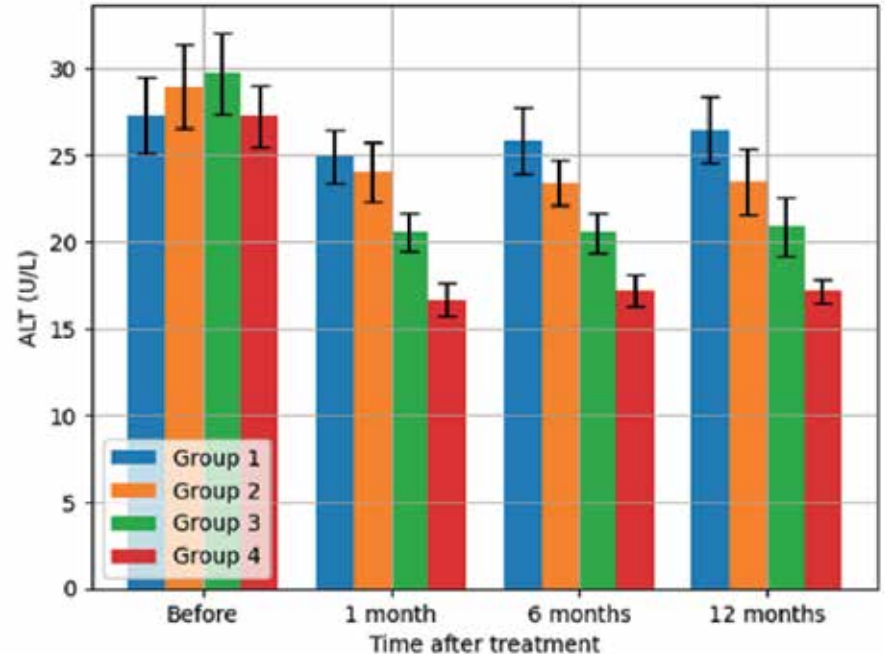

Fig. 4. Changes in serum alanine aminotransferase activity in patients with stage II-III generalized periodontitis before treatment and 1, 6, and 12 months after therapy. Data are presented as mean±SEM. The dashed horizontal line represents the mean value in healthy controls. $p<0.05$ *versus* healthy control.

Source: Own materials

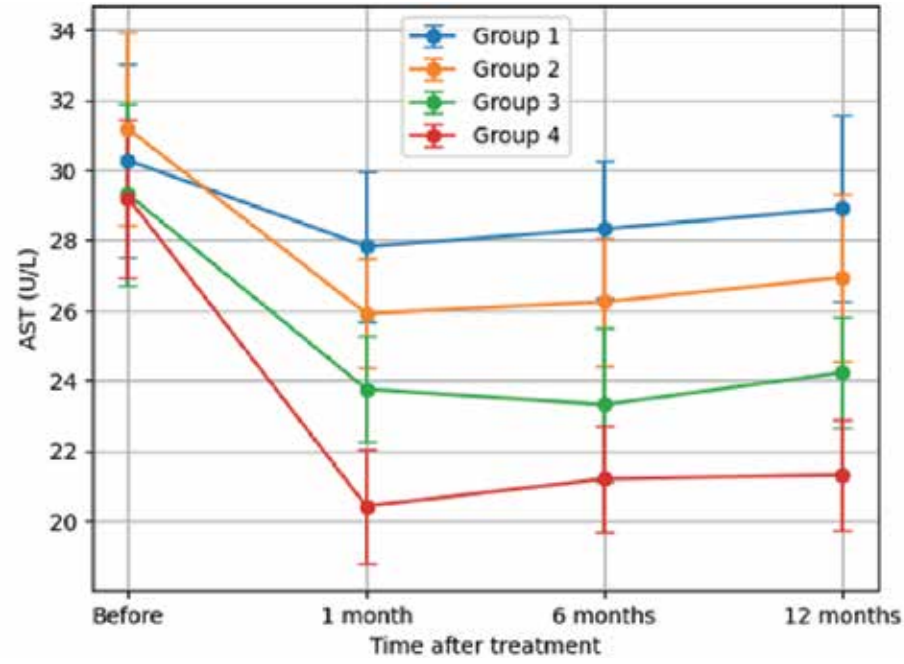

Fig. 5. Changes in serum aspartate aminotransferase activity in patients with stage II-III generalized periodontitis before treatment and 1, 6, and 12 months after therapy. Data are presented as mean±SEM. The dashed horizontal line represents the mean value in healthy controls. $p<0.05$ *versus* healthy control.

Source: Own materials

with primary closure achieved in 100% of patients by day 7, and promoted periodontal regenerative processes.

Owing to the developed mucogingival osteoplasty technique enhanced with periosteal penetrations and postoperative pharmacological management, biochemical parameters in patients with stage II-III generalized periodontitis were normalized, with ceruloplasmin activity reducing 1.49-fold and SOD activity increasing 1.21-fold ($p_1<0.05$), indicating a significant enhancement of antioxidant defense (Fig. 1, 2). A 10.46-fold reduction in CRP levels indicated a substantial decrease in inflammation (Fig. 3). Significant improvements in hepatic function were indicated by reductions in ALT and AST activities by 72.73% and 70.79%, respectively ($p_1<0.05$) (Fig. 4, 5). Values measured 1 month postoperatively showed minor changes during long-term follow-up but remained statistically significant ($p_{2/3}<0.05$).

Comprehensive treatment was significantly more effective in Group 4 patients with stage II-III generalized periodontitis than in Group 1 (controls). Specifically, ceruloplasmin activity was reduced 1.22-, 1.24-, and 1.24-fold, and ALT activity was reduced 1.49-, 1.50-, and 1.54-fold, respectively. No

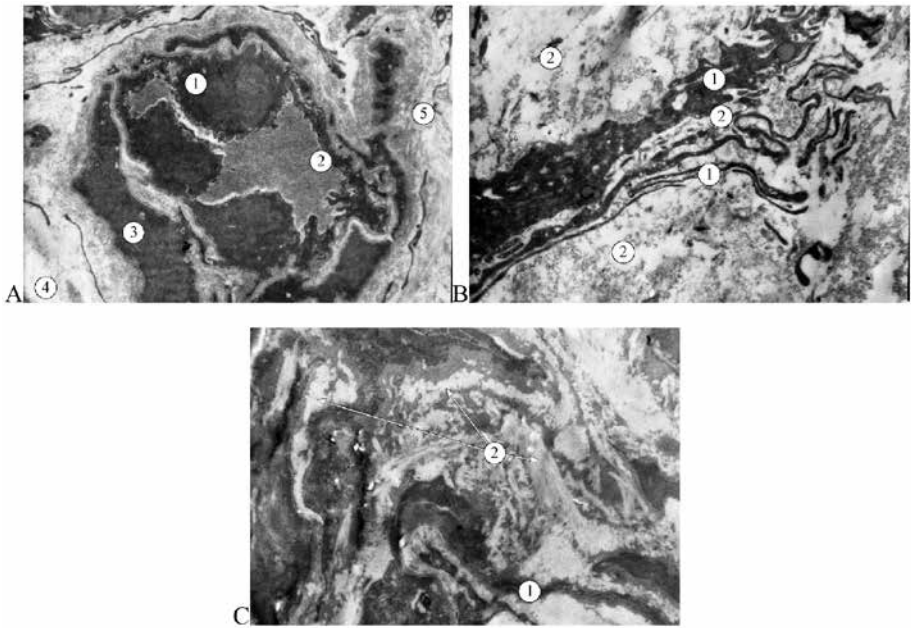

Fig. 6. Periodontal ultrastructure in patients with stage II-III generalized periodontitis.
A. 1 – endothelial cell nucleus; 2 – lumen; 3 – smooth muscle cell; 4 – extracellular matrix; 5 – disorganized collagen fibers. B. 1 – numerous fibroblast processes; 2 – amorphous extracellular matrix. C. 1 – fibroblast cytoplasm fragment; 2 – edematous collagen fibers. Electron micrographs. Magnification: A ×4,800; B, C ×8,000.

Source: Own materials

comparable results were obtained in Group 1. Therefore, the combination of periosteal penetrations, local Miramistin application, and systemic arginine glutamate administration demonstrated a clear advantage over conventional surgery combined with standard postoperative therapy.

DISCUSSION

Morphological studies revealed that in patients with stage II-III generalized periodontitis, the periodontal tissues exhibit degenerative changes in the blood vessel walls and pronounced perivascular edema, which predominate across all fields of view of the connective tissue components of the periodontium. The rising incidence of vascular catastrophes, such as myocardial infarction and stroke, as consequences of COVID-19 and wartime stress [20, 21], underscores the importance of considering the vascular etiology of periodontitis.

In this study, salivary CRP levels were 205.95±10.57 mg/mL, a 26.8-fold increase over healthy controls, indicating a pronounced inflammatory response in the periodontal tissues. Following therapy, salivary CRP levels were reduced markedly 6.27-, 6.30-, 8.29-, and 10.46-fold in Groups 1, 2, 3, and 4, respectively ($p_1<0.05$), with these reductions largely maintained during long-term follow-up. Thus, the modified mucogingival osteoplasty procedure combined with standard therapy enhanced the reduction in salivary CRP levels. A pronounced decrease in CRP levels has also been observed 1 year after minimally invasive non-surgical therapy in patients with periodontitis [22]. Overall, periodontitis treatment consistently reduces serum CRP levels during the first six months post-therapy [17].

In our study, the mean ALT and AST activities were 28.31±2.41 and 30.0±2.45 U/L, respectively, being significantly higher than in healthy controls, though still within reference ranges, with levels 1.85- and 1.84-fold higher, indicating increased functional stress on the liver in generalized periodontitis, even in systemically healthy individuals ($p<0.05$). Elevated AST levels are associated with chronic periodontitis. Salivary AST levels are higher in patients with periodontal tissue destruction, deeper periodontal pockets, and bleeding on probing [23]. Owing to comprehensive treatment, serum ALT activity was substantially reduced in Group 3 by 44.43%, 44.79%, and 42.09%, and in Group 4 by 63.49%, 58.73%, and 58.92% 1, 6, and 12 months post-therapy, respectively ($p_1<0.05$), with Group 4 reaching values comparable to healthy controls ($p >0.05$). AST activity, however, was significantly reduced at all time points only in Group 4 ($p_1<0.05$). These findings indicate that arginine glutamate significantly modulates transaminase activity in patients receiving comprehensive therapy.

Under treatment, SOD activity in Group 1 patients increased significantly only 1 month post-therapy. In Groups 2 and 3, the achieved increase was maintained for 6 and 12 months post-therapy. In Group 4, SOD activity was initially the highest (21.74% compared to 6.67%, 12.54%, and 14.82% in Groups 1, 2, and 3, respectively) and remained largely stable during long-term follow-up, showing a statistically significant difference from Groups 1-3 ($p_{4,5,6}<0.05$). An elevated salivary SOD level in patients with periodontal disease may reflect a protective tissue response against oxidative stress induced by periodontal damage [24]. Conversely, reduced salivary SOD activity may result from the chronic course of periodontitis and exhaustion of the body's defense mechanisms against reactive oxygen species [25]. The lack of a significant difference between Groups 3 and 4 at all follow-up points ($p_6>0.05$) indicates that antioxidant defense enhancement in patients with stage II-III generalized periodontitis can be achieved solely through adequate local therapy. In the context of rising antimicrobial resistance, the substantial potential of Miramistin underscores the need for further evaluation of its use [13].

All treatment approaches led to a significant reduction in salivary ceruloplasmin activity (p_1<0.05). Values observed in Groups 1 and 2 were similar 1 and 6 months post-therapy (p_4 >0.05) but differed significantly after 12 months (p_4<0.05), supporting the efficacy of the surgical approach we developed. In Groups 3 and 4, ceruloplasmin activity was lower at all follow-up points compared to Groups 1 and 2 ($p_{4,5}$<0.05). However, in Group 4, it was further reduced compared to Group 3 one and six months post-therapy by 9.94% and 11.31%, respectively (p_6<0.05), demonstrating the additional antioxidant effect of arginine glutamate. Inflammation in periodontitis can elevate ceruloplasmin levels, whereas periodontal therapy effectively reduces them [16].

L-arginine (used to modify the mesoporous bioactive glass) effectively inhibits inflammation-induced tissue destruction and stimulates osteogenesis, making it a promising multifunctional material for periodontal tissue regeneration [11]. L-arginine modulates $CD68^+$ and $CD163^+$ macrophage populations in the gingiva [12]. Its demonstrated efficacy and reported positive effects on metabolic processes underscore the importance of assessing basal metabolism in patients, which is determined by thyroid status. Iodine deficiency, accompanied by reduced thyroid hormone levels, is associated with edematous and degenerative changes in various organs and systems [26, 27].

In this study, comprehensive therapy resulted in positive changes in the periodontal tissues of all patients, with a more favorable postoperative course observed in Groups 3 and 4, as tissue edema and infiltration on day 3 were observed in only 83.33% and 70.0% of patients, respectively, compared to 96.67% and 90.0% in Groups 1 and 2. By day 7, primary wound closure was achieved in 96.67% and 100% of Group 3 and 4 patients, respectively, compared to 90.0% and 93.33% in Groups 1 and 2, suggesting better exogenous and endogenous support in Groups 3 and 4. One month after therapy, Group 3 and 4 patients showed no signs of inflammation, periodontal pockets, or tooth mobility in the treated area. In contrast, these signs were present in 24.14% and 21.43% of Group 1 and 2 patients, respectively. Six months after therapy, some negative symptoms indicative of periodontal inflammation recurrence were observed in 36.61% of patients in Group 1, 25.93% in Group 2, 18.52% in Group 3, and 7.14% in Group 4. One year after therapy, recurrence of the pathological process in the periodontium, characterized by grade I–II tooth mobility, 5–6 mm periodontal pockets with granulation tissue overgrowth, and the presence of serous/purulent exudate, indicating surgical treatment failure, was observed in 15.38% of patients in Group 1, 7.40% in Group 2, and 3.70% in Group 3. No comparable complications occurred in Group 4: 92.07% of patients exhibited satisfactory periodontal status, reflecting a "positive outcome plateau" attributable to high-quality surgical and prosthetic treatment combined with the effective pharmacological regimen we proposed; in the remaining patients, gingival inflammation recurred, requiring repeated local periodontal therapy.

Changes were also observed in the periodontal parameters. Specifically, in Groups 1, 2, 3, and 4, the OHI was reduced 2.11-, 2.14-, 2.14-, and 2.14-fold, respectively, 1 month post-therapy; 1.94-, 2.09-, 2.11-, and 2.12-fold, respectively, 6 months post-therapy; and 1.75-, 1.75-, 1.99-, and 2.05-fold, respectively, 12 months post-therapy (p<0.05). The PMA index was reduced 3.45-, 3.30-, 3.65-, and 4.10-fold, respectively, 1 month post-therapy; 3.19-, 3.06-, 3.24-, and 3.60-fold, respectively, 6 months post-therapy; and 3.12-, 2.96-, 3.31-, and 4.37-fold, respectively, 12 months post-therapy (p<0.05). Similar patterns were observed for the iodine number, BOP index, and periodontal index.

CONCLUSIONS

Surgical treatment of patients with stage II–III generalized periodontitis using modified mucogingival osteoplasty, enhanced with periosteal penetrations as proposed in this study, was successful in all patients. Adjunctive use of Miramistin during the surgical stage further accelerated postoperative wound healing. Owing to the action of the nonspecific metabolic regulator arginine glutamate, compensatory and adaptive responses were enhanced, as reflected by the normalization of biochemical parameters in saliva and blood serum. Normalization of systemic and periodontal homeostasis accelerates postoperative rehabilitation and promotes more rapid regression of clinical manifestations, thereby ensuring long-term remission.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Protective role of lycopene against cyclophosphamide-induced endocrine dysfunction in rats

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ABSTRACT

Aim: Infertility is the inability to conceive after one year of unprotected intercourse, influenced by various physiological and environmental factors, including chemical exposure. Cyclophosphamide, used for treating tumors and autoimmune diseases, is linked to reproductive toxicity and endocrine disruption via oxidative stress and cell damage in reproductive tissues. This study assesses the protective effect of lycopene, an antioxidant found in red fruits and vegetables, against cyclophosphamide-induced hormonal changes in rats.

Materials and Methods: Material and methods: Forty adult Wistar rats were assigned to four groups: a control group, a group treated with cyclophosphamide, a group treated with cyclophosphamide and lycopene, and a group treated with lycopene alone. Cyclophosphamide was administered to induce reproductive toxicity, while lycopene was administered to assess its protective role. After 29 days of treatment, samples were collected, and serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone (Pg) and estrogen (E2), were measured and statistically analysed.

Results: Administering cyclophosphamide resulted in marked changes in reproductive hormone levels compared to the control group, indicating a disruption of normal hormonal regulation. Lycopene treatment significantly improved hormonal status, maintaining follicle-stimulating hormone, luteinizing hormone, progesterone, and estrogen levels closer to physiological values compared to the cyclophosphamide-treated group.

Conclusions: The findings of this study demonstrate that lycopene significantly mitigates cyclophosphamide-induced hormonal disruption in female Wistar rats, indicating its potential as a potent protective agent against chemotherapy-associated ovarian endocrine dysfunction. Beyond its role as an antioxidant, the preservation of follicle-stimulating hormone, luteinizing hormone, progesterone, and estrogen levels suggests that lycopene may help maintain the integrity of the hypothalamic-pituitary-gonadal axis during toxic insults. These results highlight the potential of lycopene as a low-cost, non-invasive, and natural adjunctive strategy to safeguard the ovarian reserve and reduce the long-term burden of treatment-induced infertility in patients undergoing gonadotoxic therapy. This study provides a foundational basis for future clinical investigations into antioxidant-based interventions as a standard component of fertility preservation protocols in oncology.

KEYWORDS: antioxidants; carotenoids; ovarian toxicity; reproductive hormones

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INTRODUCTION

In recent years, there has been growing scientific interest in the use of natural supplements for the treatment of various diseases and disorders affecting multiple organ systems [1]. Studies have shown that antioxidants and plant-derived compounds possess significant protective and therapeutic properties against the harmful effects of toxic substances [2, 3]. For instance, curcumin has been used to alleviate testicular toxicity, while the beneficial effects of onion consumption on different organs have also been reported [4]. Cyclophosphamide is widely employed in the treatment of several conditions, including multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, connective tissue diseases associated with interstitial lung disease, neuroblastoma, bone marrow transplantation, and various cancers [5], underscoring its vital role in anticancer therapy. It is classified as an immunosuppressant and plays a significant role in managing autoimmune and inflammatory conditions [6]. Despite its broad clinical utility, cyclophosphamide is associated with substantial adverse effects, including toxicity to blood and organs [7], increased vulnerability to opportunistic infections, and potential long-term damage to tissues beyond soft tissues [8]. Prior research has demonstrated that cyclophosphamide impairs ovarian folliculogenesis and disrupts sex hormone regulation [9,10]. Oxidative stress is widely recognised as a central mechanism in cyclophosphamide-induced reproductive toxicity. Previous studies have reported increased lipid peroxidation markers such as malondialdehyde (MDA) and reduced antioxidant defenses including glutathione (GSH) and superoxide dismutase (SOD) following cyclophosphamide exposure [11]. Specifically, it inhibits granulosa cell proliferation and hormone secretion within the ovaries, leading to ovarian failure [12]. It has also been shown to reduce the number of

primordial, pre-antral, and antral follicles, as well as the levels of key reproductive hormones such as progesterone (Pg) and estrogen (E2) [13]. The primary reproductive risk for women undergoing cyclophosphamide therapy includes temporary or permanent amenorrhea, reduced fertility, or infertility [14]. Lycopene, a lipophilic, unsaturated red carotenoid pigment found in red-colored fruits and vegetables – such as tomatoes, papayas, grapefruits, and various berries – has shown promising antioxidant activity [15]. Recent studies have confirmed its protective role against multiple toxic insults, primarily through the reduction of reactive oxygen species (ROS) [16]. Lycopene has been shown to decrease lipid peroxidation and enhance endogenous antioxidant systems in several experimental models [17]. Previous research has also demonstrated lycopene's protective effects against testicular toxicity [18] and its potential in restoring ovarian tissue from chemically induced damage [19].

AIM

The present study aimed to evaluate the potential protective role of lycopene against cyclophosphamide-induced ovarian impairment in female Wistar rats. Specifically, the study investigated whether lycopene administration could mitigate alterations in key reproductive hormones, including follicle-stimulating hormone, luteinizing hormone, progesterone and estrogen, following cyclophosphamide exposure. Additionally, the study sought to determine whether the antioxidant properties of lycopene could contribute to preserving normal hormonal regulation and ovarian function.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

The current study utilised forty adults female Wistar rats. Their body weight varied from 200 to 250 grams, and they were maintained in hygienic cages under consistent environmental conditions at a daily temperature of 24±2°C, with unrestricted access to food and tap water in the animal facility.

THE DESIGN OF THE EXPERIMENT

The experimental design randomly allocated the rats into four equal cohorts: the first cohort served as the control group, receiving solely a placebo; the second cohort constituted the induction group, receiving a single intraperitoneal injection of cyclophosphamide (200 mg/kg) on the initial day of the experiment [21]; the third cohort represented the treatment group, receiving lycopene suspended in maize oil, administered via gavage at a dosage of 4 mg/kg for 28 days [20]. The fourth group, designated as the lycopene group, received lycopene suspended in maize oil via gavage at a dosage of 4 mg/kg for a duration of 28 days exclusively. Following the 29-day experimental period, we euthanised all animals in each group and collected the samples.

ACQUISITION OF ANIMAL SAMPLES

On the concluding day of the experiments, we promptly extracted blood from the heart of the sacrificed animal via puncture at specified intervals, utilising intramuscular injections of Ketamine at 90 mg/kg and Xylazine at 40 mg/kg body weight.

HORMONAL ANALYSIS

Serum samples were obtained by centrifugation of blood at 3500 rpm for 10 minutes and stored at -20 °C until analysis. Serum levels of follicle-stimulating hormone, luteinizing hormone, progesterone, and estrogen were determined using FineTest® company enzyme-linked immunosorbent assay (ELISA) kits (FineTest® Rat FSH, FineTest® Rat LH, FineTest® Rat E2 & FineTest® Rat Pg of Wuhan Fine Biotech Co., Ltd., Wuhan, Hubei, China) according to the manufacturer's instructions. The assays were performed following the standard protocols provided with the kits. Quality control samples were included in each assay batch to ensure analytical reliability.

STATISTICAL ANALYSIS

The results were evaluated utilising SPSS software version 15, with data expressed as mean±standard error of the mean (SEM). The differences among groups were analysed using one-way ANOVA, and LSD statistical tests. A p-value < 0.05 was considered statistically significant.

ETHICAL STATEMENT

The study protocol involving animal participants was reviewed and approved by the Ethics Committee of Hammurabi College of Medicine / University of Babylon (Approval No. 26 dated 30th-Jan-2025). All experimental procedures were conducted in accordance with the ethical standards of the institutional research committee and complied with the interdisciplinary principles and guidelines for the use of animals in research, as well as internationally accepted standards for laboratory animal care.

RESULTS

The results displayed the average concentrations of luteinizing hormone, follicle-stimulating hormone, progesterone and estrogen, which were used to evaluate the protective role of lycopene against the induction group by cyclophosphamide.

EFFECT OF LYCOPENE ON GONADOTROPIN HORMONES

A notable increase in luteinizing hormone levels was observed in the induction group compared to the control group (p-value <0.001). Conversely, follicle-stimulating hormone levels were markedly decreased in the induction group relative to the control group (p-value <0.001). Treatment with lycopene in combination with cyclophosphamide resulted in a marked improvement in these changes (Fig. 1). Luteinizing hormone levels in the treatment group were significantly lower than in the induction group (p-value < 0.001) and were not statistically different from the control group (p-value = 0.30). Similarly, follicle-stimulating hormone levels in the treatment group increased significantly compared to the induction group (p-value < 0.001) and approached normal levels (p-value = 0.08), (Table 1).

This group received an intraperitoneal injection of cyclophosphamide (200 mg/kg) as a signal dose on the

Table. 1. Evaluation of the protective role of lycopene against cyclophosphamide in the level of luteinizing hormone (LH) and follicle-stimulating hormone (FSH)

Groups	Luteinizing hormone (LH) (ng/mL)	Follicle stimulating hormone (FSH) (ng/mL)
Control group	11.5 ± 0.5 A	15.5± 0.3 A
Induction group	15.5 ± 1 B	7.5 ± 0.35 B
Treatment group	12.0 ± 0.5 A	14.5 ± 0.2 A
Lycopene group	11.0 ± 0.4 A	16.0 ± 0.5 A

Note: Values are presented as mean ± SEM (n =10 per group) of adults female Wistar rats.
AB Means values between groups with different superscripts are significantly different at (p <0.05).
Control group; received placebo only.
Induction group; intraperitoneal injection of cyclophosphamide (200 mg/kg).
Treatment group; intraperitoneal injection of cyclophosphamide and lycopene administered by gavage at 4mg/kg for 28 days.
Lycopene group; lycopene administered by gavage at 4 mg/kg for 28 days only
Source: Compiled by the authors of this study

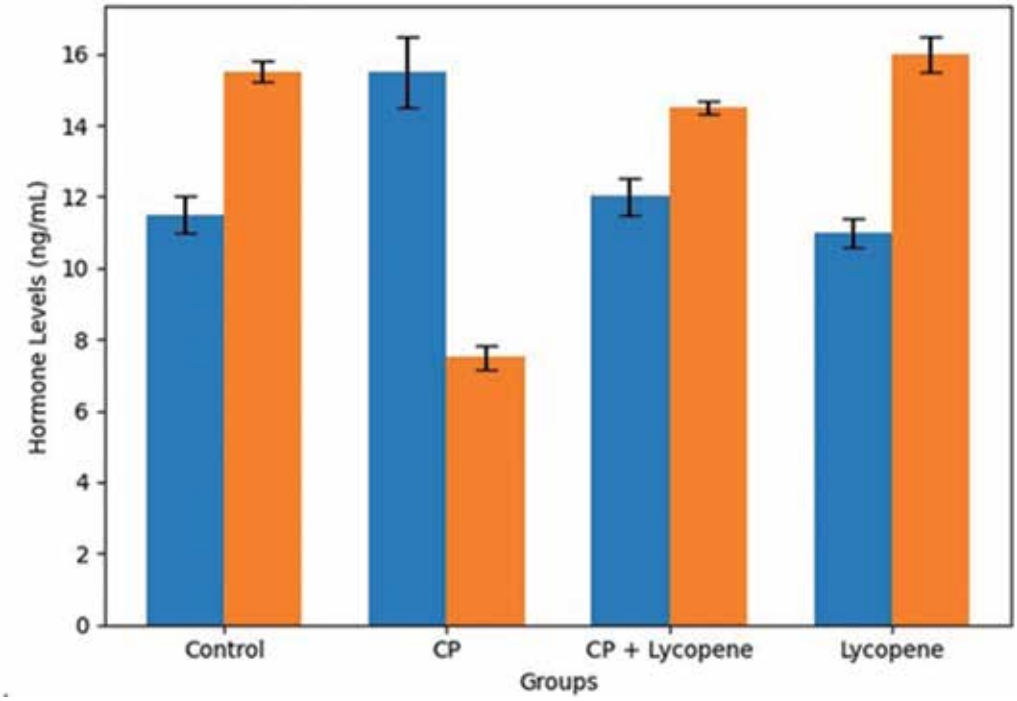

Fig. 1. Effects of cyclophosphamide and lycopene on serum gonadotropin levels (LH and FSH) in female Wistar rats.
Source: Own materials

first day of the experiment, along with a treatment of lycopene suspended in maize oil and administered by gavage at 4 mg/kg for 28 days. The control group did not show any significant differences between the treatment and control groups. Additionally, the table illustrates that lycopene has a beneficial effect on the regulation of the hormones luteinizing hormone and follicle-stimulating hormone. Furthermore, the results demonstrated that cyclophosphamide significantly reduces the level of follicle-stimulating hormone, with a significant decrease observed in the induction group compared to the control group.

EFFECT OF LYCOPENE ON OVARIAN STEROID HORMONES

The effective role of lycopene and cyclophosphamide in the levels of progesterone and estrogen were described (Table 2), which showed significant decrease in both levels of progesterone and estrogen hormone in induction group compared with control and treatment group (p-value<0.001). As well as the result illustrated that protective effect role of the lycopene against cyclophosphamide in both level of progesterone and estrogen (Fig. 2), furthermore, there is no significant difference between treatment group and control group.

Concomitant lycopene intake significantly mitigated these effects. Progesterone levels in the treatment group were significantly higher than in the induction group (p-value<0.001) and did not differ significantly from the control group (p-value=0.07). Similarly, estrogen levels in the treatment group were restored to normal levels significantly compared to the induction group (p<0.001), with no statistically significant difference from the control

Table. 2. Evaluation of the protective role of lycopene against cyclophosphamide in the level of progesterone (Pg) and estrogen (E2)

Groups	Progesterone (Pg) (ng/mL)	Estrogen (E2) (pg/mL)
Control group	38.2 ± 0.5 A	28.5 ± 1 A
Induction group	15.2 ± 0.5 B	7.2 ± 0.5 B
Treatment group	36.5 ± 0.7 A	27.0 ± 0.5 A
Lycopene group	39.0 ± 0.3 A	28.0 ± 0.2 A

Note: Values are presented as mean ± SEM (n =10 per group) of 40 adults female Wistar rats.
AB Means values between groups with different superscripts are significantly different at (p <0.05).
Control group; received placebo only.
Induction group; intraperitoneal injection of cyclophosphamide (200 mg/kg).
Treatment group; intraperitoneal injection of cyclophosphamide and lycopene administered by gavage at 4mg/kg for 28 days.
Lycopene group; lycopene administered by gavage at 4 mg/kg for 28 days only
Source: Compiled by the authors of this study

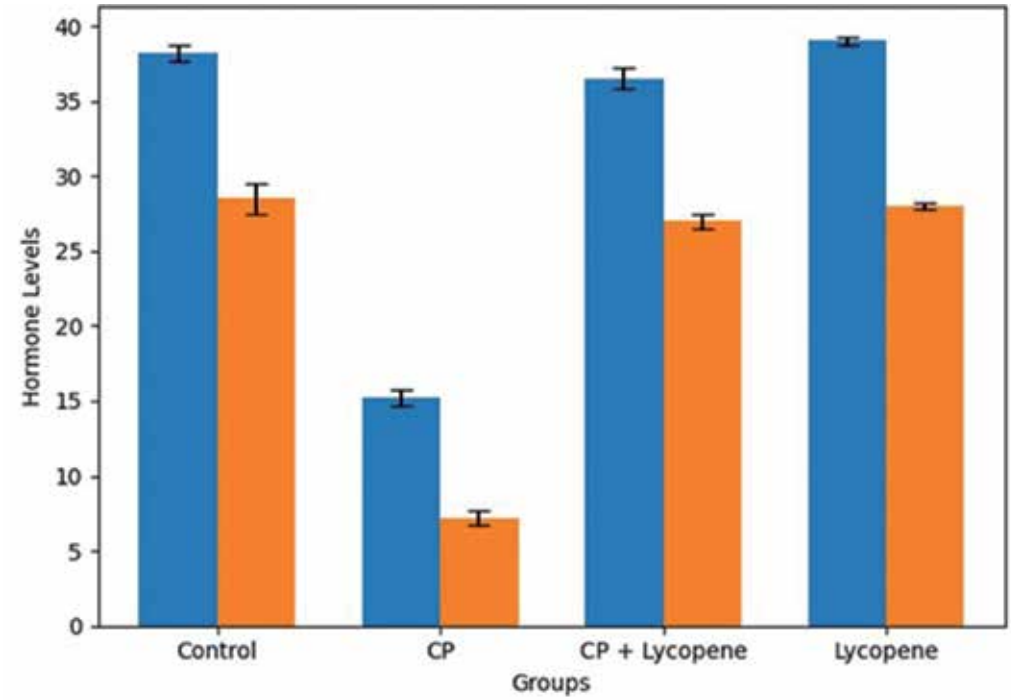

Fig. 2. Effects of cyclophosphamide and lycopene on ovarian steroid hormones (progesterone and estrogen) in female Wistar rats.
Source: Own materials

group (p-value=0.10). No statistically significant differences were observed between the lycopene-only group and the control group with respect to progesterone, confirming that lycopene alone does not alter normal ovarian endocrine function.

DISCUSSION

Infertility is a worldwide concern, prompting researchers and scientists to investigate its diverse causes and the pharmacological factors that can diminish fertility and adversely impact both male and female reproductive systems [21, 22]. Cyclophosphamide induces ovarian toxicity primarily through the formation of reactive metabolites such as phosphoramide mustard and acrolein, which promote oxidative stress and cellular apoptosis within ovarian follicles. Granulosa cells are particularly vulnerable to this damage, as they play a critical role in follicular development and steroid hormone production. Injury to these cells disrupts folliculogenesis and impairs the synthesis of estrogen and progesterone, ultimately altering the hypothalamic-pituitary-gonadal axis and affecting circulating levels of follicle-stimulating hormone, and Luteinizing hormone [23]. The findings of the present study indicate that lycopene effectively mitigates the effects of cyclophosphamide by regulating the levels of luteinizing hormone, follicle-stimulating hormone, progesterone, and estrogen. The restoration of follicle-stimulating hormone and luteinizing hormone levels observed in this study may be mechanistically explained by lycopene's ability to reduce oxidative stress within granulosa cells, thereby preserving steroidogenic enzyme activity and maintaining normal feedback regulation within the hypothalamic-pituitary-

gonadal axis. Moreover, multiple studies have shown that lycopene offers a protective effect against the detrimental effects of various substances [24]. Biological compounds with antioxidant properties can unequivocally safeguard cells and tissues from disruptions induced by reactive oxygen species, nitrogen species, and free radicals [25], primarily affecting the hypothalamic-pituitary-gonadal (HPG) axis similarly to premature ovarian failure, this severe gonadotoxicity and associated hormonal dysregulation – including reductions in anti-Müllerian hormone (AMH) expression - mimics the complex endocrine imbalances observed in conditions like polycystic ovary syndrome (PCOS) [26]. The mechanism of cyclophosphamide involves the reduction of oxidative stress, resulting in reproductive toxicity [26], while other studies indicate its capacity to generate free radicals [7]. Furthermore, additional research demonstrates that cyclophosphamide reduces sexual hormones in female granulosa cells and adversely affects Leydig and Sertoli cells in the testes of rats [7]. The present study demonstrates the effect of cyclophosphamide on steroid hormones, as shown in (Table 1 & Table 2).

Lycopene, a highly lipophilic carotenoid, can accumulate in cell membranes and act as a potent scavenger of reactive oxygen species. By reducing oxidative damage and maintaining mitochondrial integrity in granulosa cells, lycopene may preserve follicular structure and sustain normal endocrine activity of the ovary [12]. Several clinical approaches are currently used to preserve fertility in patients receiving chemotherapy, including ovarian tissue cryopreservation, oocyte or embryo freezing, and pharmacological ovarian suppression using gonadotropin-releasing hormone (GnRH) analogs [26]. GnRH analogs reduce ovarian activity during chemotherapy and may limit follicular depletion, although their effectiveness remains debated in some clinical settings [27]. Compared with these strategies, natural antioxidants such as lycopene may represent a complementary protective approach by targeting oxidative stress pathways involved in chemotherapy-induced ovarian damage [28]. The present findings suggest that lycopene may contribute to maintaining hormonal balance during cytotoxic treatment, supporting further investigation of antioxidant-based interventions alongside established fertility preservation methods. The findings correspond with prior studies from which emphasised the advantageous effects of lycopene in mitigating the detrimental impacts of toxic substances [29]. The findings indicated the advantageous effects of lycopene in relation to cyclophosphamide, an antioxidant, and improved the regulatory levels of hormones among various antioxidant compounds [30].

LIMITATION

A limitation of the present study is that oxidative stress biomarkers such as malondialdehyde (MDA), glutathione (GSH), and superoxide dismutase (SOD) were not measured. Evaluation of these biochemical parameters would provide a more direct link between lycopene's antioxidant activity and the observed preservation of hormonal balance. Future studies should incorporate oxidative stress markers and histopathological assessment of ovarian tissue to further elucidate the underlying protective mechanisms.

CONCLUSIONS

This study demonstrates that lycopene significantly attenuated cyclophosphamide-induced hormonal alterations against cyclophosphamide-induced hormonal alterations in female rats, indicating its potential to attenuate chemotherapy-associated ovarian endocrine dysfunction. The findings support the concept that antioxidant compounds may play an important role in preserving ovarian function during exposure to cytotoxic agents. Given the known contribution of oxidative stress to chemotherapy-related gonadotoxicity, lycopene may represent a promising adjunctive strategy for reducing reproductive toxicity during anticancer treatment.

Nevertheless, further mechanistic investigations and well-designed clinical studies are required to validate these observations, evaluate additional oxidative stress and ovarian reserve biomarkers, and determine the translational potential of lycopene supplementation for fertility and ovarian endocrine function preservation in women undergoing chemotherapy.

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DATA AVAILABILITY STATEMENT

On request, the data used in this investigation is available

CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Impact of multidrug resistant bloodstream infection burden, prophylactic levofloxacin and rectal swab practice on children with high-risk leukemias

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ABSTRACT

Aim: To determine the prevalence of multidrug-resistant rectal colonization and bloodstream infection in children with high-risk leukemia receiving levofloxacin prophylaxis, to assess changes in fluoroquinolone susceptibility across chemotherapy cycles, and to examine concordance between rectal surveillance cultures and bloodstream infection.

Materials and Methods: This retrospective cohort included 82 children aged 18 years or younger with acute myeloid leukemia or relapsed acute lymphoblastic leukemia treated with intensive chemotherapy and levofloxacin prophylaxis. Rectal swabs were obtained during febrile neutropenia when available, blood cultures were obtained for all febrile neutropenia episodes, and empirical meropenem was modified according to culture results. Multidrug resistance was classified according to published consensus criteria.

Results: Median age was 10 years, 67.1% were male, and 82.9% had acute myeloid leukemia. Febrile neutropenia occurred in 89.3% of chemotherapy cycles. Rectal colonization was dominated by multidrug-resistant *Klebsiella* species and multidrug-resistant *Escherichia coli*. Bloodstream infection was dominated by multidrug-resistant *Klebsiella pneumoniae* and multidrug-resistant *Escherichia coli*. Fluoroquinolone susceptibility was low in both rectal isolates (16%) and bloodstream isolates (11.8%) and decreased further across successive cycles. Mortality was associated with multidrug-resistant *Klebsiella pneumoniae* bloodstream infection and fluoroquinolone resistance in bloodstream isolates.

Conclusions: In this resistance setting, routine levofloxacin prophylaxis appears to offer limited benefit and may promote rapid expansion of resistant gram-negative flora. Rectal surveillance together with culture-directed therapy may better support antimicrobial stewardship and early risk-adapted treatment.

KEYWORDS: children; antimicrobial stewardship, colonization, *Enterobacterales,* mortality

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INTRODUCTION

Pediatric acute leukemia remains a major cause of cancer-related morbidity and mortality, and intensive chemotherapy exposes children to prolonged neutropenia and invasive infection [1, 5]. Antimicrobial resistance among gram-negative organisms, especially *Klebsiella pneumoniae* and *Escherichia coli*, has complicated empirical treatment because extended-spectrum beta-lactamase and carbapenem-resistant isolates are increasingly encountered in oncology units [2-4]. Fluoroquinolone prophylaxis can reduce febrile neutropenia and bacteremia in selected settings, but its benefit may be offset in high-resistance environments by selection of resistant flora [6, 28-30]. Rectal surveillance cultures may help identify children at risk for resistant bloodstream infection and support early escalation or de-escalation of therapy [7-9, 18, 20, 23].

AIM

This study aimed to determine the prevalence and microbiology of multidrug-resistant rectal colonization and bloodstream infection in children with high-risk leukemia receiving levofloxacin prophylaxis, to assess longitudinal fluoroquinolone susceptibility across chemotherapy cycles, and to evaluate concordance between rectal surveillance cultures and subsequent bloodstream infection.

MATERIALS AND METHODS

This retrospective observational cohort study included children 18 years old or younger with acute myeloid leukemia or relapsed acute lymphoblastic leukemia treated at the Pediatric Oncology Department, National Cancer Institute, Cairo University, between January 2022 and November 2023. Eligible patients received levofloxacin prophylaxis during intensive chemotherapy according to institutional practice; patients with incomplete records were excluded. Rectal swabs were obtained during febrile neutropenia episodes before empirical antibiotics when available, and blood cultures were obtained for all febrile neutropenia episodes or clinical suspicion of sepsis. Empirical meropenem with antipseudomonal coverage was used initially, with escalation or de-escalation guided by culture results and antimicrobial susceptibility testing.

Multidrug-resistant organisms were defined according to the international consensus criteria of Magiorakos et al.

[25]. Extended-spectrum beta-lactamase production was identified by standard phenotypic methods in the clinical microbiology laboratory. Minimum inhibitory concentration interpretation followed the Clinical and Laboratory Standards Institute criteria used by the laboratory during the study period, CLSI M100 Ed35 /2025. Levofloxacin was administered orally according to weight-based protocols, and concordance between rectal colonization and bloodstream infection was assessed.

STATISTICAL ANALYSIS

Data were analyzed with IBM SPSS Statistics for Windows, version 27.0 (IBM Corp., Armonk, NY, USA). Normality was assessed with the one-sample Kolmogorov-Smirnov test. Age showed a non-normal distribution and is therefore presented as median and interquartile range; non-parametric comparisons used the Mann-Whitney test. Categorical data were compared using the chi-square test or Fisher exact test, and logistic regression was used for univariate and multivariable analysis. A two-sided P value below 0.05 was considered statistically significant.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Institutional Review Board/ Ethics Committee of the National Cancer Institute, Cairo University (approval No. 2311-207-084; 14 November 2023). The committee reviewed the study protocol and waived written informed consent from parents or guardians because of the retrospective design and the use of anonymized routinely collected data. The study was conducted in accordance with the ethical standards of the responsible committee and the Helsinki Declaration (2013 revision).

RESULTS

Patient characteristics are summarized in (Table 1). The cohort included 82 children with a median age of 10 years (interquartile range, 5-13 years); 67.1% were male and 82.9% had acute myeloid leukemia. Outcomes included 32.9% under follow-up, 12.2% transition to palliation, and 54.9% death; septic shock accounted for 56.4% of recorded deaths.

Across 281 chemotherapy cycles, febrile neutropenia occurred in 251 (89.3%). Rectal swab cultures were available for 114 episodes, and 100 yielded bacterial growth. The most frequent rectal isolates were multidrug-resistant *Klebsiella spp.* (24.6%), multidrug-resistant *Escherichia coli* (21.9%), and extended-spectrum beta-lactamase-producing *Escherichia coli* (20.2%). Rectal isolates remained highly susceptible to colistin (98%), amikacin (72%), and tigecycline (65%), whereas levofloxacin susceptibility was only 16% (Table 2).

Blood cultures were positive in 86 of 251 febrile neutropenia episodes (34.3%), including one candidemia. Gram-negative organisms predominated (72%), and the most frequent pathogens were multidrug-resistant *Klebsiella pneumoniae* and multidrug-resistant *Escherichia coli* (29.1% each) (Table 3). Among bacterial bloodstream isolates, susceptibility was highest to colistin (85.9%), tigecycline (51.8%), and amikacin (49.4%), while levofloxacin susceptibility was 11.8% (Table 4).

Among 114 rectal swabs, the positive predictive value for subsequent bloodstream infection was 35%, whereas the negative predictive value was 78.6%. Concordance was strongest for multidrug-resistant *Klebsiella*: 39.3% of carriers later developed multidrug-resistant *Klebsiella* bloodstream infection. Among patients colonized with multidrug-resistant *Escherichia coli*, 20% developed multidrug-resistant *Escherichia coli* bloodstream infection (Table 5).

Levofloxacin resistance increased across chemotherapy cycles in both rectal and blood isolates. Rectal resistance rose from 61.8% in cycle 1 to 96.6%-100% in cycles 2-3, while blood-isolate resistance increased from 72.0% in cycle 1 to 100% in cycle 2 (Table 6).

Table 1. Demographic and clinical characteristics and outcomes

		Total no. = 82
Age	Median (IQR)	10 (5-13)
	Range	2-18
Sex	Female	27 (32.9%)
	Male	55 (67.1%)
Diagnosis	AML	68 (82.9%)
	R ALL	14 (17.1%)
Outcome	Under follow-up	27 (32.9%)
	Palliation	10 (12.2%)
	Death	45 (54.9%)
Cause of death (N=55)	Septic shock (Infection related mortality)	31 (56.4%)
	Disease progression	21 (38.2%)
	Cardiac Impairment	1 (1.8%)
	Pulmonary hemorrhage	1 (1.8%)
	Cardiac Impairment & Refractory disease	1 (1.8%)

Source: compiled by the authors of this study

Table 2. Antimicrobial sensitivity profile of rectal swab cultures

		Total no. = 100
Drug sensitivity of rectal swap culture	Amikacin	72 (72.0%)
	Colistin	98 (98.0%)
	Tigecycline	65 (65.0%)
	Meropenem	39 (39.0%)
	Imipenem	30 (30.0%)
	Ertapenem	21 (21.0%)
	Ceftazidime	10 (10.0%)
	Piperacillin/Tazobactam	12 (12.0%)
	Levofloxacin	16 (16.0%)
	Ciprofloxacin	15 (15.0%)
	Amoxicillin/clavulanate	2 (2.0%)
	Cefoxitin	12 (12.0%)
	Cefotaxime	2 (2.0%)
	Cefepime	2 (2.0%)

Source: compiled by the authors of this study

Table 3. Distribution of blood culture isolates

		Total no. = 86
Blood culture	*Klebsiella pneumoniae (MDR)*	25 (29.1%)
	Escherichia coli (MDR)	25 (29.1%)
	Klebsiella pneumoniae (ESBL)	3 (3.5%)
	Escherichia coli (ESBL)	2 (2.3%)
	Coagulase-Negative Staphylococcus (CONS)	4 (4.7%)
	Methicillin-Resistant Staphylococcus aureus (MRSA)	4 (4.7%)
	Pseudomonas aeruginosa (MDR)	2 (2.3%)
	Alpha-Hemolytic Streptococcus	3 (3.5%)
	Pseudomonas aeruginosa	1 (1.2%)
	Staphylococcus aureus	3 (3.5%)
	Staphylococcus epidermidis	2 (2.3%)
	Acinetobacter baumannii	2 (2.3%)
	Candida	1 (1.2%)
	Staphylococcus hominis	1 (1.2%)
	Staphylococcus lentus	1 (1.2%)
	Acinetobacter baumannii (MDR)	2 (2.3%)
	E.Coli (MDR), Acinetobacter (MDR)	1 (1.2%)
	E.Coli (MDR), KLB (MDR)	2 (2.3%)
	Acinetobacter baumannii (MDR), KLB (MDR)	1 (1.2%)
	Streptococcus	1 (1.2%)

Source: compiled by the authors of this study

In rectal swab isolates, susceptibility to tigecycline, meropenem, and imipenem was more frequent among survivors than nonsurvivors, although these associations did not remain significant in multivariable analysis (Table 7).

Bloodstream infection with multidrug-resistant organisms was strongly associated with adverse outcome. Multidrug-resistant *Klebsiella pneumoniae* and multidrug-resistant *Pseudomonas aeruginosa* were more common among

Table 4. Antimicrobial susceptibility patterns of blood culture isolates

		Total no. = 85 (excluding 1 Candida Bl. Culture)
Drug sensitivity of blood culture	Colistin	73 (85.9%)
	Amikacin	42 (49.4%)
	Gentamycin	16 (18.8%)
	Tigecycline	44 (51.8%)
	Tobramycin	1 (1.2%)
	Levofloxacin	10 (11.8%)
	Imipenem	12 (14.1%)
	Meropenem	17 (20.0%)
	Ertapenem	9 (10.6%)
	Ciprofloxacin	7 (8.2%)
	Cefotaxime	3 (3.5%)
	Vancomycin	17 (20.0%)
	Linezolid	6 (7.1%)
	Erythromycin	2 (2.4%)
	Clindamycin	1 (1.2%)
	Cefoxitine	4 (4.7%)
	Piperacillin/Tazobactam	8 (9.4%)
	Ceftazidim	1 (1.2%)
	Amoxicillin/Clavulanic acid	1 (1.2%)

Source: compiled by the authors of this study

nonsurvivors, whereas absence of blood-culture growth was more frequent among survivors (Table 8).

Among bloodstream isolates, susceptibility to amikacin, gentamicin, meropenem, ertapenem, vancomycin, and piperacillin/tazobactam was associated with survival; all nonsurvivors had levofloxacin-resistant bloodstream isolates (Table 9).

In logistic regression, multidrug-resistant *Klebsiella* bloodstream infection remained an independent predictor of death (odds ratio, 7.654; 95% confidence interval, 2.164-27.069; P=0.002). Multidrug-resistant *Escherichia coli* was significant in univariate analysis but lost significance after adjustment, whereas amikacin susceptibility showed a protective trend (Table 10).

DISCUSSION

Our cohort shows a heavy burden of febrile neutropenia and resistant gram-negative infection among children receiving intensive leukemia treatment. The predominance of multidrug-resistant *Klebsiella pneumoniae* and multidrug-resistant *Escherichia coli* in bloodstream infection (Table 3) is consistent with recent pediatric oncology reports from the region and from multicenter cohorts, which describe resistant *Enterobacterales* as major causes of infection-related mortality [10-20]. The mortality signal in our study was strongest for multidrug-resistant *Klebsiella* bloodstream infection, which remained independently associated with death after adjustment and mirrors findings from recent pediatric studies (Table 10) [16, 17, 24].

Rectal surveillance had practical value but imperfect discrimination. Although many colonized patients did not develop bloodstream infection, concordance between gut colonization and subsequent bacteremia was clinically relevant, especially for multidrug-resistant *Klebsiella* and multidrug-resistant *Escherichia coli* (Table 5). This supports prior work showing that surveillance cultures can help identify children at increased risk of resistant bacteremia and can inform early empirical escalation in selected neutropenic patients [7-9, 18, 20, 23]. The univariate associations between survival and rectal susceptibility to tigecycline, meropenem, and imipenem likely reflect a broader low-resistance phenotype rather than independent causal protection (Table 7).

The longitudinal susceptibility data suggest that routine levofloxacin prophylaxis may have limited utility in a setting with substantial baseline resistance. Resistance increased rapidly across chemotherapy cycles in both rectal and bloodstream isolates (Table 6), and all nonsurvivors with bloodstream infection had levofloxacin-resistant isolates (Table 9). These findings align with the ongoing debate around fluoroquinolone prophylaxis: randomized trials have shown microbiologic benefit in some low-resistance settings, but contemporary appraisals emphasize the ecological cost

Table 5. Concordance between rectal swab isolates and corresponding blood culture results

		Rectal swab culture							
		KLB	KLB (MDR)	KLB (ESBL)	*E. Coli*	*E. Coli* (MDR)	*E.Coli* (ESBL)	*Proteus*	*Enterobacter* MDR
		No=5	No=28	No=5	No=12	No=25	No=23	No=1	No=1
Blood culture	No growth	3(60%)	15(53.6%)	5(100%)	8(66.7%)	18(72%)	16(69.6%)	0(0%)	0(0%)
	KLB (MDR)	0(0%)	11(39.3%)	0(0%)	0(0%)	1(4%)	1(4.3%)	0(0%)	0(0%)
	E. Coli (MDR)	0(0%)	0(0%)	0(0%)	2(16.7%)	5(20%)	3(13%)	0(0%)	1(100%)
	KLB (ESBL)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	E. Coli (ESBL)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(4.3%)	0(0%)	0(0%)
	CONS	0(0%)	2(7.1%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	MRSA	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	Pseudomonas (MDR)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(100%)	0(0%)
	Alpha hemolytic streptococcus	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	Pseudomonas	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	Staphylococcus aureus	0(0%)	0(0%)	0(0%)	1(8.3%)	0(0%)	0(0%)	0(0%)	0(0%)
	Staphylococcus epidermidis	0(0%)	0(0%)	0(0%)	1(8.3%)	0(0%)	1(4.3%)	0(0%)	0(0%)
	Acinetobacter	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(4.3%)	0(0%)	0(0%)
	Candida	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	Staphylococcus hominis	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	Staphylococcus lentus	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	Acinetobacter (MDR)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	Acinetobacter	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	E. Coli (MDR), Acinetobacter (MDR)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	E. Coli (MDR), KLB (MDR)	1(20%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	Acinetobacter (MDR), KLB (MDR)	1(20%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	Streptococcus	0(0%)	0(0%)	0(0%)	0(0%)	1(4%)	0(0%)	0(0%)	0(0%)

Source: compiled by the authors of this study

Table 6. Levofloxacin sensitivity in rectal swab and blood culture among chemotherapy cycles

		Chemotherapy				Test value	P-value	Sig.
		Cycle 1	Cycle 2	Cycle 3	Cycle 4			
		No. (%)	No. (%)	No. (%)	No. (%)			
Levofloxacin sensitivity of rectal swab culture	Resistant	21 (61.8%)	28 (96.6%)	16 (100.0%)	19 (90.5%)	19.610*	0.000	HS
	Sensitive	13 (38.2%)	1 (3.4%)	0 (0.0%)	2 (9.5%)			
Levofloxacin sensitivity of blood culture	Resistant	18 (72.0%)	33 (100.0%)	11 (91.7%)	13 (86.7%)	10.920*	0.012	S
	Sensitive	7 (28.0%)	0 (0.0%)	1 (8.3%)	2 (13.3%)			

Source: compiled by the authors of this study

of selecting multidrug-resistant flora when background resistance is already high [21-22, 28-30].

This study should be interpreted in light of its retrospective single-center design, incomplete baseline rectal surveillance, and lack of a comparator group not exposed to levofloxacin prophylaxis. Even so, the data support a stewardship-focused strategy centered on surveillance-guided empirical therapy, rapid microbiologic confirmation, and early infection-control precautions rather than continued universal fluoroquinolone prophylaxis in this epidemiologic context [26-27].

Table 7. Association between rectal swab antimicrobial susceptibility patterns and patient outcomes

		Outcome		Test value	P-value	Sig.
		Alive	Death			
		No=85	No=15			
Drug sensitivity of rectal swab culture	Amikacin	63(74.1%)	9(60.0%)	1.261	0.262	NS
	Colistin	84(98.8%)	14(93.3%)	1.961	0.161	NS
	Tigecycline	59(69.4%)	6(40.0%)	4.848	0.028	S
	Meropenem	37(43.5%)	2(13.3%)	4.887	0.027	S
	Imipenem	29(34.1%)	1(6.7%)	4.575	0.032	S
	Ertapenem	20(23.5%)	1(6.7%)	2.185	0.139	NS
	Ceftazidim	9(10.6%)	1(6.7%)	0.218	0.641	NS
	Piperacillin/Tazobactam	11(12.9%)	1(6.7%)	0.475	0.491	NS
	Levofloxacin	15 (17.6%)	1 (6.7%)	1.144	0.285	NS
	Ciprofloxacin	14 (16.5%)	1 (6.7%)	0.961	0.327	NS
	Amoxicillin / clavulanic	2 (2.4%)	0 (0.0%)	0.360	0.548	NS
	Cefoxitine	11 (12.9%)	1 (6.7%)	0.475	0.491	NS
	Cefotaxime	2 (2.4%)	0 (0.0%)	0.360	0.548	NS
	Cefepime	2 (2.4%)	0 (0.0%)	0.360	0.548	NS

Source: compiled by the authors of this study

Table 8. Association between positive blood culture pathogens and patient outcomes

Positive blood culture	Outcome		Test value	P-value	Sig.
	Alive	Death			
	No=59	No=27			
Klebsiella pneumoniae (MDR)	11(18.6%)	14(51.9%)	9.907	0.001	HS
Escherichia coli (MDR)	17(28.8%)	8(29.6%)	0.006	0.938	NS
Klebsiella pneumoniae (ESBL)	3(5.1%)	0(0.0%)	1.423	0.232	NS
Escherichia coli (ESBL)	2(3.4%)	0(0.0%)	0.937	0.333	NS
Coagulase-Negative Staphylococcus	4(6.8%)	0(0.0%)	1.920	0.165	NS
Methicillin-Resistant Staphylococcus aureus	4(6.8%)	0(0.0%)	1.920	0.165	NS
Pseudomonas aeruginosa (MDR)	0(0.0%)	2(7.4%)	4.474	0.034	S
Alpha hemolytic streptococcus	3(5.1%)	0(0.0%)	1.423	0.232	NS
Pseudomonas aeruginosa	1(1.7%)	0(0.0%)	0.463	0.496	NS
Staphylococcus aureus	3(5.1%)	0(0.0%)	1.423	0.232	NS
Staphylococcus epidermidis	2(3.4%)	0(0.0%)	0.937	0.333	NS
Acinetobacter baumannii	2(3.4%)	0(0.0%)	0.937	0.333	NS
Candida	1(1.7%)	0(0.0%)	0.463	0.496	NS
Staphylococcus hominis	1(1.7%)	0(0.0%)	0.463	0.496	NS
Staphylococcus lentus	1(1.7%)	0(0.0%)	0.463	0.496	NS
Acinetobacter baumannii (MDR)	1(1.7%)	1(3.7%)	0.329	0.566	NS
E. Coli (MDR), Acinetobacter baumannii (MDR)	0(0.0%)	1(3.7%)	2.211	0.137	NS
E. Coli (MDR), KLB (MDR)	1(1.7%)	1(3.7%)	0.329	0.566	NS
Acinetobacter baumannii (MDR), KLB (MDR)	1(1.7%)	0(0.0%)	0.463	0.496	NS
Streptococcus aureus	1(1.7%)	0(0.0%)	0.463	0.496	NS

Source: compiled by the authors of this study

Table 9. Association between blood culture-based antimicrobial susceptibility and patient outcomes

Drug sensitivity of blood culture	Outcome		Test value	P-value	Sig.
	Alive	Death			
	No=59	No=27			
Colistin	47(81.0%)	26(96.3%)	3.539	0.060	NS
Amikacin	33(56.9%)	9(33.3%)	4.092	0.043	S
Gentamycin	15(25.9%)	1(3.7%)	5.920	0.015	S
Tigecycline	34(58.6%)	10(37.0%)	3.437	0.064	NS
Tobramycin	1(1.7%)	0(0.0%)	0.471	0.493	NS
Levofloxacin	10(17.2%)	0(0.0%)	5.276	0.022	S
Imipenem	11(19.0%)	1(3.7%)	3.539	0.060	NS
Meropenem	16(27.6%)	1(3.7%)	6.568	0.010	S
Ertapenem	9(15.5%)	0(0.0%)	4.686	0.030	S
Ciprofloxacin	7(12.1%)	0(0.0%)	3.551	0.060	NS
Cefotaxime	3(5.2%)	0(0.0%)	1.448	0.229	NS
Vancomycin	17(29.3%)	0(0.0%)	9.892	0.002	HS
Linezolid	6(10.3%)	0(0.0%)	3.005	0.083	NS
Erythromycin	2(3.4%)	0(0.0%)	0.953	0.329	NS
Clindamycin	1(1.7%)	0(0.0%)	0.471	0.493	NS
Cefoxitine	4(6.9%)	0(0.0%)	1.954	0.162	NS
Piperacillin/Tazobactam	8(13.8%)	0(0.0%)	4.111	0.043	S
Ceftazidim	1(1.7%)	0(0.0%)	0.471	0.493	NS
Amoxicillin / clavulanic	1(1.7%)	0(0.0%)	0.471	0.493	NS

Source: compiled by the authors of this study

Table 10. Predictors of mortality based on rectal swab and blood culture drug sensitivity: univariate and multivariate analysis

		Univariate				Multivariate			
		P-value	Odds ratio (OR)	95% C.I. for OR		P-value	Odds ratio (OR)	95% C.I. for OR	
				Lower	Upper			Lower	Upper
Drug sensitivity of rectal swap culture Blood culture	Tigecycline	0.034	0.294	0.095	0.911	–	–	–	–
	Meropenem	0.041	0.200	0.042	0.940	–	–	–	–
	Imipenem	0.062	0.138	0.017	1.102	–	–	–	–
	KLB (MDR)	0.000	13.109	5.259	32.679	0.002	7.654	2.164	27.069
	E. coli (MDR)	0.007	3.620	1.422	9.215	0.052	3.771	0.988	14.389
Drug sensitivity of blood culture	Amikacin	0.046	0.379	0.146	0.983	0.053	0.352	0.122	1.016
	Gentamycin	0.038	0.110	0.014	0.884	–	–	–	–

Source: compiled by the authors of this study

CONCLUSIONS

In settings with an already high prevalence of resistant *Enterobacterales*, routine levofloxacin prophylaxis may accelerate loss of fluoroquinolone activity without translating into better outcomes. A strategy based on rectal surveillance, locally tailored empirical therapy, and strict antimicrobial stewardship is more likely to improve care for children with high-risk leukemia than continued universal prophylaxis.

ABBREVIATIONS

AML: Acute myeloid leukemia
ALL: Acute lymphoblastic leukemia
BSI: Bloodstream infection
CLSI: Clinical and Laboratory Standards Institute
FN: Febrile neutropenia
MDR: Multidrug resistant
MDRO: Multidrug-resistant organism

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AVAILABILITY OF DATA AND MATERIALS

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request. Raw data are not publicly available because of patient privacy and institutional restrictions; aggregated data supporting the conclusions are presented in the manuscript.

CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Forensic medical assessment of thoracic and lumbar traumatic spinal cord injury in percutaneous and open transpedicular fixation

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ABSTRACT

Aim: To conduct a forensic medical evaluation of the severity of bodily injuries in thoracic and lumbar traumatic spinal cord injury; to assess surgical care quality indicators in percutaneous and open transpedicular fixation in the context of iatrogenic risk and prevention of medical care deficiencies; to analyse whether the surgical stabilisation method may influence parameters of forensic significance for evaluating injury outcomes.

Materials and Methods: A retrospective study of 269 patients: open fixation (215), percutaneous fluoroscopy-guided fixation (54). Assessed: Frankel scale, pelvic dysfunction, blood loss, duration of surgery and anesthesia, fentanyl dose, hyperthermia, and implant quality.

Results: Severe bodily injury (life-threatening) was established in 27.9% (Group 1) vs 7.4% (Group 2) ($p=0.001$); by ≥33% permanent disability – 22.3% vs 7.4% ($p=0.012$). Mean permanent disability: 30.1±24.2% vs 17.8±7.9% ($p<0.001$). Neurological deficit (Frankel A-D) at admission: 32.4% vs 7.7%. Moderate injuries: 63.3% vs 88.9%; no mild injuries. Percutaneous technique resulted in 16.3-fold lower blood loss (20 vs 325 mL; $p<0.001$), lower fentanyl dose, and shorter anesthesia ($p<0.001$); implant quality did not differ ($p>0.05$).

Conclusions: Injury severity is a fixed legal fact determined by the primary trauma. Surgery may affect legal qualification via: change in outcome at reassessment; qualification of care defects as independent injuries. Percutaneous fixation significantly reduces iatrogenic risk. Documentation gaps complicate forensic evaluation of care quality.

KEYWORDS: spinal cord injuries, forensic medicine, malpractice

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INTRODUCTION

Traumatic spinal cord injury (TSCI) is one of the most severe types of mechanical trauma and frequently becomes a subject of forensic medical examination. The legal framework in Ukraine comprises the Rules for Forensic Determination of the Severity of Bodily Injuries (Order of the Ministry of Health of Ukraine No. 6 of 17.01.1995; hereinafter, the Rules) and the Criminal Code of Ukraine [1, 2]. Regarding TSCI, severe bodily injury (Art. 121 of the Criminal Code) is established on the grounds of life-threatening danger (sub-paragraphs 2.1.3z and 2.1.3y of the Rules, where "z" and "y" are Latin-script transliterations of the original Ukrainian Cyrillic sub-paragraph designators: vertebral fractures or spinal cord damage with neurological deficit or severe spinal shock) or permanent loss of general working capacity ≥33% (clause 2.1.6). Moderate bodily injury (Art. 122) involves a prolonged health disorder exceeding 21 days or permanent loss of 10-33%. Minor injuries involve a health disorder of up to 21 days or a loss below 10% (Art. 125). When criteria compete, the one indicating greater severity applies.

A compressive or unstable vertebral fracture requires consolidation of at least 6-8 weeks, substantially exceeding the 21-day threshold for prolonged health disorder [3]. Surgical intervention neither replaces nor accelerates bone consolidation and therefore does not alter the duration of the health disorder caused directly by the fracture. However, surgery can substantially influence the dynamics of neurological recovery and, consequently, the final percentage of permanent loss established after treatment completion (not earlier than six months) [2, 4].

The percentage of permanent loss of general working capacity in spinal cord injuries is determined under Art. 6 of the Table of the Instructions on the Organization and Conduct of Medical-Insurance Expert Examination, based on the residual neurological deficit at the time of forensic examination. The Frankel scale (A-E) is a widely recognised standardised tool for assessing neurological deficit in TSCI [5], enabling objective correlation of the patient's clinical status with the Table criteria.

Percutaneous minimally invasive transpedicular fixation is performed through puncture incisions without separation of the paravertebral muscles under fluoroscopic control and fundamentally differs from the open technique in blood loss volume, extent of tissue trauma, and anaesthetic load [3, 6, 7]. In patients with spinal shock, these differences acquire direct forensic significance, both in evaluating patient status and in the expert assessment of care quality regarding iatrogenic risk [8, 9].

AIM

To conduct a forensic medical evaluation of the severity of bodily injuries in thoracic and lumbar TSCI; to assess surgical care quality indicators in percutaneous and open transpedicular fixation in the context of iatrogenic risk and prevention of medical care deficiencies; to analyse whether the surgical stabilisation method may influence parameters of forensic significance for evaluating injury outcomes.

MATERIALS AND METHODS

DESIGN AND CLINICAL BASE

A retrospective cohort study of 269 patients with thoracic and lumbar TSCI who underwent transpedicular stabilisation (2017-2024) at the neurosurgical department of a multidisciplinary hospital. Group 1 – open fixation (n=215, 79.9%); Group 2 – percutaneous minimally invasive fixation under fluoroscopic control (n=54, 20.1%).

DATA ANALYSE

Neurological status by Frankel scale (A-E), pelvic disorders, blood loss volume (when documented in anaesthetic records), duration of surgery and anaesthesia (min), fentanyl 0.005% dose (ml), hyperthermia > 38°C in the early postoperative period, duration of inpatient treatment, incidence of misplaced screws (postoperative CT), and early surgical complications.

STATISTICAL ANALYSIS

Data were processed using SPSS Statistics 26.0. Quantitative indicators are presented as M±SD (arithmetic mean±standard deviation) and Me [Q1-Q3] (median and interquartile range); qualitative indicators – as absolute frequencies and relative frequency (%). Normality of distribution was assessed using the Shapiro-Wilk test. Comparison of two independent groups was performed using Student's t-test (for normally distributed quantitative variables) or the Mann-Whitney test (for non-normally distributed variables). Associations between qualitative variables were assessed using Fisher's exact test. Differences were considered statistically significant at $p<0.05$.

ETHICS

The study was conducted in accordance with the provisions of the Declaration of Helsinki of the World Medical Association, the Council of Europe Convention on Human Rights and Biomedicine, and Ukrainian legislation. Aggregated statistical data without disclosure of personal information were used. Analysis of clinical cases was conducted in a generalised form, without the possibility of identifying specific individuals. The retrospective nature of the study and the anonymisation of personal data preclude the requirement for individual informed consent.

AI STATEMENT

The following artificial intelligence (AI) tool was used in this work: Gemini 3.5 Flash (Google DeepMind, May 2026). It was used for the following tasks under full author oversight:

- Formation and formatting of the bibliography under the authors' control;
- Stylistic review of the English text under the authors' supervision;
- Verification of selected statistical calculations under the authors' control.

All sections where AI was applied were reviewed and edited personally by the authors. The scientific statements, conclusions, and results are the authors' own intellectual contribution.

FRAMEWORK

The research was conducted within the framework of the Research and Development projects of the Department of Neurosurgery at Bogomolets National Medical University: „Advanced methods for predicting and surgical treatment of degenerative intervertebral disc diseases" (State Registration No. 0124U000011, 2024-2026) and „Investigation of the regeneration process in peripheral nerve and spinal cord injuries during restorative treatment in experimental settings" (State Registration No. 0124U000009, 2024-2026).

RESULTS

GENERAL GROUP CHARACTERISTICS (TABLE 1)

A total of 269 patients were included: 215 in Group 1 and 54 in Group 2. Group 2 patients were statistically significantly older (Me 54 [38-64] *vs.* 44 [32-59] years; p=0.007): the percutaneous technique is the method of choice particularly in elderly patients with osteoporosis-associated fractures. Groups did not differ by sex or spinal level.

Table 1. General characteristics of comparison groups

Parameter	Group 1 – open (n=215)	Group 2 – percutaneous (n=54)	p
Age, years (Me [Q1-Q3])	44 [32-59]	54 [38-64]	0.007
Male, N (%)	144 (67.0)	35 (64.8)	0.889
Female, N (%)	71 (33.0)	19 (35.2)	0.889
Thoracic level, N (%)	30 (14.0)	5 (9.3)	0.498
Thoracolumbar level, N (%)	141 (65.6)	38 (70.4)	0.629
Lumbar level, N (%)	44 (20.5)	11 (20.4)	1.000

Notes: Me – median; Q1-Q3 – first and third quartiles; Mann–Whitney test – for age; Fisher's exact test – for qualitative variables.

Source: compiled by the authors of this study

FORENSIC SEVERITY CLASSIFICATION AND ESTIMATED PERCENTAGE OF PERMANENT LOSS (TABLE 2, TABLE 3)

Neurological deficit (Frankel A-D) at admission – in 66/204 (32.4%) of Group 1 and 4/52 (7.7%) of Group 2 (exclusively Frankel D); Frankel data are available for 204/215 patients of Group 1 and 52/54 of Group 2. Pelvic disorders – 43/215 (20.0%) *vs.* 0/54 (p<0.001). Severe bodily injury by life-threatening danger (clauses 2.1.3z and 2.1.3y) – 60 (27.9%) in Group 1 and 4 (7.4%) in Group 2 (p=0.001). The figure of 60 includes: (a) all patients with Frankel A-D among those with available data (66 of 204), excluding 11 patients with undocumented Frankel status in whom clauses 2.1.3z and 2.1.3y are unconfirmed, and (b) Frankel E patients with pelvic disorders (a portion of the 43); patients with Frankel A–D who also have pelvic disorders are counted once. Regarding spinal shock: anti-shock therapy records are systematically absent from medical charts; direct verification is possible only indirectly from anaesthetic records.

Permanent loss ≥33% – 48/215 (22.3%) in Group 1 and 4/54 (7.4%) in Group 2 (p=0.012). 100% permanent loss (paraplegia) – 15 (7.0%) in Group 1 and 0 in Group 2 (p=0.047). All Frankel E patients without pelvic disorders – 136 (63.3%) and 48 (88.9%) – are classified as moderate severity based on duration of health disorder determined by vertebral fracture consolidation >21 days. No minor bodily injuries were identified in the cohort.

SURGICAL CARE QUALITY ASSESSMENT (TABLE 4)

Blood loss: median 325 [300-429] ml in Group 1 *vs.* 20 [20-25] ml in Group 2 – 16.3-fold less (p<0.001). Surgery duration: 160 [125-195] *vs.* 92 [75-140] min (p<0.001). Anaesthesia duration: 210 [173-260] *vs.* 142 [124-190] min (p<0.001). Total fentanyl dose: 30 [24-36] *vs.* 22 [16-28] ml (p<0.001). Hyperthermia >38°C: 69/215 (32.1%) *vs.* 2/54 (3.7%) (p<0.001). Inpatient stay: 18 [14-23] *vs.* 7 [5-11] days (p<0.001). Incidence of misplaced screws (0.5% *vs.* 1.9%; p=0.362), revision surgeries (3.7% *vs.* 1.9%; p=0.692), and early surgical complications (4.2% *vs.* 1.9%; p=0.692) did not differ between groups. The reported complications are exclusively early surgical ones within the hospitalisation

Table 2. Forensic medical severity classification of thoracic and lumbar traumatic spinal cord injury

Parameter	Group 1 – open (n=215)	Group 2 – percutaneous (n=54)	p
Neurological status by Frankel scale at admission			
Frankel A – complete paraplegia and anaesthesia, N (%)	19 (9.3)*	0	0.128
Frankel B – preserved sensation, absent movement, N (%)	5 (2.5)*	0	0.587
Frankel C – movement present, functionally useless, N (%)	20 (9.8)*	0	0.133
Frankel D – movement present, functionally useful but incomplete, N (%)	22 (10.8)*	4 (7.7)*	1.000
Frankel E – neurologically normal, N (%)	138 (67.6)*	48 (92.3)*	<0.001
Pelvic disorders, N (%)	43 (20.0)	0	<0.001
I. Severe by life-threatening danger (clauses 2.1.3z and 2.1.3y of the Rules)			
Neurological deficit (Frankel A-D) or pelvic disorders, N (%)	60 (27.9)	4 (7.4)	0.001
Δ Severe spinal shock (clauses 2.1.3z-2.1.3y): anti-shock therapy records systematically absent from medical charts – direct verification not possible, N (%)	n/a	n/a	–
II. Severe by permanent loss ≥33% (clause 2.1.6 of the Rules)			
Estimated permanent loss ≥33%, N (%)	48 (22.3)	4 (7.4)	0.012
incl. 40% (Frankel D without pelvic), N (%)	14 (6.5)	4 (7.4)	1.000
incl. 60% (Frankel C or D with pelvic), N (%)	19 (8.8)	0	0.017
incl. 100% (Frankel A or B – paraplegia), N (%)	15 (7.0)	0	0.047
Estimated % permanent loss: Me [Q1-Q3]	15 [15-40]	15 [15-15]	0.003
III. Moderate severity by duration of health disorder (clause 2.2 of the Rules)			
Frankel E without pelvic: vertebral fracture consolidation >21 days, n (%) incl. permanent loss 10-32% (Frankel E without pelvic=15%), N (%)	136 (63.3)	48 (88.9)	<0.001
IV. Minor bodily injury (clause 2.3 of the Rules)			
Not identified: vertebral fracture consolidation >21 days; permanent loss <10% not recorded, N (%)	0	0	–

*Notes: Fisher's exact test; n/a – not available; Δ – systemic documentation deficiency. *Percentages in Frankel rows (A-E) calculated from patients with available data: Group 1 – n=204, Group 2 – n=52; all other parameters – from total n=215 and n=54 respectively.*

Source: compiled by the authors of this study

Table 3. Correspondence of Frankel scale level and pelvic disorders to estimated percentage of permanent loss

Frankel	Neurological picture	Pelvic disorders	% permanent loss
A or B	Paraplegia or absent functional movement	Present	100%
C	Functionally useless movement	Any	60%
D	Functionally useful but incomplete movement	Present	60%
D	Functionally useful but incomplete movement	Absent	40%
E	Neurologically normal	Present	40%
E	Neurologically normal	Absent	15%

Notes: Estimated reference; final percentage established by the forensic expert after treatment completion, generally not earlier than 6 months post-injury

Source: compiled by the authors of this study

Table 4. Iatrogenic risk, anaesthetic load, implantation quality and inpatient stage indicators

Parameter	Group 1 – open (n = 215)	Group 2 – percutaneous (n = 54)	p
Extent of surgical intervention			
Surgery duration, min (Me [Q1-Q3])	160 [125-195]	92 [75–140]	<0.001
Blood loss volume, ml (Me [Q1-Q3]) Δ	325 [300-429] N=70	20 [20–25] N=7	<0.001
Anaesthetic load (identical protocol in both groups)			
Anaesthesia duration, min (Me [Q1-Q3])	210 [173-260]	142 [124-190]	<0.001
Total fentanyl 0.005%, ml (Me [Q1-Q3]) Δ	30 [24-36] N=204	22 [16-28] N=52	<0.001
Markers of surgical tissue trauma and inpatient stage			
Hyperthermia >38°C in early postoperative period, N (%)	69 (32.1%)	2 (3.7%)	<0.001
Inpatient stay, days (Me [Q1-Q3])	18 [14-23]	7 [5-11]	<0.001
Implantation quality and early surgical complications			
Misplaced screws (postoperative CT), N (%)	1 (0.5%)	1 (1.9%)	0.362
Revision surgeries, N (%)	8 (3.7%)	1 (1.9%)	0.692
Early postoperative complications (surgical only), N (%)	9 (4.2%)	1 (1.9%)	0.692

Notes: Δ – blood loss documented in 70/215 (32.6%) of Group 1 and 7/54 (13.0%) of Group 2; fentanyl data – 204/215 and 52/54; Me – median; Q1-Q3 – interquartile range

Source: compiled by the authors of this study

period; late somatic complications (congestive pneumonia, pressure ulcers, deep vein thrombosis) were not tracked in the retrospective database.

DISCUSSION

FORENSIC SEVERITY CLASSIFICATION: COMPARISON WITH LITERATURE

The identified incidence of severe bodily injury by life-threatening danger (27.9% in the open group) corresponds to the frequency of clinically significant neurological deficit in patients requiring decompressive surgery. Izzy [5] emphasises that the degree of primary neurological deficit does not depend on the treatment method – consistent with our conclusion about severity classification as an immutable legal fact. The between-group differences (27.9% *vs.* 7.4%) reflect different clinical selection profiles: the percutaneous technique is predominantly applied in fractures without neurological deficit [10]. Crucially, no analogous studies on forensic medical assessment of TSCI exist; the finding of a complete absence of minor bodily injuries in the cohort is a practically significant landmark for forensic experts.

EFFECT OF SURGICAL METHOD AND TIMING ON INJURY CLASSIFICATION: TWO FORENSIC MECHANISMS

Although injury severity is an immutable legal fact, surgical treatment can influence classification through two mechanisms of the Rules (Ministry of Health of Ukraine Order No. 6, 1995). The first (clause 1.3) – reclassification by outcome: the final percentage of permanent loss is established after treatment completion (not earlier than 6 months). If neurological deficit progresses due to inadequate

treatment, a moderate-severity classification may be revised to severe (clause 2.1.6), entailing reclassification from Art. 122 to Art. 121 of the Criminal Code of Ukraine. Elnahhas et al. [4] in a 2026 meta-analysis confirmed that early decompression reliably increases the probability of transition to a higher Frankel level – meaning timely surgery can prevent negative reclassification. The second mechanism (clause 1.3) – classification of the consequences of inadequate medical care as a separate bodily injury: if a surgical error caused a new functional disorder, this may be independently classified under Art. 140 or 139 of the Criminal Code of Ukraine regardless of the primary injury. Equivalent implantation quality between groups ($p>0.05$) is a key finding: neither group demonstrates elevated risk of technical errors. When both life-threatening danger and permanent loss ≥33% are concurrently present, the criterion of greater severity applies; however, both must be separately documented in the expert report.

CAUSAL RELATIONSHIP AS THE CENTRAL CATEGORY OF FORENSIC ASSESSMENT OF CARE QUALITY

Establishing a medical care deficiency in TSCI requires not only identifying a deviation from the standard but also proving a causal relationship between the deviation and an adverse outcome. Documented blood loss is a key evidence element when assessing haemodynamic load in a patient with spinal shock: without this parameter, the link between surgical tactics and the progression of hypotension cannot be retrospectively established. Anaesthesia duration and fentanyl dose, fully documented in our sample, are objective markers of anaesthetic load and can serve as an evidentiary basis when assessing care quality. The absence of Frankel data at discharge and follow-up is an independent documentation deficiency that complicates the establishment of a relationship between surgical quality and the final percentage of permanent loss. As emphasised in our prior work [8, 9], incomplete medical documentation is interpreted against the medical institution in commission-based forensic expert assessments of care quality.

BLOOD LOSS, ANAESTHESIA, AND IATROGENIC RISK: COMPARISON WITH INTERNATIONAL DATA

The identified 16.3-fold difference in blood loss volume is the primary forensically significant parameter in the context of care quality assessment. Luo et al. [10], Daher et al. [11], and Esposito et al. [12] in meta-analyses confirmed significantly lower blood loss with the percutaneous technique (4-8-fold difference); Wajid et al. [7] also established a lower incidence of wound complications. The greater magnitude observed in our study (16.3-fold) may be explained by a more severely affected clinical cohort in the open group. The forensic significance of this parameter increases sharply in patients with spinal shock: blood loss of 300-400+ ml superimposes a hypovolaemic component onto neurogenic hypotension, which in patients with cardiovascular comorbidity may precipitate acute coronary syndrome or multi-organ failure. Incomplete documentation of blood loss (32.6% and 13.0%) substantially complicates retrospective forensic assessment of care quality [8, 9].

ANAESTHETIC LOAD AND MARKERS OF SURGICAL TISSUE TRAUMA

An identical anaesthesia protocol with a statistically significantly greater total fentanyl dose (30 *vs.* 22 ml; $p<0.001$) and anaesthesia duration (210 *vs.* 142 min; $p<0.001$) in the open group represents an independent iatrogenic factor. Dandurand et al. [6] emphasised that reducing anaesthetic load is a key advantage of the percutaneous approach for patients with reduced cardiovascular reserve. Hyperthermia in 32.1% of open *vs.* 3.7% of percutaneous surgeries ($p<0.001$) reflects a greater extent of surgical trauma to paravertebral tissues; Enache et al. [3] confirmed a greater inflammatory response with the open technique. Direct inflammatory markers (CRP, CPK) were not measured – a limitation and a subject for future research.

IMPLANTATION QUALITY: COMPARISON WITH INTERNATIONAL DATA

The equivalent incidence of misplaced screws (0.5% *vs.* 1.9%; $p=0.362$) and absence of significant differences in revision surgery rates ($p=0.692$) are consistent with Luo et al. [10], where the meta-analysis found no significant differences in technical complications between methods. Wajid et al. [7] also confirmed comparable accuracy of both methods when using fluoroscopic control. This is a key forensic conclusion: the use of the percutaneous technique under fluoroscopic control does not increase the risk of technical errors that could be classified as medical care deficiencies.

PERSPECTIVES

The priority direction is transition to a prospective design with mandatory long-term follow-up at 6- and 12-months post-surgery. This will allow objective evaluation of Frankel scale recovery dynamics and determination of the final percentage of permanent loss of general working capacity, which is critically important for forensic expert assessment of injury outcomes. Monitoring of biochemical markers of surgical trauma (C-reactive protein, etc.) is also planned for more precise iatrogenic risk verification.

CONCLUSIONS

Severe bodily injury by life-threatening danger was established in 27.9% of patients in the open group and 7.4% in the percutaneous group ($p=0.001$); permanent loss ≥33% was observed in 22.3% versus 7.4% ($p=0.012$). These differences reflect distinct clinical selection profiles rather than the influence of the surgical method on primary injury severity. Injury severity in the vast majority of cases is an immutable legal fact determined by the primary trauma.

All Frankel E patients without pelvic disorders (63.3% and 88.9%) were classified as moderate severity based on the duration of health disorder determined by vertebral fracture consolidation exceeding 21 days. No minor bodily injuries were identified in the cohort: permanent loss below 10% was not recorded, which represents a practically significant finding for forensic experts.

Surgical treatment may influence forensic classification through two mechanisms: reclassification of primary injury severity based on outcome, and classification of consequences

of medical care deficiency as a separate bodily injury. Timely and technically correct surgery potentially prevents both risks. When severity criteria overlap, each must be separately documented in the expert report.

Percutaneous stabilisation was associated with a 16.3-fold lower blood loss (20 *vs.* 325 mL; p<0.001), lower fentanyl dose (p<0.001), and shorter anaesthesia duration (p<0.001), with equivalent technical implantation quality (p>0.05). In patients with spinal shock and comorbidities, excessive blood loss and prolonged anaesthetic load are direct iatrogenic factors that may exacerbate a life-threatening condition.

A systematic absence of blood loss documentation in surgical and anaesthetic records, as well as a lack of records of anti-shock therapy, was identified. Both factors preclude direct retrospective verification of iatrogenic risk and the presence of spinal shock in forensic expert assessments of care quality; however, they may be classified by the expert as deficiencies in medical documentation.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Detoxification of some heavy metals by GST level and null genotypes of GST (GSTM1 and GSTT1) in car repairing workshops workers

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ABSTRACT

Aim: To evaluated efficiency of heavy metals detoxification by GST level and null genotypes of GST in car repairing workshops workers

Materials and Methods: Samples included 32 male workers with an average age 34 year. Level of heavy metals (Cd and Pb) and GST were measured, GSTT1 and GSTM1 null genotyping were determined by PCR.

Results: significant association were found between socidemographic variables. Significant higher concentration of Pb, Cd and GST in case group compared to control group $p<0.05$, no-significant effect of disease present or smoking in Pb, Cd and GST levels, in the case group, significant inverse correlation in age with Cd. A Significant inverse association between Cd and Pb in case group but in control group, no significant correlation among other variables. Linear regression of GST level on Pb and Cd showed non-sig effect ($b=0.041$, $p=0.884$ of Cd and $b=-0.957$, $p=0.973$ of Pb) in case group, significant association of GSTT1 null genotyping (OR=34.7561, CI95%=1.9448 to 621.1255 at $p=0.0159$) with risk of toxicity, the null of GSTT1 also showed sig significant impact on Pb and Cd levels in the case group ($p= 0.000$ and 0.006 respectively), while GSTM1 showed non -significant.

Conclusions: Results suggested elevation of heavy metals PB, Cd levels and GST activity in case group may be significantly influence by the GSTT1 null genotyping, indicating a potential role in reduce detoxification capacity and increase health risk in exposed worker.

KEYWORDS: detoxification, heavy metals, gst level, null genotypes, gst, car repairing workshops workers

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INTRODUCTION

Pollution refer to increase in the concentration of materials-whether solid, liquid or gaseous in the environment, it may contribute the introduction of different types of energy, like radiation, thermal or sound that negatively impact ecosystems and living organism [1]. Pollution is one of the most pressing challenges faced by modern societies, impacting all forms of life, such as humans, plants and animal. Some countries practically in the Arab world and other developing regions are string to identify the causes of pollution and implement effective solutions [2]. The heavy metals term refer to a group of metallic elements with relatively high density (typically five times greater than that of water). These metals pose significant health and environmental risk bases on their toxicity, bioaccumulation, persistence and their ability to enter the food chain [3]. Heavy metals contaminate all components of the ecosystem, like soil, water and air, human activities especially since the industrial revolution- have intensified environmental metal pollution, as well as through mining, electroplating, steel production, machinery manufacturing and agriculture [4-6]. Heavy metal toxicity often results from long or chronic exposure through environmental media like air, water, food and numerous consumer products. Prolong is linked to different chronic disease and health complications. Workers in the automotive repair industry are particularly vulnerable to such exposures, they frequently come into contact with particulate matter, carbon monoxide, inorganic solvent, welding fumes, isocyantes in paints and heavy metals, these molecules are inhaled and over time interact with body tissue potentially leading to sever health outcome [7].

MATERIALS AND METHODS

STUDY DESIGN

Setting and duration: A case – control design was conducted between December 2023 and January 2024 in various automobile repairing centers located in Baghdad province, Iraq.

STUDY POPULATION

The study subjects comprised from 32 case worked as car mechanics with different duration of working range from (1-10 years), the control group study included 30 male apparently healthy and this group matched with case study group, all participant on condition that informed consent prior to their inclusion in the study.

DATA COLLECTION

Data collection was provided using structured Questionnaire that including information name, age, occupation, duration of working, presence and duration of disease, medication intake, weight, height, alcohol drinking, smoking habit and social status.

BLOOD SAMPLES COLLECTION

Blood samples were drawn from workers and control individuals in the sitting position. Three ml of blood were divided to one ml for serum isolation and the remaining 2 ml stored in the EDTA tubes at- 20˚C which used for heavy metal determination and DNA extraction, sera stored at -20˚C until GST level detection.

LEAD AND CADMIUM MEASUREMENT

Lead and cadmium determined by acid digestion method (Inyengar et al., 1998). About 10 ml of HNO3 was added to 1 ml of blood, treated with heat until being colorless, the liquids was completed to 25 ml with de-ionized water after being cool. Then analyzed with a calibrated (Buck 205 flame atomic absorption spectrophotometer) in college of science, Babylon University. DNA isolation was implemented by extraction kit the target sites of GST genes were amplified using primers that previous mention by [8] the deletion mutation was detected by failed in amplification target site belong to the deletion sequence in GSTT1 and GSTM1 genes. The GST level was detected using colorimetric method.

STATISTICAL ANALYSIS

Data was presented as mean ± SD, statistical analysis carried out SPSS 26 version. Independent sample T-test, odd ratio, CI95% and linear regression model was used to evaluate the significant changes between the groups. P values less than 0.05 is statistically significant.

RESULTS AND DISCUSSION

THE DISTRIBUTION OF STUDY GROUPS (CASES *VS* CONTROL)

This study revealed that there were significant difference between the case and control group regarding the presence of chronic disease, social status, having children and smoking habit. In contrast, no significant association was found between the two groups concerning alcohol consumption as present in Table 1.

The significant difference between cases and control group regarding the prevalence chronic disease may be attributed to severity of adverse health effects associate with the chemical structure of heavy metals as well as the duration and dose of exposure. Heavy metals intoxication can have both acute and chronic impacts on human health and environment, while the toxicity of heavy metals at high exposure levels is well established, there is growing concern over the health risk of chronic exposure to low level, which may still lead to serious health consequences, one of the emerging areas of interest is the contribution

Table 1. Distribution of study groups (cases vs control) according to study variables

Variable	Control N (%)	Cases N (%)	Chi2	P-value
Chronic disease				
Yes	2 (6.6 %)	8 (25 %)	3.847	0.04983*
No	28 (93.3 %)	24 (75 %)		
Social status				
Married	0 (0 %)	24 (75 %)	33.048	0.000*
Single	30 (100%)	8 (25 %)		
Have children				
Yes	0 (0%)	24 (75 %)	33.04	0.000*
No	30 (100%)	8 (25 %)		
Smoking habit				
Yes	24 (80 %)	15 (46.8 %)	7.28085	0.0069*
No	6 (20 %)	17 (53.1 %)		
Alcohol drinking				
Yes	5 (16.6 %)	1 (3.12 %)	3.24839	0.07149
No	25 (83.3 %)	31 (96.8 %)		

**: significant at p value ≤0.05*

Source: compiled by the authors of this study

of heavy metals to cardiovascular disease although this relationships remains incompletely understood, recent researches suggested that the vascular effect of heavy metals may have impact in different pathological conditions like hypertension and diabetes mellitus [9-10]. In addition a study implemented by [11] found lead poisoning is associated with iron deficiency anemia. Individuals with iron deficiency tend to absorb more lead, making them more susceptible to its toxic effect. The prevalence of chronic obstructive pulmonary disease is also strongly linked to tobacco smoking, however, in many regions outdoor, occupational and indoor air pollution particularly from the burning of biomass fuels are also major risk factors [12-13]. Smoking has long be recognized as a major health hazards, it increased the risk of various disease including many kinds of cancer, COPD, coronary heart disease, stroke, peripheral vascular disease, and peptic ulcer disease [14-15]. This study also demonstrated that there were valuable mean differences $p \leq 0.05$ in age between cases and the control groups, furthermore, significant differences $p \leq 0.05$ were found in the level of heavy metals as summarized in Table 2.

Most of participants had not heard about lead poisoning, indication that automobile mechanics in the study area have limited had little or no knowledge about lead and its toxic effects. This highlight a critical gab in awareness regarding the health risk associated with lead exposure and routes through that it enters the body. Regrettably, this lack of knowledge is concerning and contradicts the finding [16] where inhalation of lead particles was reported more frequently than dermal absorption as the primary route of exposure, without proper awareness, these workers are at increased risk of developing serious health complications related to chronic lead exposure, moreover, a high percentage of participant reported not using personal protective equipment like face mask, hand gloves or protective garment while at work. This suggests that their limited knowledge is reflected in poor preventive practice, further increasing their vulnerability to lead related health hazards. In study [17], how found that personal habits in the workplace have major role in increasing exposure to lead smelters, automobile mechanics, and gasoline retails, some studies have linked elevated blood lead level to poor workplace habits [18-19]. This may be attributed to the ingestion of lead through contaminated hands or enhanced absorption of inhaled lead molecules due to inadequate protective practices, (contamination from hands during work) or increased absorption of inhaled lead. Interestingly, this study observed that majority of the automobile mechanics were non-smokers with only 2(5.4%) reporting daily smoking. Despite significant progress in reducing lead exposure through test like elimination of leaded gasoline, removal of lead in paint, food can soldering and glazed ceramics, lead contamination remains a major environmental health issue in specific communities and high – risk population [20]. Cadmium accumulates in the human body is especially concerning due to its persistence, as the body cannot effectively eliminate it, cadmium fumes are known to irritate the mucous membranes of the respiratory tract, potentially leading to that respiratory complications [21]. The significantly elevated blood cadmium concentration observed in the occupationally exposed automobile repair workers compared to non-exposed controls highlight the increased risk of cadmium related health hazards among these workers this finding aligns with previous reports that have recorded elevated cadmium levels in the blood of auto mechanics [22-23], spray painters, and battery recyclers [24]. Furthermore, this study showed that the mean blood cadmium levels in the exposed group were above the WHO's permissible range of 0.03-0.12 µg/dl of Cd [25]. Underscoring the need for intervention and occupational safety measures. Literatures on the exposure pathway to cadmium, chromium, and nickel indicates that individuals may come in to contact with these heavy metals through scrapped paint dust by multiple ways, like ingestion, inhalation of airborne molecules and dermal absorption such findings are consistent with the exposure routes observed in our study, as well as ingestion exposure has been documented by [26-27], while dermal and inhalational (nasal) exposure have been highlighted in studies by [28, 30-31]. These studies underscore the potential health risk associated with occupational environments where heavy metal laden dust is prevent Heavy metals can accumulate and persist in different tissue like cells, bones, glands and hair [29], Scrap soil aerosols, car paint dusts, and fugitive dust laden heavy metals especially particles smaller than 10 µml are lightweight and microscopically allowing them to travel considerable distances, dependent on the meteorological conditions of area, upon inhalation, these particles can become trapped in the tracheobronchial and alveolar- bronchial region of the respiratory system, notably, the penetration of inhalator molecules in to lower airways is inversely related to their size, with particles larger than 0.5 µm showing reduced infiltrations [30]. Biologic and physicochemical

Table 2. Difference in age, heavy metals and GST concentrations between case and control groups

Parameters	Case	Control	P
Age	23.1000±.42168	34.6875±1.64821	0.000
Cadmium (cd)	84.24±20.58	26.72±24.84	0.000
Lead (pb)	3.2328±.11845	2.8969±.03485	0.006
GST Level	128.18±3.015	119.30±3.40	0.05

Source: compiled by the authors of this study

factors significant effects the movement and behavior of settled particulates within the respiratory tract, these factors affect the dissolution of particles, their entry in to body fluid and blood and their clearance through phagocytosis, additionally, the mobility of these particles with mucus, as well as their adhesion and dispersion various across different sections of the respiratory. This variation in particles dynamic reflect the complex interaction between the respiratory environment and contaminations [31]. Heavy metals Level rates and GST concentrations in control group and case study according to the presence of chronic disease: According to the statistical analysis of cars repairing mechanics data, the finding showed that there were significant differences between group with the presence of chronic diseases and the other group without chronic disease according to age, while there were no significant differences between other parameters (Lead and Cadmium) concentrations, while the statistical analysis of control group presented there were no significant difference according to age and heavy metals concentration between group with chronic disease and the other group without chronic disease and significant differences in GST level in control and non-sig in case group as demonstrated in Table 3.

Level rates of heavy metals and GST concentrations in control and case study subjects according to smoking habit: According to the statistical analysis of control group and cars repairing mechanics data, the results showed that there were no significant changes between smokers and non-smokers groups in both control group and case study group according to heavy metals and GST concentrations as explorer in Table 4

Level rates of heavy metals and GST concentrations in control and case study subjects according to smoking habits showed significant inverse correlation in age with cadmium. Significant inverse association of Cd with pb in case group, while in case study subjects the correlation analysis showed no significant correlation among variables (Table 5).

A study conducted on workers in cars repair who deal with chemicals, oils and car paints. etc., showed that there was no significant inverse correlation with cadmium and no significant positive correlation with lead for workers in this field with experience years (duration of work) [32] and It agrees with the findings of the current study conducted on car repair workers. Another report found that individual over than 11 years of occupational exposure exhibit significantly

Table 3. Level rates of heavy metals concentration in case study according to the presence of chronic diseases

Parameters	Control		Case study	
	Disease yes	Disease No	Disease yes	Disease No
Age	24.0000±1.4142	23.0357±2.36459	46.2500±4.97853	30.8333±6.90096
P	0.577		0.000*	
Cadmium (cd)	38.5000±4.94975	25.8519±2.54288	78.7500±15.56324	89.8750±21.51706
P	0.497		0.189	
Lead (pb)	3.2450±0.04884	3.2319±0.66190	2.9163±0.23525	2.8904±0.18802
P	0.978		0.754	
GST level	82.30±1.58	123.57±23.03	128.56±7.79	128.05±3.148
P	0.00		0.942	

Source: compiled by the authors of this study

Table 4. Level rates of heavy metals and GST concentrations in control and case study subjects according to smoking habit

Parameters	Control		Cases	
	Smoking	Non smoking	Smoking	Non smoking
Cadmium (cd)	23.12±20.65	44.00±37.66	89.0±22.494	85.41±19.20
P-value within group	0.087		0.630	
Lead (pb)	3.20±0.698	3.35±0.132	2.92±0.192	2.87±0.204
P-value within group	0.643		0.519	
GST level	119.0±4.02	120.72±4.65	125.00±3.74	130.87±4.57
P-value within group	0.853		0.340	

Source: compiled by the authors of this study

Table 5. Correlation analysis among variables in control and subject cases (white color for case and pink foe control group)

Variables		age	duration	cd	pb	GST level
Age	r	1	0.718**	-0.343*	0.141	-0.141
	p		0.000	0.043	0.419	0.419
Duration	r	nd	1	-0.247	0.062	0.098
	p	nd		0.152	0.722	0.574
cd	r	-0.136	nd	1	-0.792**	0.069
	p	0.456	nd		0.000	0.693
pb	r	-0.042	nd	0.030	1	-0.082
	p	0.819	nd	0.871		0.640
GST level	r	-0.301	nd	-0.022	0.122	1
	p	0.113	nd	0.911	0.530	

***significant at the 0.01*

**significant at the 0.05*

Source: compiled by the authors of this study

Table 6. Effect of GST level with Cd and pb

	Cases		Control	
Heavy metals	Beta	Sig	Beta	Sig
cd	0.041	0.884	0.016	0.894
pb	-0.957	0.973	3.345	0.464
Duration	0.425	0.577	-	-

Source: compiled by the authors of this study

higher blood lead levels compared to those 6-10 years of service. This finding aligns with the results by [33] who found a gradual increase in blood lead concentration correlated with longer duration of occupational exposure. These results are consistent with the current study's finding, further emphasizing the cumulative effect of prolong exposure to lead in occupational setting. Cadmium is a prevalence component in welding fumes, spray paint pigments (e. g. cadmium yellow), cadmium based batteries, and tobacco products. These sources ma account for the increased blood cadmium levels observed among the auto repair workers in the present study. The primary route of cadmium exposure is inhalation, particularly through occupational activities like welding and spray painting, however, additional exposure pathways include cigarette smoking and ingestion contaminated food, often facilitated by poor personal hygiene practices in the workplace. at work due to poor personal hygiene at the place of work. The health hazards linked to cadmium toxicity are well documented and include obstructive lung disease and lung cancer [34-35]. The effect of GST level with Cd and pb was evaluate by linear regression model, the results showed non-significant effect of GST in these metals in case and control group (Table 6). Figure 1 showed study samples distribution according to linear regression model, GST level is dependent variables.

The GST (T1 and M1) genotyping, results demonstrated significant association of GSTT1 null genotyping with case than control group p=0.015 and non-significant differences in GSTM1 (Fig. 2).

The level of heavy metals and GST levels according to GSTT1 and GSTM1 null genotyping's in case and control group were demonstrated in table 7, the results found significant effect of GSTT1 in cd and Pb levels while non-significant in GST level, other genotyping like null genotyping in the control group in both GST types were didn't report, and GSTM1 null genotyping in case group also.

The null genotyping of GSTT1 and GSTM1 have a crucial role in detoxification of heavy metals such as Cd and Pb by conjugation them with glutathione to facilitating excretion out of body, the deletion or null genotyping in these genes can impair this processing and increased toxicity by heavy metals [35, 36]. The GSTT1 null genotyping have been observed to exhibit higher concentration of Pb and Cd in the blood in the children exposed to polluted environment, they found positive correlation between GSTT1 deletion and high level of heavy metals ($\beta = 0.11$ for Pb, $p = 0.02$; $\beta = 0.10$ for Cd, $p = 0.01$), moreover, the combination between GST (T1 and P1) have been associated with risk of toxicity by pb as a synergism effect of these genes variations in detoxification [35], on the other hand the

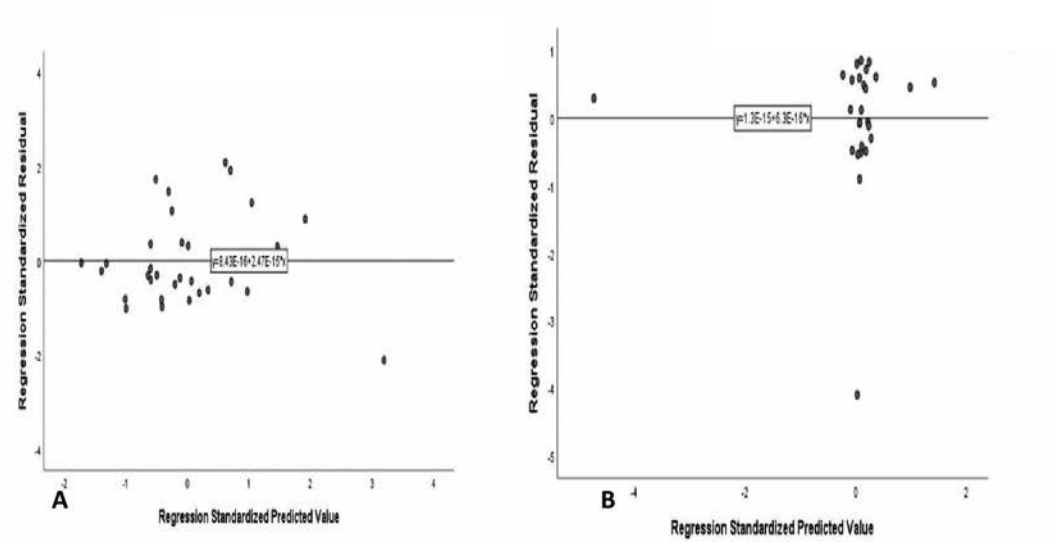
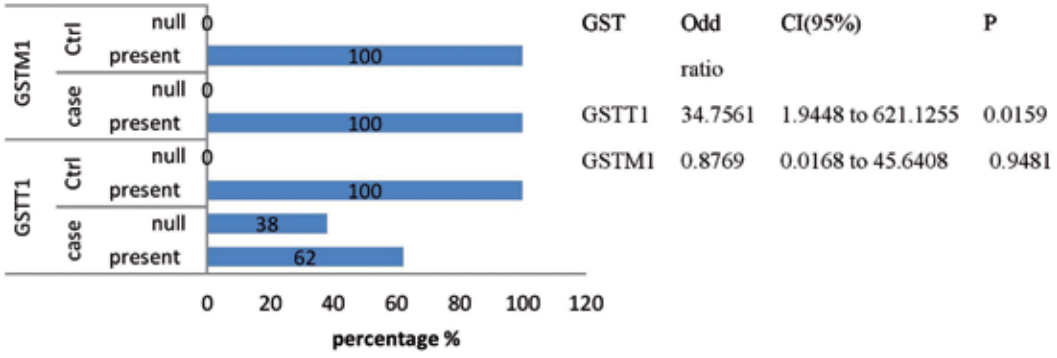


Fig. 1. Study samples distribution according to linear regression model (A, case; B control)

Source: Own materials



GST	Odd ratio	CI(95%)	P
GSTT1	34.7561	1.9448 to 621.1255	0.0159
GSTM1	0.8769	0.0168 to 45.6408	0.9481

Fig. 2. Percentage of study samples (case and control group) according to GSTT1 and GSTM1 null genotyping (odd ratio, CI95%)

Source: Own materials

Table 7. Level of heavy metals and GST levels according to GSTT1 and GSTM1 null genotyping's in case and control group

Variables	Case			Control		
GSTT1	Present	null	sig	Present	null	sig
Cd	79.70±19.51	99.41±16.33	0.006	26.72±24.84	-	nd
Pb	2.98±0.167	2.74±0.150	0.000	3.23±0.637	-	nd
GST level	125.78±16.77	132.00±18.8	0.315	121.40±14.80	-	Nd
GSTM1	Present	null	sig	Present	null	sig
Cd	84.24±20.58	-	nd	26.72±24.84	-	nd
Pb	2.92±0.198	-	nd	3.23±0.63	-	nd
GST level	127.80±17.43	-	nd	121.38±14.74	-	nd

Source: compiled by the authors of this study

prolong exposure to Cd has been found to decrease total GST activity in the organs as well as liver, heart, and ling, which lead to impair in body ability to Cd detoxification and increased oxidative stress and tissue injury. The GST enzyme facilitate the conjugation of GSH with electrophilic compound as well as heavy metals, rendering them more water soluble and easer to excrete, this a vital mechanism to protect cell from oxidative damage by heavy metals like Cd and Pb, the efficiency of detoxification pathway was impaired by null genotyping of GST genes lead to high retention of heavy metals in the body and increased side effects [35].

CONCLUSIONS

The conclusion of this study revealed that there was significant association of heavy metals (Cd and Pb) level and GST concentration with case subjects, no significant effects of disease and smoking habit in the Pb, Cd and GST levels, in case there was significant correlation between Pb and Cd in case, no-sig effect of GST level in the Cd and Pb in case and control group, moreover, significant association of GSTT1 null genotyping with case group that impact in the Cd and Pb levels in case group.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Factors associated with ocular structural changes inherent to pseudoexfoliation syndrome among Ukrainian patients

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ABSTRACT

Aim: To identify factors associated with ocular structural changes inherent to PEX among Ukrainian patients.

Materials and Methods: The study was based on a clinical examination of 56 patients (112 eyes) aged 14 to 88 years. The main group included 39 patients (78 eyes) with ocular structural changes confirming the diagnosis of PEX: 14 patients (28 eyes) and 25 patients (50 eyes) with uni- and bilateral lateral involvement, respectively. The control group consisted of 17 patients (34 eyes) without any signs of PEX. The follow-up period was 5 years.

Results: The findings of the study support the concept of PEX as a systemic fibrillopathy, accompanied by a significant association with chronic coronary syndrome (64%), joint pathology (72%), diabetes mellitus (40%), and central nervous system (56%) disorders compared with the control group. Environmental factors, particularly urbanization (52%) and increased radiation background (64%), are likely additional contributors to disease development. The study demonstrated that bilateral PEX is accompanied by changes in eye structures: pronounced trabecular pigmentation (48%), a critical decrease in corneal endothelial cell density (80%), progression of age-related macular degeneration (40-44%), and the development of posterior subcapsular cataract (96%). In contrast, patients with unilateral PEX demonstrated a significantly higher long-term risk of nuclear cataract progression.

Conclusions: Managing patients with PEX necessitates a comprehensive multidisciplinary approach in view of the associated comorbidities and contributing factors. The clinical course of unilateral PEX is strongly linked to exposure to exogenous triggers (urbanization, increased background radiation, and tobacco smoking). The absolute majority of eyes with bilateral PEX showed biomechanical weakness of the sclera and cornea.

KEY WORDS: pseudoexfoliation syndrome, disease laterality, cataract

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INTRODUCTION

Pseudoexfoliation syndrome (PEX) is a systemic ocular disease characterised by the production and accumulation of abnormal extracellular material in multiple tissues. According to the literature, PEX is one of the most common causes of secondary open-angle glaucoma (SOAG) and is associated with a higher risk of vision loss, higher maximum and mean intraocular pressure (IOP), and a wider range of IOP fluctuations compared with primary open-angle glaucoma (POAG) [1]. Despite significant advances in understanding genetic factors, particularly the lysyl oxidase-like 1 (LOXL1) gene marker found in over 90% of pseudoexfoliation glaucoma (PEXG) cases [2], the role of environmental and comorbid factors in the development of PEX remains an important unresolved issue in modern ophthalmology and a subject of active debate.

PEXG is a particularly aggressive type of SOAG that exhibits more rapid progression and a poorer response to medical therapy than POAG. Multiple genetic and environmental factors are involved in the pathogenesis of PEXG; although significant progress has been made in understanding these factors in recent years, this area of research remains far from conclusive.

Studies have identified mutations in the LOXL1 gene as a risk factor for PEXG [3]. The pathognomonic sign of PEX is the presence of white material on the lens capsule and other anterior chamber structures. The systemic nature of PEX is evidenced by alterations in the extracellular matrix of various organs and tissues, including the eyelid skin, heart, lungs, liver, kidneys, gallbladder, meninges, and vascular endothelium [4]. Currently, PEX is recognised as the most common identifiable cause of glaucoma. It has been shown that this syndrome leads to the development of SOAG, specifically PEXG, which poses a significantly greater risk of irreversible vision loss compared with POAG [4].

Observational data indicate that the presence of PEX significantly increases the frequency and likelihood of developing glaucoma [4]. Various genetic and environmental factors are associated with the onset of PEXG; however, their exact significance and impact on the development of the pathological process have not yet been fully elucidated [4, 5].

According to recent literature, the most substantiated theory of PEX pathogenesis is based on four key mechanisms: altered microRNA expression, autophagy dysfunction, probable mitochondrial mutations, and disruption of the blood-ocular barrier [4].

Other studies suggest that genetic alterations, such as polymorphisms in the LOXL1 gene, must also be considered. Patients with this syndrome have a tenfold higher risk of developing glaucoma [1]. However, according to these studies, assessment of LOXL1 is limited to detecting the presence of this marker in the aqueous humour, which consequently imposes significant limitations on its widespread application in clinical practice.

Thus, analysis of the current literature indicates that PEX is a key contributing factor to the development of systemic and ocular diseases. The majority of available research highlights the urgency of this issue and the need for further investigation into the primary factors driving the development and progression of PEX [2-5]. This is particularly important for the Ukrainian population, given that glaucoma has remained the leading cause of disability and blindness in Ukraine for the past five years. The need to elucidate the mechanisms underlying PEX-related complications, as well as to establish the causes of the development and progression of PEXG in this patient cohort, underscores the relevance of this study and defines its aim and objectives.

AIM

The aim of this study was to identify factors associated with ocular structural changes inherent to PEX among Ukrainian patients.

MATERIALS AND METHODS

The study was based on a clinical examination of 56 patients (112 eyes) aged 14 to 88 years, including 30 women and 26 men. The wide age range of the study participants was intentionally selected to determine the earliest age at which clinical signs appear and to analyze the current state of ocular structural and biomechanical parameters. The main group consisted of 39 patients (78 eyes) with ocular structural changes confirming the diagnosis of PEX. During the study, the main group was divided into two subgroups. The first subgroup included 14 patients (28 eyes) with unilateral involvement, while the second subgroup comprised 25 patients (50 eyes) with bilateral involvement. The control group consisted of 17 patients (34 eyes) without any signs of PEX. Patients were followed up for 5 years.

The inclusion criteria for the study were as follows: a clinically confirmed diagnosis of PEX (for the study group) or its absence (for the control group); written informed consent from the patient (or their legal representative) to participate in the study and undergo follow-up monitoring; and the patient's ability to adequately cooperate during the study procedures.

The exclusion criteria for the study were: patient refusal to undergo specific stages of diagnostic testing or failure to adhere to the examination schedule.

Each participant was provided with a questionnaire that included the following items: smoking status; alcohol consumption; absence of harmful habits; permanent place of residence; residence in an area with elevated background radiation; the presence of chronic diseases (diabetes mellitus [DM], central nervous system]CNS] diseases, chronic coronary syndrome [CCS], joint disorders, ear, nose and throat [ENT] diseases, thyroid disorders, obesity); and a family history of glaucoma.

To achieve the study objectives, all patients underwent a comprehensive ophthalmological examination, which included: visual acuity assessment; automated perimetry (Humphrey 750i, Zeiss); tonometry (Topcon, Japan; Icare IC200); gonioscopy (Ocular, USA); biomicroscopy (Ocular MaxField® 78D, USA); optical coherence tomography (OCT) and OCT angiography (OCTA) (REVO NX Version 10.0.0 Device SN: 2570793/06; Cirrus HD-OCT 5000, Zeiss); confocal imaging using the Heidelberg Retina Tomograph (HRT) II with the Rostock Cornea Module (Heidelberg Engineering Inc., Germany) to determine corneal endothelial cell density (ECD) and determination of ocular biomechanical properties, including the measurement of corneal (KER) and scleral (KES) rigidity coefficients. The biomechanical properties were measured according to the method described by N. Sergiyenko and I. Shargorodska [6]. The presence and severity of age-related macular degeneration (AMD) were assessed and categorized according to the Age-Related Eye Disease Study (AREDS) classification system.

Statistical data analysis was performed using EZR software, version 1.70. Quantitative variables were presented as median and interquartile range, whereas categorical variables were summarized as absolute numbers and percentages. Quantitative variables across the three independent groups were compared using the Kruskal–Wallis test, followed by post hoc pairwise comparisons with the Mann–Whitney U test. Categorical variables were compared using Fisher's exact test, with post hoc pairwise comparisons performed using the same test. A p-value of <0.05 was considered statistically significant, with Bonferroni correction applied for multiple comparisons.

ETHICS

The clinical studies were conducted in accordance with fundamental bioethical standards and the requirements of the Declaration of Helsinki, the Council of Europe Convention on Human Rights and Biomedicine (1977), relevant provisions of the World Health Organization (WHO), the Council for International Organizations of Medical Sciences (CIOMS), the International Code of Medical Ethics (1983), and Order No. 690 of the Ministry of Health of Ukraine dated September 23, 2009. All patients were fully informed about the study procedures and provided written informed consent prior to participation.

FRAMEWORK

The work was carried out at the Department of Ophthalmology and Optometry of Postgraduate Education of the Institute of Postgraduate Education of Bogomolets National Medical University and is a fragment of the initiative-research project «Theoretical and practical aspects of improving clinical and experimental methods of diagnosis, treatment and prevention of diseases and injuries of the organ of vision and their complications» (state registration number 0123U104207; term: 2023-2026).

AI STATEMENT

The author states that no AI tools were used in writing this article.

RESULTS

The results of the analysis of clinical and anamnestic characteristics in patients with PEX and the control group are presented in Table 1.

The analysis of the results (Table 1) revealed no statistically significant differences in baseline demographic characteristics among the study groups. No significant difference in age was observed among patients without PEX, with unilateral PEX, and with bilateral PEX. Regarding gender distribution, no significant differences were observed; however, a trend toward male predominance was noted in the bilateral PEX group (60%) compared to the control group without PEX, where females constituted the absolute majority (77%).

Furthermore, the analysis of environmental factors confirmed (Table 1) that urban residence was significantly more associated with unilateral PEX (79%) compared to the control group (29%). It was found that exposure to elevated background radiation was significantly more frequent in the unilateral PEX group (86%) compared to the control group (35%). Regarding the impact of active smoking, a trend toward a higher frequency was noted among patients with unilateral PEX (57%) compared to the non-PEX group (18%), as no significant differences were detected in multiple comparisons (Table 1).

It was found that CCS was present in 79% of patients with unilateral PEX and 64% of those with bilateral PEX, which was significantly higher than the rate observed in the control group (18%) (Table 1).

The analysis of concomitant joint pathology revealed statistically significant differences among the studied groups. Joint diseases were the least prevalent in patients without PEX, occurring in only 12% of cases. In contrast, the presence of PEX was associated with a significantly higher frequency of joint pathology: 64% in the unilateral PEX group and up to 72% in the bilateral PEX group when compared to the non-PEX control group.

The analysis of the prevalence of DM demonstrated a similar pattern: a trend toward a higher frequency of this pathology was observed in patients with unilateral PEX (43%) compared to the control group (6%). Meanwhile, a statistically significant difference was recorded between the bilateral PEX group (40%) and the control group (6%).

The results regarding the prevalence of CNS disorders revealed a statistically significant difference in patients with unilateral PEX (64%) compared to the control group (18%). Meanwhile, the frequency of this pathology among patients with bilateral PEX (56%) demonstrates a trend toward an increase relative to the control group (18%).

The prevalence of concomitant ENT disorders and comorbid thyroid pathology did not differ significantly among the study groups.

Although a history of glaucoma was quantitatively more frequent in patients with unilateral (29%) and bilateral involvement (28%), these data did not demonstrate a statistically significant difference relative to the control group (Table 1).

Notably, the evaluation of the anterior chamber angle revealed a significant difference regarding the presence of pronounced trabecular pigmentation (Grade III), which was observed in 48% of patients with bilateral PEX, compared to none in the control group. When comparing patients with bilateral (48%) and unilateral PEX (7%), a trend toward a higher prevalence of this feature was recorded in bilateral involvement (Table 1). Furthermore, trabecular pigmentation (Grade II) was identified in 28% of cases with bilateral PEX and in 36% of cases with unilateral involvement, alongside an absence of trabecular pigmentation (Grade I) in the bilateral PEX group.

The assessment of reduced corneal ECD (<2000 cells/mm^2) revealed a significant difference in patients with bilateral PEX (80%) compared to the control group (6%). Meanwhile, the difference in the frequency of this condition between patients with bilateral (80%) and unilateral PEX (29%) demonstrates a trend toward an increase in bilateral involvement (Table 1).

It was found that the prevalence of AMD (AREDS Category 2) was significantly higher in patients with bilateral PEX (44%) compared to the control group. Besides, the AMD (AREDS Category 3) was presented in 40% of patients with bilateral PEX (40%), as opposed to its absence among PEX free patients (Table 1). Furthermore, only 4% of patients with bilateral PEX and 64% of those with unilateral PEX had AMD (AREDS Category 1).

The analysis of biomechanical properties (Table 1) revealed that 100% of PEX patients, including both unilateral and bilateral cases, exhibited impaired biomechanical properties of the ocular fibrous capsule: KER > (+)1.2 and KES > (+)0.12.

Baseline lens assessment demonstrated that the prevalence of posterior subcapsular cataract was significantly higher in patients with bilateral PEX (96%) compared to the control group (29%). Furthermore, a significant difference was observed between patients with bilateral involvement (96%) and those with unilateral PEX (50%), confirming substantially more severe local lens changes specifically in bilateral involvement (Table 1).

The analysis of long-term disease outcomes warrants special attention. According to the results of a 5-year follow-up, nuclear cataract progression was recorded in 36% of patients with unilateral PEX. This rate was significantly higher than that observed in the bilateral PEX group, in which progression occurred in only 4% of cases (Table 1).

DISCUSSION

The current scientific paradigm considers PEX to be a degenerative multisystem pathology extending well beyond the ophthalmological domain. According to studies by N. Yüksel et al. [1] and M. Tomczyk-Socha et al. [4], PEX is associated with pathological changes in the vascular extracellular matrix and in various internal organs.

The results of our study in the Ukrainian population provide confirmation of this concept, demonstrating a

Table 1. Demographic, clinical and ophalamological characteristics of patients with and without PEX

Parameter		Without PEX n=17	Unilateral PEX n=14	Bilateral PEX n=25	p
Demographics					
Age, years		60 (45-68)	66 (53-73)	67 (51-77)	0.308
Sex, N (%)	Males	4 (23)	7 (50)	15 (60)	0,073
	Females	13 (77)	7 (50)	10 (40)	
Males <60 years, N (%)		3 (18)	3 (21)	7 (28)	0.725
Males ≥60 years, N (%)		1 (6)	4 (29)	8 (32)	0.108
Females <60 years, N (%)		4 (23)	2 (14)	3 (12)	0.585
Females ≥60 years, N (%)		9 (53)	5 (36)	7 (28)	0.243
Urban dwelling, N (%)		5 (29)	11 (79)	17 (68)	p1-2=0.034
Lifestyle & Environment					
High background radiation, N (%)		6 (35)	12 (86)	16 (64)	p1-2=0.028
Active smoking, N (%)		3 (18)	8 (57)	13 (52)	0.044
Adipositas, N (%)		5 (29)	5 (36)	11 (44)	0.685
Alcohol abuse, N (%)		3 (18)	6 (43)	11 (44)	0.194
Comorbidities					
CCS, N (%)		3 (18)	11 (79)	16 (64)	p1-2=0.003 p1-3=0.014
Joint disorders, N (%)		2 (12)	9 (64)	18 (72)	p1-2=0.020 p1-3<0.001
DM, N (%)		1 (6)	6 (43)	10 (40)	p1-3=0.048
CNS disorders, N (%)		3 (18)	9 (64)	11 (56)	p1-2=0.036
ENT disorders, N (%)		5 (29)	4 (29)	6 (24)	0.930
Thyroid disorders, N (%)		10 (59)	3 (21)	10 (40)	0.110
History of glaucoma, N (%)		3 (18)	4 (29)	7 (28)	0.730
Ocular Clinical Findings					
Trabecular pigmentation, N (%)	Absent	15 (88)	0	6 (24)	p1-2<0.001 p1-3<0.001
	Grade I	2 (12)	8 (57)	0	p2-3<0.001
	Grade II	0	5 (36)	7 (28)	p1-3=0.035
	Grade III	0	1 (7)	12 (48)	p1-3=0.002 p2-3=0.039
ECD (<2000 cells/mm2), N (%)		1 (6)	4 (29)	20 (80)	p1-3<0.001 p2-3=0.039
AMD	Absent	14 (82)	0	1 (4)	p1-2<0.001 p1-3<0.001
	AREDS Category 1	3 (18)	9 (64)	1 (4)	p1-2=0.036 p2-3<0.001
	AREDS Category 2	0	5 (36)	11 (44)	p1-2=0.035 p1-3=0.004
	AREDS Category 3	0	0	10 (40)	p1-3=0.008 p2-3=0.020
	AREDS Category 4	0	0	2 (8)	0.497
KER > (+)1,21		1 (6)	14 (100)	25 (100)	p1-2<0.001 p1-3<0.001
KES > (+)0,12		2 (11)	14 (100)	25 (100)	p1-2<0.001 p1-3<0.001

Table 1. Cont.

	Lens Status			
Posterior subcapsular cataract, N (%)	5 (29)	7 (50)	24 (96)	p1-3<0.001 p2-3=0.004
Nuclear opacity progression*, N (%)	4 (23)	5 (36)	1 (4)	p2-3=0.049

*Notes: * – at 5-year follow-up; p1-2 – the significance of difference between patients without PEX and unilateral PEX; p1-3 – the significance of difference between patients without PEX and bilateral PEX; p2-3 – the significance of difference between patients with uni- and bilateral PEX*

Source: compiled by the authors of this study

significant association between PEX and CCS. However, in contrast to literature data that predominantly emphasise age and sex – as reported by M. Tomczyk-Socha et al. [4] – our analysis revealed a specific regional factor, namely exposure to elevated background radiation associated with the development of PEX. While this association was statistically significant primarily when comparing the unilateral PEX subgroup with the control group, it is important to note that this environmental factor was also highly prevalent, occurring in 64% of patients with bilateral PEX.

It should be noted that the genetic determination of PEX, particularly through LOXL1 gene polymorphisms, as reported by S. Miglior and F. Bertuzzi [3], leads to profound structural changes in the fibrous tunic of the eye. While the global literature places significant emphasis on blood–ocular barrier disruption and autophagy mechanisms, as evidenced by the publication of M. Tomczyk-Socha et al. [4], our results expand this understanding from a clinical biomechanics perspective.

Thus, pathological alterations in the rigidity coefficients of the cornea (KER > +1.2) and sclera (KES > +0.12) were established in 100% of the patients with PEX in the study group. Our findings, which indicate a correlation between impaired rigidity of the ocular tunics and the development of PEX, are consistent with the classical hypothesis of N. Sergiyenko and Y. Kondratenko (1998) regarding the role of hydrodynamic disturbances as a trigger for decompensation in structural refractive anomalies of the eye – a mechanism that assumes a particularly aggressive course in the presence of the pseudoexfoliative process [7-10].

Our results regarding the predominance of posterior subcapsular cataract in patients with bilateral PEX and its accelerated maturation compared to the control group are associated with the findings of M. Tomczyk-Socha et al. [4] regarding the high risk of blindness associated with this pathology.

An interesting clinical finding of our study is the significantly higher rate of nuclear cataract progression in patients with unilateral PEX (36%) compared to those with bilateral PEX (4%). This paradox could be explained by a combination of metabolic and chronological factors. Pathophysiologically, unilateral PEX may represent an actively progressive, asymmetrical phase of the disease characterised by an acute breakdown of the blood-aqueous barrier. This leads to profound local oxidative stress and a rapid decline in aqueous humor antioxidants, accelerating lens opacification in the affected eye. Chronologically, bilateral PEX typically indicates a more chronic and advanced systemic manifestation of the syndrome. Patients in the bilateral group may have already experienced maximum densitometry changes in the lens core before the follow-up period, or the lens changes had already stabilised. Further longitudinal biochemical studies of the aqueous humour in unilateral versus bilateral PEX are needed to fully elucidate these mechanisms.

The obtained results indicate the need for further in-depth research, as the identified triggers may have a profound impact on the development of secondary optic neuropathy and the likelihood of PEX progressing to PEXG. Furthermore, we consider it appropriate to conduct further investigation of the LOXL1 gene to determine the extent of its influence on the development of PEX and PEXG within the Ukrainian population.

Several limitations of this study should be acknowledged. First, the relatively small sample size of 56 patients indicates the need for larger-scale studies to enable more robust statistical analysis, particularly for the detection of rare complications. Second, because the study focused specifically on the Ukrainian population, extrapolation of these results to other ethnic groups may be limited due to the known genetic heterogeneity of PEX. Furthermore, it is challenging to completely isolate the independent effect of PEX from the influence of concurrent systemic diseases on ocular biomechanical properties, particularly corneal and scleral rigidity.

Despite these limitations, the presented results justify the need to evaluate specific systemic markers and biomechanical parameters (KER and KES) in the Ukrainian patient population. This comprehensive approach is essential for optimising surgical strategies for cataract treatment and identifying potential pathways for preventing the development of SOAG.

Changes in ocular rigidity represent important clinical factors that may affect diagnostic accuracy and surgical outcomes. Therefore, they should be considered when interpreting tonometric measurements and calculating intraocular lens (IOL) power. Further studies are warranted to clarify their clinical significance.

Perspectives of subsequent scientific research

The results obtained in this clinical study are the basis for a more detailed study of the possibilities of diagnosing glaucoma at preclinical stages and determining the possibilities of stabilizing its progression.

CONCLUSIONS

The present study demonstrated that the most significant factors associated with the development of PEX and subsequent ocular biomechanical changes in the Ukrainian population are CCS, joint disorders, and residence in areas with elevated background radiation.

The severity of PEX, compounded by these systemic factors, directly determine the progression of specific intraocular structural alterations, including corneal endothelial decompensation, the development of posterior subcapsular cataracts, and the deterioration of the biomechanical properties of the fibrous tunic.

The changes in ocular rigidity represent important clinical factors that may affect diagnostic accuracy and surgical outcomes. Further studies are warranted to clarify their clinical significance.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Biopsychosocial model of inflammatory bowel diseases: The impact of multisystem comorbidity and mental health on patients' quality of life

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ABSTRACT

Aim: To determine the association between the presence of comorbid pathology, the frequency and nature of extraintestinal manifestations, and changes in different health-related quality-of-life domains in patients with inflammatory bowel diseases.

Materials and Methods: A total of 248 patients with inflammatory bowel disease were examined, including 117 with Crohn's disease (Group I) and 131 with ulcerative colitis (Group II). The control group consisted of 82 apparently healthy individuals. Patients received inpatient treatment at healthcare institutions in Ivano-Frankivsk. The diagnosis was established in accordance with current clinical guidelines. Quality of life was assessed using the IBDQ questionnaire, while comorbidity was evaluated using the Cumulative Illness Rating Scale (CIRS) and the Charlson Comorbidity Index.

Results: Patients with IBD demonstrated a statistically significant decrease in the overall IBDQ quality-of-life index compared with the control group (119.3±10.48 in Crohn's disease and 127.4±8.3 in ulcerative colitis versus 198.5±12.6; $p<0.001$), corresponding to a reduction of 39.9% and 35.9%, respectively. The most pronounced impairments were observed in the systemic and social domains, particularly in patients with Crohn's disease. A high prevalence of comorbid pathology was identified (73% in Crohn's disease and 82% in ulcerative colitis), along with a substantial burden of extraintestinal manifestations, characterized by predominant multisystem involvement in Crohn's disease and mental and vascular disorders in ulcerative colitis. The Charlson Comorbidity Index was higher in Crohn's disease (3.14±0.27) compared with ulcerative colitis (2.93±0.14; $p\leq0.005$), indicating a less favorable prognosis in this patient group.

Conclusions: Inflammatory bowel diseases are associated with a marked deterioration in quality of life and a high comorbid burden, substantiating the need for a comprehensive multidisciplinary approach to the management of such patients.

KEYWORDS: Inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, quality of life, comorbidity, extraintestinal manifestations, IBDQ

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INTRODUCTION

Inflammatory bowel diseases (IBD), including Crohn's disease and ulcerative colitis, are chronic immune-mediated inflammatory disorders characterized by a relapsing-remitting course and represent a significant medical and socio-economic burden in contemporary medicine [1]. Epidemiological data indicate that approximately 2.2 million people in Europe and over 6 million individuals worldwide are affected by IBD [2]. Despite relatively low mortality rates, these conditions are associated with a substantial non-fatal burden due to their chronic course, frequent relapses, development of complications, and the need for long-term therapy. Another important aspect is the considerable economic burden of treatment, particularly driven by the increasing use of biologic therapies, as well as the risk of disability among young, economically active patients [3].

According to recent international studies, direct annual costs per patient vary considerably across regions but consistently exceed those observed in the general population. A large systematic review covering the past decade reported that the average annual direct costs per patient with Crohn's disease were approximately USD 4,417 (1,230-31,161) in Asia, EUR 12,439 (7,694-15,807) in Europe, and USD 17,495 (14,454-20,535) in North America. For patients with ulcerative colitis, costs were somewhat lower but remained substantial: USD 1,606 in Asia, EUR 7,224 in Europe, and USD 13,559 in North America [2]. Regional studies further confirm these trends. In a population-based study conducted by Brunet-Mas et al. in Catalonia (Spain), direct costs per patient with IBD ranged from EUR 4,200 to 7,200. Although Asia demonstrates lower overall costs, substantial variability is observed [3].

Pharmacotherapy represents the main cost driver, accounting for more than 60% of total treatment expenditures. A similar trend has been observed in Sweden, where between 2007 and 2020 a marked increase in medication-related costs was reported, primarily driven by biologic therapies, with a 274% increase for Crohn's disease and a 638% increase for ulcerative colitis.

A characteristic feature of contemporary clinical practice is the frequent coexistence of multiple pathological conditions in a single patient. Current evidence indicates that the clinical course of IBD is not limited to gastrointestinal

involvement alone. A substantial proportion of patients develop extraintestinal manifestations (EIMs), defined as systemic involvement of organs and systems driven by increased antigenic load, dysregulated immune responses, and impaired intestinal mucosal barrier integrity. EIMs may affect multiple organ systems, contribute significantly to morbidity, and occur both concurrently with IBD flares and independently of intestinal disease activity [1]. Their prevalence across studies ranges from 6% to 50% [4].

At present, EIMs are regarded as systemic manifestations of IBD, as their presentation is not confined to the gastrointestinal tract; in approximately 25% of cases, multiple organ systems are involved simultaneously. Their pathogenesis may result from propagation of intestinal immune responses, represent independent inflammatory processes associated with IBD, or share common genetic and environmental risk factors. According to a population-based study by Hsun Y-C et al., the prevalence of EIMs in European populations ranges from 16% to 43% in Crohn's disease (CD) and from 7% to 31% in ulcerative colitis (UC), while in Asian populations the average prevalence is approximately 19%, including 11.9% in Taiwan. The same authors reported a rising trend in EIM prevalence over the past 14 years, increasing from 2.8% to 25.9% in CD and from 2.8% to 26.6% in UC. The most commonly affected systems include the joints, skin, eyes, and hepatobiliary tract, and these manifestations are frequently associated with intestinal inflammatory activity. Notably, arthritic manifestations are more frequent in CD (12.9%) than in UC (8.1%) [5].

Furthermore, current evidence indicates that arthritis and erythema nodosum are closely associated with intestinal disease activity, whereas uveitis and ankylosing spondylitis are not dependent on IBD activity [6]. Essex M. et al. reported that genetic susceptibility may underlie the simultaneous development of intestinal and articular inflammation. The presence of spondyloarthritis increases the likelihood of developing IBD, and conversely, approximately one-fifth of patients with IBD subsequently develop spondyloarthritis, indicating a bidirectional pathophysiological relationship [7].

Additional systemic involvement may include glomerulonephritis, tubulointerstitial nephritis, amyloidosis, bronchiectasis, chronic bronchitis, cryptogenic organizing pneumonia, pericarditis, myocarditis, thromboembolic events, and arrhythmias [8].

Given that small intestinal Crohn's disease is associated with impaired absorption of iron and zinc, deficiencies of these and other micronutrients may contribute to dermatological changes in affected patients. Terminal ileitis leads to impaired bile acid circulation and enterohepatic recycling, which may result in xerosis and nonspecific eczema [9].

In addition, patients with inflammatory bowel diseases (IBD) are characterized by a high prevalence of comorbid conditions. Comorbidity refers to diseases and conditions that are frequently observed in individuals with IBD but do not have a confirmed direct pathogenetic link with the primary disease. These include various immune-mediated inflammatory diseases (IMIDs) as well as other conditions that occur more frequently in IBD patients due to shared risk factors or dysregulation of immune homeostasis. Comorbid conditions are known to modify the clinical presentation, complicate diagnosis, influence therapeutic decision-making and treatment efficacy, and significantly reduce patients' quality of life. Similar patterns of the negative impact of somatic comorbidity on social functioning, levels of frustration, and perceived quality of healthcare have also been demonstrated in other chronic psychosomatic conditions [10].

According to contemporary studies, the prevalence of comorbid pathology in the general adult population is approximately 7%. In patients with IBD, this proportion is substantially higher. In particular, a Swiss study demonstrated that 78% of patients with IBD had at least one comorbid chronic condition, with a median of three comorbidities per patient. Another study conducted in Italy reported that approximately 25% of patients with IBD had at least one IMID, further confirming the high burden of comorbid chronic diseases in this patient population [11].

Psychiatric disorders, particularly anxiety and depression, are also frequently observed in patients with IBD and are associated with a significant reduction in quality of life (QoL). The presence of these psychiatric comorbidities has been shown to be associated with poorer social functioning and overall health status [12]. Conditions such as generalized anxiety disorder, social phobia, and post-traumatic stress disorder (PTSD) have a particularly strong negative impact on QoL. Therefore, systematic assessment of mental health represents an essential component of a comprehensive multidisciplinary approach to the management of IBD patients.

The contemporary concept of biopsychosocial medicine emphasizes the integration of biological, psychological, and social dimensions in the understanding and management of chronic somatic diseases, which is particularly relevant for personalized patient care [13].

Furthermore, recent studies indicate that physical frailty, psychosocial maladaptation, and reduced mental reserve are important predictors of impaired quality of life in patients with IBD [15].

At the same time, the role of comorbid pathology and extraintestinal manifestations, as well as their frequency and phenotypic characteristics, in determining changes across different domains of health-related quality of life (HRQoL) in IBD patients remains insufficiently investigated. This approach requires not only control of intestinal inflammation, but also a comprehensive assessment of psychological status, social functioning, and adaptive capacity, which is fully consistent with the contemporary biopsychosocial model of chronic disease management [13]. Therefore, the question of whether comorbid conditions and the frequency and nature of extraintestinal manifestations in patients with inflammatory bowel disease are associated with changes in different HRQoL domains remains highly relevant.

AIM

The aim of this study was to determine the association between the presence of comorbid pathology, the frequency

and nature of extraintestinal manifestations, and changes in different domains of health-related quality of life in patients with inflammatory bowel diseases.

MATERIALS AND METHODS

The study was conducted at the Departments of Propedeutics of Internal Medicine named after Professor M.M. Bereznytskyi, General Practice-Family Medicine and Rehabilitation, and Psychiatry, Narcology, and Medical Psychology of Ivano-Frankivsk National Medical University, Ministry of Health of Ukraine.

A total of 248 patients with inflammatory bowel diseases (IBD) were enrolled, including 117 patients with Crohn's disease (CD) (Group I) and 131 patients with ulcerative colitis (UC) (Group II). All participants underwent inpatient treatment in somatic departments of healthcare institutions in Ivano-Frankivsk, including: Communal Non-Commercial Enterprise "Regional Clinical Hospital of Ivano-Frankivsk Regional Council" (CNPE "RCH IFR C"); CNPE "Central City Clinical Hospital of Ivano-Frankivsk City Council" (CNPE "CGCH IF CC"); CNPE "City Clinical Hospital No. 1 of Ivano-Frankivsk City Council" (CNPE "CCH No. 1 IF CC"); and the Center for Clinical Medicine of the University Clinic of Ivano-Frankivsk National Medical University. On average, each included patient had 2-3 hospitalizations per year due to the underlying disease or its complications. The control group consisted of 82 apparently healthy volunteers without IBD or related diseases in their family history.

Patients aged 18-65 years were included, consistent with the standard age range used in contemporary clinical studies. The mean age was 40.32±21.74 years in the CD group and 43.34±21.57 years in the UC group. The diagnosis of IBD (Crohn's disease or ulcerative colitis) was established based on clinical, laboratory, radiological, and endoscopic criteria in accordance with the Unified Clinical Protocol of Primary and Specialized Medical Care "Inflammatory Bowel Diseases (Crohn's disease, ulcerative colitis)" (approved by Order of the Ministry of Health of Ukraine No. 1742 dated October 6, 2023) and the clinical guidelines "Inflammatory Bowel Diseases" and "Management of Crohn's Disease in Adults." These clinical guidelines represent an adaptation of international clinical recommendations for the healthcare system of Ukraine (MHU, 2023).

Exclusion criteria included the presence of comorbid conditions not specified in the protocol that could affect data interpretation, the presence of concomitant neuropsychiatric disorders potentially influencing patient compliance, and refusal to participate in the study.

For comprehensive assessment, all patients were evaluated using the following instruments: the Inflammatory Bowel Disease Questionnaire (IBDQ), the Cumulative Illness Rating Scale (CIRS), the Personal and Social Performance Scale (PSP), and the Medical Outcomes Study Short Form-36 (MOS SF-36), which includes eight subscales. In addition, the Charlson Comorbidity Index (CCI) was calculated for all participants [16, 17].

The Inflammatory Bowel Disease Questionnaire (IBDQ) is a disease-specific instrument consisting of 32 items grouped into four domains reflecting intestinal, systemic, emotional, and social aspects of the disease. The total score ranges from 32 to 224, with each item rated on a 7-point Likert scale, where higher scores indicate better quality of life. The maximum scores for each domain are as follows: intestinal symptoms – 70 points, systemic symptoms – 35 points, social function – 35 points, and emotional status – 84 points. The items are designed to comprehensively assess the impact of each domain on the quality of life of patients with IBD. Consideration of not only local intestinal symptoms but also systemic manifestations, emotional well-being, and social functioning is essential for a complete evaluation of patients' health status. In particular, assessment of psychological disturbances, which frequently accompany chronic disease progression, as well as social dimensions reflecting the patient's ability to fulfill social roles and maintain social participation, is of key importance. The IBDQ-32 has been shown to possess one of the strongest measurement properties among instruments used in IBD research [18].

The Cumulative Illness Rating Scale (CIRS) allows for the assessment of both the number and severity of chronic diseases within a patient's comorbidity profile. Administration of the CIRS requires a comprehensive clinical evaluation, including detailed medical history and results of instrumental and laboratory investigations, such as complete blood count, biochemical profile (including electrolytes, cholesterol levels, serum vitamin B12), liver and kidney function tests, thyroid function tests, and electrocardiography [19]. The scale evaluates 14 organ systems without requiring disease-specific diagnoses. Each system is rated on a 5-point Likert scale from 0 to 4, where 0 indicates no pathology; 1 – past or mild disease; 2 – disease requiring pharmacological treatment; 3 – severe disease causing disability; and 4 – extremely severe disease requiring urgent intervention. The total CIRS score ranges from 0 to 56, although maximal values are rarely observed in clinical practice due to the incompatibility of severe multi-organ failure with survival.

In data interpretation, the total score (sum across all 14 categories), the number of affected organ systems, and comorbidity indices were analyzed. Thus, this instrument provides a structured quantitative approach to assessing disease burden and identifying potential health risks associated with cumulative chronic pathology [17]. The use of two indices-the Cumulative Illness Rating Scale-Comorbidity Index (CIRS-CI) and the Cumulative Illness Rating Scale-Severity Index (CIRS-SI) – allows for a multidimensional assessment of patient status. The CIRS-CI reflects the burden of comorbidity, defined as the number of organ systems with at least one clinically significant condition (score ≥2), whereas the CIRS-SI represents the overall severity of somatic burden and the intensity of disease involvement across systems.

According to contemporary evidence, the CIRS is a reliable and validated tool for comprehensive clinical assessment of patients both at the time of examination and with consideration of medical history. Due to its structured design, it enables not only quantitative evaluation of

existing pathological conditions but also identification of subclinical or oligosymptomatic chronic syndromes. These conditions are often overlooked by both clinicians and patients, which underscores the clinical value of the CIRS in the early detection of complex multisystem disorders.

Based on the assessed comorbid conditions, the Charlson Comorbidity Index (CCI) was calculated. A practical advantage and distinctive feature of this index is its ability to estimate the 10-year mortality risk in percentage terms. In the absence of comorbidity, the estimated 10-year mortality is approximately 12%; with 1-2 points, it increases to 26%; with 3-4 points, to 52%; and with more than 5 points, to 85%.

The study was conducted in accordance with the fundamental principles of Good Clinical Practice (GCP, 1996). All ethical principles for biomedical research involving human participants, as defined by the Declaration of Helsinki of the World Medical Association (2000 revision) and the provisions of the Council of Europe Convention on Human Rights and Biomedicine (04.04.1997), were strictly observed. The study protocol was approved by the Bioethics Committee of Ivano-Frankivsk National Medical University (protocol No. 153/25, September 17, 2025).

Statistical analysis of the obtained data was performed using "STATISTICA 8.0" software (StatSoft, serial number STA862D175437Q) and Microsoft Excel 2016 statistical functions. The significance of the results was assessed by calculating the standard error of relative values. Between-group comparisons were performed using Student's t-test, with statistical significance determined using the corresponding critical values to ensure reliable inference. Quantitative variables were presented as mean values (M) with standard error (±m), allowing for accurate assessment of variability and reliability of the results.

AI STATEMENT

The author states that no AI tools were used in writing this article.

RESULTS

Analysis of the sex distribution of patients, presented in Fig. 1, showed that among patients with Crohn's disease (CD), females predominated, accounting for 54.7% (64 individuals), whereas males accounted for 45.3% (53 individuals). In contrast, among patients with ulcerative colitis (UC), males predominated, accounting for 59.5% (78 individuals), while females represented 40.5% (53 individuals).

As shown in Table 1, educational attainment differed between the study groups. In Group I, 5.98% of patients had secondary education, 27.35% had secondary specialized education, 38.46% had incomplete higher education, and 28.21% had completed higher education. In Group II, the corresponding proportions were 8.39%, 21.37%, 37.40%, and 32.84%, respectively. In the control group, 50.0% of participants had completed higher education.

The occupational structure also differed between the groups. High-skilled employment was reported in 36.75% of patients in Group I and 42.75% in Group II, compared with 58.54% in the control group. Low-skilled work was observed in 17.95% of Group I patients, 9.93% of Group II patients, and 7.32% of controls. Intermittent employment was recorded in 24.78% of Group I, 34.35% of Group II, and 21.95% of the control group.

The level of disability was higher in Group I (20.52%) compared with Group II (12.97%). Across all groups, married individuals predominated; however, their proportion was lower in Crohn's disease (50.43%) compared with ulcerative colitis (60.31%) and the control group (62.20%). The proportion of single individuals was 33.3% in Group I, 19.08% in Group II, and 9.76% in the control group.

The IBDQ questionnaire results demonstrated a marked reduction in quality of life in both study groups.

As shown in Fig. 2, the total IBDQ score in Group I was 119.3±10.48, while in Group II it was 127.4±8.3. Compared with the control group (198.5±12.6 points), these values were 39.9% and 35.9% lower, respectively. When comparing mean total scores between the two patient groups, the IBDQ score was significantly lower in Group I than in Group II (119.3±10.48 vs 127.4±8.3; t=-6.36, p<0.001).

The domain structure of quality of life in the examined patients, assessed using the IBDQ, is presented in Fig. 3 and Table 2. The most pronounced differences were observed in the systemic and social subscales, indicating a stronger

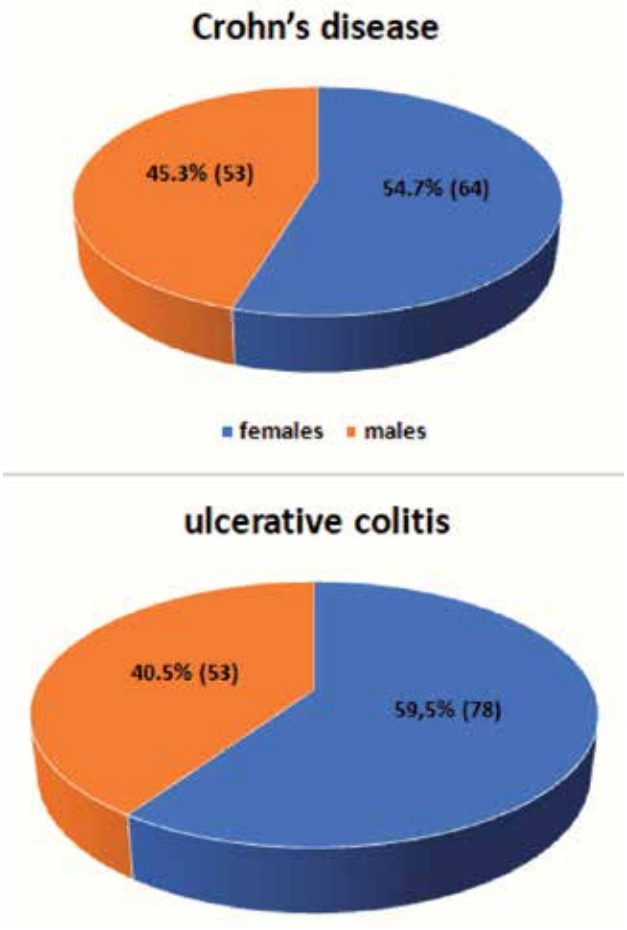

Fig. 1. Sex distribution of the examined patients
Picture taken by the authors

Table 1. Socio-demographic characteristics of the examined patients

Indicator	Group I (n=117)		Group II (n=131)		Control group (n=82)	
Level of education						
Secondary education	7	5.98 %	11	8.39 %	-	-
Specialized secondary education	32	27.35 %	28	21.37 %	14	17.07 %
Incomplete higher education	45	38.46 %	49	37.40 %	27	32.93 %
Higher education	16	28.21 %	43	32.84 %	41	50.0 %
Occupational status						
Low-skilled employment	21	17.95 %	13	9.93 %	6	7.32 %
Highly skilled employment	43	36.75 %	56	42.75 %	58	70.73 %
Intermittent employment	29	24.78 %	45	34.35 %	18	21.95 %
Disabled and not employed	24	20.52 %	17	12.97 %	-	-
Marital status						
Married	59	50.43 %	79	60.31 %	51	62.20 %
Divorced	11	9.40 %	20	15.27 %	12	14.63 %
Single	39	33.33 %	25	19.08 %	8	9.76 %
Widowed	8	6.84 %	7	5.34 %	11	13.41 %

Source: compiled by the authors of this study

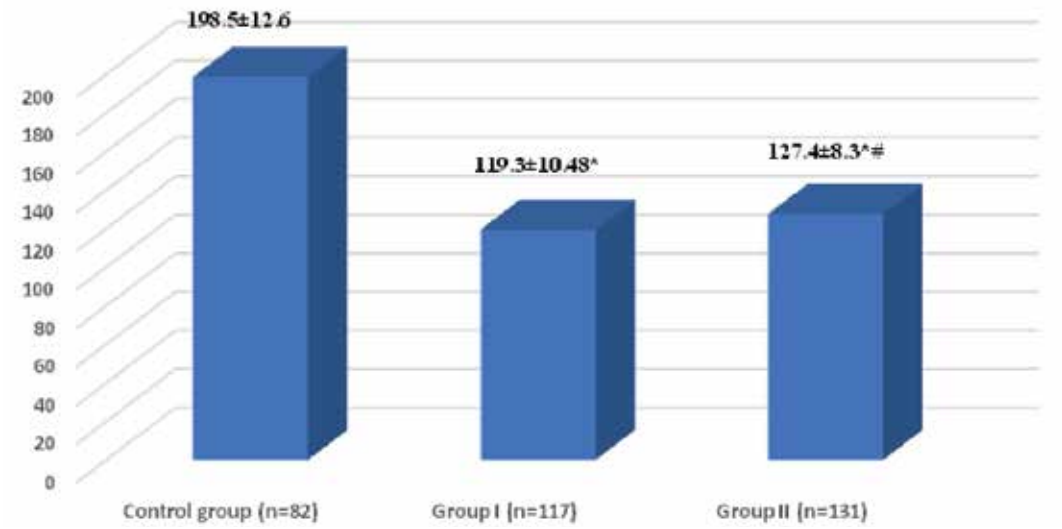

Fig. 2. Quality of life in the examined patients according to the IBDQ questionnaire (total score)

Notes:
** – (p < 0.05) statistically significant differences compared with the control group*
 # - ((p<0.05) statistically significant differences between the study groups

Picture taken by the authors

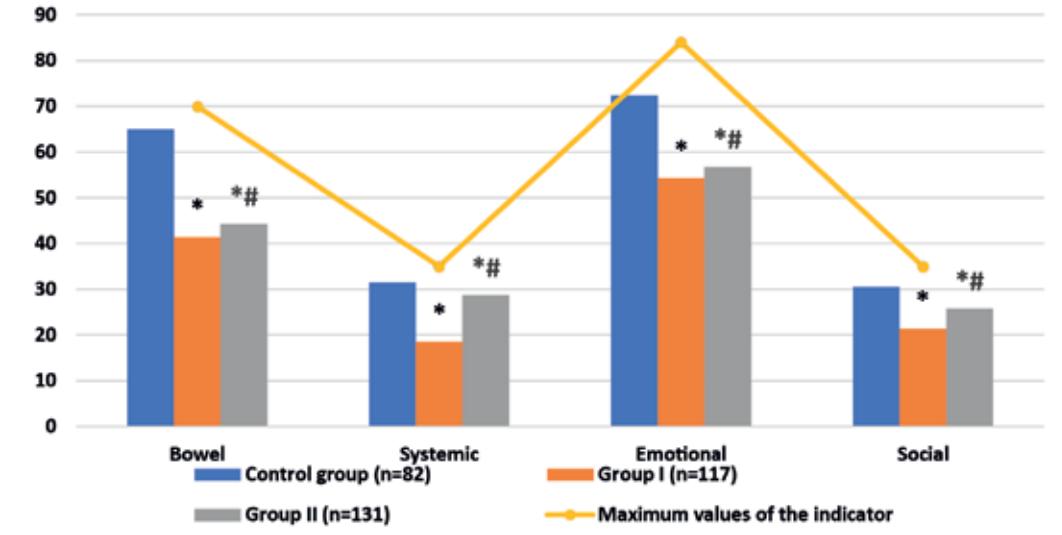

Fig. 3. Domain structure of quality of life in the examined patients according to the IBDQ questionnaire

Notes:
** – (p < 0.05) statistically significant differences compared with the control group*
 # - ((p<0.05) statistically significant differences between the study groups

Picture taken by the authors

Table 2. Comparative characteristics of the main domains of quality of life in the examined patients according to the IBDQ questionnaire

	Group I (n=117)	Group II (n=131)	Control group (n=82)
Bowel	65±5.2	41.4±3.12*	44.2±8.4*#
Systemic	31.5±4.3	18.5±2.08*	28.7±6.3*#
Emotional	72.4±6.9	54.3±1.27*	56.7±9.5*#
Social	30.5±6.9	21.4±1.43*	25.8±5.4*#
Total score	198.5±12.6	119.3±10.48*	127.4±8.3*#

Notes:
** – (p < 0.05) statistically significant differences compared with the control group*
 # - ((p<0.05) statistically significant differences between the study groups
Source: compiled by the authors of this study

negative impact of inflammatory bowel disease on patients' physical condition and social functioning. Differences in emotional and bowel symptom domains were also statistically significant, although less pronounced.

As shown in Table 2, bowel symptom scores in Group I were 41.4±3.12 points, and in Group II 44.2±8.4 points, corresponding to reductions of 36.3% and 32.0%, respectively, compared with the control group. Notably, this parameter was 6.8% lower in patients with Crohn's disease compared with those with ulcerative colitis (t=-3.55, p<0.001).

Systemic manifestations averaged 18.5±2.08 points in Crohn's disease and 28.7±6.3 points in ulcerative colitis, corresponding to reductions of 41.3% and 8.9%, respectively, compared with controls. Comparison between the study groups revealed a more than 1.55-fold difference in favor of ulcerative colitis (t=-17.5, p<0.001).

Emotional domain scores were 54.3±1.27 points in patients with Crohn's disease and 56.7±9.5 points in those with ulcerative colitis, corresponding to decreases of 25.0% and 21.7% relative to the control group. Intergroup comparison demonstrated a statistically significant difference of 1.04-fold in favor of ulcerative colitis (t=-2.87, p=0.005).

Social functioning scores were 21.4±1.43 points in Crohn's disease and 25.8±5.4 points in ulcerative colitis, corresponding to reductions of 29.8% and 15.4% compared with controls. A significant intergroup difference of 1.21-fold was observed in favor of ulcerative colitis (t=-9.39, p<0.001).

The frequency of comorbid conditions assessed using the CIRS scale in the examined patients is presented in Figure 4. For clarity of data visualization, only 13 categories are shown in the figure. Diseases of the small and large intestine were present in 100% of the examined patients by default, as they constituted one of the principal inclusion criteria for the study.

The CIRS-based assessment demonstrated the presence of comorbid conditions in 73% of patients in Group I and in 82% of patients in Group II, whereas in the control group

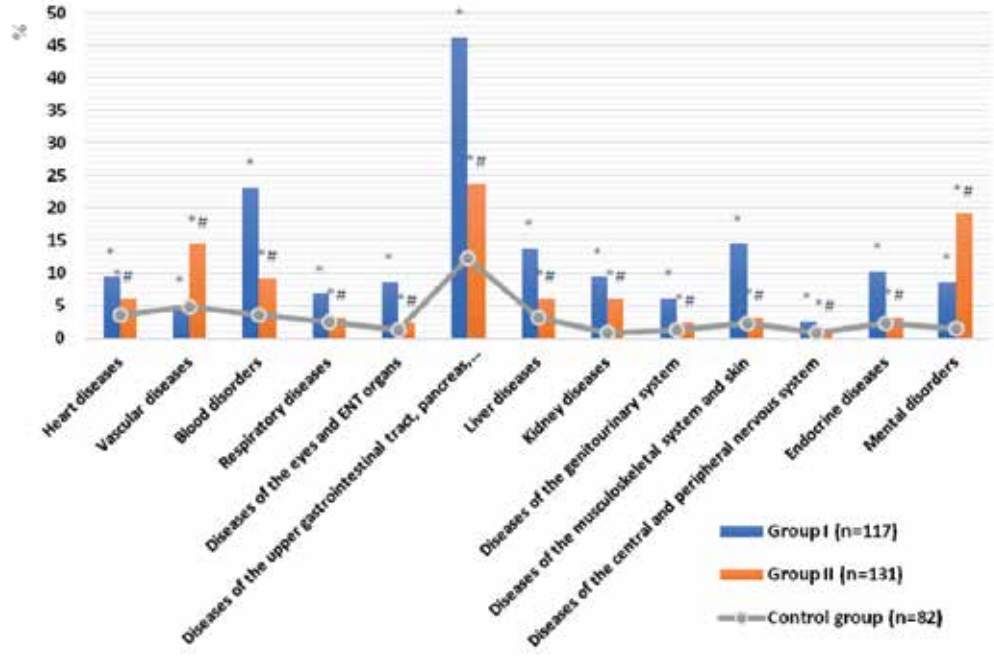

Fig. 4. Frequency of comorbid conditions according to the CIRS scale in the examined patients
Notes:
** – (p < 0.05) statistically significant differences compared with the control group*
 # - ((p<0.05) statistically significant differences between the study groups
Picture taken by the authors

this indicator was only 28%.

As shown in Fig. 4, patients with Crohn's disease (Group I) had significantly higher frequencies of hematological, hepatic, musculoskeletal, endocrine, and upper gastrointestinal tract disorders compared with patients with ulcerative colitis (Group II). Specifically, hematopoietic system involvement was observed in 23.1% of Group I patients, compared with 9.2% in Group II and 3.65% in the control group. Liver disorders were present in 13.7% of Group I patients, 6.1% of Group II patients, and 1.2% of controls.

Endocrine disorders were recorded in 10.3% of patients with Crohn's disease, which was nearly four times higher than in the control group (2.43%) and more than three times higher than in ulcerative colitis (3.1%). Upper gastrointestinal tract involvement, including pancreatic pathology, was observed twice as often in Crohn's disease (46.2%) compared with ulcerative colitis (23.7%) and almost four times more frequently than in healthy controls (12.1%).

In contrast, patients with ulcerative colitis more frequently exhibited psychiatric disorders (19.1% vs 8.5% vs 3.65%) and vascular pathology (14.5% vs 5.1% vs 2.43% in controls). A similar pattern was observed for genitourinary system disorders, with a prevalence of 11.5% in the ulcerative colitis group compared with 6.8% in Crohn's disease and 1.2% in the control group.

Regarding other clusters, cardiac diseases were observed in 9.4% of patients with Crohn's disease (CD), 6.01% of patients with ulcerative colitis (UC), and only 3.65% in the control group. Respiratory tract pathology was most prevalent in the CD group (6.8%), whereas its frequency in UC patients and controls was 3.1% and 2.43%, respectively. Renal diseases were more common in patients with CD (9.4%) and UC (6.1%), while in the control group they accounted for only 0.8%.

Diseases of the ocular and ENT systems were present in 8.5% of patients with CD, which is approximately seven times higher than in the control group. In UC patients, this pathology was observed in 2.3%, which is nearly twice as high as in controls (1.2%).

The results of the Charlson Comorbidity Index (CCI) – a prognostic tool used to estimate the impact of comorbidity on 10-year survival – are presented in Fig. 5. As shown in the data, the mean CCI score in Group I was 3.14±0.27, whereas in Group II it was 2.93±0.14 ($p \leq 0.005$). The interquartile range (Q1-Q3) in Group I was 2.0-4.0, and in Group II it was 2.0-3.0, corresponding to an estimated 10-year survival probability ranging from 45% to 79% in patients with Crohn's disease and from 52% to 79% in patients with ulcerative colitis.

Compared with the control group (1.69±0.68; IQR 1.0-2.0), the CCI was 1.86-fold higher in Crohn's disease (an increase of 85.8%) and 1.73-fold higher in ulcerative colitis (an increase of 73.4%).

DISCUSSION

The observed sex-related differences partially correspond to global patterns in the distribution of inflammatory bowel diseases. In particular, the predominance of women among patients with Crohn's disease (CD) and men among those with ulcerative colitis (UC) is consistent with findings from several international studies reporting gender-specific variations depending on disease phenotype, geographic region, and age at disease onset. These findings may reflect the contribution of hormonal, immunological, and behavioral factors to the clinical presentation of inflammatory bowel diseases (IBD).

Socio-demographic analysis demonstrated that patients with IBD were characterized by lower educational and occupational levels compared with the control group, as well as higher rates of disability and social isolation, particularly in CD. These findings may be explained by the chronic relapsing nature of the disease, the need for long-term treatment, frequent hospitalizations, and reduced work capacity. In turn, reduced social activity and occupational functioning may further exacerbate psychological burden, creating a vicious cycle between disease severity and social maladjustment. Similar trends have been reported in international studies linking IBD

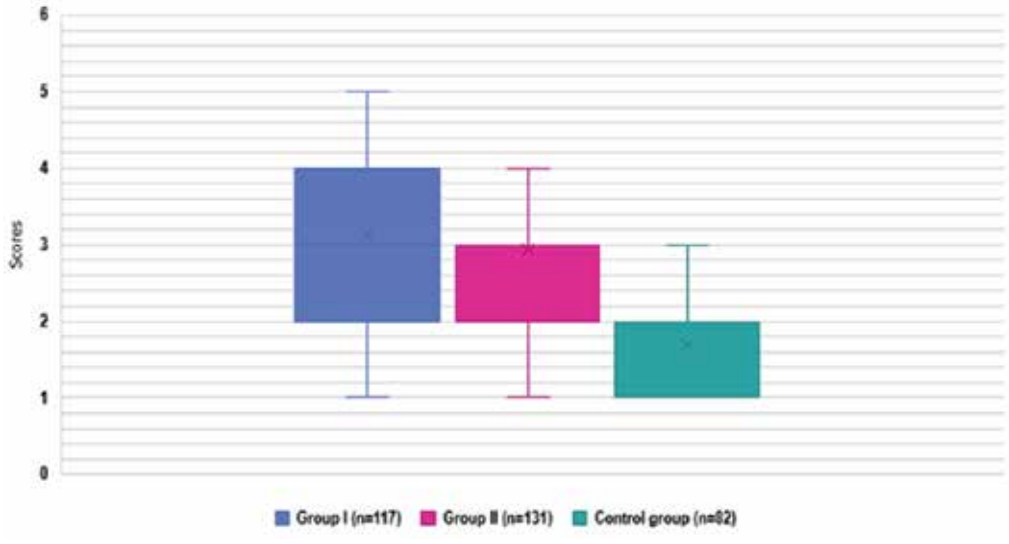

Fig. 5. Interquartile ranges of the Charlson Comorbidity Index in the examined patients

Picture taken by the authors

with reduced work productivity, higher unemployment rates, and impaired social functioning.

The results of the IBDQ assessment demonstrated a marked reduction across all quality-of-life domains in both patient groups compared with controls. More pronounced impairments were observed in patients with CD, particularly in the systemic and social domains. This may be attributed to the transmural nature of inflammation in CD, a higher frequency of complications, extraintestinal manifestations, and the need for more aggressive therapy. These findings confirm that CD is associated with a greater multisystem burden and more substantial impairment of daily functioning.

The modern concept of health-related quality of life (HRQOL) considers it a key outcome in chronic disease management. In IBD, HRQOL assessment is particularly important, as clinical remission does not necessarily translate into full recovery of physical, psychological, and social well-being. The observed reductions in emotional and social functioning highlight the importance of a comprehensive approach to patient management that incorporates not only inflammatory activity but also psychological status, social support, and adaptive capacity.

The findings are consistent with those reported by Gao et al., who demonstrated that the main predictors of impaired HRQOL in patients with CD include disease activity, anxiety and depressive disorders, nutritional deficiencies, and complications. Thus, psychological disturbances and systemic disease manifestations may act as interrelated factors that substantially influence patients' subjective perception of health status and treatment effectiveness [14].

The high prevalence of comorbid conditions observed in patients with IBD supports the systemic nature of this disease. According to the CIRS assessment, comorbidities were present in the majority of patients in both study groups, more frequently in UC. These results are consistent with the findings of Lenti et al., who reported a high prevalence of comorbidity among both hospitalized and non-hospitalized IBD patients [20]. The authors emphasized that chronic systemic inflammation itself is a key driver of comorbid conditions, independent of disease severity or hospitalization status.

The comorbidity profile differed between CD and UC. CD was characterized by a predominance of somatic involvement (hematopoietic system, liver, endocrine system, and upper gastrointestinal tract), which may reflect deeper transmural inflammation. In contrast, UC was more frequently associated with psychiatric and vascular disorders, potentially reflecting different pathogenetic mechanisms or the influence of chronic stress and systemic inflammation. These findings are partially consistent with a 2023 meta-analysis, which found that approximately one-quarter of IBD patients present with at least one extraintestinal manifestation involving the joints, skin, or eyes.

At the same time, the comorbidity profile differed substantially between CD and UC. In CD, hematological, hepatic, endocrine, renal, and upper gastrointestinal involvement was more prominent. This pattern may be explained by transmural inflammation, malabsorption syndrome, chronic anemia, impaired nutritional status, and systemic immune activation. Notably, pancreatic and upper gastrointestinal involvement was particularly frequent in CD. These findings are consistent with recent Mendelian randomization studies; Fang et al. (2025) demonstrated an increased risk of acute pancreatitis in both CD and UC, whereas Fu et al. (2024) confirmed a causal association predominantly for CD [21, 22]. This may suggest shared immune-mediated mechanisms underlying gastrointestinal organ involvement.

In UC patients, psychiatric and vascular disorders were more prominent. The higher frequency of psychiatric comorbidity may be related to chronic psychological stress, persistent pain, fear of relapse, and the impact of symptoms on social functioning. Additionally, systemic inflammation in IBD has been linked to dysregulation of the gut–brain axis, contributing to anxiety and depressive disorders. The increased vascular pathology likely reflects a prothrombotic state and endothelial dysfunction characteristic of chronic inflammation.

Findings related to extraintestinal manifestations are consistent with international data. A meta-analysis conducted by researchers from the United Kingdom and Australia reported that approximately one-quarter of IBD patients experience at least one extraintestinal manifestation, most commonly affecting joints, skin, or eyes. The high frequency of musculoskeletal, ophthalmological, and ENT involvement observed in the present study further supports the multisystem nature of IBD and underscores the need for a multidisciplinary approach to patient management.

Elevated Charlson Comorbidity Index scores in IBD patients indicate a substantial impact of comorbidity on long-term prognosis and survival. Higher CD values may reflect a more severe systemic course, a higher prevalence of metabolic and nutritional disturbances, and a broader spectrum of extraintestinal involvement. These findings emphasize the importance of early identification and management of comorbid conditions to improve prognosis and quality of life.

Overall, the results of this study confirm that IBD is associated with significantly reduced quality of life, a high prevalence of comorbid conditions, and substantial multisystem involvement. CD is characterized by a more pronounced somatic and multisystem burden, whereas UC appears to be more strongly associated with psychiatric and vascular disorders. These findings highlight the need for a comprehensive, personalized approach to IBD management involving gastroenterologists, psychiatrists, psychologists, endocrinologists, and other specialists. Beyond the direct effects of chronic inflammation, the long-term psychological and social consequences of living with a chronic disease may further contribute to maladaptation and deterioration in well-being. As emphasized by Matiashova et al., prolonged exposure to stressful and traumatic experiences may have enduring effects on mental health, social functioning, and quality of life. Similarly, Kang et al. highlighted the substantial psychological burden associated with prolonged exposure to chronic stressors, emphasizing the importance of mental

health support and resilience-oriented interventions in vulnerable populations [23,24]. This perspective aligns with modern Healthcare 4.0 principles, which emphasize multidisciplinary care, personalized medicine, and the consideration of biological, psychological, and social determinants of health in the management of chronic diseases [25].

LIMITATIONS OF THE STUDY

The study has several limitations, including its single-center, cross-sectional design, which may limit the generalizability of the findings and preclude the assessment of long-term causal relationships. In addition, quality of life was assessed using the self-reported IBDQ questionnaire, whose results may be partly influenced by patients' psychological state at the time of evaluation. Nevertheless, the comprehensive analysis of comorbid conditions, extraintestinal manifestations, and quality-of-life domains yielded clinically meaningful results that may serve as a basis for future multicenter prospective studies in patients with inflammatory bowel disease (IBD).

PERSPECTIVES FOR FUTURE RESEARCH

The present study opens new perspectives for further in-depth investigation of the relationships between clinical manifestations of inflammatory bowel disease, comorbid pathology, quality-of-life parameters, and molecular-biological mechanisms of systemic inflammation. Of particular scientific interest is the further analysis in the studied cohort of the role of BDNF, serotonin, MMP-9, markers of oxidative protein modification, and lipid peroxidation processes, including MDA and TBA-reactive products, as potential biomarkers of disease activity, oxidative stress, psycho-emotional disturbances, and the development of extraintestinal manifestations in Crohn's disease and ulcerative colitis. Exploring the relationships between these parameters, disease severity, hospitalization frequency, and treatment effectiveness may provide a foundation for improving personalized approaches to prognosis and management of patients with IBD.

CONCLUSIONS

In patients with inflammatory bowel disease, a significant reduction in quality of life across all IBDQ domains was observed compared with the control group, more pronounced in Crohn's disease. A high prevalence of comorbid conditions and extraintestinal manifestations was identified, confirming the systemic nature of IBD and demonstrating distinct clinical profiles in Crohn's disease and ulcerative colitis. Elevated Charlson Comorbidity Index values indicate a less favorable prognosis in patients with Crohn's disease and a generally negative impact of comorbidity on disease course. The obtained results emphasize the need for a comprehensive approach to the management of patients with IBD, taking into account systemic manifestations and comorbid conditions.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Comparison of steroidal and nonsteroidal mineralocorticoid receptor antagonists on the incidence of new-onset cardiac arrhythmias following myocardial infarction: A retrospective cohort study

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ABSTRACT

Aim: The purpose of this study was to compare the incidence of new-onset cardiac arrhythmias in post–myocardial infarction (MI) patients treated with nonsteroidal versus steroidal MRAs.

Materials and Methods: A retrospective cohort study was conducted using the TriNetX database, including MI patients without a prior history of cardiac arrhythmias who received either nonsteroidal or steroidal MRAs. Propensity score matching was performed across 28 baseline variables, including demographics, cardiovascular risk factors, medication use, and laboratory values. The primary outcome was the incidence of new-onset cardiac arrhythmia, while secondary outcomes included the individual components of the primary outcome (atrial and ventricular arrhythmias) as well as cardiac arrest events.

Results: After matching, 861 patients were included in each cohort (nonsteroidal and steroidal mineralocorticoid). New-onset cardiac arrhythmia occurred in 62 patients (9.1%) in the nonsteroidal group and 97 patients (15.6%) in the steroidal group. However, the risk of developing new-onset cardiac arrhythmia did not differ significantly between the two cohorts (HR: 0.856; 95% CI: 0.614-1.193; P=0.284). Secondary outcomes also showed no significant differences: atrial arrhythmia (HR: 0.752; 95% CI: 0.520-1.089; P=0.125), ventricular arrhythmia (HR: 1.016; 95% CI: 0.558-1.850; P=0.647), and cardiac arrest (HR: 0.851; 95% CI: 0.465-1.558; P=0.081).

Conclusions: Our findings showed no difference in the incidence of new-onset cardiac arrhythmias or in secondary outcomes between patients treated with nonsteroidal versus steroidal MRAs. Prospective, standardized studies are needed to validate these results.

KEYWORDS: cardiac arrhythmia, post-myocardial infarction, mineralocorticoid receptor antagonists, finerenone, spironolactone

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INTRODUCTION

Mineralocorticoid receptor antagonists (MRAs) are class of drugs that are classified to two types, based on their structure, to steroidal MRAs (spironolactone and eplerenone), and nonsteroidal MRAs (Finerenone and Esaxerenone) [1]. In general all MRAs exert their action by competitive antagonists effect on mineralocorticoid receptors, which are mainly found in the epithelial cells of the collecting ducts on the kidney, as well as in non-epithelial cells of blood vessels, heart, fibroblasts and immune cells [1, 2].

The antagonistic action of MRAs inhibits activation of the sodium transport cascade involving epithelial sodium channels (ENaC) and the sodium-potassium pump (Na^+/K^+-ATPase), thereby abolishing the sodium-retaining effects of mineralocorticoids [3]. In addition, MRAs suppress aldosterone-mediated inflammation, fibrosis, and oxidative stress in cardiac and renal tissues [1, 4]. Currently, MRAs have been demonstrated to be effective therapeutic agents in the management of heart failure with reduced ejection fraction (HFrEF), type 2 diabetes mellitus associated with chronic kidney disease, resistant hypertension, and primary hyperaldosteronism [5-7].

Steroidal MRAs have affinity to sex hormone receptors, an effect observed more in spironolactone compared to eplerenone, which leads to undesired side effects such as impotence, gynecomastia, and menstrual irregularities. However, nonsteroidal MRAs have less effect on sex hormone receptors and are therefore associated with less effect of these side effects [5, 8].

Up to the present, the only FDA-approved nonsteroidal MRAs is Finerenone, it is approved and indicated to decrease the risk of nonfatal myocardial infarction, eGFR decline,

cardiovascular-related death,and hospitalization for heart failure in adults with chronic kidney disease associated with type 2 diabetes mellitus [9]. More recently, Finerenone was also approved to lower the risk of cardiovascular death, heart failure hospitalization, and urgent heart failure visits in adults with heart failure with a left ventricular ejection fraction (LVEF) ≥40% [9].

Despite the well-recognized effects of the MRAs on patients with established cardiovascular disease, the literature is limited regarding comparing the steroidal and the non steroidal MRAs in post-myocardial infarction patients. This study aims to address this gap by evaluating the impact of steroidal and non steroidal MRA on cardiac arrhythmia outcome in post-myocardial infarction patients.

AIM

The primary endpoint for this study is to compare the risk of new onset cardiac arrhythmia after MI between non-steroidal and steroidal MRAs receiving patients. Secondary endpoints included individual elements of the primary endpoint: atrial arrhythmia and ventricular arrhythmia, and occurrence of cardiac arrest. The Supplemental Appendix elaborates on outcome definitions and ICD-10 codes.

MATERIALS AND METHODS

DATA SELECTION

This study was conducted using the TriNetX research network, a federated database providing access to electronic health records (EHRs) from 162 healthcare organizations (HCOs). A total of 70 providers responded with patients. The TriNetX platform aggregates deidentified patient data, ensuring compliance with HIPAA deidentification standards (§164.514(a)).

PATIENT POPULATION

We conducted a retrospective observational multicentric cohort study including patients with MI without previous history of cardiac arrhythmia, categorized based on the MRAs type they were receiving, either steroidal MRAs (spironolactone and eplerenone) or non-steroidal MRAs (Finerenone and Esaxerenone). Cohort 1 (non-steroidal MRAs group) included patients with MI without previous history of cardiac arrhythmia who were receiving non-steroidal MRAs without receiving steroidal MRAs, while Cohort 2 (steroidal MRAs group) included patients with MI without previous history of cardiac arrhythmia who were receiving steroidal MRAs without receiving non-steroidal MRAs. The index event was defined as the initiation of receiving the MRAs, identified using Rxnorm codes: 2562811 (Finerenone), OMOP5040499 (Esaxerenone), 298869 (eplerenone), and 9997 (spironolactone), marked the beginning of the observation window for evaluating outcomes. Cardiac arrhythmia and MI diagnosis were identified using ICD-10 codes. Additional details on cohort identification, and study window definitions, including the relevant ICD-10, RxNorm, and Current Procedural Terminology codes, are provided in the Supplemental Appendix.

STATISTICAL ANALYSIS

Continuous variables are presented as mean ± standard deviation (SD), whereas categorical variables are presented as number (percentage), as appropriate. Baseline characteristics were compared between the non-steroidal and steroidal MRAs groups using independent samples Student's t-tests for continuous variables and chi-square tests for categorical variables. To mitigate baseline differences between cohorts, 1:1 propensity score matching was performed using greedy nearest neighbor matching with a caliper of 0.1 times the pooled SD of the linear propensity scores. Variables included in the matching process were age, sex, race, comorbidities (diabetes mellitus, hypertension, hyperlipidemia, chronic kidney disease, cardiomyopathy, heart failure, ischemic heart disease, cerebrovascular disease,pulmonary disease, obesity, nicotine dependence), medication use (antilipemic agents, ACE inhibitors, angiotensin receptor inhibitors, sacubitril and B-blocker agents) and laboratory values (cholesterol level, hemoglobin A1C, urea, and creatinine). The standardized mean difference represents the difference between the means of two groups in terms of SD units, and is used to assess balance in measured variables in the sample weighted by the inverse probability of treatment. Variables were selected based on their potential impact on overall and cardiovascular outcomes.

After propensity score matching (PSM), adjusted outcomes were compared between cohorts using hazard ratios (HRs) and 95% confidence intervals (CIs) derived from Cox proportional hazards regression models. Kaplan–Meier survival analysis was used to assess time-to-event outcomes, with differences between cohorts evaluated using the log-rank test. A P-value<0.05 was considered statistically significant. All statistical analyses were conducted using integrated R (The R Foundation) within the TriNetX platform.

ETHICS APPROVAL

This study was conducted using de-identified data from the TriNetX research network. In accordance with U.S. federal regulations, studies using only de-identified data are not considered human subjects research and are exempt from institutional review board (IRB) approval. TriNetX, LLC has received a waiver from the Western IRB and complies with the Health Insurance Portability and Accountability Act (HIPAA), with de-identification confirmed through a qualified expert determination as defined in Section §164.514(b)(1) of the HIPAA Privacy Rule.

RESULTS

STUDY POPULATION

This retrospective cohort study identified a total of 160,719 post MI patients receiving non-steroidal or steroidal MRAs and had no prior history of cardiac arrhythmia. Among them, 861 patients received non-steroidal MRAs, and 159,858 patients received steroidal MRAs (Fig. 1A). After applying 1:1 propensity score matching (PSM) to balance baseline characteristics, 861 patients were included in each cohort (non-steroidal MRAs group and steroidal MRAs group) for the final analysis (Fig. 1B).

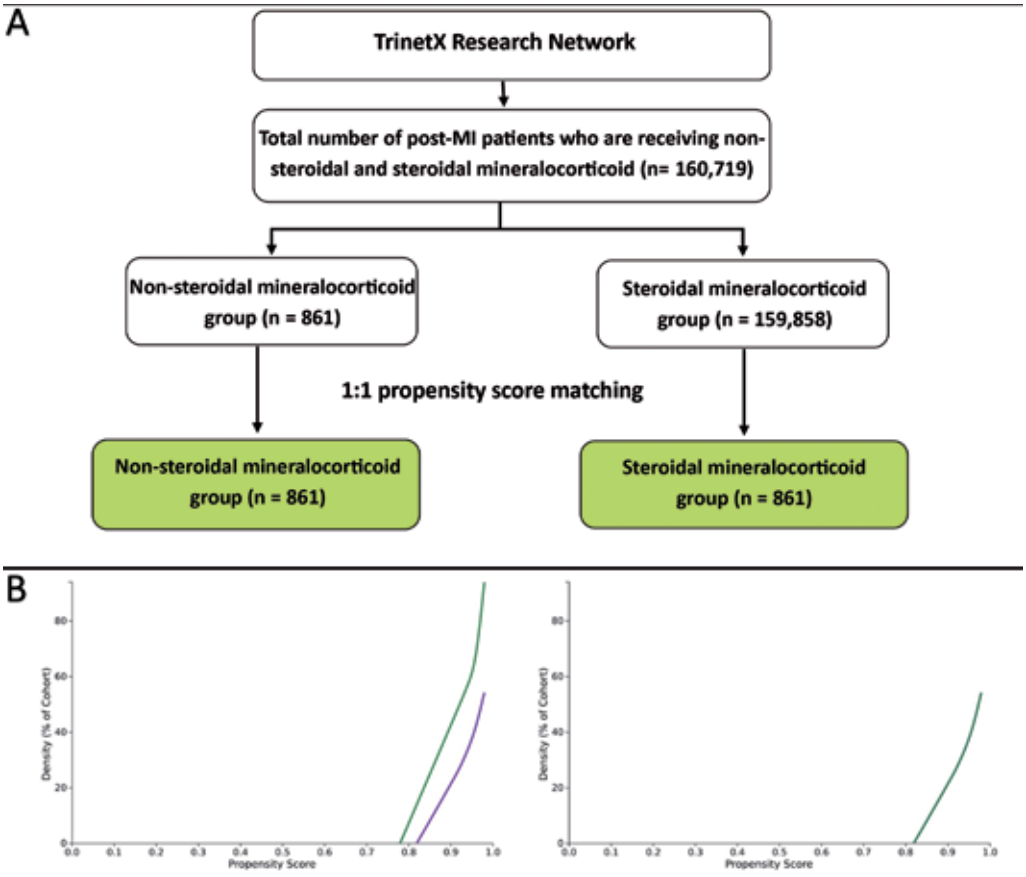

Fig. 1. A: Flow diagram of patient selection and cohort derivation following propensity score matching. B: Propensity score density distributions before and after weighting

Source: Own materials

PATIENT CHARACTERISTICS

The baseline characteristics of the study cohorts, before and after propensity score matching (PSM), are shown in Table 1. In the unmatched cohort, patients receiving non-steroidal MRAs were older at index (mean age: 68.8 ±11.0 years vs. 65.7±13.5 years, P<0.001) compared to those receiving steroidal MRAs. The proportion of male patients was slightly higher in the non-steroidal MRAs group (63.3% vs. 60.6%, P=0.104), while the steroidal MRAs group had a higher proportion of female patients (39.4% vs. 36.7%, P=0.105). Regarding racial distribution, the steroidal MRAs group was more likely to be White (56.3% vs. 41.5%, P<0.00) compared to the non-steroidal MRAs group.

Before matching, the non-steroidal MRAs group exhibited a higher prevalence of cardiovascular risk factors, including hypertension (88.5% vs. 65.2%, P<0.001), ischemic heart disease (84.2% vs. 74.2%, P<0.001), diabetes mellitus (86.9% vs. 36.7%, P<0.001), dyslipidemia (80.3% vs. 50.4%, P<0.001), chronic kidney disease (56.9% vs. 20.3%, P<0.001), overweight and obesity (35.3% vs. 22.7%, P<0.001), pulmonary disease (60.3% vs. 50.4%, P<0.001), and cerebrovascular disease (29.0% vs. 16.3%, P<0.001). Conversely, cardiomyopathy (15.6% vs. 8.0%, P<0.001), heart failure (48.2% vs. 42.2%, P<0.001) and nicotine dependence (19.0% vs. 17.5%, P=0.281), were more frequently observed in the steroidal MRAs group (Fig. 2).

After propensity score matching (PSM), the two cohorts were well balanced across key baseline characteristics, including age, sex, race, comorbidities, medication use, and laboratory values with standardized mean differences (SMDs)<0.1 for most variables, indicating a well-matched cohort. The final matched cohort consisted of 861 patients in the non-steroidal MRAs group and 861 patients in the steroidal MRAs group.

For each outcome, patients with a prior history of the event before the start of the time window were excluded, resulting in outcome-specific cohort sizes. Consequently, the reported event rates (percentages) are calculated using these adjusted denominators rather than the overall matched cohort size. This approach follows the TriNetX analytic framework and ensures accurate estimation of incident events.

Primary outcome: Incident of new-onset cardiac arrhythmia

After matching, during the follow-up period, a total of 62 patients (9.1%) in the non-steroidal MRAs cohort experienced new-onset cardiac arrhythmia, compared to 97 patients (15.6%) in the steroidal MRAs cohort. Receiving non-steroidal or steroidal MRAs showed no difference in the risk of development of new onset cardiac arrhythmia with a hazard ratio of 0.856 (95% CI: 0.614 - 1.193, P=0.284); (Table 2, Fig. 3).

Secondary Outcome: Atrial arrhythmia, ventricular arrhythmia and cardiac arrest events:

Table 1. Baseline characteristics of patients in steroidal vs. non-steroidal groups before and after propensity score matching (PSM)

	Before PSM			After PSM		
	Before Matching (Non-steroidal group, n=861)	Before Matching (Steroidal group, n=159,858)	Standardized Difference	After Matching (Non- steroidal group, n=861)	After Matching (Steroidal group, n=861)	Standardized Difference
Demographics						
Age at Index (Mean±SD)	68.8±11.0	65.7±13.5	0.257	68.8±11.0	69.2±11.5	0.024
Female (%)	316 (36.7%)	63,002 (39.4%)	0.056	316 (36.7%)	296 (34.4%)	0.049
Male (%)	545 (63.3%)	96,843 (60.6%)	0.056	545 (63.3%)	565 (65.6%)	0.049
White (%)	357 (41.5%)	89,921 (56.3%)	0.299	357 (41.5%)	355 (41.2%)	0.005
Comorbid conditions						
Hypertension	762 (88.5%)	104,209 (65.2%)	0.575	762 (88.5%)	754 (87.6%)	0.029
Dyslipidemia	691 (80.3%)	80,497 (50.4%)	0.662	691 (80.3%)	699 (81.2%)	0.024
Ischemic heart diseases	725 (84.2%)	118,576 (74.2%)	0.249	725 (84.2%)	705 (81.9%)	0.062
Cardiomyopathy	69 (8.0%)	25,002 (15.6%)	0.238	69 (8.0%)	62 (7.2%)	0.031
Chronic kidney disease	490 (56.9%)	32,387 (20.3%)	0.813	490 (56.9%)	490 (56.9%)	<0.001
Overweight, obesity and other hyperalimentation	304 (35.3%)	36,347 (22.7%)	0.280	304 (35.3%)	296 (34.4%)	0.020
Diseases of the respiratory system	519 (60.3%)	80,632 (50.4%)	0.199	519 (60.3%)	513 (59.6%)	0.014
Heart failure	363 (42.2%)	77,093 (48.2%)	0.122	363 (42.2%)	367 (42.6%)	0.009
Cerebrovascular diseases	250 (29.0%)	26,028 (16.3%)	0.308	250 (29.0%)	245 (28.5%)	0.013
Nicotine dependence	151 (17.5%)	30,347 (19.0%)	0.037	151 (17.5%)	158 (18.4%)	0.021
Diabetes mellitus	748 (86.9%)	58,631 (36.7%)	1.206	748 (86.9%)	755 (87.7%)	0.024
Medication use						
ACE inhibitors	336 (39.0%)	62,892 (39.3%)	0.007	336 (39.0%)	320 (37.2%)	0.038
Angiotensin II inhibitors	538 (62.5%)	51,982 (32.5%)	0.629	538 (62.5%)	505 (58.7%)	0.078
Antilipemic agents	740 (85.9%)	103,627 (64.8%)	0.506	740 (85.9%)	732 (85.0%)	0.026
Beta-blockers	658 (76.4%)	105,730 (66.1%)	0.229	658 (76.4%)	664 (77.1%)	0.017
Sacubitril	62 (7.2%)	10,224 (6.4%)	0.032	62 (7.2%)	72 (8.4%)	0.043
Laboratory						
Cholesterol, mg/dL	153.7±49.1	158.0±51.5	0.086	153.7±49.1	153.3±49.0	0.008
Hemoglobin A1c, %	7.4±1.7	6.8±1.9	0.336	7.4±1.7	7.4±2.0	0.027
Blood urea nitrogen, mg/dL	26.5±13.3	22.2±14.4	0.311	26.5±13.3	28.4±19.8	0.112
Creatinine, mg/dL	1.6±3.9	1.5±6.1	0.022	1.6±3.9	1.9±4.7	0.064

Source: Compiled by the authors of this study

After matching, the secondary outcomes demonstrated no statistical significant differences between the non-steroidal and steroidal MRAs groups. Atrial arrhythmia was statistically not significant, with a hazard ratio of 0.752 (95% CI: 0.520-1.089). Ventricular arrhythmia was also statistically insignificant between the two groups with a hazard ratio of 1.016 (95% CI: 0.558-1.850).Cardiac arrest events were statistically insignificant, with a hazard ratio of 0.851 (95% CI: 0.465- 1.558); (Table 2, Fig. 3).

DISCUSSION

In this large, real-world, propensity score matched cohort study of post-myocardial infarction patients without a prior history of cardiac arrhythmia, treatment with nonsteroidal mineralocorticoid receptor antagonists (nsMRAs) was not associated with a statistically significant reduction in the incidence of new-onset cardiac arrhythmias or cardiac arrest events compared with steroidal MRAs (sMRAs). These findings suggest that, in the post-MI setting, nsMRAs and

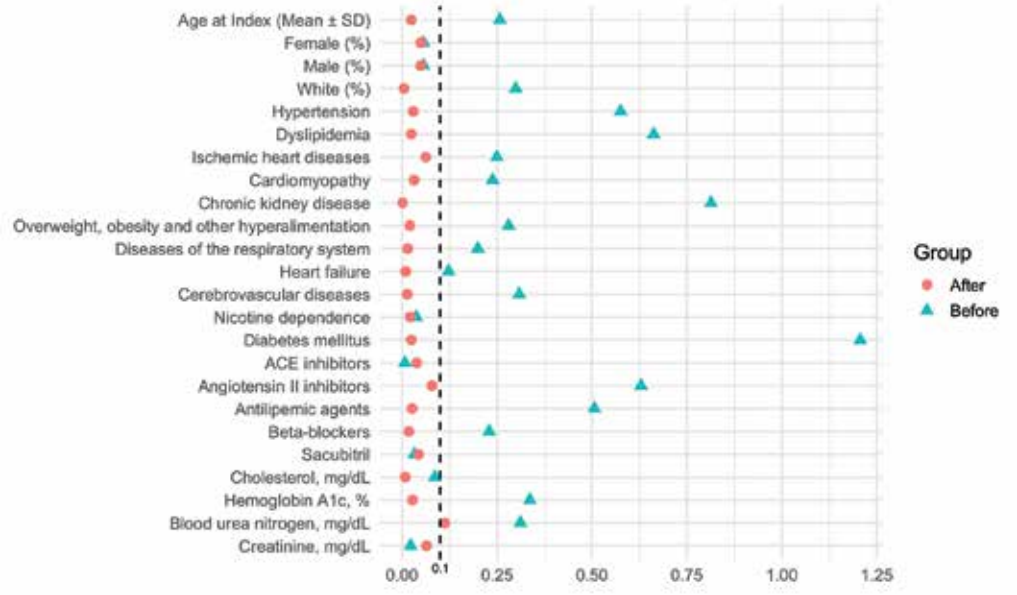

Fig. 2. Baseline characteristics and cardiovascular risk factor prevalence in steroidal vs. non-steroidal groups before and after propensity score matching

Source: Own materials

Table 2. Primary and secondary clinical outcomes: Steroidal vs. non-steroidal users

Outcome	Risk of Event, n (%) (Steroidal mineralocorticoid group)	Risk of Event, % (Non-steroidal mineralocorticoid group)	Hazard Ratio (95% CI)	P Value
Primary Outcome				
New Onset Cardiac Arrhythmia	97 (15.6%)	62 (9.1%)	0.856 (0.614–1.193)	P=0.284
Secondary Outcome				
Atrial Arrhythmia	86 (13.1%)	47 (6.6%)	0.752 (0.520-1.089)	P=0.125
Ventricular Arrhythmia	28 (3.4%)	20 (2.4%)	1.016 (0.558-1.850)	P=0.647
Cardiac Arrest	18 (2.1%)	34 (4.0%)	0.851 (0.465-1.558)	P=0.081

Source: Compiled by the authors of this study

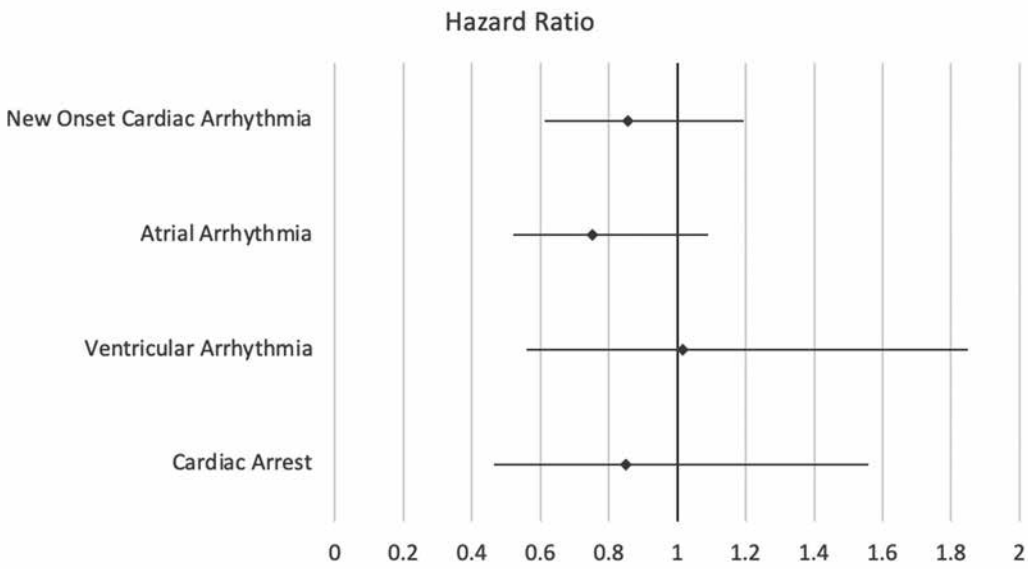

Fig. 3. Forest plot of hazard ratios for primary and secondary outcomes

Source: Own materials

sMRAs have comparable arrhythmic safety profiles. While direct head to head comparative trials or cohort studies between nonsteroidal and steroidal MRAs for arrhythmia outcomes are lacking. One study showed Nonsteroidal MRAs are associated with a statistically significant reduction in new-onset atrial fibrillation/atrial flutter compared with placebo (HR: 0.83; 95% CI: 0.71-0.97; P=0.019) [10]. A meta-analysis of 20 trials demonstrated that patients with established cardiovascular diseases/risk factors treated with MRAs benefit from a significant reduction in the risk of AF events (3.8% vs. 5.0%; RR=0.76: 95% CI: 0.67-0.87) as compared with placebo/usual care. Another meta-analysis of 20 trials demonstrated that MRAs as a class reduce atrial fibrillation events (risk ratio=0.76; 95% CI: 0.67- 0.87) in patients with and without prior atrial fibrillation [11].

MRAs remain clinically relevant after MI because mineralocorticoid receptor activation contributes to adverse remodeling, inflammation, and fibrosis, pathways that plausibly promote both atrial and ventricular arrhythmogenesis [12]. Still, the post-MI randomized evidence suggests that the clinical impact of MR blockade depends strongly on patient phenotype and baseline risk, and that arrhythmia-specific endpoints are often not the main drivers of trial results. In EPHESUS, eplerenone improved outcomes in patients with MI complicated by left ventricular dysfunction and heart failure, including reductions in sudden cardiac death [13]. By contrast, when aldosterone blockade is introduced very early or applied to broader MI populations without established heart failure, benefits have been less consistent [14]. In REMINDER, early eplerenone in STEMI patients without known heart failure improved a composite endpoint largely influenced by biomarker changes rather than hard arrhythmic events [14]. Similarly, ALBATROSS did not show benefit of an early MRA regimen initiated irrespective of heart failure or left ventricular dysfunction on a composite endpoint that included clinically significant ventricular arrhythmia-related outcomes [15]. This trial landscape makes it plausible that, even when MR blockade is beneficial in selected post-MI populations, any incremental effect on incident arrhythmias, especially differences between MRA classes, may be modest and difficult to detect without systematic rhythm surveillance.

Evidence syntheses point in a similar direction: MRAs after MI appear to deliver their most consistent clinical value through heart-failure and remodeling pathways, while signals for arrhythmia prevention are less uniform. A systematic review and meta-analysis of randomized trials in acute MI reported non-significant reductions in several short-term outcomes and likewise a non-significant reduction in ventricular arrhythmia, with cardiovascular mortality benefits emerging more clearly after longer follow-up in some analyses [16]. Additional meta-analytic work has shown reductions in all-cause mortality and new or worsening heart failure after MI with MRA therapy, including among patients without baseline heart failure, but without a clear, consistent reduction in potentially lethal ventricular arrhythmias across studies [17, 18]. Taken together, this broader evidence base fits with what we observed, class selection did not show a detectable association with incident arrhythmia risk in our post-MI cohort.

Within steroidal MRAs, outcome data are robust in heart failure populations and support biologic plausibility for antiarrhythmic effects in some settings. Spironolactone improved major outcomes in systolic heart failure [19], and eplerenone reduced new-onset atrial fibrillation/flutter in patients with systolic heart failure and mild symptoms [20]. In acute MI, some observational work has suggested lower rates of life-threatening ventricular arrhythmias and cardiac arrest with early aldosterone blockade, though such findings remain vulnerable to confounding and should be interpreted cautiously [21]. This mixed pattern suggests strong benefits in selected high-risk phenotypes, less consistent signals on incident arrhythmia endpoints, again providing a reasonable context for a neutral class comparison in routine post-MI care.

For non-steroidal MRAs, most randomized evidence comes from cardiorenal metabolic populations rather than dedicated post-MI cohorts. Finerenone reduced cardiovascular and heart-failure events in patients with type 2 diabetes and chronic kidney disease [22]. Importantly, pooled participant level data have also suggested a reduction in new-onset atrial fibrillation/flutter with Finerenone across trials with adjudicated events [10]. Meta-analytic evidence similarly reports improvements in major cardiovascular outcomes and heart-failure–related hospitalizations with Finerenone versus control, alongside a higher risk of hyperkalemia, while myocardial infarction risk and overall adverse events were broadly comparable [23]. These results support the concept that non-steroidal MR blockade may influence atrial remodeling pathways, but they do not directly answer whether class choice changes arrhythmia risk in a post-MI population, especially when event capture relies on routine clinical practice rather than structured monitoring.

Real-world comparative studies have tried to address that evidence gap, with some suggesting lower hyperkalemia risk and improved cardiovascular outcomes, including lower arrhythmia incidence and mortality with non-steroidal MRAs compared with steroidal MRAs in selected high risk populations [24, 25]. Those findings are clinically interesting, but they arise in different patient mixes (often enriched for diabetes, CKD, and heart failure), and are sensitive to confounding by indication, differences in background therapies, and how outcomes are captured. Against that backdrop, our neutral post-MI class comparison may reflect true similarity in net electrophysiologic effects, differences in phenotype and baseline risk, and/or the reality that modest class effects on incident arrhythmias are hard to detect without systematic rhythm surveillance.

A neutral class effect is biologically plausible. Mineralocorticoid receptor activation has been linked to atrial and ventricular arrhythmias through direct effects on cardiac electrical activity, particularly calcium handling and action potential properties with fibrosis likely contributing in some settings [12]. Because both steroidal and non-steroidal MRAs ultimately block the same receptor pathway,

any differences in incident arrhythmias between classes may be small, context dependent, or difficult to detect in routine practice. At the same time, both classes can influence potassium homeostasis, an important determinant of cardiac electrophysiology and hyperkalemia remains a key tradeoff in Finerenone trials [23]. In practice, the net observed arrhythmia risk may therefore reflect a balance between substrate modifying effects of MR blockade and real-world factors such as potassium dynamics, dose intensity, adherence, and monitoring, which could blunt modest class-related differences.

Clinically, our findings suggest that arrhythmia risk, by itself, should probably not be the primary determinant of choosing a non-steroidal versus steroidal MRA after MI when the goal is prevention of new-onset arrhythmias. Instead, class selection can be guided by the broader clinical picture such as renal function and potassium trajectory, comorbid diabetes or chronic kidney disease, tolerability (including endocrine adverse effects), and access or cost, while remaining aligned with phenotype specific evidence supporting MRA therapy after MI and across cardiorenal metabolic syndromes. Ultimately, prospective head to head comparisons with systematic rhythm surveillance and adjudicated endpoints will be required to determine whether small, clinically meaningful class differences exist in post-MI patients, particularly for ventricular arrhythmias where event rates are lower and precision is often limited.

LIMITATIONS OF THE STUDY

Our study has limitations like other observational retrospective studies. It cannot establish causality. Despite propensity score matching helped reduce confounding, unmeasured factors such as the dose and the frequency of the MRAs use, physical activity, health care accessibility and socioeconomic status may still have influenced the results. Therefore, our findings cannot establish a dose response relationship. Furthermore, post-myocardial infarction, incidence of arrhythmia and type of MRAs use were diagnosed based on International Classification of Diseases (ICD) codes, which carries the risk of misclassification or coding errors and biases common in real-world databases..

In regard to these limitations, our study has notable strengths. It is considered one of the first studies to compare steroidal MRAs to non steroidal MRAs receiving patients using a large, real-world dataset spanning diverse healthcare settings, thereby investigating the relationship between use and cardiac arrhythmias across a diverse, multicenter patient population. We employed rigorous propensity score matching to enhance comparability between the steroidal and nonsteroidal MRAs cohorts across a wide range of demographic and clinical variables. Also, our methodology allowed exploration of potential associations between steroidal or non steroidal MRAs use and arrhythmia risk within a large, diverse post-myocardial infarction patients population.

CONCLUSIONS

In conclusion, in a large, propensity-matched cohort of post-MI patients without prior arrhythmia, nsMRAs were not associated with a statistically significant reduction in new-onset cardiac arrhythmias compared with sMRAs. These results suggest comparable arrhythmic outcomes between MRA subclasses in the post-MI setting. Prospective randomized studies and mechanistic investigations are warranted to further elucidate whether specific patient subgroups may derive differential arrhythmic benefit from nsMRAs.

LIST OF ABBREVIATIONS

ACE: Angiotensin-Converting Enzyme
AF: Atrial Fibrillation
ALBATROSS: Acute Left Ventricular Function During Beta-Blocker Therapy After Acute Myocardial Infarction Study
CKD: Chronic Kidney Disease
CI: Confidence Interval
EHR: Electronic Health Record
ENaC: Epithelial Sodium Channel
eGFR: Estimated Glomerular Filtration Rate
FDA: Food and Drug Administration
HCO: Healthcare Organization
HFrEF: Heart Failure with Reduced Ejection Fraction
HR: Hazard Ratio
ICD-10: International Classification of Diseases, 10th Revision
LVEF: Left Ventricular Ejection Fraction
MI: Myocardial Infarction
MR: Mineralocorticoid Receptor
MRA: Mineralocorticoid Receptor Antagonist
nsMRA: Non-Steroidal Mineralocorticoid Receptor Antagonist
sMRA: Steroidal Mineralocorticoid Receptor Antagonist
PSM: Propensity Score Matching
REMINDER: Early Mineralocorticoid Receptor Blockade in STEMI Study
RR: Risk Ratio
SD: Standard Deviation
STEMI: ST-Elevation Myocardial Infarction
SMD: Standardized Mean Difference
Tx: Treatment

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Appendix

Category	Type	Coding System	Code	Description
Demographics	Age	—	—	Age ≥ 18 years
Diagnosis	Myocardial Infarction	ICD-10-CM	I21.0	STEMI myocardial infarction of anterior wall
Diagnosis	Myocardial Infarction	ICD-10-CM	I21.09	STEMI myocardial infarction involving other coronary artery of anterior wall
Diagnosis	Myocardial Infarction	ICD-10-CM	I21.02	STEMI myocardial infarction involving left anterior descending coronary artery
Diagnosis	Myocardial Infarction	ICD-10-CM	I21.01	STEMI myocardial infarction involving left main coronary artery
Diagnosis	Myocardial Infarction	ICD-10-CM	I21.1	STEMI myocardial infarction of inferior wall
Diagnosis	Myocardial Infarction	ICD-10-CM	I21.19	STEMI myocardial infarction involving other coronary artery of inferior wall
Diagnosis	Myocardial Infarction	ICD-10-CM	I21.11	STEMI myocardial infarction involving right coronary artery
Diagnosis	Myocardial Infarction	ICD-10-CM	I21.2	STEMI myocardial infarction of other sites
Diagnosis	Myocardial Infarction	ICD-10-CM	I21.29	STEMI myocardial infarction involving other sites
Diagnosis	Myocardial Infarction	ICD-10-CM	I21.21	STEMI myocardial infarction involving left circumflex coronary artery
Diagnosis	Myocardial Infarction	ICD-10-CM	I21.3	STEMI myocardial infarction of unspecified site
Diagnosis	Myocardial Infarction	ICD-10-CM	I21.4	NSTEMI myocardial infarction

Category	Type	Coding System	Code	Description
Diagnosis	Myocardial Infarction	ICD-10-CM	I21.9	Acute myocardial infarction, unspecified
Diagnosis	Myocardial Infarction	ICD-10-CM	I22.0	Subsequent STEMI myocardial infarction of anterior wall
Diagnosis	Myocardial Infarction	ICD-10-CM	I22.1	Subsequent STEMI myocardial infarction of inferior wall
Diagnosis	Myocardial Infarction	ICD-10-CM	I22.2	Subsequent NSTEMI myocardial infarction
Diagnosis	Myocardial Infarction	ICD-10-CM	I22.8	Subsequent STEMI myocardial infarction of other sites
Diagnosis	Myocardial Infarction	ICD-10-CM	I22.9	Subsequent STEMI myocardial infarction of unspecified site
Diagnosis	Arrhythmia	ICD-10-CM	I49	Other cardiac arrhythmias
Diagnosis	Arrhythmia	ICD-10-CM	I49.9	Cardiac arrhythmia, unspecified
Medication	MRA (Steroidal)	RxNorm	9997	Spironolactone
Medication	MRA (Steroidal)	RxNorm	298869	Eplerenone
Medication	MRA (Non-steroidal)	RxNorm	2562811	Finerenone
Medication	MRA (Non-steroidal)	OMOP	5040499	Esaxerenone
Diagnosis	Ventricular Arrhythmia	ICD-10-CM	I49.3	Ventricular premature depolarization
Diagnosis	Ventricular Arrhythmia	ICD-10-CM	I47.2	Ventricular tachycardia

Category	Type	Coding System	Code	Description
Diagnosis	Ventricular Arrhythmia	ICD-10-CM	I47.20	Ventricular tachycardia, unspecified
Diagnosis	Ventricular Arrhythmia	ICD-10-CM	I49.0	Ventricular fibrillation and flutter
Diagnosis	Ventricular Arrhythmia	ICD-10-CM	I49.01	Ventricular fibrillation
Diagnosis	Ventricular Arrhythmia	ICD-10-CM	I49.02	Ventricular flutter
Diagnosis	Atrial Arrhythmia	ICD-10-CM	I48	Atrial fibrillation and flutter
Diagnosis	Atrial Arrhythmia	ICD-10-CM	I48.91	Unspecified atrial fibrillation
Diagnosis	Atrial Arrhythmia	ICD-10-CM	I48.0	Paroxysmal atrial fibrillation
Diagnosis	Atrial Arrhythmia	ICD-10-CM	I48.1	Persistent atrial fibrillation
Diagnosis	Atrial Arrhythmia	ICD-10-CM	I48.92	Unspecified atrial flutter
Diagnosis	Atrial Arrhythmia	ICD-10-CM	I48.9	Unspecified atrial fibrillation and flutter
Diagnosis	Atrial Arrhythmia	ICD-10-CM	I48.2	Chronic atrial fibrillation
Diagnosis	Atrial Arrhythmia	ICD-10-CM	I48.20	Chronic atrial fibrillation, unspecified
Diagnosis	Atrial Arrhythmia	ICD-10-CM	I48.19	Other persistent atrial fibrillation

Category	Type	Coding System	Code	Description
Diagnosis	Atrial Arrhythmia	ICD-10-CM	I48.21	Permanent atrial fibrillation
Diagnosis	Atrial Arrhythmia	ICD-10-CM	I48.11	Longstanding persistent atrial fibrillation
Diagnosis	Atrial Arrhythmia	ICD-10-CM	I48.3	Typical atrial flutter
Diagnosis	Atrial Arrhythmia	ICD-10-CM	I48.4	Atypical atrial flutter
Diagnosis	Supraventricular Arrhythmia	ICD-10-CM	I47.1	Supraventricular tachycardia
Diagnosis	Supraventricular Arrhythmia	ICD-10-CM	I47.19	Other supraventricular tachycardia
Diagnosis	Cardiac Arrest	ICD-10-CM	I46	Cardiac arrest

AVAILABILITY OF DATA AND MATERIAL
The data supporting the findings of this study are available through the TriNetX research network but are subject to licensing restrictions. Access to TriNetX data can be obtained upon reasonable request and with permission from TriNetX, LLC.

CONFLICT OF INTEREST
The authors declare no conflict of interest

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ORIGINAL ARTICLE

Structural remodeling of the glomerular filtration barrier and renal tubular structures during experimental alcohol intoxication

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ABSTRACT

Aim: To study changes in the structural components of the glomerular filtration barrier and renal tubular structures following exposure to alcoholic beverages of varying strength and quality in an experimental model.

Materials and Methods: This experimental study was conducted on 130 sexually mature male Wistar albino rats. The animals were divided into four experimental groups of 30 each, according to the type of alcoholic beverage administered: vodka (40% alcohol by volume, ABV), home-distilled spirits (40% and 50% ABV), and wine (17% ABV). Each group was further subdivided into three subgroups of 10 animals according to the first (5 days), second (9 days), and third (12 days) stages of alcohol administration. Ten animals served as controls. All procedures involving animals were performed in accordance with bioethical standards. Morphological analysis was carried out using histological, electron microscopic, and morphometric methods.

Results: Administration of 40% ABV vodka, 40% ABV home-distilled spirits, and 17% ABV wine for 5 days did not induce noticeable morphological changes in the components of the glomerular filtration barrier or renal tubular structures. At this stage, administration of 50% ABV home-distilled spirits induced functional stress. On day 9, administration of 40% ABV vodka and 17% ABV wine resulted in hypertrophy and hyperplasia of both the components of the glomerular filtration barrier and renal tubular structures. Administration of 40% ABV home-distilled spirits during this period induced initial signs of destruction. Administration of 50% ABV home-distilled spirits over the same period led to an overload of adaptive and protective mechanisms. By day 12, administration of 40% ABV and 50% ABV home-distilled spirits caused pronounced destructive changes, whereas 40% ABV vodka and 17% ABV wine induced functional stress.

Conclusions: The most pronounced destructive changes in the components of the glomerular filtration barrier and renal tubular structures were observed in animals administered 50% ABV home-distilled spirits, whereas the least pronounced changes were associated with 17% ABV fortified wine. The severity of destructive changes progressively increased with the duration of alcohol administration.

KEYWORDS: kidney, glomerular filtration barrier, renal tubular structures, alcohol intoxication, experiment.

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INTRODUCTION

Globally, approximately 3 million deaths per year are attributable to alcohol consumption, accounting for 5.3% of all mortality [1]. In Ukraine, over 40,000 deaths annually are alcohol-related, with women comprising a larger proportion of these cases [2, 3].

Long-term alcohol consumption is associated with social dysfunction and mental disorders, as well as damage to multiple organs and body systems, including the brain, heart, and liver [4]. Renal damage in chronic alcohol intoxication is significantly less common than alcoholic hepatitis or involvement of the pancreas and heart. However, findings from numerous clinical and experimental studies suggest that different types of alcoholic beverages play a substantial etiological role in the development of kidney injury [5, 6].

Several studies have investigated morphological changes in the glomerular filtration barrier (GFB) components and renal tubular structures in the context of excessive alcohol use. However, researchers have primarily focused on changes induced solely by ethanol and its metabolites. The impact of different alcoholic beverages, in particular home-distilled spirits and fortified wines, has not received sufficient attention. Therefore, we aimed to experimentally investigate, at the light microscopic and ultrastructural levels, changes in the GFB components and renal tubular structures in animals subjected to alcohol administration, using vodka (40% alcohol by volume, ABV), home-distilled spirits (40% and 50% ABV), and fortified wine (17% ABV).

AIM

To investigate structural remodeling of the GFB components and renal tubular structures at the histological and ultrastructural levels in animals subjected to alcohol administration using alcoholic beverages of varying strength and quality.

MATERIALS AND METHODS

The experiment was conducted on 130 sexually mature male Wistar albino rats weighing between 180 and 210 g, 10 of which served as controls. The animals were divided into four groups of 30 animals each. Each group was further subdivided into three subgroups of 10 animals according to the first (5 days), second (9 days), and third (12 days) time points of experimental alcohol exposure.

Alcohol administration was carried out with consideration of the ethanol-oxidizing systems of the animals, via intragastric gavage four times daily at 8:00 a.m., 2:00 p.m., 6:00 p.m., and 10:00 p.m. Animal care, all procedures, and euthanasia were conducted in strict adherence to the general bioethical standards and the regulations on animal research.

Sampling for light and electron microscopy, block preparation, sectioning, and staining were performed using standard methods [7]. To objectively assess the damaging effects of alcohol on the GFB components and renal tubular structures, histometric analysis of their structural elements was performed [8] using a modified approach [9].

RESULTS

Five days after administration of 40% ABV vodka, the GFB components and renal tubular structures exhibited a typical architecture, consistent with that of the control group and well documented in the literature. Therefore, a detailed descriptive characterization of these structures was deemed unnecessary.

With prolonged alcohol administration for 9 days, initial structural changes in the structural components of the GFB were observed, including enlarged nuclei of epithelial and endothelial cells, swollen mitochondria with an electron-lucent matrix, and an irregularly thickened basement membrane of the glomerular capillaries. Vacuolization of podocyte cytoplasm and pronounced nuclear invagination were also noted. In the cytoplasm of podocytes and their primary processes, the rough endoplasmic reticulum exhibited dilated cisternae and tubules.

The surface areas of the vascular glomeruli and renal corpuscles at the present and subsequent stages of the experiment are presented in Table 1.

In renal tubular structures, granular degeneration in epithelial cells, deformation of the brush border microvilli in the proximal convoluted tubules, and mitochondrial swelling were observed. The epithelial cells of the distal convoluted tubules had a few microvilli in their apical region. Their mitochondria were enlarged with an electron-lucent matrix.

Changes in the morphometric parameters of the proximal and distal convoluted tubules at all experimental stages are presented in Tables 2 and 3, respectively.

By the end of the 12-day period of 40% ABV vodka administration, morphological changes progressed (Fig. 1A). The thickness of the glomerular capillary basement membrane increased, with fusion of individual podocyte foot processes leading to narrowing of the filtration slits. Podocytes and glomerular capillary endothelial cells exhibited noticeable nuclear envelope invagination, an increase in the number of rough endoplasmic reticulum and Golgi apparatus profiles, and mitochondrial swelling with a reduction in cristae. Administration of 40% ABV vodka for 12 days resulted in ultrastructural damage to epithelial cells of the proximal and distal convoluted tubules of the nephron (Fig. 2A). In epithelial cells of the proximal convoluted tubules, features of granular degeneration prevailed, whereas hydropic degeneration predominated in the distal tubules. Peritubular capillary endothelial cells were swollen, with enlarged nuclei exhibiting deep nuclear envelope invaginations. Mitochondria were swollen with reduced cristae. The number of fenestrae in the cytoplasm of endothelial cells increased, and pores appeared in some

Table 1. Surface area of vascular glomeruli and renal corpuscles at the experimental stages

Alcohol administration	Vascular glomerulus (μm²)	Renal corpuscle (μm²)
Control	4.008±140	6.315±202
40% ABV vodka; 5 days	4.113±147	6.343±214
40% ABV vodka; 9 days	4.203±151	6.543±218
40% ABV vodka; 12 days	4.465±178	6.789±291
40% ABV home-distilled spirits; 5 days	4.196±144	6.325±370
40% ABV home-distilled spirits; 9 days	4.357±182	6.864±242
40% ABV home-distilled spirits; 12 days	4.456±143	7.035±211*
50% ABV home-distilled spirits; 5 days	4.165±166	6.415±192
50% ABV home-distilled spirits; 9 days	4.635±139*	7.025±210*
50% ABV home-distilled spirits; 12 days	4.925±147*	7.937±238*
17% ABV wine; 5 days	4.152±120	6.234±219
17% ABV wine; 9 days	4.305±137	6.445±206
17% ABV wine; 12 days	4.475±134*	6.955±208

*Note: *statistically significant difference compared to the control group; n=10 for each experimental time point; data are presented as mean±SD; ABV, alcohol by volume.*

Table 2. Changes in morphometric parameters of the proximal convoluted tubules in cross-section at the experimental stages

Alcohol administration	External diameter (μm)	Luminal diameter (μm)	Wall thickness (μm)
Control	46.50±1.63	7.58±0.26	38.92±1.36
40% ABV vodka; 5 days	46.85±1.64	7.30±0.25	39.55±1.38
40% ABV vodka; 9 days	47.15±1.62	5.75±0.20*	41.4±1.32
40% ABV vodka; 12 days	48.60±1.55	4.65±0.16*	43.95±1.40*
40% ABV home-distilled spirits; 5 days	46.90±1.59	7.20±0.25	39.70±1.38
40% ABV home-distilled spirits; 9 days	48.25±1.68	5.15±0.18*	43.10±1.50
40% ABV home-distilled spirits; 12 days	48.95±1.71	4.05±0.14*	44.90±1.57*
50% ABV home-distilled spirits; 5 days	47.15±1.79	7.05±0.27	40.10±1.52
50% ABV home-distilled spirits; 9 days	48.25±1.78	4.85±0.17*	43.40±1.64
50% ABV home-distilled spirits; 12 days	49.55±1.73	3.95±0.15*	45.60±1.45*
17% ABV wine; 5 days	46.60±1.95	7.40±0.29	39.20±1.56
17% ABV wine; 9 days	47.30±1.98	6.05±0.24*	41.25±1.57
17% ABV wine; 12 days	48.15±1.92	4.90±0.19*	43.25±1.73

*Note: *statistically significant difference compared to the control group; n=10 for each experimental time point; data are presented as mean±SD; ABV, alcohol by volume.*

Table 3. Changes in morphometric parameters of the distal convoluted tubules in cross-section at the experimental stages

Alcohol administration	External diameter (μm)	Luminal diameter (μm)	Wall thickness (μm)
Control	39.15±1.62	10.67±0.44	28.48±0.99
40% ABV vodka; 5 days	39.35±1.37	10.56±0.36	28.79±1.07
40% ABV vodka; 9 days	41.56±1.66	8.75±0.35*	32.81±1.31*
40% ABV vodka; 12 days	42.65±1.70	5.15±0.20*	37.5±1.50*
40% ABV home-distilled spirits; 5 days	40.25±1.52	9.75±0.34	30.50±1.15
40% ABV home-distilled spirits; 9 days	41.95±1.46	8.65±0.29*	33.30±1.09*
40% ABV home-distilled spirits; 12 days	44.25±1.77	4.95±0.19*	39.30±1.37*
50% ABV home-distilled spirits; 5 days	40.95±1.47	9.35±0.35	31.60±1.26
50% ABV home-distilled spirits; 9 days	42.05±1.68	7.85±0.29*	34.20±1.33*
50% ABV home-distilled spirits; 12 days	44.75±1.87	4.35±0.16*	40.40±1.57*
17% ABV wine; 5 days	39.40±1.49	10.45±0.37	28.95±1.01
17% ABV wine; 9 days	41.45±1.57	8.85±0.35*	32.60±0.97*
17% ABV wine; 12 days	42.50±1.61	5.30±0.16*	37.20±1.11*

*Note: *statistically significant difference compared to the control group; n = 10 for each experimental time point; data are presented as mean ± SD; ABV, alcohol by volume.*

areas. These changes were accompanied by alterations in the morphometric parameters of the proximal and distal convoluted tubules of the kidney at the experimental stages (Tables 2, 3).

Alcohol administration of 40% ABV home-distilled spirits for 5 days did not induce pronounced morphological changes. The morphological state of the GFB components was similar to that observed at the corresponding stage of the experiment with 40% ABV vodka administration. This suggests that, at the early stage of alcohol-related pathology, the kidney exhibits well-developed adaptive and protective mechanisms against alcohol exposure.

With prolonged administration of 40% ABV home-distilled spirits for 9 days, hypertrophy and hyperplasia of podocytes and glomerular capillary endothelial cells were observed. The nuclei were enlarged, exhibiting pronounced invaginations of the nuclear envelope. Mitochondria were swollen with a homogeneous matrix. Endothelial pores and fenestrae were dilated. In the epithelial cells of the proximal convoluted tubules, features of granular degeneration were observed, whereas hydropic degeneration predominated in the distal tubules. In the cytoplasm of epithelial cells in

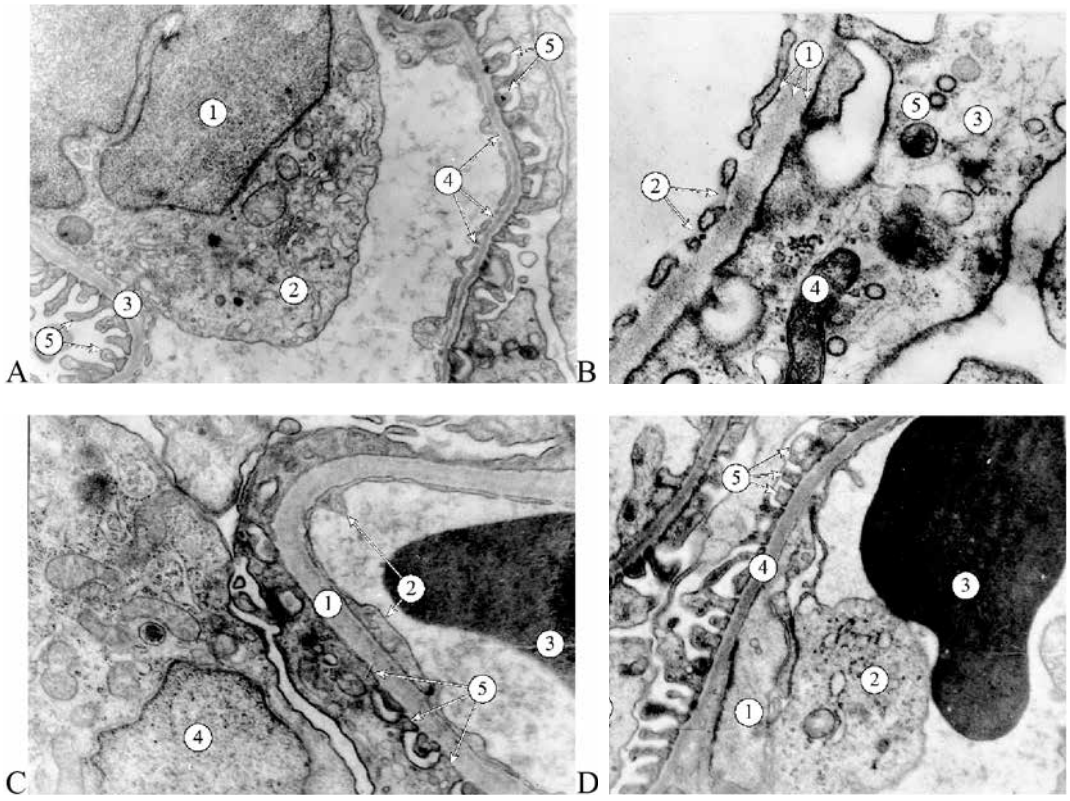

Fig. 1. Ultrastructure of the glomerular filtration barrier at different stages of alcohol administration. A. After 12 days of 40% ABV vodka administration: 1 – endothelial cell nucleus; 2 – endothelial cell cytoplasm; 3 – basement membrane; 4 – fenestrae; 5 – podocyte foot processes. B. After 12 days of 40% ABV home-distilled spirit administration: 1 – basement membrane; 2 – fenestrae; 3 – podocyte primary process; 4 – mitochondria; 5 – lysosomes. C. After 12 days of 500% ABV home-distilled spirit administration: 1 – basement membrane; 2 – endothelial cell; 3 – erythrocyte in the lumen of a capillary; 4 – podocyte nucleus; 5 – podocyte foot processes. D. After 12 days of 17% ABV wine administration: 1 – vacuolated endothelial cell; 2 – activated platelet with processes; 3 – erythrocyte; 4 – basement membrane; 5 – podocyte foot processes; 6 – podocyte primary process. Electron microscopy. Magnification: A ×6,000, B ×12,500, C ×7,000, D ×6,000.

the proximal and distal convoluted tubules, the number of vacuoles increased. The rough endoplasmic reticulum exhibited dilated cisternae. Mitochondria were swollen. Alcohol administration at this stage led to ultrastructural remodeling of the peritubular capillaries.

With prolonged administration of 40% ABV home-distilled spirits for 12 days, organelle disintegration and destructive changes were observed (Fig. 1B). Morphometric analysis revealed a statistically significant increase in the surface area of the renal corpuscles and a non-significant increase in the surface area of the glomeruli (Table 1). Comparison of the findings obtained after administration of 40% ABV vodka and 40% ABV home-distilled spirits indicated that changes in the tubular epithelium were more pronounced under chronic intoxication with the latter.

At the first stage of the experiment (5 days), during administration of 50% ABV home-distilled spirits, glomerular capillary endothelial cells were characterized by cytoplasmic swelling, the presence of micropinocytotic vesicles, and dilation of pores and fenestrae. Podocytes and their primary processes exhibited organelle hypertrophy and hyperplasia. Notably, even at early stages, chronic alcohol intoxication with 50% ABV home-distilled spirits induced granular degeneration in the proximal and distal convoluted tubules of the nephron.

Further administration of 50% ABV home-distilled spirits for 9 days induced destruction of the basement membrane of glomerular capillary endothelial cells and deformation of podocyte primary and foot processes in the visceral layer of the glomerular capsule. Morphometric analysis revealed a statistically significant increase in the surface area of the renal corpuscles and glomerular capillaries (Table 1). Disintegration of cytoplasmic structures and the nuclear apparatus was observed in epithelial cells of the proximal and distal convoluted tubules. Necrosis was observed in the epithelial cells of the distal convoluted tubules. Morphometric analysis revealed a decrease in luminal diameter and an increase in wall thickness of the proximal and distal convoluted tubules (Tables 2, 3). In the peritubular capillaries, pronounced hypertrophy and hyperplasia were observed. In some areas, endothelial cells detached from the basement membrane. After 12 days of alcohol administration, destructive changes predominated in the structural components of the GFB and epithelial cells of the proximal and distal convoluted tubules of the nephron (Fig. 1C, 2B, 2C).

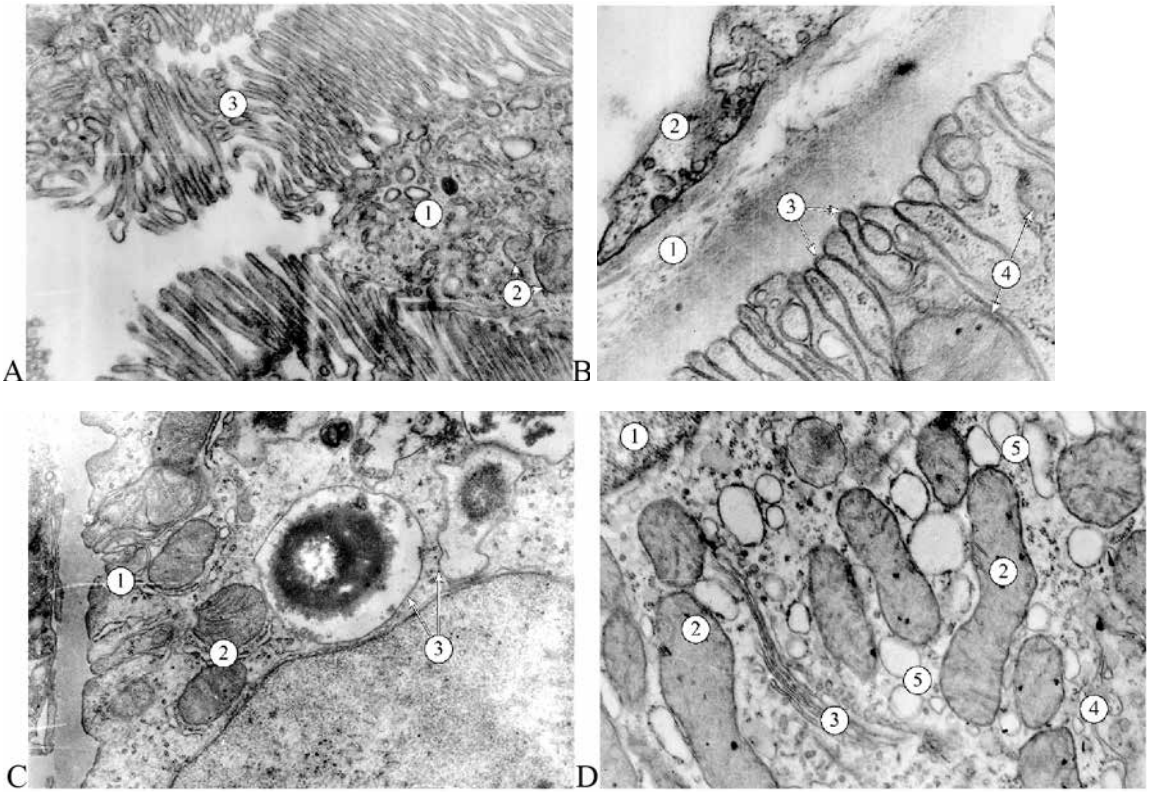

Fig. 2. Ultrastructure of the tubular epithelium at different stages of alcohol administration. A. Proximal tubule of the nephron after 12 days of 40% ABV vodka administration: 1 – epithelial cell cytoplasm; 2 – mitochondrion; 3 – brush border. B. Proximal tubule of the nephron after 12 days of 50% ABV home-distilled spirit administration: 1 – basement membrane; 2 – endothelial cell; 3 – invaginations of the epithelial cell plasma membrane; 4 – mitochondria. C. Distal tubule of the nephron after 12 days of 50% ABV home-distilled spirit administration: 1 – folds of the epithelial cell plasma membrane; 2 – mitochondria; 3 – autophagolysosomes. D. Distal tubule of the nephron after 12 days of 17% ABV wine administration: 1 – epithelial cell nucleus; 2 – mitochondria; 3 – rough endoplasmic reticulum; 4 – Golgi apparatus; 5 – vacuoles. Electron microscopy. Magnification: A ×8,000, B ×8,500, C ×10,000, D ×20,000.

Comparison of the results obtained after administration of 40% and 50% ABV home-distilled spirits revealed more pronounced morphological changes in animals receiving 50% ABV home-distilled spirits. This indicates that, for beverages of similar quality, the severity of alcohol intoxication depends on the ethanol concentration.

Alcohol intoxication with 17% ABV wine for 5 days did not induce cellular or subcellular changes. Alcohol administration for 9 days did not result in morphological alterations at the microscopic level as well. Electron microscopy revealed only mild organelle hypertrophy and hyperplasia in glomerular capillaries.

Twelve days after 17% ABV wine administration, pronounced hypertrophic changes in intracellular structures of podocytes and glomerular endothelial cells were observed (Fig. 1D). The basement membrane was thickened and homogeneous. Morphometric analysis revealed a statistically significant increase in the surface area of the vascular glomeruli and a non-significant increase in the surface area of the renal corpuscles (Table 1). Pronounced hypertrophic changes in organelles of the proximal and distal convoluted tubules of the nephron were observed. Histologically, degenerative changes predominated (Fig. 2D). Morphometric analysis revealed a statistically significant decrease in luminal diameter and an increase in wall thickness of the distal convoluted tubules (Table 3). In the proximal convoluted tubules, the increase in wall thickness was not statistically significant (Table 2).

Thus, at the late stage of 17% ABV wine administration, morphological changes were comparable to those observed at the corresponding stage of administering 40% ABV vodka and 40% ABV home-distilled spirits.

DISCUSSION

In this study, the authors aimed to comprehensively investigate the microscopic and ultrastructural remodeling of the GFB components and renal tubular structures in rats exposed to chronic alcohol consumption with beverages of varying strength and quality. The results obtained in this study, as well as those reported by other authors who conducted similar investigations at both the light microscopic [10-16] and electron microscopic [17-20] levels, are highly variable. This variability is primarily associated with differences in the duration of alcohol exposure. In some studies, alcohol was administered for 7–28 days [11, 13-15, 20], whereas in others, exposure lasted 2-11 months [12, 16, 18, 19], and in some cases, up to 472 days [10]. The variability is further increased

by differences in experimental design, including the use of not only Wistar rats but also laboratory mice of various strains [10, 15, 20], as well as ethanol of varying concentrations, not only 40% ABV [11, 12, 14, 17-19], but also beverages containing 10%, 16%, 18%, and 20% ethanol [10, 15, 16, 20].

Despite the above, we identified specific remodeling features of the GFB components. Administration of 40% ABV vodka, 40% ABV home-distilled spirits, and 17% ABV wine over a 5-day period did not induce morphological changes. This finding suggests a high compensatory capacity of the GFB. To the best of our knowledge, alcohol exposure of this duration has not been previously investigated.

On day 9 of the experiment, edema of the glomerular capillary endothelial cells was observed, confirming endothelial dysfunction as a manifestation of ethanol-induced microcirculatory injury. An increase in the number of pores and fenestrae indicates that the GFB has become pathologically permeable, as further evidenced by deformation of podocyte primary and foot processes [20]. Reports indicate endothelial dysfunction in the early stages of ethanol intoxication [19]. In contrast, damage, edema, and glomerular fragmentation in later stages have been described by numerous researchers [11, 13, 14, 18]. Some authors have also reported glomerular condensation [14], as well as retraction and atrophy [10, 12, 15, 19]; however, such changes were not observed in the present study. We found a significant increase in the surface area of the vascular glomeruli at the intermediate and late stages of administering 50% ABV home-distilled spirits and 17% ABV wine. This indicates compensatory hypertrophy and hyperfiltration, which may lead to GFB cell depletion.

Chronic overload of the GFB is indirectly evidenced by hypertrophy and hyperplasia of both tissue and intracellular structures, as reported in our study and by other researchers [11, 12, 15, 18, 19]. This may result in cellular depletion, degenerative changes, or even cell death. The chronic pathological process is further supported by thickening and homogenization of the glomerular basement membrane, as observed both in our study and in previous reports [11, 18, 19].

In chronic alcohol intoxication, renal tubular structures are also affected; however, these changes become evident only after 9 days of alcohol administration. In contrast, intoxication with 50% ABV home-distilled spirits, which contain both ethanol and methanol, leads to earlier alterations observed after 5 days of alcohol exposure. The negative effects of liquors containing both ethanol and methanol on renal tubular structures have also been reported by other authors [13], who observed such changes somewhat later, after 14 days of alcohol administration.

By measuring the wall thickness of the proximal and distal convoluted tubules, we morphometrically established that the earliest and most frequent sign of damage to renal tubular structures under all types of alcohol administration is swelling (edema) of the cells of these nephron tubules, indicative of hydropic or vacuolar degeneration. These findings correlate with the results of numerous studies [10, 11, 15, 18, 19] and indicate direct ethanol-induced toxic injury to the convoluted tubules of the nephron, leading to disruption of ion exchange.

Our findings are consistent with previous studies demonstrating that common features of damage to renal tubular structures include granular [10, 14] and hyaline droplet [11, 16, 18, 19] degeneration of the convoluted tubules, as well as loss of brush border microvilli in the proximal convoluted tubules [15, 20].

Importantly, in rats exposed to ethanol during the prenatal period, ultrastructural analysis revealed mitochondrial atrophy and vacuolar degeneration of epithelial cells in the distal convoluted tubules, findings characteristic of fetal alcohol syndrome that contribute to impaired renal function [17].

To date, several pathogenetic mechanisms underlying alcohol-induced damage to the GFB and renal tubular structures have been proposed. Some authors consider ethanol to exert a direct nephrotoxic effect [14, 21], whereas others argue that it is harmful to the kidney only in the context of hepatorenal syndrome [19].

The kidney is highly sensitive to reactive oxygen species [19], the levels of which increase significantly during chronic alcohol intoxication, thereby contributing to the development of oxidative stress [23-26]. The transport of synthetic folic acid and natural forms of vitamin B9 (circulating folates) across the brush border membrane of the proximal convoluted tubules is essential for the normal tubular reabsorption function [19]. Chronic alcohol intoxication reduces plasma folate levels and increases their urinary excretion, thereby leading to folate deficiency [27, 28]. Alcohol abuse may also induce metabolic rhabdomyolysis, a process characterized by the metabolic destruction of skeletal muscles and the release of intracellular toxic contents into the bloodstream, which, in turn, can result in acute renal failure and multiple tubular injuries [29].

CONCLUSIONS

1. Alcohol intoxication with 40% ABV vodka, 40% ABV home-distilled spirits, and 17% ABV fortified wine for 5 days does not induce pronounced morphological changes in the GFB components and renal tubular structures. However, exposure to 50% ABV home-distilled spirits over the same period results in functional strain on both filtration and reabsorption.
2. Alcohol intoxication with 40% ABV vodka for 9 days induces pronounced hypertrophy and hyperplasia of the GFB components and renal tubular structures and remodeling of glomerular capillary basement membranes, indicating that adaptive and protective mechanisms are under strain. In contrast, administration of 40% ABV home-distilled spirits over the same period results in early destructive changes in the GFB components and renal tubular structures.
3. On day 9 of chronic alcohol intoxication with 50% ABV home-distilled spirits, an overload of adaptive and protective mechanisms occurs, the morphological correlate of which is partial structural disruption

of the GFB components and renal tubular structures, along with remodeling of nephron capillary basement membranes.

4. Chronic alcohol intoxication with 40% ABV and 50% ABV home-distilled spirits for 12 days leads to depletion of adaptive and protective mechanisms in the glomerular capillaries and convoluted tubules of the nephron, accompanied by pronounced destructive changes. The surface area of the glomerular capillaries and renal corpuscles increases compared to earlier stages of alcohol exposure. Administration of 40% ABV vodka and 17% ABV fortified wine during this period results in signs of structural damage to the GFB components and renal tubular structures.
5. The severity of damage to cellular organelles in the GFB and renal tubular epithelium (nuclei, mitochondria, Golgi apparatus, and rough endoplasmic reticulum) varies across stages of chronic alcohol intoxication. The rough endoplasmic reticulum and mitochondria are the most sensitive to the toxic effects of alcohol. The extent of morphological changes in the GFB components and renal tubular structures during chronic alcohol intoxication depends on the quality and strength of the alcoholic beverage.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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REVIEW ARTICLE

Increased cellular stress responses, neuroinflammation, and altered neuronal signaling in the pathophysiology of PTSD

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ABSTRACT
Post-traumatic stress disorder (PTSD) is associated with persistent dysregulation of cellular stress pathways and altered neuronal signaling within limbic and prefrontal circuits. Increasing evidence indicates that activation of endoplasmic reticulum stress responses, oxidative imbalance, and inflammasome signaling contribute to sustained neurobiological alterations underlying PTSD symptomatology. Stress-induced activation of inositol-requiring enzyme 1 (IRE1) and activating transcription factor 6 (ATF6) interacts with inflammatory mediators such as interleukin-18 (IL-18) and interleukin-1β (IL-1β), amplifying neuroimmune responses. Activation of the NOD-like receptor family pyrin domain containing 3 inflammasome (NLRP3 inflammasome) and caspase-1 promotes cytokine maturation and microglial activation, leading to impaired synaptic plasticity. These mechanisms are accompanied by alterations in glutamatergic and gamma-aminobutyric acid (GABA) signaling, oxidative stress imbalance, and dysregulation of the hypothalamic–pituitary–adrenal axis. Neuroimaging data demonstrate volumetric and microstructural changes in the hippocampus, amygdala, and prefrontal cortex in chronic PTSD, suggesting structural correlates of prolonged inflammatory and stress-related activity. Biomarker studies further indicate associations between inflammatory mediators, coping strategies, sleep disturbances, and illness duration. These findings support the concept that increased cellular stress responses and altered neuronal signaling are central components of PTSD pathophysiology rather than secondary epiphenomena. Understanding the interaction between endoplasmic reticulum stress, inflammasome activation, oxidative imbalance, and neurotransmitter dysregulation may improve biomarker-based diagnostics and facilitate development of targeted therapeutic interventions.

KEYWORDS: endoplasmic reticulum stress, inflammasome, microglia, neuronal signaling, oxidative stress, post-traumatic stress disorder

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INTRODUCTION

Post-traumatic stress disorder (PTSD) is a trauma-related psychiatric condition characterized by intrusive recollections, avoidance, negative alterations in cognition and mood, and persistent hyperarousal. Beyond its psychological manifestations, PTSD is increasingly recognized as a disorder involving sustained molecular and cellular dysregulation. Converging evidence indicates that chronic activation of cellular stress pathways and altered neuronal signaling contribute substantially to its pathophysiology [1, 2].

Exposure to severe or prolonged stress activates intracellular adaptive mechanisms designed to restore homeostasis. However, when stress is persistent, these responses may become maladaptive. One important mechanism involves endoplasmic reticulum (ER) stress and activation of unfolded protein response (UPR) pathways. Increased activity of inositol-requiring enzyme 1 (IRE1) and activating transcription factor 6 (ATF6) has been associated with inflammatory signaling and altered emotional regulation. These pathways interact with oxidative stress mechanisms and inflammatory cascades, amplifying cellular dysfunction [3].

Neuroinflammation represents another central component of PTSD. Activation of the NOD-like receptor family pyrin domain-containing 3 inflammasome (NLRP3 inflammasome) leads to caspase-1 activation and maturation of interleukin-1 beta (IL-1β) and interleukin-18 (IL-18). Elevated concentrations of these cytokines have been linked to symptom severity, impaired emotional control, and illness chronicity. Sustained microglial activation and disrupted neuron–glia communication may impair synaptic plasticity and reduce neurogenesis, particularly in the hippocampus [4, 5].

Altered neuronal signaling further contributes to network instability. Dysregulation of glutamatergic and gamma-aminobutyric acid (GABA) transmission has been described in PTSD, reflecting imbalance between excitatory and inhibitory systems. This imbalance may reflect biologically distinct PTSD phenotypes characterized by differential excitatory–inhibitory dysregulation. Oxidative stress and mitochondrial dysfunction may enhance excitotoxic vulnerability and amplify inflammatory responses [6].

Recent biomarker studies demonstrate associations between inflammatory mediators, coping strategies, sleep disturbances, and neuroendocrine alterations involving the hypothalamic–pituitary–adrenal axis (HPA axis). Importantly, combined biomarker profiles may enable identification of biologically distinct PTSD subtypes, including neuroinflammatory-dominant, stress-axis dysregulation-dominant, and excitotoxicity-related phenotypes, which may have direct implications for personalized treatment strategies [7].

This paper is intended as a narrative review with a structured literature search aimed at integrating molecular, biomarker, and neuroimaging evidence relevant to PTSD pathophysiology.

AIM

The aim of this review is to critically summarize and integrate current evidence on increased cellular stress responses and altered neuronal signaling in PTSD, with particular emphasis on ER stress pathways, inflammasome activation, oxidative imbalance, and dysregulation of glutamatergic and GABA signaling. In addition, this review examines the relationships between these molecular processes, structural and functional brain alterations, and the clinical manifestations of PTSD. Special attention is also given to the potential diagnostic relevance of inflammatory and stress-related biomarkers and to the therapeutic implications of targeting neuroinflammation, oxidative stress, and cellular stress pathways in PTSD.

MATERIALS AND METHODS

This article is a narrative review based on a structured literature analysis focusing on cellular stress responses, neuroinflammatory signaling, and altered neuronal communication in PTSD. The review was designed to summarize current evidence on the molecular and neurobiological mechanisms linking ER stress, inflammasome activation, oxidative imbalance, and neurotransmitter dysregulation with the clinical manifestations and neuroimaging correlates of PTSD.

A literature search was conducted using the PubMed, Scopus, and Web of Science databases. The search included studies published in English between 2016 and 2025. Earlier publications were also considered if they were regarded as foundational for understanding the biological background of ER stress, inflammasome activation, oxidative stress, and neurotransmitter imbalance.

The search strategy was based on combinations of the following keywords: „post-traumatic stress disorder", „PTSD", „endoplasmic reticulum stress", „ER stress", „inositol-requiring enzyme 1", „IRE1", „activating transcription factor 6", „ATF6", „unfolded protein response", „UPR", „NLRP3 inflammasome", „caspase-1", „interleukin-1β", „interleukin-18", „oxidative stress", „microglia", „neuroinflammation", „glutamate", „gamma-aminobutyric acid", „GABA", „hypothalamic–pituitary–adrenal axis", „HPA axis", „hippocampus", „amygdala", „prefrontal cortex", „functional magnetic resonance imaging", „fMRI", „diffusion tensor imaging", and „DTI". Additional relevant studies were identified by screening the reference lists of selected articles.

The inclusion criteria comprised: (1) original experimental studies, clinical studies, systematic reviews, meta-analyses, and narrative reviews; (2) publications addressing PTSD or trauma-related chronic stress in relation to molecular stress pathways, neuroinflammation, or altered neuronal signaling; and (3) studies examining associations between biomarkers, brain structure or function, and clinical manifestations.

The exclusion criteria included: (1) publications not directly related to PTSD or trauma-related stress pathology; (2) studies not addressing the molecular, inflammatory, oxidative, neuroimaging, or neurotransmission-related mechanisms discussed in this review; (3) conference abstracts without sufficient methodological data; and (4) duplicate or low-relevance publications.

The final literature was analyzed qualitatively and organized into thematic sections covering ER stress, inflammasome-mediated cytokine maturation, oxidative stress, neurotransmitter imbalance, structural and functional brain alterations, and clinical correlates. Based on the applied methodology, the present paper should be regarded as a narrative review with a structured literature search rather than a systematic review.

REVIEW AND DISCUSSION

ENDOPLASMIC RETICULUM STRESS AND CELLULAR ADAPTATION IN PTSD

Exposure to severe psychological trauma activates intracellular stress-response systems that aim to preserve cellular homeostasis. One of the most important mechanisms involved is ER stress.

The ER is responsible for protein folding, calcium homeostasis, and lipid synthesis. ER stress occurs when protein misfolding or metabolic imbalance disrupts its normal function. In such conditions, the UPR is activated to restore ER homeostasis [8].

In PTSD, persistent stress exposure may lead to prolonged activation of ER stress pathways. Among the principal signaling branches of the UPR are IRE1 and ATF6. Activation of IRE1 promotes transcription of stress-related and inflammatory genes, while ATF6 regulates protein quality control mechanisms. Under chronic stress conditions, sustained activation of these pathways may shift from adaptive regulation to maladaptive signaling [9].

ER stress is closely linked to inflammatory processes. IRE1 signaling can enhance nuclear factor kappa B (NF-κB) activation, leading to increased transcription of pro-inflammatory cytokines. This interaction connects cellular stress responses with immune activation. Elevated levels of IL-1β and IL-18 observed in PTSD may therefore reflect, in part, upstream activation of ER stress pathways [10, 11].

Oxidative stress further amplifies ER dysfunction. Increased production of reactive oxygen species (ROS) disrupts protein folding and calcium homeostasis, strengthening UPR signaling. Mitochondrial dysfunction may also contribute to this process by impairing energy metabolism and increasing oxidative burden. These mechanisms create a feedback loop in which cellular stress enhances inflammation, and inflammation further destabilizes cellular homeostasis [12, 13].

In neuronal cells, prolonged ER stress can impair synaptic protein synthesis and plasticity-related signaling. Altered phosphorylation of extracellular signal-regulated kinase (ERK) has been associated with dysregulated neuronal adaptation. Disruption of these pathways may weaken synaptic connectivity in the hippocampus and prefrontal cortex, regions critically involved in memory processing and emotional regulation.

Persistent ER stress may also increase susceptibility to apoptosis when adaptive mechanisms fail. Although widespread neuronal loss is not a defining feature of PTSD, subtle cellular dysfunction may accumulate over time, contributing to structural and functional alterations detected in neuroimaging studies.

Importantly, from a pathophysiological perspective, ER stress should not be interpreted solely as a downstream consequence of trauma exposure. Increasing evidence suggests that it may act as an upstream regulator integrating inflammatory signaling, oxidative imbalance, and synaptic dysfunction. This integrative role positions ER stress as a potential central node in PTSD pathophysiology rather than an isolated molecular phenomenon.

Furthermore, variability in ER stress activation may contribute to inter-individual differences in disease progression. Patients with stronger or prolonged UPR activation may exhibit greater vulnerability to chronic neurobiological dysregulation, which may partly explain heterogeneity in clinical presentation and treatment response.

Overall, sustained activation of ER stress pathways represents an important molecular link between chronic psychological trauma and long-term neuronal dysfunction in PTSD [14, 15].

INFLAMMASOME ACTIVATION AND NEUROINFLAMMATORY SIGNALING IN PTSD

Chronic psychological stress activates innate immune mechanisms within the central nervous system (CNS). In PTSD, increasing evidence indicates that inflammasome activation contributes to sustained neuroinflammatory signaling. The NLRP3 inflammasome plays a central role in this process [16].

The NLRP3 inflammasome is a multiprotein complex composed of the sensor molecule NLRP3, the adaptor protein apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), and pro-caspase-1. Its activation requires two signals. The priming signal increases transcription of inflammasome components and pro-inflammatory cytokines via NF-κB. The second signal is triggered by cellular stressors such as mitochondrial dysfunction, ROS production, potassium efflux, or extracellular adenosine triphosphate (ATP) release. These stimuli promote assembly of the inflammasome complex and activation of caspase-1 [17, 18].

Active caspase-1 cleaves pro-interleukin-1 beta (pro-IL-1β) and pro-interleukin-18 (pro-IL-18) into their mature forms, IL-1β and IL-18. These cytokines amplify inflammatory signaling and enhance microglial activation. Elevated levels of IL-1β and IL-18 have been associated with symptom severity, impaired emotional regulation, and illness chronicity in PTSD. Persistent cytokine release may interfere with synaptic plasticity and long-term potentiation (LTP), particularly in the hippocampus [19].

Microglia are central mediators of inflammasome-driven neuroinflammation. Under prolonged stress exposure, microglia shift toward a pro-inflammatory phenotype characterized by increased cytokine production and reduced neurotrophic support. This state may impair neuron–glia communication and contribute to altered network activity in limbic structures [20].

Inflammasome activation is closely linked to oxidative stress. ROS promote NLRP3 activation, while inflammatory cytokines further increase oxidative burden. This reciprocal interaction sustains a pro-inflammatory environment within the CNS.

Importantly, inflammasome activation in PTSD should be interpreted as a dynamic and self-perpetuating process rather than a transient immune response. The bidirectional interaction between NLRP3 activation and oxidative stress suggests the existence of a feed-forward loop in which cellular stress promotes inflammation, and inflammation further enhances cellular dysfunction. This mechanism may contribute to the persistence of neurobiological alterations long after the traumatic exposure has ceased.

A critical aspect concerns the functional consequences of IL-1β signaling on synaptic plasticity. Experimental data indicate that IL-1β may impair LTP and facilitate long-term depression (LTD), thereby shifting synaptic plasticity toward a state favoring memory stabilization rather than adaptive modification. In PTSD, such a shift may promote consolidation and persistence of trauma-related memory traces while impairing extinction learning.

Another important issue is regional specificity. Neuroinflammatory signaling does not affect all brain structures equally. The hippocampus appears particularly vulnerable to inflammation-induced impairment of neurogenesis and synaptic plasticity, whereas the amygdala may exhibit increased excitatory responsiveness. This regional asymmetry may explain the coexistence of impaired contextual memory and exaggerated emotional reactivity characteristic of PTSD.

Furthermore, inflammasome activation may interact with stress-axis dysregulation. Glucocorticoid signaling associated with HPA axis dysfunction may modulate inflammatory responses, potentially altering the threshold for NLRP3 activation. This interaction suggests that neuroendocrine and immune mechanisms jointly contribute to sustained neurobiological dysregulation in PTSD.

From a clinical perspective, variability in inflammasome activation may define biologically distinct PTSD subtypes. A subgroup characterized by high inflammatory activity may present with increased symptom severity, greater cognitive impairment, and reduced responsiveness to standard psychotherapeutic interventions. In contrast, patients with lower neuroinflammatory burden may exhibit a more adaptive course of the disorder.

This observation supports the concept of biomarker-guided stratification. Measurement of IL-1β, IL-18, and NLRP3-related signaling components may contribute to identifying patients with dominant neuroimmune dysregulation. Such stratification could be clinically relevant for selecting targeted therapeutic approaches and improving treatment outcomes.

Targeting inflammasome-related pathways represents a promising therapeutic strategy. Potential approaches include direct inhibition of NLRP3 activation, modulation

Table 1. Cellular stress pathways in PTSD: molecular mechanisms, neurobiological effects, and main clinical manifestations

Pathway	Molecular Components	Biological Effect	Neural Consequence	Main Clinical Manifestation
Endoplasmic reticulum stress (ER stress)	Inositol-requiring enzyme 1 (IRE1), activating transcription factor 6 (ATF6)	Increased inflammatory gene expression	Impaired synaptic protein synthesis	Emotional dysregulation, impaired stress adaptation
NOD-like receptor family pyrin domain-containing 3 inflammasome (NLRP3 inflammasome)	NLRP3, apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), caspase-1	Interleukin-1 beta (IL-1β) and interleukin-18 (IL-18) maturation	Microglial activation	Hyperarousal, persistent inflammatory burden
Oxidative stress	Reactive oxygen species (ROS), mitochondrial dysfunction	Redox imbalance	Synaptic damage	Cognitive impairment, reduced resilience to stress
Neurotransmitter imbalance	Glutamate ↑, gamma-aminobutyric acid (GABA) ↓	Excitatory-inhibitory instability	Network hyperreactivity	Hypervigilance, sleep disturbance, emotional instability
Hypothalamic-pituitary--adrenal axis dysregulation (HPA axis dysregulation)	Cortisol variability	Glucocorticoid resistance	Sustained inflammation	Chronic symptom persistence, maladaptive stress response

Source: Compiled by the author of this study

of caspase-1 activity, and indirect attenuation of oxidative stress. Additionally, anti-inflammatory interventions may help restore synaptic plasticity and reduce neuronal vulnerability.

However, current evidence is largely derived from preclinical and early translational studies. The clinical efficacy, safety, and long-term impact of inflammasome-targeted therapies in PTSD remain to be established. Future clinical trials should incorporate biomarker-based patient stratification and longitudinal assessment to determine the therapeutic relevance of these mechanisms.

Overall, inflammasome-mediated cytokine maturation represents an important mechanism linking cellular stress responses with altered neuronal signaling in PTSD. Persistent activation of this pathway may contribute to structural brain changes and long-term symptom maintenance [21, 22].

The principal molecular mechanisms linking cellular stress responses with neuroinflammatory signaling and neuronal dysfunction in PTSD are summarized in Table 1.

ALTERED NEURONAL SIGNALING AND NEUROTRANSMITTER IMBALANCE IN PTSD

PTSD is associated with disturbances in neuronal signaling within circuits responsible for fear processing, memory, and emotional regulation. These alterations involve imbalance between excitatory and inhibitory neurotransmission, impaired synaptic plasticity, and disrupted intracellular signaling pathways [23].

Glutamate is the principal excitatory neurotransmitter in the CNS. In PTSD, excessive glutamatergic activity has been reported, particularly in limbic structures such as the amygdala and hippocampus. Increased glutamate release and enhanced receptor activation may promote excitotoxic stress, leading to impaired synaptic function. Prolonged activation of N-methyl-D-aspartate (NMDA) receptors can increase intracellular calcium levels, contributing to mitochondrial dysfunction and oxidative stress [24, 25].

GABA is the main inhibitory neurotransmitter and plays a critical role in stabilizing neuronal networks. Reduced GABAergic signaling has been associated with heightened arousal and impaired inhibitory control over stress responses. Decreased inhibitory tone may facilitate persistent activation of fear circuits and contribute to hypervigilance and emotional instability observed in PTSD [26].

Inflammatory mediators influence neurotransmitter systems. IL-1β and tumor necrosis factor alpha (TNF-α) can modulate glutamate receptor trafficking and reduce GABA receptor expression. These interactions link immune activation with neuronal imbalance. Sustained neuroinflammation may therefore destabilize excitatory-inhibitory equilibrium and impair LTP, a cellular correlate of learning and memory.

Intracellular signaling pathways are also affected. Altered phosphorylation of ERK and other synaptic plasticity-related proteins may disrupt memory consolidation and extinction processes. Dysregulated signaling in the prefrontal cortex can weaken top-down control over the amygdala, reinforcing exaggerated threat perception [27, 28].

Oxidative stress further contributes to neuronal dysfunction. Increased production of ROS damages membrane lipids and synaptic proteins, impairing transmission efficiency. Mitochondrial dysfunction may reduce energy availability, limiting adaptive synaptic remodeling.

Importantly, neurotransmitter imbalance in PTSD should not be interpreted solely as increased excitation or reduced inhibition. Rather, it reflects a circuit-specific disruption of excitatory-inhibitory regulation. Increased glutamatergic activity within amygdala-centered fear networks may facilitate threat overencoding and hyperarousal, whereas reduced inhibitory control in prefrontal and hippocampal circuits

may impair contextual regulation and extinction learning.

This mechanism may also contribute to the biological heterogeneity of PTSD. Patients with dominant excitatory dysregulation may present with intrusive memories, heightened startle responses, and hypervigilance, while those with more pronounced inhibitory dysfunction may show impaired emotional regulation, sleep disturbance, and reduced stress tolerance. Such differences may be clinically relevant for future biomarker-guided stratification and treatment personalization.

Together, altered neurotransmitter balance, inflammatory signaling, oxidative stress, and intracellular pathway disruption form a mechanistic network underlying impaired neuronal communication in PTSD. These disturbances provide a biological basis for persistent emotional dysregulation, intrusive memories, and cognitive impairment.

STRUCTURAL AND FUNCTIONAL BRAIN ALTERATIONS ASSOCIATED WITH CELLULAR STRESS IN PTSD

PTSD is associated with measurable structural and functional changes in brain regions involved in memory, emotional regulation, and executive control. These alterations are closely linked to prolonged cellular stress responses, neuroinflammation, and disrupted neuronal signaling [29].

The hippocampus is particularly vulnerable to chronic stress. Persistent exposure to elevated stress hormones and pro-inflammatory cytokines such as IL-1β and IL-18 may impair neurogenesis and synaptic plasticity. Reduced dendritic branching and altered spine density have been described in experimental models of chronic stress. Neuroimaging studies consistently report decreased hippocampal volume in individuals with long-standing PTSD. These structural changes are associated with deficits in contextual memory processing and impaired fear extinction [30].

The amygdala plays a central role in threat detection and emotional reactivity. Functional imaging studies demonstrate increased amygdala activation in response to trauma-related stimuli in PTSD. Chronic inflammatory signaling and excessive glutamatergic activity may enhance excitatory transmission within amygdala circuits, reinforcing hyperarousal and exaggerated stress responses. Structural findings vary, but altered connectivity between the amygdala and prefrontal cortex is frequently observed [31, 32].

The prefrontal cortex is responsible for executive control and regulation of emotional responses. In PTSD, reduced cortical thickness and microstructural abnormalities have been reported, particularly in medial and dorsolateral regions. Sustained oxidative stress and microglial activation may disrupt white matter integrity and weaken fronto-limbic connectivity. Impaired top-down regulation contributes to persistent fear responses and reduced cognitive flexibility [33, 34].

Diffusion tensor imaging (DTI) studies reveal decreased white matter coherence in pathways connecting the hippocampus, amygdala, and prefrontal cortex. These findings suggest long-term effects of inflammatory and stress-related mechanisms on axonal structure and myelination.

From a mechanistic perspective, these structural and functional alterations may represent the cumulative consequence of prolonged molecular dysregulation rather than isolated anatomical abnormalities. Neuroinflammation, oxidative stress, and excitatory–inhibitory imbalance may jointly impair synaptic remodeling, dendritic stability, and white matter organization. This provides a biologically plausible explanation for why chronic PTSD is often associated with persistent disturbances in memory, emotional regulation, and executive control.

The regional pattern of brain alterations may also support the concept of biologically defined PTSD subtypes. For example, a hippocampal-dominant profile may be associated with contextual memory impairment and poor fear extinction, whereas an amygdala-dominant profile may be linked to hyperarousal and exaggerated threat reactivity. A prefrontal-dominant profile may manifest primarily as impaired cognitive control and emotional inhibition.

Overall, structural and functional brain alterations in PTSD reflect the cumulative impact of chronic cellular stress, neuroinflammation, and neurotransmitter imbalance. These changes provide a neurobiological substrate for persistent symptoms and may represent potential targets for early therapeutic intervention [35, 36].

CLINICAL CORRELATES, DIAGNOSTIC RELEVANCE, AND THERAPEUTIC IMPLICATIONS

The molecular and cellular alterations described in PTSD have important diagnostic and therapeutic implications. Increased cellular stress responses, inflammasome activation, oxidative imbalance, and altered neuronal signaling are associated not only with symptom severity and illness duration, but also with measurable biological abnormalities that may contribute to clinically meaningful patient stratification [37].

Elevated concentrations of IL-1β and IL-18, as well as markers related to ER stress and oxidative imbalance, may represent candidate biomarkers of disease activity and chronicity. Although none of these markers can currently be considered sufficiently specific to serve as standalone diagnostic tools, their combined assessment together with psychometric and neuroimaging findings may improve biological characterization of PTSD. In this context, inflammatory and cellular stress markers may be particularly useful in identifying subgroups of patients with pronounced neuroimmune dysregulation, persistent sleep disturbance, maladaptive coping, or increased risk of chronic symptom persistence [38, 39].

A more clinically useful approach may involve defining biomarker-informed PTSD profiles rather than relying on single markers. Potential profiles may include: a neuroinflammatory phenotype characterized by elevated IL-1β, IL-18, and NLRP3 inflammasome activity; an oxidative-stress phenotype characterized by increased ROS and mitochondrial dysfunction; a stress-axis phenotype characterized by HPA axis dysregulation and cortisol variability; and an excitatory–inhibitory imbalance phenotype characterized by excessive glutamatergic signaling and reduced GABAergic inhibition.

Such profiles may support precision-oriented clinical decision-making. For example, patients with a neuroinflammatory phenotype may be candidates for anti-inflammatory or immunomodulatory approaches, whereas those with dominant excitatory–inhibitory dysregulation may benefit from interventions targeting glutamatergic or GABAergic signaling. Patients with prominent sleep-related inflammatory activation may require integrated treatment focused on sleep normalization, inflammation reduction, and trauma-focused psychotherapy.

From a therapeutic perspective, the described mechanisms suggest several potential intervention targets. Modulation of neuroinflammatory pathways, especially those related to NLRP3 inflammasome activation and caspase-1-dependent cytokine maturation, may help reduce excessive production of IL-1β and IL-18. Similarly, strategies aimed at restoring redox balance and reducing oxidative stress may indirectly attenuate inflammasome activation and improve neuronal resilience [40].

Interventions influencing ER stress and UPR signaling may also be relevant, particularly in patients in whom persistent cellular stress contributes to impaired synaptic adaptation. In addition, therapeutic approaches targeting glutamatergic overactivity and reduced GABAergic inhibition may help stabilize neural circuits involved in fear processing, emotional regulation, and stress reactivity [37].

The clinical significance of these mechanisms extends beyond pharmacotherapy. Biomarker-guided approaches may in the future support treatment personalization, help monitor treatment response, and identify patients who could benefit from multimodal interventions combining psychotherapy, pharmacological treatment, sleep normalization, and strategies aimed at reducing inflammation and oxidative burden [18, 21]. Therefore, the integration of molecular biomarkers with clinical and neuroimaging assessment may become an important element of precision-oriented PTSD management [6, 23, 31].

However, it should be emphasized that the clinical application of these biomarkers remains at an early stage. Current evidence supports their potential utility in research-based stratification, but not yet as routine diagnostic tests. Future validation requires large longitudinal cohorts, standardized assay protocols, replication across trauma populations, and integration with clinical severity scales and neuroimaging markers.

The relationships between molecular biomarkers, structural brain alterations, and clinical manifestations in PTSD are presented in Table 2.

Recent neuroimaging studies using functional magnetic resonance imaging (fMRI) and DTI provide additional support for the concept that PTSD is associated with altered fronto-limbic network organization. Functional MRI findings indicate abnormal activation and connectivity patterns involving the amygdala, hippocampus, medial prefrontal cortex, and salience-related networks, particularly during emotional processing and trauma-related recall. DTI studies further demonstrate reduced white matter integrity in pathways linking limbic and prefrontal regions, suggesting that chronic cellular stress, neuroinflammation, and oxidative imbalance may contribute not only to volumetric changes but also to impaired neural connectivity. These findings strengthen the translational relevance of combining biomarker-based and neuroimaging-based approaches in PTSD research [21, 40].

CONCLUSIONS

The evidence reviewed in this paper indicates that PTSD is associated with a persistent interaction between cellular stress responses, neuroinflammation, oxidative imbalance, and altered neuronal signaling. Among the best documented mechanisms are dysregulation of inflammatory pathways, increased activity of IL-1β- and IL-18-related signaling, microglial activation, altered glutamatergic and GABAergic balance, and structural or connectivity abnormalities involving the hippocampus, amygdala, and prefrontal cortex. These findings strongly support the view that PTSD is not solely a psychological disorder, but also a condition involving measurable neurobiological dysregulation.

Table 2. Biomarker profiles, neuroimaging correlates, and clinical significance in PTSD

Biomarker	Associated Mechanism	Structural/Functional Correlate	Clinical Significance
Interleukin-1 beta (IL-1β)	Inflammasome activation	Hippocampal volume reduction	Intrusive memories, impaired fear extinction
Interleukin-18 (IL-18)	Neuroinflammatory signaling	Amygdala hyperreactivity	Hyperarousal, emotional dysregulation
Endoplasmic reticulum stress markers (IRE1, ATF6)	UPR activation	Prefrontal dysfunction	Emotional inhibition, reduced adaptive control
Oxidative markers	ROS imbalance	White matter alterations	Cognitive decline, reduced neuronal resilience
Cortisol variability	HPA axis dysregulation	Fronto-limbic imbalance	Chronic symptom persistence, stress maladaptation
Sleep-related cytokines	Inflammatory amplification	Hippocampal vulnerability	Sleep disturbance, impaired recovery processes

Source: Compiled by the author of this study

At the same time, some mechanisms remain insufficiently clarified and should still be considered partly hypothetical or incompletely understood. These include the exact causal role of ER stress pathways such as IRE1 and ATF6 in PTSD progression, the temporal sequence linking inflammasome activation with structural brain alterations, and the extent to which oxidative stress directly drives chronic symptom persistence in human patients. Similarly, although multiple biomarkers show promising associations with PTSD severity and chronicity, their specificity and predictive value in clinical practice remain to be fully established.

The most robust conclusions can therefore be drawn for the presence of neuroimmune activation, excitatory-inhibitory imbalance, and fronto-limbic network alterations in PTSD. In contrast, biomarker-defined PTSD subtypes, ER-stress-directed therapeutic strategies, and inflammasome-targeted interventions should currently be regarded as promising but still emerging translational concepts.

The most important future research directions include longitudinal studies integrating inflammatory, oxidative, and ER stress markers with multimodal neuroimaging and detailed clinical phenotyping. Further work is also needed to determine whether biomarker-defined subgroups of PTSD can be identified and whether they differ in prognosis or treatment response. In addition, translational studies should evaluate whether interventions targeting neuroinflammation, redox imbalance, or altered excitatory–inhibitory signaling can modify the course of PTSD and reduce long-term neural consequences.

Overall, increased cellular stress responses and altered neuronal signaling should be regarded as central components of PTSD pathophysiology. A more precise integration of molecular biomarkers, neuroimaging data, and clinical assessment may improve diagnostic refinement and support the development of more individualized therapeutic strategies.

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CONFLICT OF INTEREST
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REVIEW ARTICLE

Children on the brink: Mental health, long-term social consequences, and psychosocial support for Ukrainian children in war

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ABSTRACT

Aim: To systematically analyse empirical data on the mental health of Ukrainian children in the context of armed conflict; to conduct a comparative analysis with research on the consequences of the Second World War; to examine long-term social consequences; and to analyse the state of the psychosocial support system, with particular attention to the trauma-informed approach as its methodological foundation.

Materials and Methods: Narrative review with elements of comparative analysis. Databases searched: PubMed/MEDLINE, Scopus, Web of Science, PsycINFO. Included: empirical studies and systematic reviews/meta-analyses (2022-2026) on children and adolescents (0-18 years) in war or post-conflict settings with measurable mental health, behavioural, or developmental outcomes; comparative studies on children from the Second World War era, with no time restrictions. Supplemented by institutional reports and national Ukrainian research.

Conclusions: Ukraine is facing a psychiatric and social crisis of historic proportions for European children. An effective response requires long-term systemic investment in psychosocial support grounded in the trauma-informed approach, with priority for underserved groups (the youngest children, orphans, children of the mobilised), deinstitutionalisation, anti-stigma education, and urgent funding for longitudinal research.

KEYWORDS: stress disorders; post-traumatic; child; intergenerational trauma; trauma-informed care

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INTRODUCTION

The full-scale invasion of Ukraine by the Russian Federation on 24 February 2022 triggered one of the worst humanitarian crises in Europe since the Second World War. According to UNICEF, as of early 2025, over 6.5 million Ukrainians had become external refugees, 3.7 million were internally displaced persons (IDPs), and approximately 2.9 million people continued to live in the immediate vicinity of the front line [1]. Women and children constitute 90% of external refugees [2]. It is precisely this demographic structure of displacement – driven by the prohibition on men of conscription age leaving the country – that defines the distinctive character of the trauma: millions of children found themselves in a situation of simultaneous military danger and separation from a parent.

The link between armed conflict and mental disorders in childhood and adolescence is well established. The landmark meta-analysis by Attanayake et al. [3], covering 7,920 children from various conflict zones, reported a pooled prevalence of PTSD at 47%, depression at 43%, and anxiety disorders at 27%. A more recent meta-analysis by Zasiekina et al. [4], based on studies conducted between 2013 and 2023, recorded a mean prevalence of PTSD among adolescents in conflict zones of 29.4% (95% CI: 18.7-43.0%). A systematic review by Blackmore et al. [5] found rates of 22.7% for PTSD, 13.8% for depression, and 15.8% for anxiety disorders among refugee and asylum-seeking children. The systematic reviews of Carpiniello [6] and Badanta et al. [7] further corroborate the profound and wide-ranging mental health burden borne by children in zones of armed conflict. These established figures provide an indispensable reference point for assessing the scale of the crisis unfolding in Ukraine.

Equally significant is the fact that this crisis is not confined to the present. Research into the consequences of the Second World War (WWII) provides compelling evidence that the psychological effects of childhood wartime experience persist throughout adult life and can be transmitted to subsequent generations. Akbulut-Yuksel et al. [8], analysing the long-term impact of wartime trauma on children, demonstrated that the psychological burden endures into late adulthood. A population-based registry study by Santavirta et al. [9] (n=93,391) found a twofold higher risk of psychiatric hospitalisation among the daughters of mothers who had been evacuated as children during WWII (HR=2.04; 95% CI: 1.04-4.01) – the first large-scale, population-level evidence of intergenerational transmission of psychiatric risk arising from childhood wartime experiences. The paradigm of intergenerational trauma, grounded in epigenetic research [10], provides a biological basis for these observations.

Understanding the scale of the crisis is, however, only the starting point. The genuine scientific and practical

challenge lies in understanding what constitutes an adequate response – and what must be done today to prevent the conclusion, in twenty or thirty years' time, that this burden could have been substantially reduced.

AIM

The present article sets four interrelated objectives: (1) to synthesise the empirical evidence on the mental health of different categories of Ukrainian children affected by the conflict; (2) to conduct a comparative analysis with WWII research; (3) to examine the social dimension of the problem from a long-term perspective; and (4) to analyse the current state and priorities of the psychosocial support system, with particular attention to the trauma-informed approach as its methodological core.

MATERIALS AND METHODS

STUDY DESIGN

This article is a narrative review incorporating elements of comparative analysis. The narrative review format was selected as the most appropriate methodology for synthesising a heterogeneous body of evidence spanning multiple disciplines – child psychiatry, developmental psychology, social epidemiology, and conflict studies – and two distinct historical periods (the current conflict in Ukraine and the aftermath of the Second World War). Given the rapid pace of publication in this area since February 2022 and the diversity of study designs represented in the literature, a narrative approach enables integrative synthesis that a formal systematic review with meta-analysis would not readily accommodate without substantial loss of contextual nuance.

SEARCH STRATEGY

The literature search was conducted between January 2025 and April 2026 across four electronic databases: PubMed/MEDLINE, Scopus, Web of Science, and PsycINFO. The search employed the following key terms and their Boolean combinations: 'Ukraine war children mental health', 'Ukrainian refugee children PTSD', 'internally displaced children Ukraine', 'children military families war', 'transgenerational trauma war', 'World War II children long-term outcomes', 'trauma-informed approach children war', 'psychosocial support armed conflict', and their Ukrainian-language equivalents. All search terms were applied to titles, abstracts, and keywords. Reference lists of included articles were manually screened to identify additional relevant sources.

For studies relating to the current conflict in Ukraine, the search was restricted to the period 2022-2026. Comparative studies examining the long-term consequences of the Second World War, as well as foundational meta-analyses and systematic reviews on children in conflict settings, were included without chronological restrictions.

Supplementary sources included institutional reports and datasets from UNICEF, the World Health Organization (WHO), Human Rights Watch (HRW), the Yale Conflict Observatory, and Disability Rights International, as well as the results of the first nationwide study of children from military and veteran families conducted by the NGO 'Free Choice' and the Fama Research Agency (2026).

INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria: (1) original empirical studies or systematic reviews and meta-analyses; (2) sample comprising children and/or adolescents aged 0–18 years, or their parents and primary caregivers; (3) study conducted in a wartime or post-conflict context; (4) reporting measurable psychological, behavioural, or developmental outcomes. Studies examining adult populations where relevant paediatric subgroup data were reported were also eligible.

Exclusion criteria: isolated clinical case reports without comparative data; non-peer-reviewed publications (including preprints without peer review); duplicate publications; and studies for which full-text access could not be obtained. Methodological quality was not used as an exclusion criterion in keeping with the narrative review design; however, methodological limitations of individual studies are noted where relevant in the text.

DATA EXTRACTION AND SYNTHESIS

Data were extracted independently by the first author and cross-checked by the second author. For each included study, the following information was recorded: authors and year; country and setting; study design; sample size and participant characteristics (age range, vulnerability category, displacement status); outcome measures and instruments; and key findings. Discrepancies were resolved through discussion.

Studies were grouped thematically according to the vulnerability category of the child population examined (refugees abroad; IDPs; children of mobilised military personnel; orphans and children in residential care) and according to analytical domain (acute mental health outcomes; longitudinal and cumulative effects; social consequences; psychosocial support systems). Findings from the Ukrainian context were systematically juxtaposed with comparable evidence from the WWII literature to identify structural parallels and points of divergence.

ETHICAL CONSIDERATIONS

This article is a narrative review based exclusively on published peer-reviewed empirical studies, systematic reviews, and official reports from international and national organisations. No new data collection, recruitment of participants, or experimental procedures were undertaken. All primary data cited were collected by the original study teams under their respective institutional ethical frameworks. The article does not identify any individuals, does not reproduce raw personal data, and does not make clinical recommendations directed at specific persons. It therefore does not require independent ethical review.

FRAMEWORK

The study was conducted within the research project of the Department of Sociology and Social Work, Lviv Polytechnic National University, titled: „Research Initiative and Practical Implementation of Socio-Political Projects

Aimed at Resolving Social Problems in Ukraine Under Wartime Conditions and During Post-War Recovery" (State Registration No. 0123U103018,01.09.2023 – 30.09.2028.

AI STATEMENT

The author states that no AI tools were used in writing this article.

REVIEW AND DISCUSSION

1. ACUTE MENTAL HEALTH OUTCOMES: PREVALENCE AND SEVERITY

The empirical evidence accumulated since February 2022 presents a consistent and deeply troubling picture. The first large-scale nationwide study was conducted by McElroy et al. [11] among 1,238 parents surveyed between July and September 2022, which recorded a statistically significant increase in all three symptom clusters on the PSC-17 scale – internalising, externalising, and attention-related – with the most pronounced increases observed in anxiety (over 35% of children) and sadness (over 25%). Using the Child and Adolescent Trauma Screen (CATS) instrument, validated for the Ukrainian context by Redican et al. [12], Martsenkovskyi et al. [13] found that 17.5% of pre-schoolers and 12.6% of school-age children met the full DSM-5 criteria for PTSD just six months after the start of the full-scale invasion – compared with a peacetime prevalence of 1-3% in the general child population.

The AUDRI cohort (Adolescents of Ukraine During the Russian Invasion), studied by Goto et al. [14] across 8,096 adolescents in Ukraine and abroad, found that 32.0% screened positive on psychiatric assessment – a rate that underscores the pervasive reach of the crisis. Lotzin et al. [15], in a study of Ukrainian university students in Kyiv, reported that 91.5% had been exposed to at least one war-related stressor, with 12.4% meeting criteria for PTSD and 11.2% for complex PTSD under ICD-11.

Naeem et al. [16], studying parents of children aged 3-17 years (n=858) one year after the invasion, recorded a substantial burden of ADHD symptoms, behavioural disorders, and anxiety-depressive states among pre-schoolers, confirming the particular vulnerability of the youngest age group, whose actively developing brains are most sensitive to chronic stress. These findings are consistent with the pattern identified by Martsenkovskyi et al. [13], who established that developmental delay is the strongest paediatric predictor of PTSD (AOR=2.38): children whose development is already compromised carry a disproportionately elevated risk.

2. LONGITUDINAL AND CUMULATIVE EFFECTS OF PROLONGED CONFLICT

The longitudinal dimension is of particular importance for understanding the full psychiatric impact of the war. Sourander et al. [17] compared two waves of school-based surveys in the Donetsk and Kirovohrad regions – conducted in 2016-2017 (n=2,766) and 2023–2024 (n=2,720) – and documented a powerful cumulative effect of sustained conflict: adolescents who had experienced both the 2014 and 2022 invasions showed a 14.08-fold higher risk of PTSD (OR=14.08; 95% CI: 8.36-23.72) and an almost five-fold higher risk of major depression (OR=4.83; 95% CI: 3.28-7.11) compared with peers without wartime experience, alongside significantly elevated rates of suicidal ideation.

Serdiuk et al. [18], in a study of 2,086 young people aged 10-26 in eastern Ukraine, found that 23.49% met the clinical threshold for PTSD on the UCLA PTSD Reaction Index for DSM-5; notably, interpersonal trauma – particularly sexual and physical violence – was associated with a higher risk than direct exposure to combat. This finding points to the complex, multi-layered structure of traumatic experience in conflict settings, in which collateral violence and social destabilisation function as independent pathogenic factors.

3. VULNERABILITY CATEGORIES AND RISK PROFILES

3.1 Refugees Abroad. Evacuation abroad confronted women and children with what might be termed a 'double bereavement': the simultaneous experience of loss of home and the necessity of constructing a new everyday existence in an unfamiliar cultural environment. Rozynek et al. [19] documented clinically significant post-traumatic stress among Ukrainian refugee minors and their caregivers in Polish refugee camps within the first months of displacement. In a study of 250 Ukrainian children in Poland, Urbański et al. [20] demonstrated that depression and anxiety were significantly higher among those relying on avoidance-based coping strategies and reporting lower resilience. Childress et al. [21], in a qualitative study of Ukrainian refugees in the United States, identified systemic barriers to adaptation: language barriers, legal and financial instability, cultural alienation, and family conflict. The linguistic isolation of children in host-country schools emerges as an independent source of chronic stress, compounding existing war-related trauma.

3.2 Internally Displaced Persons. IDPs are perhaps the least visible category in public discourse, as they remain within Ukraine's borders and are formally regarded as 'safe'. This safety is relative. IDP families arrive in unfamiliar cities without permanent housing, employment, or established social ties, whilst remaining in a country where hostilities continue. Naeem et al. [16] found a direct association between the intensity of drone strikes in an area of residence and the level of parental PTSD: 28.9% in high-intensity areas versus 25.0% and 20.5% in areas of moderate and low intensity, respectively. The chronic stress arising from nocturnal air-raid alerts constitutes an independent pathogenic factor that disrupts sleep, cognitive development, and the capacity for emotional regulation [22]. Tsybuliak and Suchikova [23] describe a distinctive sense of 'social invisibility' experienced by IDPs within their own country – a condition that is especially painful for adolescents, whose sense of identity depends most heavily on social belonging.

3.3 Children of Mobilised Military Personnel. The general mobilisation of men of conscription age has given rise to a phenomenon with no precedent in modern psychiatry at this scale. The first nationwide Ukrainian study of children from military and veteran families, conducted by the NGO 'Free Choice' and the Fama Research Agency [24] (n=2,504 participants from 1,252 families, including 40 families for

in-depth qualitative interviews), reveals a complex picture. On one hand, 69% of children rate their own psychological distress as minimal, 96% feel safe at home, and 87% feel understood within the family. On the other hand, 55% of children report constant fear or sadness related to a parent's military service (a further 24% experience such feelings intermittently), 53.2% report heightened psychoemotional tension, and 48% avoid news and conversations about the war.

Particularly significant is the gap between need and provision: approximately 9% of children display signs of an anxiety disorder, 7% of depression, yet only 9% are receiving professional psychological support, whilst 20% express a wish for such support that their parents are typically unaware of [24]. Martsenkovskyi et al. [13] lend clinical weight to these findings: having a father in the armed forces or emergency services is one of the strongest predictors of childhood PTSD (AOR=2.13; 95% CI: 1.28-3.53). Erlewein et al. [25] emphasise that in conditions of active conflict, a child's own war-related traumatisation compounds anxiety about the absent parent, producing a cumulative risk that exceeds any documented in comparative studies of military families in peacetime settings.

3.4 Orphans and Children in Residential Care. Whilst for other categories the conflict has disrupted an otherwise normal childhood, for orphans and children in residential care it has struck at what remained of an already precarious existence. According to official data, over 13,000 children have acquired the status of orphan or child deprived of parental care since the start of the full-scale invasion [26]. Human Rights Watch [27] documented a troubling paradox: contrary to the declared deinstitutionalisation strategy, the number of children's residential institutions in Ukraine increased from 663 in 2015 to 727 in 2022. Fronek et al. [28], working in cooperation with the Ukrainian NGO 'Magnolia', documented the systematic deportation of children from occupied institutions to Russia. The Yale Conflict Observatory [29] identified 314 children across three Russia-linked databases for forced adoption – evidence of a deliberate, state-coordinated programme of identity erasure. Disability Rights International [30] recorded discriminatory practices whereby children with disabilities were frequently left behind in occupied territories whilst children without special needs were evacuated. For those who remain in institutional settings, the findings of the Bucharest Early Intervention Project serve as a sobering reference point: prolonged institutional upbringing in early childhood is associated with lower IQ, elevated rates of behavioural disorders, and persistent difficulties in emotional regulation and attachment formation – deficits that manifest in adulthood as higher rates of unemployment, criminal behaviour, and psychiatric morbidity.

4. THE ROLE OF PARENTAL AND CAREGIVER MENTAL HEALTH

A particularly consequential finding is the close interdependence between parental and child mental health. Naeem et al. [16] established that parental PTSD increases the risk of mental disorder in children aged 7-17 by 1.85 times, and parental depression by 1.99 times. This relationship gains added significance in light of findings by Chemerys et al. [31], who recorded an overall parental burnout rate of 58% in Ukraine – compared with 11% prior to the invasion and 3% in the global population – with the highest rate (70%) found in households where the primary caregiver is a grandmother, a pattern directly linked to the mass mobilisation of men and the large-scale emigration of women. Burnout manifests in reduced emotional availability and harsher parenting – conditions under which the child is deprived of the stable, predictable caregiving environment that constitutes the most important protective factor against trauma.

5. LONG-TERM SOCIAL CONSEQUENCES

The mental health consequences described do not exist in a social vacuum: they are woven inextricably into the fabric of the systemic social upheaval wrought by the conflict. Education, family income, social networks, cultural identity, and environmental stability form the developmental substrate for every child's psychological growth. When armed conflict destroys these foundations, it inflicts injuries that frequently defy psychiatric classification yet determine a person's life trajectory for decades to come.

5.1 Educational Disruption and Human Capital. As of early 2025, 1.2 million Ukrainian children were not receiving full in-person schooling [1]; three years of conflict have meant three years of interrupted, stress-saturated education – or no education at all [22]. Research drawing on WWII data [8, 32] allows us to anticipate concrete long-term costs: reduced educational attainment, lower wages, diminished entrepreneurial activity, and weakened social trust – playing out over a 15-25-year horizon. An additional burden is the phenomenon of 'double-schooling' affecting children abroad: simultaneous enrolment in host-country schools and Ukrainian online provision generates chronic cognitive overload and social isolation.

5.2 Material Deprivation. Material deprivation is not a remote 'social factor' but a direct activator of the chronic stress response. According to UNICEF [1], 85% of families with three or more children in Ukraine are currently living in poverty. Bürgin et al. [33] demonstrated that lack of basic resources is among the most consistent predictors of adverse mental health outcomes in children in conflict zones. For IDP families, this deprivation is often especially acute: loss of housing, assets, and employment in an unfamiliar city without social networks creates what researchers describe as 'psychological paralysis' – an inability to reconstitute normal daily life when all available resources are consumed by survival and grief.

5.3 Disruption of Social Networks and Identity. Armed conflict systematically severs social ties: friendship circles disintegrate as some children leave and others remain; family relationships are broken by displacement and death; schools and communities change. UNICEF [1] estimates that one in five children in Ukraine has lost a relative or friend since February 2022. Kalmijn [34] showed, on the basis of WWII research, that the destruction of social ties in childhood reduces the capacity for trusting interpersonal

relationships in adulthood and adversely affects parenting in the next generation. Sourander et al. [17] document identity fragmentation among displaced adolescents – a painful condition of being suspended between two environments, unable to belong fully to either.

5.4 Disruption of Parenthood as an Institution. The most systemic and durable social consequence of the conflict is the disruption of parenthood. The father is at the front or has been killed; the mother is exhausted and overburdened as the sole caregiver; the grandmother is frequently the only stable figure in the household, yet is herself traumatised and depleted. Chemerys et al. [31] recorded an overall caregiver burnout rate of 58% – against 11% pre-invasion and 3% globally – with the highest rate (70%) in households where a grandmother serves as the primary caregiver. Kalmijn [34] documented the long-term effects of this mechanism in the WWII context: traumatised parents formed less supportive, less emotionally available, and more hostile relationships with their children, generating insecure attachment that shaped the mental health and social functioning of the next generation. Given the scale of parental dysfunction in contemporary Ukraine, there is every reason to anticipate an analogous dynamic in the generation that follows.

The aggregate prognosis is sobering: elevated rates of mental disorder in affected cohorts will increase the burden on healthcare systems in 15-20 years; the educational and cognitive crisis will affect labour markets in 15-25 years; parental dysfunction and attachment disruption will amplify social problems among the children of this generation – that is, the intergenerational impact will be not only psychological but concretely measurable in social and economic terms. At the same time, resilience research consistently demonstrates that the presence of even a single stable caregiver, a supportive community, and access to targeted psychosocial support are powerful protective factors capable of substantially altering this trajectory [33]. The scale of the risk defines the scale of the required response – but does not determine its outcome in advance.

6. COMPARATIVE ANALYSIS: LESSONS FROM THE SECOND WORLD WAR

To assess the future dimensions of this crisis, it is necessary to engage seriously with the extensive literature on the long-term consequences of the Second World War – the only event in modern European history comparable in terms of the scale of displacement and childhood loss. Akbulut-Yuksel et al. [8] demonstrated that the psychological impact of childhood war trauma persists into late adulthood and old age, mediated by the collapse of healthcare infrastructure, prolonged family economic hardship, and the long-term erosion of social trust. Kesternich et al. [32], in a pan-European analysis, showed that the WWII experience reduced educational attainment, employment rates, and health outcomes throughout the working life of those affected. Rusby and Tasker [35], studying 870 British children evacuated during WWII, found that the highest rates of depression and clinical anxiety in adulthood occurred among those evacuated at the youngest ages who had experienced poor-quality foster care – a pattern that resonates directly with contemporary Ukrainian data, in which pre-schoolers show higher PTSD rates than school-age children [13] and the youngest children are especially vulnerable to the psychosocial effects of displacement [16].

The most compelling population-based evidence for intergenerational transmission remains the Finnish registry study by Santavirta et al. [9]: daughters of mothers evacuated to Sweden in childhood showed a twofold higher risk of psychiatric hospitalisation (HR=2.04; 95% CI: 1.04-4.01) and an almost fourfold higher risk of affective disorders (HR=4.68). Kalmijn [34] documented the mechanism of secondary traumatisation in Dutch WWII survivors: parents who had survived wartime trauma formed less supportive relationships with their children and displayed higher levels of aggression – an almost exact description of the mechanism now empirically confirmed for Ukraine [13, 16].

The concept of intergenerational trauma – the transmission of psychological consequences from parents to children who have had no direct traumatic experience of their own – is directly relevant here. Yehuda et al. [10] identified alterations in the methylation of the FKBP5 gene in the offspring of Holocaust survivors – constituting the first direct molecular evidence that traumatic experiences can leave a biological imprint on subsequent generations. Yehuda and Lehrner [36] caution, however, that the epigenetic hypothesis requires further verification; what is not in question are the psychosocial transmission mechanisms – disrupted parenting styles, shifts in value systems, and collective memory – which are well documented and practically inevitable in the absence of targeted intervention. Jawaid et al. [37] explicitly call for the study of these mechanisms in the specific Ukrainian context. The Deutsches Zentrum für Psychische Gesundheit [38] includes ‚refugees from Ukraine' among the groups for whom intergenerational trauma transmission has been empirically documented. It must also be recognised that Ukraine enters the current conflict bearing an existing stratum of collective trauma: the intergenerational consequences of the 1932-1933 Holodomor, WWII, and Soviet-era repressions – a 'foundational trauma' onto which new wounds are superimposed.

The systemic implication of the intergenerational perspective is stark: children who do not receive timely psychosocial support today are the potential parents of the next generation, with impaired parenting capacities of their own. This transforms investment in children's mental health from an acute-phase response into the prevention of a psychiatric crisis among the generation of the 2040s and 2050s – extending the significance of this issue far beyond the present emergency.

7. PSYCHOSOCIAL SUPPORT: TRAUMA-INFORMED APPROACH AS A SYSTEMIC NECESSITY

Understanding the nature and magnitude of these risks is a necessary but insufficient condition for an effective response. Such a response requires a conceptual foundation:

the trauma-informed approach (Trauma-Informed Care, TIC) – a systemic reorientation from 'what is wrong with you?' to 'what has happened to you?' – structured around the six principles established by SAMHSA: safety; trust and transparency; peer support; collaboration and mutuality; empowerment; and sensitivity to cultural, gender, and historical context. In the setting of armed conflict, these principles acquire concrete and urgent meaning. Safety must encompass not only physical protection but psychological security: a child seeking help must not be re-traumatised by questioning that forces a re-living of the most painful experiences. Trust is especially critical where authority figures have been discredited or are absent – where the father is at the front, the school is in a shelter, and the state feels unreliable. Cultural sensitivity is indispensable in a context where the social construction of the ‚strong man', the ‚resilient mother', and the ‚brave child' actively conflicts with the recognition of vulnerability and the legitimacy of seeking help.

Bürgin et al. [33] argue that only a multi-level, needs-based, and trauma-informed approach can provide an effective systemic response – one that differentiates between universal psychosocial support for all those affected; targeted interventions for those displaying symptoms; and specialised clinical care for children with diagnosed disorders. Shkoliar and Vus [39] emphasise that trauma-informed practice must permeate not only the work of clinical psychologists and psychiatrists but the daily activity of teachers, social workers, paediatricians, and institutional administrators. The authors also emphasise the need to 'localise' the approach: integrating evidence-based methods with traditional community practices that restore identity and social bonds. They argue that communities of practice – structured networks for peer exchange among practitioners – are a key mechanism for sustaining trauma-informed approaches in chronic conflict settings, where resources are persistently constrained and staff turnover is high. Yelizarova et al. [40], in a nationwide study (N=7,551 parents and caregivers, 2022-2025), found that teachers who systematically applied psychological support practices were more effective at preventing escalating anxiety in their pupils.

8. SYSTEM RESPONSE: ACHIEVEMENTS AND DEFICITS

The system's response to the crisis has demonstrated both considerable mobilisation capacity and serious structural limitations. UNICEF reports that in 2024, mental health and psychosocial support (MHPSS) services reached 760,000 children, adolescents, and caregivers in Ukraine. Coordinated MHPSS activities reached over 1.2 million people in 2023, with the introduction of telemedicine and mobile outreach teams demonstrating adaptive system-level resilience. Redlener et al. [41] describe an integrated psychosocial recovery camp model implemented in Lviv between September 2022 and July 2024, combining play and art therapy, music, breathing practices, PTSD risk screening, and specialist referral, delivered jointly to mothers and children. Pukhnyak et al. [42], in their evaluation of the 'Children and War' project of the International Charitable Foundation 'Caritas Ukraine', recorded significant positive changes in children's psychoemotional wellbeing and improved parental competence.

The Network of Resilience Centres, launched by government decree in January 2024, represents an important systemic step, although its full national rollout is ongoing. Yelizarova et al. [40] found that 12.0% of parents believed their child needed psychological support and 9.9% had sought help from a psychologist; however, even technically accessible services were frequently perceived as unreachable due to geographic distance, lack of trust, or unclear referral pathways.

The deficits are multidimensional and demand honest acknowledgement. Geographic: the children most severely affected by the conflict, in frontline areas, have the least access to support. Professional: the number of trained specialists in childhood traumatic stress remains critically inadequate relative to need. Temporal: most programmes address the acute phase; long-term supportive psychotherapy for children with established clinical diagnoses is not yet operating at scale. Informational: large numbers of families do not seek available help, either because they are unaware of it or because stigma acts as a prohibitive barrier [24]. Stigmatisation remains one of the principal barriers. Stigma around mental disorders is particularly pronounced in rural areas and in communities characterised by traditional gender norms. Addressing stigma requires sustained, targeted public education delivered through trusted intermediaries – paediatricians, school staff, local community leaders – and must be treated as a structural component of any effective support system, not an afterthought.

The situation of children deprived of parental care is particularly acute: despite policy commitments to deinstitutionalisation, the majority remain in residential settings rather than family care. Beyond the institutional failures described above, the deportees – children enrolled in Russian programmes of forced adoption and identity replacement – will require a lengthy, specialised, and culturally sensitive reintegration process for which the Ukrainian system is not yet prepared. The WWII literature suggests three enduring lessons. First, time is critical but not limiting: the psychological impact persists for decades, and support cannot be restricted to the acute phase; long-term investment in children's mental health yields measurable social returns through lower crime rates, higher productivity, and reduced healthcare burden 20-30 years hence [8]. Second, supporting caregivers is no less important than supporting children: effective help for a child is, first and foremost, help for the carer. Third, scaling up through trained non-specialist practitioners – teachers, nurses, general practitioners, social workers – is the only realistic path to reaching millions of affected people in conditions of specialist scarcity, provided that such scaling is grounded in trauma-informed principles and supplements rather than substitutes for specialised clinical care.

9. METHODOLOGICAL LIMITATIONS

A number of methodological limitations warrant acknowledgement. Most existing studies are cross-sectional in design, precluding causal inference. Samples are often convenience-based, systematically under-representing the most vulnerable children – those in the hardest-hit areas or in residential institutions. The use of heterogeneous measurement instruments complicates direct comparison across studies. These limitations underscore the urgent need for genuine longitudinal cohort designs, population-representative samples, and standardised measurement protocols. The comparison with WWII, for all its heuristic value, also requires interpretive caution: contemporary Ukraine is considerably more urbanised, technologically connected, and psychologically literate than mid-twentieth-century Europe. The chronic media exposure that characterises the current conflict represents a new and distinct form of secondary traumatisation for which the WWII literature provides no established analogue. As for intergenerational trauma: its psychosocial transmission mechanisms in the Ukrainian context are practically inevitable without targeted intervention. This shifts the question from ‚will it occur?' to ‚how can it be prevented?' – and makes the case for a trauma-informed approach not as a methodological preference but as a structural necessity.

CONCLUSIONS

The first and most fundamental conclusion of this review is simultaneously the most straightforward: the scale of the crisis is real and vast. Between 12.6% and 32% of affected children exhibit clinically significant PTSD symptoms, depending on their vulnerability category and degree of war exposure; the cumulative effect of prolonged conflict has been empirically confirmed; and the most vulnerable – the youngest children, those in residential institutions, and children from the families of mobilised personnel – are, paradoxically, among those receiving the least adequate support.

Second, the mental health crisis is inextricably bound up with a social one. A disrupted education system, material deprivation, the collapse of social networks, impaired parenting, and incomplete deinstitutionalisation together constitute a 'social trauma of a generation', whose concrete and measurable consequences will unfold over the next 15 to 30 years. Framing this not as an inevitable catastrophe but as a map of risks pointing to specific sites of necessary systemic intervention is essential for effective long-term planning.

Third, the experience of the Second World War is simultaneously a warning and a source of hope: a warning because psychological wounds persist across decades and are transmitted to subsequent generations; a source of hope because timely, systemic, and culturally embedded intervention has the demonstrated capacity to substantially alter this trajectory.

Fourth, a trauma-informed approach is not a methodological option among others but a prerequisite for any effective psychosocial support system. It must permeate every level – from specialist clinical practice to the school classroom, from social care to legislative drafting.

Fifth, significant research gaps remain. These include, most urgently: genuine longitudinal cohort studies; systematic data on children aged 0–5 and on orphans; primary research in frontline areas; and investigation of intergenerational transmission mechanisms in the specific Ukrainian cultural and historical context. These gaps must be addressed with the active participation of Ukrainian researchers – not as subjects of internationally led research agendas, but as primary agents in the scientific understanding of their own society's experience.

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REVIEW ARTICLE

Altered glutamatergic neurotransmission in the development and maintenance of PTSD

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ABSTRACT
Post-traumatic stress disorder (PTSD) is a multifactorial neuropsychiatric condition characterized by persistent disturbances in fear processing, emotional regulation, and memory. Increasing evidence indicates that dysregulation of glutamatergic neurotransmission plays a crucial role in both the development and persistence of PTSD. Glutamate, the primary excitatory neurotransmitter in the central nervous system (CNS), is essential for synaptic plasticity and the formation of trauma-related memories. The aim of this study was to provide an integrated and critical synthesis of current knowledge on glutamatergic dysfunction in PTSD, with particular emphasis on its neurobiological, clinical, and therapeutic implications. This article is a narrative review of the literature published between 2017 and 2025 was conducted. Databases such as PubMed, Scopus, and Web of Science were searched for experimental, clinical, and neuroimaging studies investigating glutamatergic signaling in PTSD. The reviewed studies indicate that chronic stress disrupts glutamate homeostasis, leading to increased extracellular glutamate concentrations and impaired synaptic clearance. These alterations result in excessive activation of ionotropic receptors, particularly N-methyl-D-aspartate (NMDA) receptors and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, promoting calcium influx and neuronal dysfunction. Such processes affect key brain regions, including the amygdala, hippocampus, and prefrontal cortex, contributing to impaired fear extinction and heightened emotional reactivity. Importantly, glutamatergic dysregulation does not occur in isolation but interacts dynamically with neuroinflammatory pathways, altered inhibitory transmission, and dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis. In conclusion, altered glutamatergic neurotransmission represents a central mechanism in PTSD pathophysiology. Its role in synaptic plasticity, neuronal vulnerability, and network dysregulation highlights its potential relevance as a diagnostic and therapeutic target. Future research should focus on integrating glutamate-related biomarkers with mechanism-based therapeutic interventions in order to improve diagnostic precision and treatment outcomes in PTSD.

KEYWORDS: AMPA receptor, excitotoxicity, glutamate, glutamatergic neurotransmission, HPA axis, NMDA receptor, neuroinflammation, post-traumatic stress disorder, synaptic plasticity

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INTRODUCTION

Post-traumatic stress disorder (PTSD) is a severe and chronic neuropsychiatric condition that develops following exposure to traumatic events such as life-threatening situations, violence, or disasters. It is characterized by intrusive recollections, avoidance behaviors, negative alterations in cognition and mood, and persistent hyperarousal. Although PTSD has traditionally been conceptualized within psychological and behavioral frameworks, increasing evidence supports its strong neurobiological basis, involving complex interactions between neurotransmitter systems, neuroendocrine regulation, immune signaling, and structural and functional brain alterations [1].

Among the neurochemical systems implicated in PTSD, particular attention has recently been directed toward glutamatergic neurotransmission. Glutamate is the primary excitatory neurotransmitter in the central nervous system (CNS) and plays a fundamental role in synaptic plasticity, learning, and memory. These processes are especially relevant in the context of PTSD, as the disorder is closely linked to maladaptive encoding, consolidation, and retrieval of traumatic memories. Dysregulation of glutamate signaling may therefore represent a key mechanism underlying both the development and persistence of PTSD symptoms [2].

The neurocircuitry of PTSD primarily involves interconnected brain regions responsible for emotional processing, threat detection, and cognitive control, including the amygdala, hippocampus, and prefrontal cortex. The amygdala is critically involved in fear conditioning and emotional salience and often exhibits hyperactivity in individuals with PTSD. In contrast, the hippocampus, which is essential for contextual memory and discrimination between safe and threatening environments, frequently shows reduced volume and impaired function. The prefrontal cortex, particularly the medial and ventromedial regions, is responsible for top-down regulation of emotional responses and fear extinction. Dysfunction within this network contributes to the persistence of fear responses and impaired extinction of traumatic memories [3, 4].

Glutamatergic neurotransmission regulates activity within these neural circuits. At the synaptic level, glutamate exerts its effects through ionotropic receptors, including N-methyl-D-aspartate (NMDA) receptors and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors,

as well as metabotropic glutamate receptors (mGluRs). NMDA receptors are particularly important for long-term potentiation, a cellular mechanism underlying learning and memory, whereas AMPA receptors mediate fast excitatory synaptic transmission. Proper balance and regulation of these receptor systems are essential for adaptive neural plasticity [5].

In PTSD, chronic stress and trauma exposure can disrupt glutamate homeostasis, leading to excessive synaptic release and impaired reuptake. Astrocytes, which play a key role in glutamate clearance via excitatory amino acid transporters (EAATs), may become dysfunctional under conditions of prolonged stress. As a result, elevated extracellular glutamate levels can lead to overstimulation of NMDA receptors, excessive calcium influx, and activation of intracellular pathways that promote neuronal damage. This process, known as excitotoxicity, contributes to synaptic loss, dendritic remodeling, and impaired connectivity within key brain regions [6, 7].

Moreover, glutamatergic dysfunction is closely linked to neuroinflammatory processes. Activation of microglia, the resident immune cells of the CNS, can influence glutamate signaling by releasing pro-inflammatory cytokines and modulating synaptic transmission. Inflammatory mediators such as interleukin-1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) have been shown to affect glutamate receptor expression and function, further exacerbating synaptic dysregulation. This bidirectional interaction between glutamatergic and immune systems represents an important component of PTSD pathophysiology [8, 9].

Another critical aspect is the interaction between glutamate and the hypothalamic–pituitary–adrenal (HPA) axis, which regulates the stress response. Dysregulation of the HPA axis in PTSD, often reflected by altered cortisol levels, may influence glutamate release and receptor sensitivity. Chronic exposure to glucocorticoids can impair synaptic plasticity and promote neurotoxicity, particularly in the hippocampus, thereby contributing to cognitive deficits and memory disturbances observed in PTSD [10, 11].

Recent advances in neuroimaging and molecular research have provided further insights into glutamatergic abnormalities in PTSD. Proton magnetic resonance spectroscopy (^{1}H-MRS) studies have demonstrated altered glutamate concentrations in specific brain regions, while preclinical models have highlighted changes in receptor expression and synaptic function. These findings support the hypothesis that glutamatergic dysregulation is not only a consequence of trauma but may also participate in maintaining pathological stress responses [12].

Importantly, the glutamatergic system has emerged as a promising target for novel therapeutic interventions. Pharmacological agents that modulate NMDA receptor activity, such as ketamine, have shown rapid antidepressant and anti-PTSD effects in clinical studies. Similarly, compounds targeting AMPA receptors or mGluRs are being investigated for their potential to restore synaptic balance and improve clinical outcomes. These approaches represent a shift toward mechanism-based treatments that address the underlying neurobiology of PTSD rather than solely managing symptoms [13, 14].

Taken together, current evidence indicates that altered glutamatergic neurotransmission should be considered not only as a molecular abnormality but also as a mechanism linking traumatic memory, neuroinflammation, endocrine stress responses, and structural brain changes. This integrated perspective provides the rationale for examining glutamatergic dysregulation as a central pathway in the development and maintenance of PTSD.

AIM

The aim of this study was to provide an integrated overview of current knowledge on glutamatergic neurotransmission alterations in PTSD, focusing on underlying neurobiological mechanisms, their role in synaptic plasticity and trauma-related memory, interactions with neuroinflammation and HPA axis dysregulation, as well as their relevance for biomarker identification and glutamate-targeted therapeutic strategies.

REVIEW

GLUTAMATERGIC SYSTEM – NEUROBIOLOGICAL FOUNDATIONS

Glutamate is the principal excitatory neurotransmitter in the CNS and plays a fundamental role in synaptic transmission, plasticity, and neuronal communication. It is primarily synthesized in presynaptic terminals from glutamine through the action of phosphate-activated glutaminase. Additionally, glutamate can be generated from glucose via the tricarboxylic acid cycle, linking neuronal activity with cellular energy metabolism [15, 16].

Following synthesis, glutamate is packaged into synaptic vesicles by vesicular glutamate transporters (VGLUTs) and released into the synaptic cleft in response to neuronal depolarization and calcium influx. Once released, glutamate binds to ionotropic and metabotropic receptors on postsynaptic neurons, mediating fast excitatory transmission and activating intracellular signaling pathways involved in synaptic plasticity [17, 18].

To maintain neurotransmitter balance and prevent excessive stimulation, extracellular glutamate is rapidly cleared from the synaptic space. This process is mediated by EAATs, which are expressed on both neurons and glial cells. Among these, excitatory amino acid transporter 1 (EAAT1) and excitatory amino acid transporter 2 (EAAT2), predominantly localized on astrocytes, are responsible for the majority of glutamate uptake in the brain. A key mechanism regulating glutamate homeostasis is the glutamate–glutamine cycle, which involves a close metabolic interaction between neurons and astrocytes. After uptake by astrocytes, glutamate is converted into glutamine by the enzyme glutamine synthetase. Glutamine is then transported back to neurons, where it serves as a precursor for the resynthesis of glutamate, thereby sustaining neurotransmission [19, 20].

Astrocytes play a central role in regulating extracellular glutamate concentrations and protecting neurons from excitotoxic damage. Beyond neurotransmitter clearance,

they contribute to synaptic modulation, metabolic support, and maintenance of the extracellular environment, ensuring proper synaptic function and neuronal survival [21].

From the perspective of PTSD, these mechanisms are clinically important because chronic stress may weaken astrocytic glutamate clearance and disturb neuron–glia metabolic coupling. Consequently, glutamate homeostasis may shift from adaptive synaptic signaling toward prolonged extracellular accumulation, increasing the risk of excitotoxicity and maladaptive plasticity. Thus, glial regulation of glutamate should be interpreted as an active component of PTSD pathophysiology rather than as a passive supportive process.

GLUTAMATERGIC RECEPTORS

Glutamatergic neurotransmission is mediated by two main classes of receptors: ionotropic and metabotropic receptors, which differ in structure, signaling mechanisms, and functional roles within neural circuits. Ionotropic glutamate receptors are ligand-gated ion channels responsible for fast excitatory synaptic transmission. These include NMDA receptors, AMPA receptors, and kainate receptors. AMPA receptors mediate rapid depolarization through sodium influx and are essential for the initiation of excitatory postsynaptic potentials. Kainate receptors contribute to synaptic modulation and presynaptic regulation of neurotransmitter release, although their role is less prominent compared to other ionotropic subtypes [6, 22-24].

NMDA receptors exhibit unique biophysical properties, including voltage-dependent magnesium block and high calcium permeability. Activation of NMDA receptors requires both glutamate binding and sufficient postsynaptic depolarization, allowing them to function as molecular coincidence detectors. This feature is critical for activity-dependent synaptic plasticity, particularly long-term potentiation (LTP), which underlies learning and memory processes [25-27].

mGluRs are G protein-coupled receptors that modulate neuronal excitability and synaptic transmission through intracellular signaling pathways. They are divided into three groups based on structure and function. Group I receptors enhance excitatory transmission, whereas groups II and III generally exert inhibitory effects by reducing presynaptic glutamate release. These receptors play an important role in fine-tuning synaptic responses and maintaining network stability [28, 29].

NMDA receptors are especially important for synaptic plasticity and emotional memory formation. Through calcium-dependent signaling cascades, they regulate gene expression, dendritic remodeling, and strengthening of synaptic connections. In circuits involving the amygdala and hippocampus, NMDA receptor activity is essential for the encoding and consolidation of emotionally salient experiences. Dysregulation of these receptors may lead to impaired extinction of fear memories and persistence of maladaptive responses, which are characteristic features of stress-related disorders.

Overall, glutamatergic receptors coordinate rapid excitatory transmission with long-term synaptic modifications, forming a neurobiological basis for adaptive and maladaptive learning processes [30-32].

In PTSD, the functional significance of these receptors depends not only on their expression level but also on their regional distribution, receptor subunit composition, and interaction with stress-related mediators. Excessive NMDA receptor activation may promote pathological consolidation of traumatic memories, whereas impaired receptor-dependent plasticity in prefrontal circuits may reduce the capacity for fear extinction. This dual role helps explain why glutamatergic signaling may contribute both to the formation of intrusive traumatic memories and to the failure of adaptive recovery mechanisms.

The key neurobiological mechanisms underlying glutamatergic dysregulation in PTSD are summarized in Table 1.

GLUTAMATE AND TRAUMATIC MEMORY

Glutamatergic neurotransmission contributes to the formation, storage, and persistence of traumatic memories. Glutamate regulates synaptic plasticity within neural circuits involved in fear processing, particularly in the amygdala, hippocampus, and prefrontal cortex. These processes are essential for encoding emotionally salient experiences and contribute to the development of persistent trauma-related memory traces.

LTP and LTD represent key cellular mechanisms underlying synaptic plasticity. LTP involves a sustained increase in synaptic strength following high-frequency stimulation, primarily mediated by NMDA receptors and AMPA receptors. In contrast, LTD leads to a reduction in synaptic efficacy, contributing to synaptic remodeling and adaptation. The balance between these processes determines the strength and persistence of memory traces [33-35].

Memory formation involves distinct stages, including consolidation and reconsolidation. Consolidation refers to the stabilization of newly acquired information into long-term memory, a process dependent on glutamate receptor activation and intracellular signaling pathways. Reconsolidation occurs when previously stored memories are reactivated and temporarily destabilized, allowing for modification before being stored again. This dynamic process is particularly relevant in traumatic memory, as it contributes to the persistence and potential updating of fear-related memories [36-38].

Engram formation refers to the physical and functional representation of memory within specific neuronal ensembles. Glutamatergic signaling supports the strengthening of synaptic connections within these networks, facilitating the long-term storage of information. In traumatic contexts, enhanced glutamatergic activity may lead to overconsolidation of memory traces, making them resistant to extinction [39, 40].

The amygdala plays a critical role in emotional memory processing, particularly in fear conditioning. It integrates sensory and emotional information and modulates synaptic plasticity through glutamatergic mechanisms. Increased activity within the amygdala enhances memory encoding and promotes the persistence of emotionally charged experiences.

Table 1. Neurobiological mechanisms of glutamatergic dysregulation in PTSD

Domain / Mechanism	Neurobiological Description	Relevance in PTSD
Glutamatergic neurotransmission	Glutamate as the primary excitatory neurotransmitter; activation of NMDA receptors, AMPA receptors, and mGluRs	Central role in traumatic memory formation and synaptic plasticity
NMDA receptors	High Ca^{2+} permeability; involvement in LTP	Overactivation may promote persistent fear memory and impaired extinction
AMPA receptors	Mediate fast excitatory synaptic transmission	Regulation of synaptic strength and network remodeling
mGluRs	Modulate presynaptic glutamate release and neuronal excitability	Dysregulation may disturb excitation-inhibition balance
Astrocytes	Responsible for glutamate uptake from the synaptic cleft through EAAT1/EAAT2	Dysfunction may increase extracellular glutamate and excitotoxicity
Glutamate–glutamine cycle	Recycling between neurons and astrocytes	Disruption may impair neurotransmitter homeostasis
Excitotoxicity	Excessive NMDA receptor activation leading to Ca^{2+} influx	Neuronal damage and synaptic loss
Synaptic plasticity	LTP and long-term depression (LTD)	Pathological consolidation of traumatic memories
Memory engram	Neural networks encoding memory traces	Overconsolidation of trauma-related memories
Interaction with HPA axis	Cortisol modulates glutamate release and receptor function	Chronic stress may promote synaptic dysfunction
Neuroinflammation	Cytokines modulate glutamate signaling	Feedback loop between inflammation and excitotoxicity

Source: Compiled by the authors of this study

In contrast, effective recovery from trauma requires not only the weakening of fear-related associations but also the formation of new safety memories. This process depends on coordinated glutamatergic plasticity in the hippocampus and prefrontal cortex. Therefore, PTSD may be understood as a disorder in which glutamate-dependent plasticity becomes biased toward the persistence of threat memories while mechanisms supporting extinction and contextual regulation remain insufficient.

This interpretation is important clinically because it suggests that glutamatergic dysfunction is not limited to excessive excitation. Rather, it reflects a maladaptive redistribution of plasticity across neural circuits: enhanced fear encoding within amygdala-centered networks and weakened regulatory plasticity within hippocampal–prefrontal pathways.

An integrated overview linking glutamatergic alterations with clinical manifestations, biomarkers, and therapeutic targets in PTSD is presented in Table 2.

GLUTAMATERGIC EXCITOTOXICITY

Glutamatergic excitotoxicity is a pathological process resulting from excessive stimulation of glutamate receptors, leading to neuronal injury and loss of cellular integrity. It represents a key mechanism linking altered neurotransmission with neurodegeneration in stress-related conditions [26].

A central feature of excitotoxicity is the overactivation of NMDA receptors, which are highly permeable to calcium ions. Under conditions of excessive glutamate release or impaired clearance, prolonged receptor activation results in sustained depolarization and disruption of ionic homeostasis. This leads to a significant influx of calcium ions into the postsynaptic neuron [25-27].

Elevated intracellular calcium concentration acts as a critical trigger for a cascade of neurotoxic processes. Calcium-dependent enzymes, including proteases, phospholipases, and endonucleases, become activated and contribute to structural damage of cellular components. These processes affect cytoskeletal elements, membrane lipids, and nuclear material, leading to progressive impairment of neuronal function.

Mitochondria play a central role in calcium buffering and energy production; however, excessive calcium uptake disrupts mitochondrial function. Mitochondrial membrane potential becomes destabilized, impairing oxidative phosphorylation and reducing adenosine triphosphate synthesis. In parallel, mitochondrial dysfunction enhances the generation of ROS, further amplifying oxidative damage and cellular stress [28, 29].

The combination of calcium overload and mitochondrial impairment leads to activation of intrinsic cell death pathways. Release of pro-apoptotic factors, such as cytochrome c, initiates a cascade of intracellular signaling events that culminate in programmed cell death. Apoptosis is characterized by chromatin condensation, DNA fragmentation, and eventual loss of neuronal viability [6].

In addition to direct cellular damage, excitotoxic processes disrupt synaptic connectivity and network function. Neuronal loss and dendritic retraction impair communication within neural circuits, particularly in regions involved in emotional regulation and memory processing.

Table 2. Integrated model of glutamatergic dysfunction in PTSD: from molecular changes to clinical implications

Level of Organization	Neurobiological Alterations	Clinical Manifestations	Representative Biomarkers	Therapeutic Implications
Molecular	Increased extracellular glutamate; NMDA receptor overactivation	Hyperarousal, anxiety, heightened stress reactivity	Glutamate levels measured by ^{1}H-MRS; pro-inflammatory cytokines	NMDA receptor modulation
Cellular	Intracellular Ca^{2+} overload; oxidative stress; mitochondrial dysfunction	Cognitive impairment, neuronal vulnerability	Reactive oxygen species (ROS); mitochondrial markers	Neuroprotective and antioxidant strategies
Synaptic	Dysregulated LTP/LTD	Persistent traumatic memory traces, impaired fear extinction	Synaptic proteins such as postsynaptic density protein 95 (PSD-95) and synaptophysin	AMPA receptor modulation, synaptic plasticity enhancement
Neural circuit	Functional dysconnectivity within amygdala--hippocampus-prefrontal cortex networks	Intrusive memories, emotional dysregulation, impaired executive control	Functional neuroimaging and spectroscopy	Combined pharmacotherapy and psychotherapy
Neuroendocrine	Dysregulation of the HPA axis	Chronic stress response, impaired stress adaptation	Baseline and stress-induced cortisol levels	Stress-targeted interventions
Neuroimmune	Microglial activation; increased pro-inflammatory cytokines	Neuroinflammation--associated cognitive and emotional disturbances	Cytokine profiles; inflammasome components such as NOD-like receptor family pyrin domain-containing 3 (NLRP3)	Anti-inflammatory and immunomodulatory therapies
Structural	Reduced hippocampal volume; amygdala hyperactivity; prefrontal cortex alterations	Memory deficits, exaggerated fear response, impaired emotional regulation	Structural magnetic resonance imaging (MRI)	Neurorehabilitation and plasticity-enhancing interventions
System-level	Imbalance between excitatory and inhibitory neurotransmission	Network hyperexcitability, emotional instability	Indirect neurochemical markers	Restoration of excitation-inhibition balance

Source: Compiled by the authors of this study

In PTSD, excitotoxicity should be considered a mechanism that links repeated stress exposure with long-term alterations in brain structure and function. However, it is important to note that excitotoxic damage is unlikely to occur uniformly across the brain. Regions with high plasticity and high sensitivity to stress hormones, such as the hippocampus and prefrontal cortex, may be particularly vulnerable. At the same time, amygdala-centered circuits may show functional hyperreactivity rather than simple structural decline. This regional specificity may explain the coexistence of impaired contextual memory, reduced executive control, and exaggerated fear responses.

Overall, glutamatergic excitotoxicity represents a critical link between neurotransmitter imbalance and neurodegeneration. Its involvement in calcium dysregulation, mitochondrial dysfunction, and activation of apoptotic pathways underscores its importance in the progression of neuronal damage and functional brain alterations [30, 32].

STRUCTURAL AND FUNCTIONAL BRAIN CHANGES

Chronic stress and trauma exposure are associated with significant structural and functional alterations in key brain regions involved in emotional processing, memory, and cognitive control. These changes primarily affect the hippocampus, prefrontal cortex, and amygdala, forming a dysfunctional neural network underlying persistent stress responses [3].

The hippocampus plays a critical role in contextual memory formation and the integration of spatial and temporal information. Prolonged exposure to stress hormones, particularly glucocorticoids, contributes to hippocampal atrophy through mechanisms involving reduced neurogenesis, dendritic retraction, and synaptic loss. Structural changes in this region are associated with impaired contextual memory, leading to difficulties in distinguishing between safe and threatening environments. This dysfunction contributes to inappropriate activation of fear responses in non-threatening situations.

The prefrontal cortex, especially its medial and ventromedial regions, is responsible for executive functions, including cognitive control, decision-making, and regulation of emotional responses. Structural alterations in this region include reduced gray matter volume and disrupted synaptic connectivity. Functionally, these changes result in impaired top-down regulation of subcortical structures, particularly the amygdala. As a consequence, individuals exhibit deficits in cognitive flexibility, attention regulation, and inhibitory control, which contribute to the persistence of maladaptive behavioral patterns [3, 4].

The amygdala is a central structure involved in emotional processing, threat detection, and fear conditioning. In contrast to the hippocampus and prefrontal cortex, the amygdala often demonstrates increased activity and, in some cases, structural enlargement. Heightened amygdala responsiveness leads to exaggerated emotional reactivity and enhanced encoding of fear-related stimuli. This hyperactivity is closely linked to persistent anxiety, hypervigilance, and intrusive recollections.

The interaction between these regions is essential for adaptive emotional regulation. Disruption of connectivity within this network results in an imbalance between bottom-up emotional processing and top-down cognitive control. Reduced inhibitory influence of the prefrontal cortex over the amygdala, combined with impaired contextual processing in the hippocampus, promotes sustained activation of fear circuits [3, 4].

Glutamatergic dysregulation provides a plausible molecular explanation for these circuit-level abnormalities. Excessive excitatory signaling may strengthen amygdala-dependent fear associations, while stress-related synaptic loss in the hippocampus and prefrontal cortex may weaken contextual discrimination and emotional inhibition. Therefore, the structural and functional changes observed in PTSD may reflect the cumulative impact of altered glutamate signaling on network organization.

Overall, structural and functional brain changes contribute to persistent alterations in memory, emotion, and cognition. These neurobiological adaptations play a central role in maintaining maladaptive responses to stress and trauma.

INTERACTIONS WITH OTHER SYSTEMS

Glutamatergic neurotransmission operates within a complex network of interacting biological systems, including the HPA axis, inhibitory gamma-aminobutyric acid (GABA) signaling, and neuroimmune pathways. These interactions are essential for maintaining neural homeostasis but become dysregulated under conditions of chronic stress.

The HPA axis is a central regulator of the stress response. Activation of this system leads to the release of glucocorticoids, primarily cortisol, which modulate neuronal activity and synaptic function. Cortisol influences glutamate release, receptor expression, and synaptic plasticity. Prolonged elevation of glucocorticoid levels may enhance glutamate release and impair its reuptake, contributing to excitatory imbalance and increased neuronal vulnerability, particularly in the hippocampus and prefrontal cortex [1, 4, 9].

The GABA system represents the main inhibitory neurotransmitter system in the CNS. A functional balance between glutamatergic excitation and GABA-mediated inhibition is necessary for stable neuronal activity. Disruption of this balance, often characterized by reduced inhibitory signaling, leads to excessive excitatory transmission. This imbalance may facilitate hyperexcitability within neural circuits involved in fear processing and emotional regulation, contributing to persistent stress responses.

Neuroinflammatory processes play an important role in modulating glutamatergic neurotransmission. Activation of microglia, the resident immune cells of the CNS, occurs in response to stress and cellular damage. Activated microglia release pro-inflammatory cytokines, including IL-1β, interleukin-6 (IL-6), and TNF-α [38]. These signaling molecules influence glutamate metabolism by altering transporter function, receptor sensitivity, and synaptic signaling. Cytokines can increase extracellular glutamate levels by reducing its uptake and enhancing its release, thereby promoting excitatory activity. At the same time, inflammatory mediators may affect the expression and function of glutamate receptors, further contributing to synaptic dysregulation.

These interactions suggest that glutamatergic abnormalities in PTSD should not be interpreted as an isolated neurotransmitter disturbance. Instead, they appear to emerge from reciprocal interactions between stress hormones, inhibitory control, glial activation, and immune signaling. Such a model helps explain why PTSD is associated with heterogeneous clinical symptoms, including hyperarousal, intrusive memories, sleep disturbances, cognitive impairment, and affective dysregulation.

Overall, the interplay between the HPA axis, inhibitory GABA neurotransmission, and neuroinflammatory processes significantly influences glutamatergic signaling. Disruption of these interactions contributes to altered synaptic function, increased neuronal excitability, and impaired regulation of emotional and cognitive processes [11, 14].

GLUTAMATERGIC BIOMARKERS IN PTSD

Alterations in glutamatergic neurotransmission have prompted increasing interest in identifying reliable biomarkers that reflect underlying neurobiological changes in post-traumatic stress disorder. These biomarkers may support diagnosis, characterize disease severity, and provide insight into treatment response [8, 13, 15].

One of the most widely studied approaches involves the assessment of glutamate concentrations in the brain using ^{1}H-MRS. This non-invasive imaging technique enables in vivo quantification of glutamate and related metabolites in specific brain regions. Studies have reported region-dependent alterations in glutamate levels, particularly in the prefrontal cortex, hippocampus, and anterior cingulate cortex. These changes are associated with disturbances in synaptic activity and may reflect impaired excitatory-inhibitory balance within neural circuits involved in emotional regulation and memory processing.

In addition to neurochemical measurements, synaptic markers provide valuable information on structural and functional integrity of neuronal connections. Proteins associated with synaptic density, vesicular release, and receptor function may serve as indirect indicators of glutamatergic activity. Alterations in synaptic protein expression can reflect changes in synaptic plasticity, dendritic architecture, and connectivity, which are commonly observed in stress-related conditions [28, 31, 35].

NMDA receptors have also been proposed as potential diagnostic and functional biomarkers. Due to their central role in synaptic plasticity and calcium signaling, changes

in NMDA receptor expression or function may indicate disrupted neural processing. Functional abnormalities in these receptors may contribute to impaired learning, altered memory consolidation, and reduced capacity for fear extinction. Imaging and pharmacological studies suggest that NMDA receptor-related mechanisms are closely linked to symptom severity and treatment responsiveness.

Furthermore, glutamatergic biomarkers may interact with other biological systems, including neuroinflammatory pathways and neuroendocrine regulation. This integrative perspective supports the concept that glutamate-related measures should be interpreted within a broader biological context.

However, the clinical use of glutamatergic biomarkers remains limited by several methodological challenges. Glutamate concentrations may vary depending on brain region, illness stage, medication status, comorbid depression or substance use, and technical parameters of imaging protocols. Therefore, a single glutamate-related marker is unlikely to provide sufficient diagnostic specificity. A more realistic approach may involve multimodal biomarker panels combining spectroscopy, inflammatory markers, endocrine measures, and clinical phenotyping.

Overall, glutamatergic biomarkers represent a promising area of research in PTSD. Their potential to reflect synaptic function, neuronal integrity, and circuit-level alterations may contribute to the development of more precise diagnostic tools and individualized therapeutic strategies.

THERAPEUTIC IMPLICATIONS

Advances in understanding glutamatergic dysregulation have opened new directions for targeted interventions in post-traumatic stress disorder. Modulation of glutamate signaling is increasingly recognized as a promising approach for restoring synaptic function and improving clinical outcomes.

NMDA receptor antagonists, such as ketamine, have demonstrated rapid and sustained effects on mood and stress-related symptoms. By transiently blocking NMDA receptors, ketamine enhances downstream synaptic plasticity through increased glutamate release and activation of AMPA receptors. This mechanism promotes synaptogenesis, strengthens neural connectivity, and may facilitate the reorganization of maladaptive memory networks.

Direct modulation of AMPA receptors represents another therapeutic strategy aimed at enhancing synaptic efficacy. Positive modulation of AMPA receptor activity supports LTP and improves communication within neural circuits responsible for cognitive control and emotional regulation. Such approaches may counteract synaptic deficits associated with chronic stress and trauma exposure [13, 32].

mGluRs have emerged as important pharmacological targets due to their regulatory role in synaptic transmission. Modulation of these receptors can influence presynaptic glutamate release and postsynaptic excitability. Depending on the receptor subtype, activation or inhibition may help restore excitatory–inhibitory balance and reduce neuronal hyperexcitability.

Combined therapeutic approaches integrating pharmacological and psychotherapeutic interventions offer additional benefits. Pharmacological agents targeting glutamatergic pathways may enhance neuroplasticity, creating a neurobiological window that supports the effectiveness of psychotherapy, particularly in fear extinction and cognitive restructuring. This synergy may improve long-term outcomes by addressing both biological and psychological aspects of the disorder.

The development of personalized medicine strategies represents an important future direction. Individual variability in glutamatergic function, receptor expression, and treatment response highlights the need for tailored interventions. Biomarker-guided approaches may enable more precise selection of therapies, optimizing efficacy and minimizing adverse effects [11, 17, 24, 32].

Despite these promising findings, glutamate-targeted therapies require careful clinical evaluation. PTSD is a heterogeneous disorder, and not all patients are likely to benefit equally from the same pharmacological intervention. Moreover, modulation of glutamatergic signaling may produce different effects depending on treatment timing, symptom profile, comorbidities, and concurrent psychotherapy. Therefore, future clinical trials should include biomarker-based stratification and long-term follow-up to determine which patients may benefit most from glutamatergic interventions.

Overall, targeting the glutamatergic system provides a mechanistically grounded framework for novel treatments, with the potential to improve therapeutic effectiveness and support long-term recovery.

DISCUSSION

The evidence summarized in this review indicates that glutamatergic dysregulation is a multidimensional mechanism involved in the development and maintenance of PTSD. Importantly, glutamate-related abnormalities should not be reduced to a simple model of increased excitatory activity. Rather, they involve disturbances in synaptic plasticity, receptor function, glial clearance, neuroimmune regulation, stress-axis signaling, and circuit-level connectivity [6, 30].

A key issue emerging from the literature is whether glutamatergic alterations represent a primary pathogenic factor or a secondary consequence of chronic stress exposure. Available findings suggest a bidirectional relationship. Trauma and chronic stress may disrupt glutamate homeostasis through glucocorticoid-mediated effects, astrocytic dysfunction, and inflammatory activation. At the same time, persistent glutamatergic imbalance may reinforce pathological neural circuits, promote traumatic memory consolidation, and impair extinction learning. This creates a self-perpetuating mechanism in which stress exposure and glutamatergic dysfunction mutually amplify each other [2, 36].

Another important aspect concerns the regional specificity of glutamatergic alterations. Increased excitatory signaling within the amygdala may support fear conditioning, hypervigilance, and exaggerated emotional reactivity.

In contrast, impaired glutamate-dependent plasticity in the hippocampus and prefrontal cortex may weaken contextual memory, cognitive control, and extinction learning. This regional imbalance may explain why PTSD is characterized by both excessive threat detection and insufficient regulatory control [3, 4].

The interaction between glutamatergic signaling and neuroinflammation represents a particularly important amplifying mechanism. Activated microglia and pro-inflammatory cytokines may impair glutamate uptake, modify receptor function, and increase extracellular glutamate concentrations. Conversely, excessive glutamatergic activity may promote cellular stress and further stimulate inflammatory pathways. This feedback loop provides a biologically plausible explanation for the persistence of neuronal vulnerability and emotional dysregulation in chronic PTSD [21, 29].

The reviewed findings also indicate that glutamatergic dysfunction may be relevant for understanding individual differences in PTSD course and treatment response. Patients with dominant hyperarousal, intrusive memories, or cognitive impairment may present different patterns of glutamate-related abnormalities. Therefore, future studies should move beyond group-level comparisons and focus on biologically defined patient subgroups. Such an approach may be especially useful for developing personalized therapeutic strategies [12, 13].

From a therapeutic perspective, the rapid clinical effects of NMDA receptor modulators, including ketamine, support the view that glutamatergic mechanisms are clinically relevant in stress-related disorders. However, these findings should be interpreted cautiously. The long-term safety, optimal dosing, durability of response, and interaction with psychotherapy remain areas requiring further investigation. Moreover, glutamate-targeted treatment should not be considered a replacement for trauma-focused psychotherapy but rather a potential adjunct capable of enhancing neuroplasticity and improving treatment responsiveness [26, 35].

Overall, the integration of molecular, neuroimaging, and clinical data supports the hypothesis that glutamatergic dysregulation is both a marker and a mechanism of PTSD. Its central role in synaptic plasticity, traumatic memory, excitotoxicity, neuroinflammation, and network dysfunction makes it a promising target for future research. Nevertheless, further longitudinal and multimodal studies are needed to clarify causal relationships and translate these findings into clinically applicable diagnostic and therapeutic tools [1, 40].

CONCLUSIONS

Alterations in glutamatergic neurotransmission represent a central component of the neurobiological mechanisms underlying post-traumatic stress disorder. Rather than acting as an isolated pathway, glutamatergic dysregulation integrates molecular, cellular, and network-level disturbances that contribute to persistent emotional and cognitive dysfunction. Excessive activation of glutamate receptors, particularly NMDA receptors, leads to calcium overload, mitochondrial dysfunction, and neuronal damage. These processes are closely associated with structural and functional alterations in the hippocampus, prefrontal cortex, and amygdala. The persistence of traumatic memories appears to be strongly linked to dysregulated synaptic plasticity and maladaptive stabilization of fear-related memory engrams, rather than solely to increased glutamate levels. Emerging evidence suggests that glutamatergic biomarkers, including regional glutamate concentrations and receptor-related changes, may provide valuable insights into disease mechanisms and support individualized diagnostic and therapeutic strategies. Future studies should focus on translating these findings into clinically applicable models by integrating glutamate-related biomarkers, neuroimaging data, inflammatory and endocrine markers, and clinical symptom profiles. Such an approach may facilitate the development of targeted, mechanism-based interventions and improve long-term outcomes in patients with PTSD.

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REVIEW ARTICLE

Training of nursing personnel for the public health system

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ABSTRACT

Aim: To analyse contemporary global approaches to training nursing personnel for the public health sector, focusing on the integration of competency-based, interprofessional, and practice-oriented models, and to evaluate their strategic relevance to the transformation of national health systems.

Materials and Methods: A systematic literature review and narrative synthesis were conducted using international scientific databases (PubMed/Medline, Scopus, Web of Science) covering 2010-2026, with an analytical focus on publications from 2021-2026. The search strategy was guided by Boolean logic and targeted MeSH terms. Of the 120 records initially identified, 35 core sources, including global strategic guidelines from the WHO and ICN, met the strict inclusion and exclusion criteria and were selected for final qualitative analysis.

Conclusions: Transitioning from traditional clinical education to integrated instructional models (CBE, IPE, and the ADDIE framework) is crucial for developing a resilient workforce. For transforming national systems, such as Ukraine's, managing this transition through adaptive, asynchronous e-learning under emergency conditions ensures the attainment of critical population-based competencies, with a focus on epidemiological surveillance and community-based preventive care.

KEYWORDS: preventative medicine, medical education, workforce, competencies

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INTRODUCTION

Nursing personnel are a foundational component of the contemporary public health system, delivering a substantial share of primary prevention, health education, screening, and health promotion services directly to the population [1, 2]. Under highly volatile global conditions, characterised by emerging pandemics, rapid demographic ageing, an escalating burden of chronic non-communicable diseases (NCDs), and widening health inequities, the strategic training of the nursing workforce has become unprecedentedly urgent [3]. Modern public health emergencies require a workforce capable of swift operational responses, systemic crisis mitigation, and flexible professional adaptation [4, 5].

The traditional, legacy model of nursing education, which remains predominantly clinically oriented and focused on secondary or tertiary hospital-based care, no longer aligns with the macro-level needs of the public health infrastructure [6, 7]. Today's public health nurse is expected to work as an autonomous professional with advanced analytical capabilities, epidemiological literacy, leadership skills, and the capacity to facilitate intersectoral and interprofessional collaboration [8, 9]. Consequently, there is an essential need to systematically evaluate current educational pathways, identify structural barriers, and establish integrated frameworks that bridge the gap between academic preparation and real-world health demands [10-12].

AIM

To analyse contemporary global approaches to training nursing personnel for the public health sector, focusing on the integration of competency-based, interprofessional, and practice-oriented models, and to evaluate their strategic relevance to the transformation of national health systems.

MATERIALS AND METHODS

To rigorously synthesise current educational approaches, the authors conducted a systematic review. The research involved a comprehensive evaluation of peer-reviewed literature, institutional reports, and global strategic guidelines published between 2010 and 2026, with a specialised analytical focus on high-impact literature from the past five years (2021-2026) to ensure maximal temporal relevance.

The authors compiled the information base through targeted queries on international scientific and medical databases, including PubMed/Medline, Scopus, and Web of Science. The search syntax used controlled Medical Subject Headings (MeSH) and Boolean operators, incorporating key terms such as: „public health nursing education", „competency-based nursing curricula", „interprofessional education in public health", „community-based nursing practice", and „ADDIE instructional design in healthcare education". The inclusion criteria required: (a) peer-reviewed publications examining theoretical, conceptual, or empirical models of nursing education tailored to public health; (b) official policy documents, action frameworks, and declarations issued by the World Health Organisation (WHO) and the International Council of Nurses (ICN); and (c) national regulatory instruments defining professional qualification standards. Exclusion criteria applied to non-peer-reviewed data, conference abstracts without full text, localised

clinical case studies lacking educational generalisability, and articles focusing solely on specialised hospital nursing sub-specialities.

The quantitative results of the selection process comprised a „primary" macro-array of 120 publications identified in the initial database search. After removing duplicates and applying strict thematic screening, the authors selected a final array of 35 core sources for in-depth narrative synthesis, structural analysis, and conceptual modelling.

ETHICS

The authors conducted this review study solely through secondary analysis of publicly available scientific data from peer-reviewed journals, official clinical guidelines, and institutional databases. It did not utilise any private, confidential, or identifiable patient data, involve any new clinical interventions or animal testing, or require the primary collection of human subject data. Consequently, formal approval from a local institutional review board or bioethics committee was not required.

The authors strictly adhered to the ethical principles of the Declaration of Helsinki of the World Medical Association and to international standards of publication ethics, including the comprehensive recommendations of the International Committee of Medical Journal Editors (ICMJE). This work contains no plagiarism, data fabrication, or falsification. All secondary sources of information, data points, and conceptual models used in this review have been rigorously and transparently cited and properly listed in the bibliographic block, thereby ensuring complete academic integrity.

AI STATEMENT

During the preparation of this work, the authors used Gemini (Google) solely for basic language quality checks and technical reference formatting.

Detailed description of the use of AI:

- formatting and mechanical alignment of the bibliographic list;
- proofreading for minor grammatical and spelling accuracy.

The artificial intelligence tool was not used to generate, rewrite, or synthesise any scientific text, data, or core ideas. The entire manuscript was conceived, written, and approved solely by the human authors, who accept full intellectual responsibility for its content.

REVIEW AND DISCUSSION

THE SHIFT TOWARD COMPETENCY-BASED PUBLIC HEALTH NURSING

The competency-based education (CBE) model is the contemporary international baseline for modernising healthcare curricula. Unlike traditional models that prioritise instructional hours, CBE focuses directly on the verifiable attainment of multidimensional learning outcomes. These outcomes encompass specific cognitive knowledge, practical psychomotor skills, professional attitudes, and ethical behaviours [13]. Within the public health domain, the CBE model organises professional training into three distinct tiers of competencies:

General Core Competencies: advanced critical thinking, ethical reasoning, transcultural communication, and digital data literacy.

Professional Core Competencies: Epidemiological risk modelling, community health assessment, biostatistical analysis, design of primary-prevention interventions, emergency preparedness, and health policy development.

Personal/Leadership Competencies: adaptive leadership, systemic management, emotional intelligence, and continuous professional development (CPD) [14, 15].

The authors deliberately mapped these competency streams against the Essential Public Health Functions (EPHFs), which encompass surveillance, health protection, disease prevention, and governance. Global architectural benchmarks – such as the World Health Organisation (WHO) nursing guidelines, the Centres for Disease Control and Prevention (CDC) core competency matrices, and the Quad Council competencies in the United States – demonstrate a high degree of structural granularity. These frameworks place strong emphasis on a population-based approach, shifting the locus of care from individual clinical diagnostics to community-wide macro-surveillance. Within statutory national health frameworks, establishing clearly defined professional boundaries and functions remains a structural necessity for personnel deployment. Comparative international analysis shows that aligning national qualification standards with global competency frameworks significantly shortens the adaptation period for nursing graduates entering the primary care sector [16-18].

This alignment is critical for Ukraine, where the nursing education model is currently hybrid. It dynamically balances traditional hospital-based training with the newly mandated functions under the Law of Ukraine ‚On the Public Health System' (2022) [5], requiring rapid capacity building in epidemiological surveillance and community-based prevention.

INTERPROFESSIONAL EDUCATION AND COLLABORATIVE PRACTICE (IPCP)

Public health challenges are complex and multifaceted, making it difficult for any single medical discipline to operate effectively in isolation. Interprofessional Education (IPE) is the primary mechanism for breaking down professional silos, enabling students from nursing, medicine, epidemiology, social work, and healthcare management to learn with, from, and about one another. The IPE model is structured around three functional layers:

1. Educational Layer: Implemented through joint academic modules, Problem-Based Learning (PBL) seminars, and high-fidelity simulation scenarios focused on outbreak containment or community health crises.
2. Competency Layer: Focused on mastering shared decision-making models, cross-disciplinary communication tools, team-based conflict resolution, and mutual professional respect.
3. Practical Layer: Delivered through immersive clinical rotations within multidisciplinary public health teams, joint field epidemiological investigations, and collaborative community health promotion initiatives [19].

4. Empirical evidence indicates that institutionalising IPE significantly reduces fragmentation in healthcare services, minimises communication errors, and optimises resource allocation during epidemiological emergencies. In highly developed healthcare systems – such as those in Canada, the United Kingdom, and the Netherlands – interprofessional core competencies are fully embedded in university accreditation frameworks, ensuring that graduating nurses are thoroughly prepared for Interprofessional Collaborative Practice (IPCP) [20-24].

PRACTICE-ORIENTED EDUCATION AND COMMUNITY HEALTH IMMERSIONS

The practice-oriented educational model is grounded in community-based learning. It marks a paradigm shift from a clinical, hospital-centric training environment to an expansive, population-focused practical setting. The authors base this model on sustainable academic-practice partnerships that link higher education institutions with primary health networks, non-governmental organisations, and regional public health centres. This framework comprises three core elements:

1. The Dual/Integrated Curricular Pathway: This approach ensures that theoretical university lectures are immediately followed by simulation labs and field practice in local communities.
2. The „Community-Hospital-Community" Cycle: This practical model prioritises the early initiation of home health visits. Under this model, students conduct community screenings and directly participate in immunising and educating populations.
3. The Lifelong Learning Continuum: This component integrates structured, continuous professional development (CPD) with specialised postgraduate certifications in public health and community care nursing [25-27].

This model has reached a high level of operational maturity in the Scandinavian countries, Canada, and the United Kingdom, where public health nurses enjoy a high degree of clinical autonomy, leading nurse-led community health centres and actively managing regional preventive programmes. By immersing students in real-world socioeconomic contexts, this model ensures that future nurses can identify and mitigate the social determinants of health affecting diverse populations [28-31].

INSTRUCTIONAL DESIGN AND ADAPTIVE E-LEARNING: THE ADDIE FRAMEWORK

To ensure that nursing curricula remain structurally coherent, evidence-based, and highly responsive to evolving health data, a systematic framework for educational programme planning is necessary. The ADDIE model (Analysis, Design, Development, Implementation, Evaluation) is the premier instructional design framework used globally for designing public health curricula. The linear and iterative stages of ADDIE proceed as follows:

1. Analysis: Involves conducting an exhaustive gap analysis between current population health indicators (e.g., the rise in NCDs and pandemic threats) and the existing competency levels of the nursing workforce, while assessing the educational needs of the target student audience.
2. Design: Focused on translating identified needs into clear, verifiable learning outcomes, structuring the competency framework, and selecting the most appropriate instructional methods (such as hybrid learning or IPE simulation).
3. Development: Dedicated to building tangible educational infrastructure, including interactive digital modules, validated clinical case studies, public health field protocols, and Learning Management System (LMS) content.
4. Implementation: Launching the finalised programme across higher education institutions or on continuous professional development platforms, with interprofessional training tracks.
5. Evaluation: Measuring the programme's educational efficacy using Key Performance Indicators (KPIs), analysing students' competency acquisition, and tracking graduates' long-term impact on community health indicators [32, 33].

The primary advantage of the ADDIE methodology is its exceptional structural flexibility. It enables academic institutions to rapidly update and pivot educational content in response to sudden healthcare disruptions or changes in institutional policies. Modern nursing education must be flexible and adaptable, particularly when facing disruptive events such as pandemics or security and military restrictions [3, 34].

In the specific case of Ukraine, systemic disruptive events – including continuous air-raid alerts, damage to critical infrastructure, and safety restrictions – have significantly accelerated reliance on the ADDIE framework. It serves as a vital methodological baseline for Ukrainian medical universities to design resilient, asynchronous E-learning environments that maintain high standards despite external disruptions [35].

SYNTHESIS, LIMITATIONS AND PERSPECTIVES

The results of this narrative synthesis indicate that no single educational model is sufficient when used in isolation. The most effective paradigm is a multi-dimensional, integrated educational model in which CBE establishes rigorous professional standards, IPE builds collaborative team capacity, practice-oriented training ensures an immediate connection to community needs, and the ADDIE framework provides the operational mechanism for systemic curriculum engineering and continuous evaluation [7, 8, 11].

For Ukraine, this integrated paradigm marks a strategic shift from the legacy model of the nurse as a purely clinical executor to that of an autonomous public health professional. Embedding interprofessional collaborative practice (IPCP) and structured instructional design within national curricula is essential to prepare the workforce for post-war healthcare reconstruction and emergency management. Modernising workforce policies requires embedding digital technologies, strengthening biostatistical and analytical education, and establishing independent, simulation-based and community-based training pathways [1, 2, 5].

POTENTIAL LIMITATIONS AND PERSPECTIVES

This review acknowledges limitations, as it relies on secondary data synthesis across diverse national education contexts with highly varied infrastructures. Future empirical research should focus on implementing longitudinal cohort studies to track long-term public health outcomes directly linked to integrated, nurse-led community health interventions and localised preventive frameworks.

CONCLUSIONS

1. Modern public health demands a clear shift away from isolated, hospital-centred nursing education. To build a resilient workforce, training must pivot towards population-wide thinking, primary prevention, data-driven epidemiology, and systemic crisis management.
2. Merging competency-based frameworks, interprofessional modules, and hands-on community practice is the most effective way to build workforce capacity. This practical combination ensures that graduates can immediately join complex, multidisciplinary primary care teams and collaborate effectively.
3. Relying on structured instructional design, particularly the ADDIE model, gives medical universities the operational agility they need. It enables educators to quickly adapt and rebuild curricula in response to evolving global health threats, technological advances, and institutional disruptions.
4. For Ukraine's healthcare system, adopting this integrated, ADDIE-managed approach is an urgent necessity amid ongoing wartime and emergency conditions. This methodology provides a practical blueprint for keeping nursing programmes resilient and ensuring that new graduates possess the precise analytical, digital, and preventive skills required to sustain public health on the ground.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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CASE STUDY

Glioblastoma mimicking acute stroke: A case report and diagnostic challenges

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ABSTRACT

Glioblastoma is the most common malignant primary brain tumor in adults. Gliomas infiltrate and destroy surrounding brain tissue, leading to neurological deficits such as speech and visual disturbances, hemiparesis, cranial nerve palsies, personality changes, and impaired consciousness. These symptoms may mimic acute stroke; however, 20-40% of patients initially diagnosed with stroke in the emergency department (ED) are later found not to have experienced an acute cerebrovascular event. Brain tumors account for approximately 5% of such stroke mimics. The aim of the study was to present a clinical case of brain tumor mimicking stroke.

We present a 71-year-old woman admitted with suspected stroke due to impaired verbal communication, confusion, disorientation, and transient mild right-sided weakness. Symptoms had progressed over one week. Brain computed tomography revealed a hypodense lesion in the medial left temporal lobe, and magnetic resonance imaging suggested a high-grade glioma infiltrating deep structures. Intravenous dexamethasone was initiated. Navigated biopsy confirmed glioblastoma on histopathological examination. The patient was not eligible for oncological treatment and underwent supportive care, including rehabilitation. At discharge, she was ambulatory with caregiver assistance and remained on long-term dexamethasone with proton pump inhibitor (PPI) prophylaxis. Despite advances in therapy, glioblastoma carries a poor prognosis, with approximately 10% five-year survival.

KEYWORDS: glioma, glioblastoma, brain tumors, stroke mimics

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INTRODUCTION

Gliomas represent the most common primary CNS tumors in adults and account for a substantial proportion of brain tumors across all age groups, comprising approximately 24.5% of cases in individuals aged 15-39 years, 23.1% in those aged 40-64 years, and 21.4% in older adults [1]. Gliomas originate from glial progenitor cells, and their pathogenesis is a multi-step process resulting from the accumulation of specific genetic and epigenetic changes. Mutations in the isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) genes are important in adult diffuse gliomas [2].

The best-documented risk factors for glioma development are exposure to ionizing radiation and the coexistence of genetic syndromes (such as Li-Fraumeni syndrome, neurofibromatosis types 1 and 2, Turcot syndrome, and Cowden syndrome). Gliomas are more common in men, and the risk increases with age. Pesticides, heavy metals, and certain viral infections, such as cytomegalovirus and Epstein-Barr virus, are also significant factors. Primary prevention may include a diet rich in antioxidants, vitamin D supplementation, and reduced exposure to air pollution [3, 4].

GBM can arise from neural stem cells (NSCs), oligodendrocyte progenitor cells (OPCs), and astrocytes, which transform into cancer cells due to the accumulation of somatic mutations. This process is associated with genetic factors, such as mutations in the TP53, phosphatase and tensin homolog (PTEN), and neurofibromin 1 (NF1) genes, and microenvironmental factors, such as interactions with growth factors and the vascularization of the subventricular zone [5].

Gliomas occur in various grades of malignancy, differing in aggressiveness, growth rate, and response to treatment. There are four grades (from 1 to 4), with higher-grade gliomas being more aggressive and more difficult to treat. Low-grade gliomas include grades 1 and 2, and high-grade gliomas include grades 3 and 4. GBM is always assigned the highest grade, grade 4. Another type is astrocytoma, which is classified as a high-grade tumor in adult grades 3 and 4. Oligodendroglioma is classified as a high-grade tumor when it exhibits malignancy characteristics corresponding to World Health Organization (WHO) grade 3 [2]. The most common locations for gliomas are the frontal (28.0%) and

temporal (27.5%) lobes, while locations in the parietal (12.1%) and occipital (3.8%) lobes are much less common. Temporal lobe gliomas are associated with the lowest survival rate from diagnosis, both at 12 months (41.8%) and 24 months (13.4%) [6]. The incidence of GBM in the right and left hemisphere is similar, but the prognosis may be poorer for left hemisphere tumors due to the higher incidence of unfavorable molecular alterations in this region, such as TP53 mutations, PTEN loss, or epidermal growth factor receptor (EGFR) amplification [7]. Clinical symptoms are related to the tumor's growth rate and anatomical location. Frontal lobe lesions may manifest as motor deficits, subtle cognitive and personality disorders, while temporal lobe gliomas may manifest as short-term memory deficits, seizures, visual field defects, and behavioral and affective changes [8]. Treatment for diffuse gliomas begins with a safe surgical resection, which aims to remove as much of the tumor as possible while preserving the patient's neurological function. For lower-grade gliomas (G2/G3), adjuvant radiotherapy and chemotherapy are used, while for patients with the most aggressive glioblastoma multiforme, radiochemotherapy with temozolomide is the standard treatment. In older patients or those in poor general condition, the choice between radiotherapy and chemotherapy is determined by the genetic profile of the tumor, which allows for a better adjustment of the treatment intensity [9].

CASE REPORT

A 71-year-old patient presented to the neurology emergency room due to deterioration in verbal communication, confusion, and disorientation, which had been preceded by a headache with a temporary loss of contact the day before. A similar episode had occurred approximately a year earlier. The family also reported a temporary, discrete right-sided weakness, which appeared during the current incident. Twelve years earlier, the woman had been treated for right breast cancer (mastectomy with subsequent chemotherapy and radiotherapy). She also had a history of hypertension, type 2 diabetes, and gout. She denied substance use or allergies. Her family history was unremarkable.

On admission, the patient was conscious, had limited verbal communication, and was disoriented both physically and mentally. A neurological examination revealed no other abnormalities.

A stroke was initially suspected. On admission, a computed tomography (CT) scan of the head was performed before and after the administration of iodinated contrast material, which revealed a hypodense area in the medial part of the left temporal lobe, encompassing the anterior hippocampus and partially the cortex (Fig. 1). Persistent vascular degenerative changes in the supratentorial region and cortical atrophy were also noted. Atherosclerotic plaques were visible in the intracranial segments of both internal carotid arteries. The CT scan image indicated a low probability of a stroke.

Next day, the diagnostic workup was expanded to include a contrast-enhanced magnetic resonance imaging (MRI) of the head to more precisely assess the previously detected abnormalities in the left temporal lobe (Fig. 2). The MRI was performed in the following sequences: T1-weighted, T2-weighted, diffusion-weighted imaging (DWI) with apparent diffusion coefficient (ADC) map, susceptibility-weighted angiography (SWAN), and fluid-attenuated inversion recovery (FLAIR). After intravenous administration of contrast material, T1-weighted images were obtained. An irregular area of increased signal intensity with poorly defined borders, approximately 5 cm in diameter, was revealed. The pathological infiltration involved the pole of the left temporal lobe, the hippocampus, the inferior frontal gyrus of the left frontal lobe, and the head of the caudate nucleus on the left side. It did not significantly enhance or cause increased restriction of water molecules in the extracellular space. It also did not cause a significant mass effect. After contrast agent administration, areas of hyperperfusion were noticeable beyond the area of altered signals. The entire image of altered signals showed moderate edema with effacement of the cerebral sulci in the affected area. The obtained image suggested a high-grade glial tumor.

The patient also underwent a Doppler ultrasound examination (UDP) of the intracerebral arteries, which revealed no flow disturbances in the assessed vessels. A blood test package was performed, including: complete blood count, activated partial thromboplastin time (APTT), prothrombin time (PT), international normalized ratio (INR), fibrinogen, albumin, glucose, creatinine, urea, sodium, potassium, C-reactive protein (CRP), and a lipid profile. Hyperglycemia was noted (serum glucose concentration was 243 mg/dL). Other parameters were within the reference range.

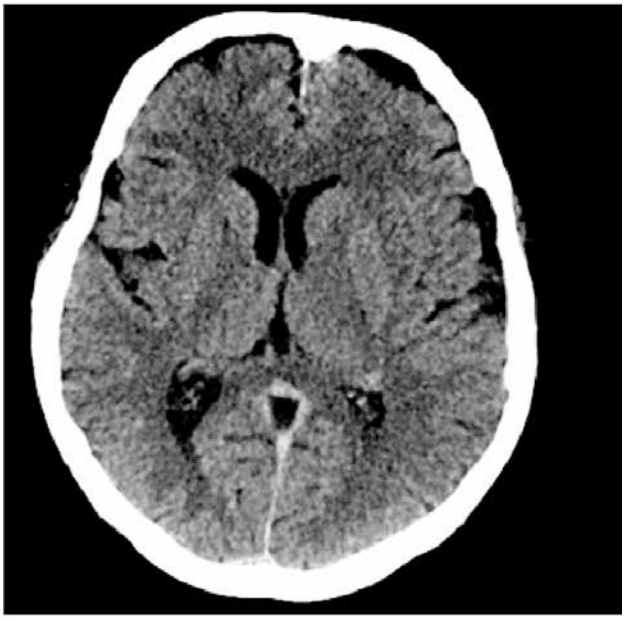

Fig. 1. Non-contrast CT scan. Hypodense area (42 x 32 x 26 mm) in the medial part of the left temporal lobe and hippocampus. No mass effect or evident tumoral mass at this stage (25.11.2025)

Source: Own materials

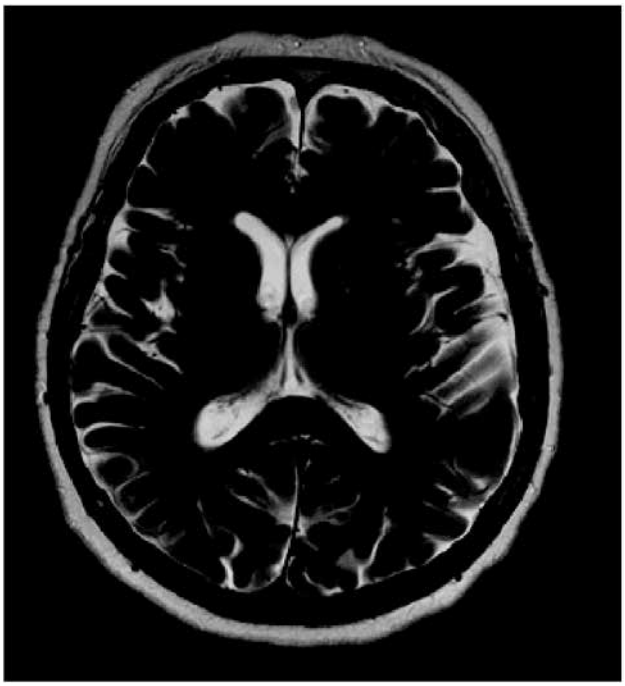

Fig. 2. MR T2/FLAIR and Perfusion. Extensive hyperintense area (approx. 5 cm) involving the left temporal pole, frontal lobe, and caudate nucleus. Noteworthy hyperperfusion relative cerebral blood volume (rCBV) 1.8-2.2, despite the lack of significant contrast enhancement (26.11.2025)

Source: Own materials

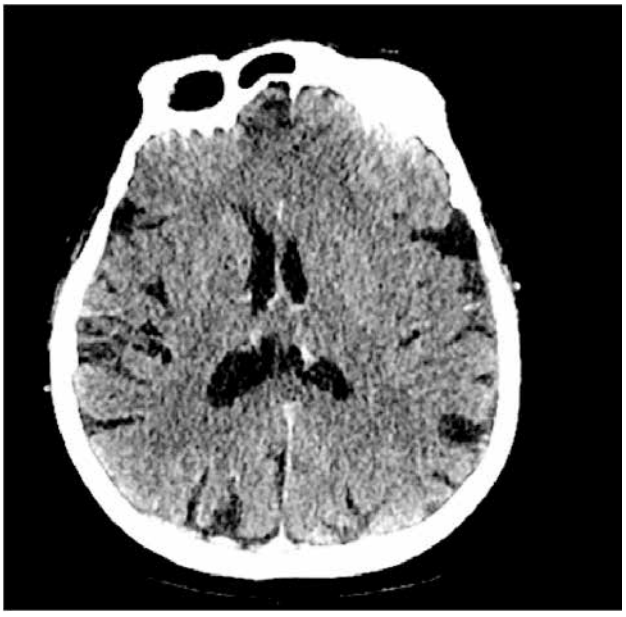

Fig. 3. Multiphase CT scan for neuronavigation. Hypodense lesion in the left cerebral hemisphere involving the medial temporal region and adjacent left frontal lobe, without visible post-contrast enhancement. Mild sulcal effacement and slight asymmetry of the supratentorial ventricular system were noted, with mild narrowing of the anterior part of the left lateral ventricle. No intracranial hemorrhage was seen (09.12.2025)

Source: Own materials

Due to the findings of cerebral edema, the patient was treated with intravenous steroids (dexamethasone).

During her hospital stay, the patient received a consultation with an internal medicine specialist. Aside from obesity, no abnormalities were found on the physical examination. The consulting neurosurgeon qualified the patient for a biopsy. Two weeks later, a multiphase CT scan for neuronavigation was performed, showing a hypodense lesion in the left cerebral hemisphere involving the medial temporal region and the adjacent part of the left frontal lobe, without visible post-contrast enhancement (Fig. 3).

Ultimately, the patient was diagnosed with a brain and central nervous system tumor of uncertain or unknown nature according to the International Classification of Diseases, 10th Revision (ICD-10: D43.0). Two months later, follow-up CT showed lesion progression with internal hyperdense hemorrhages, significant mass effect, an 8 mm midline shift, and compression of the left lateral ventricle (Fig. 4). Follow-up post-contrast MRI and susceptibility-weighted imaging (SWI) demonstrated strong peripheral enhancement, infiltration of the corpus callosum and right hemisphere, hemosiderin deposits, and progression of the midline shift to 10–11 mm (Fig. 5).

The patient was transferred to the neurosurgical department, where the procedure was performed and the specimen was sent for histopathological evaluation. Control CT after navigated biopsy showed post-biopsy changes with gas collections in the lesion area and stable mass effect with a 9–10 mm midline shift (Fig. 6). The resulting morphological pattern was consistent with diffuse astrocytic glioma. Immunohistochemical examination

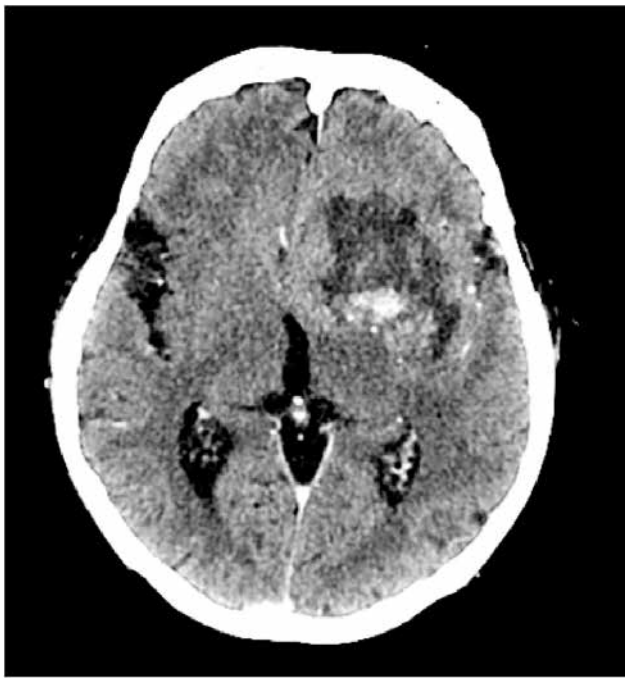

Fig. 4. CT scan. Lesion progression with internal hyperdense hemorrhages up to 70 Hounsfield units (HU). Significant mass effect with an 8 mm midline shift and compression of the left lateral ventricle (02.02.2026)

Source: Own materials

revealed positive expression of glial fibrillary acidic protein (GFAP) and S100 in the tumor cells. Molecular analysis of the tumor material revealed methylation of the O6-methylguanine-DNA methyltransferase (MGMT) gene promoter and amplification of the EGFR gene. No

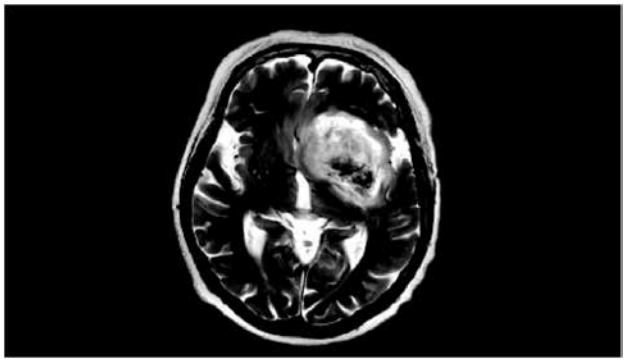

Fig. 5. Post-contrast and SWI MR images. Strong peripheral enhancement and infiltration of the corpus callosum and right hemisphere. SWI shows hemosiderin deposits; midline shift progressed to 10-11 mm (03.02.2026)

Source: Own materials

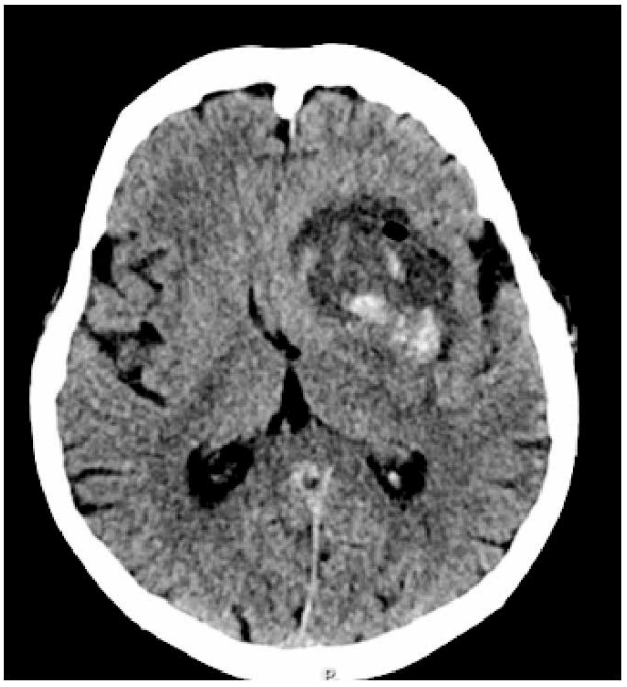

Fig. 6. Control CT after navigated (NAVI) biopsy. Post-biopsy changes with gas collections in the lesion area. Stable morphology and mass effect with a 9-10 mm midline shift (05.02.2026).

Source: Own materials

mutations in the IDH1 and IDH2 genes or co-deletions of chromosome 1p/19q fragments were detected. Based on the diagnostic workup, the final diagnosis was a primary malignant brain tumor - glioblastoma, not otherwise specified (NOS), CNS, WHO grade IV International Classification of Diseases for Oncology, 3rd Edition (ICD-O-3: M-9440/3, ICD-10: C71._). The patient was not eligible for oncological treatment and underwent supportive care, including rehabilitation. At discharge, she was ambulatory with caregiver assistance and remained on long-term dexamethasone with proton pump inhibitor (PPI) prophylaxis. Palliative care and follow-up in a neurology outpatient clinic were recommended

DISCUSSION

During a neurological emergency, rapid diagnosis or exclusion of suspected ischemic stroke is particularly important for the application of reperfusion therapy. According to the „2024 Guideline for the Primary Prevention of Stroke: A Guideline from the American Heart Association/ American Stroke Association," a rapid differential diagnosis is crucial in patients with acute neurological disorders. Identifying symptoms that mimic stroke is also essential. Such measures help prevent ineffective treatment and enable timely implementation of appropriate medical procedures. Additionally, they significantly reduce the likelihood of long-term neurological complications [10]. A significant proportion of patients hospitalized for suspected stroke experience so-called stroke mimics. These are conditions that present with symptoms similar to those of stroke. Brain tumors are among the potential causes of such stroke-like presentations [11, 12]. Gliomas, which are tumors of CNS, can be difficult to initially differentiate from an ischemic stroke. They manifest with the rapid development of focal neurological symptoms, including hemiparesis or aphasia. It is estimated that as many as 15-19% of patients with suspected stroke ultimately receive a different diagnosis [12, 13]. The first-line diagnostic method used to diagnose acute stroke is non-contrast computed tomography. However, it has limited sensitivity, particularly in the differential diagnosis of ischemic and neoplastic pathologies. Therefore, in cases of clinical uncertainty, magnetic resonance imaging (MRI) of the brain plays a key role [10, 14]. Using MRI, the nature of the lesion and its extent can be assessed in more detail, and the presence of infiltration can be ruled out or confirmed. This is crucial in the differential assessment of brain tumors [14, 15]. Furthermore, advanced magnetic resonance imaging techniques, such as perfusion MRI, enable precise determination of tumor vascularity. This is particularly important in differentiating high-grade gliomas from brain metastases. Gliomas are characterized by increased relative cerebral blood volume (rCBV) and infiltrative growth within the brain tissue. Metastases, on the other hand, are characterized by a significantly demarcated nature. In this patient, despite the initial suspicion of stroke, hyperperfusion on MRI (rCBV 1.8-2.2) played a significant role in the differential diagnosis. Based on current scientific reports, this indicates the presence of a high-grade tumor [14, 16]. In the described case, a positive history of breast cancer should raise diagnostic alertness. Brain metastases are common in this condition. It is estimated that they may be present in as many as 20-40% of patients with this type of cancer [17, 18, 19]. Breast cancer remains one of the most common types of cancer characterized by metastasis to CNS. Factors influencing its occurrence include the time since diagnosis of the underlying disease and the specific molecular subtype of the cancer [17, 19]. For this reason, in patients with a history of breast cancer, a detailed differential diagnosis should be performed if a fresh focal lesion appears. This requires distinguishing a primary brain tumor from a metastasis [15, 17].

Another important aspect to consider is the likelihood of a second primary malignancy in women with a history of breast cancer. This is crucial because population-based studies indicate an increased risk of subsequent malignancies in this group of patients. In the patient presented with a history of breast cancer, this possibility was raised during clinical management. Therefore, glioma was suggested as the second primary malignancy [20, 21].

Another key issue was the presence of hyperglycemia in laboratory tests, which required correction. This was caused by the introduction of necessary steroid therapy (dexamethasone), which was expected to reduce cerebral edema. This phenomenon is described in the medical literature as steroid-induced hyperglycemia. In this case, close observation and treatment modification are often necessary. Due to this phenomenon, the patient was switched from oral antidiabetic medications to insulin therapy [22].

The described case highlights the importance of comprehensive differential diagnosis and specialized neuroimaging techniques. Such procedures are essential to determine the cause of acute neurological symptoms and establish a correct diagnosis.

CONCLUSIONS

The presented case highlights the diagnostic difficulties encountered when distinguishing stroke from neoplastic lesions of the central nervous system, as brain tumors may closely mimic the clinical and radiological features of cerebrovascular events, leading to potential delays in establishing the correct diagnosis and initiating appropriate treatment.

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CONFLICT OF INTEREST
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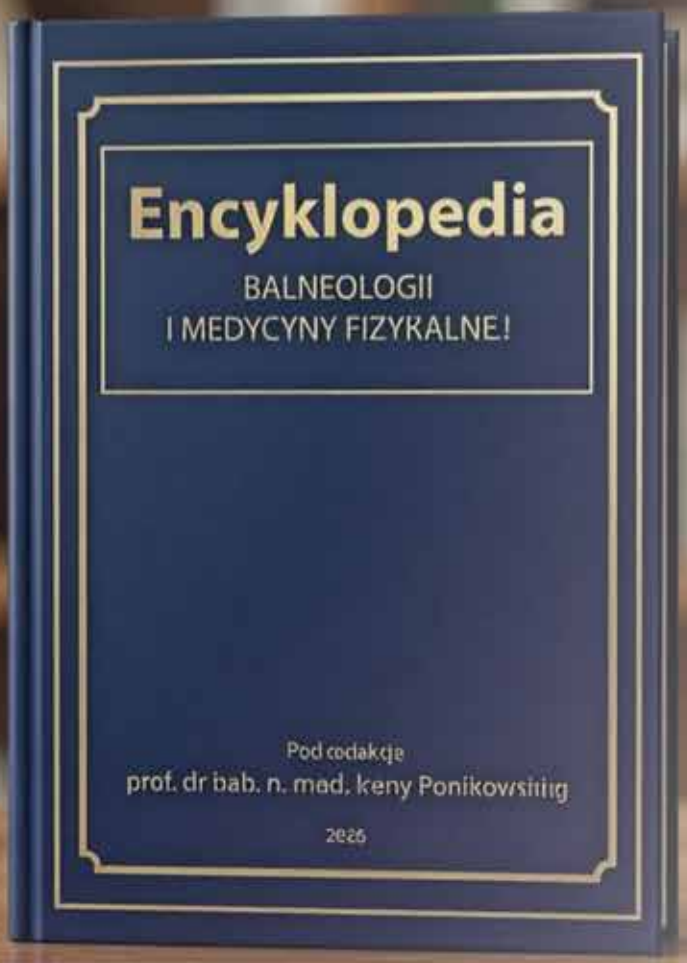

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